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STUDIES ON EUGENOL

*Celestine Varghese, Anita Yadav and A. P. Jadhav**

ABSTRACT

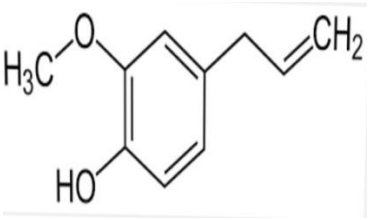
Today there is greater scope of herbal medicine in the whole world due to there less or negligible side effect. But the scarcity of data regarding the parameters and method employed for assessing the quality of medicines prevent lots of medicine to be formed using herbs and spices. The spices like cloves, tejpatta, nutmeg, tulsi all of them contain an active component eugenol.The spices are rich in volatile oil content. This can be monoterpene alcohol or phenyl propanoid derivative or their combination.

Eugenol is a volatile component which posses many other properties like they are antiseptic, antioxidant, anti -inflammatory as well as they are analgesic. For our research we studied eugenol by aid of thin layer chromatography.

INTRODUCTION

The spices form inseparable part of Indian food. They are dried parts of plants which have been used as diet component mainly to improve colour, aroma, and palatability. Some of the spices and herbs contain a bioactive component such as eugenol.

Eugenol is a phenyl propene, an allyl chain-substituted guaiacol. Eugenol is a member of the phenylpropanoids class of chemical compounds. It is a colourless to pale yellow, aromatic oily liquid 6extracted from certain essential oil especially from clove, nutmeg, tulsi and bay leaf.It is present in concentrations of 80–90% in clove bud oil and at 82–88% in clove leaf oil.



CHARACTERISTIC FEATURES

Chemical formula	C10H12O14
Acidity (pka)	10.19 at 25°C
Boiling point	254

PLANTS CONTAINING EUGENOL

Common Name	Scientific name	Family
Clove	*Syzygiumaromaticum*	Myrataceae
Worm wood	*Artemisiaabsinthium*	Asteraceae
Indian bay leaf	*Cinnamomumtamala*	Lauraceae
Nutmeg	*Myristicafargrans*	Myristicaceae
Sweet basil	*Ocimumbasilicum*	Labiateae
Holy basil	*Ocimumsanctum*	Labiateae

METHODOLOGY

- Definition -thin layer chromatography is a seperation technique where various components are seperated based on their affinity towards mobile phase and station phase

PLATE PREPARATION

- The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases. Because of its simplicity and speed TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products. Plates can be labeled before or after the chromatography process using a pencil or other implement that will not interfere or react with the process.
- To run a thin layer chromatography plate, the following procedure is carried out
- Using a capillary, a small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent it is allowed to completely evaporate off to prevent it from interfering with sample's interactions with the mobile phase in the next step. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber .This step is often repeated to ensure there is enough analyte at the starting spot on the plate to obtain a visible result. Different samples can be placed in a row of spots the same distance from the bottom edge, each of which will move in its own adjacent lane from its own starting point.
- A small amount of an appropriate solvent is poured into a glass beaker or any other suitable transparent container to a depth of less than 1 centimeter. A strip of filter paper is put into the chamber so that its bottom touches the solvent and the paper lies on the chamber wall and reaches almost to the top of the container. The container is closed with a cover glass or any other lid and is left for a few minutes to let the solvent vapors ascend the filter paper and saturate the air in the chamber.
- The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The solvent moves up the plate by capillary action meets the sample mixture and carries it up the plate The plate should be removed from the chamber before the solvent front reaches the top and air dry it
- And for detection of results the below listed method were used
- UV cabinet (254nm)
- Iodine chamber
- Visualising agents (vanillin and sulphuric acid)

DRUG USED IN RESEARCH

Fig. 1 Crude drug and Formulations used in research Clove, Cinnamon, Tulsi, Colgate pain out formulation, Jolly Tulsi drops 51.

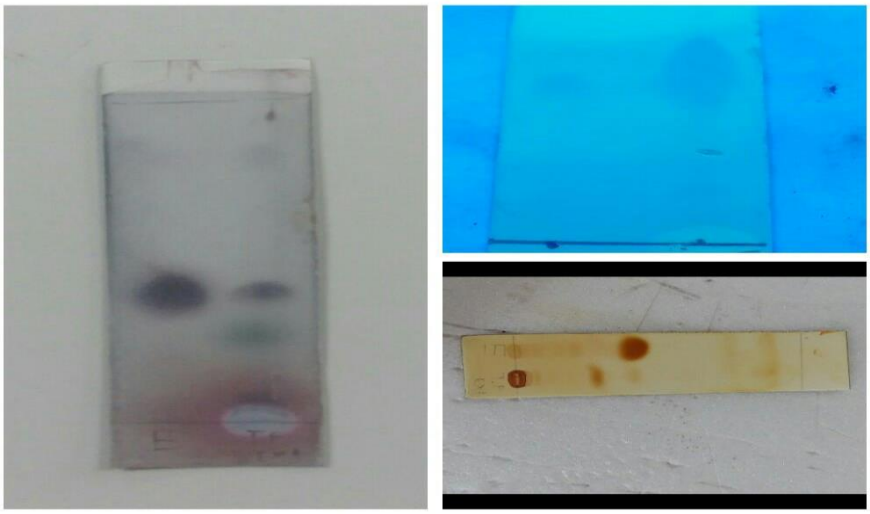


Fig. 2 Thin layer chromatography of methanolic extract of clove.

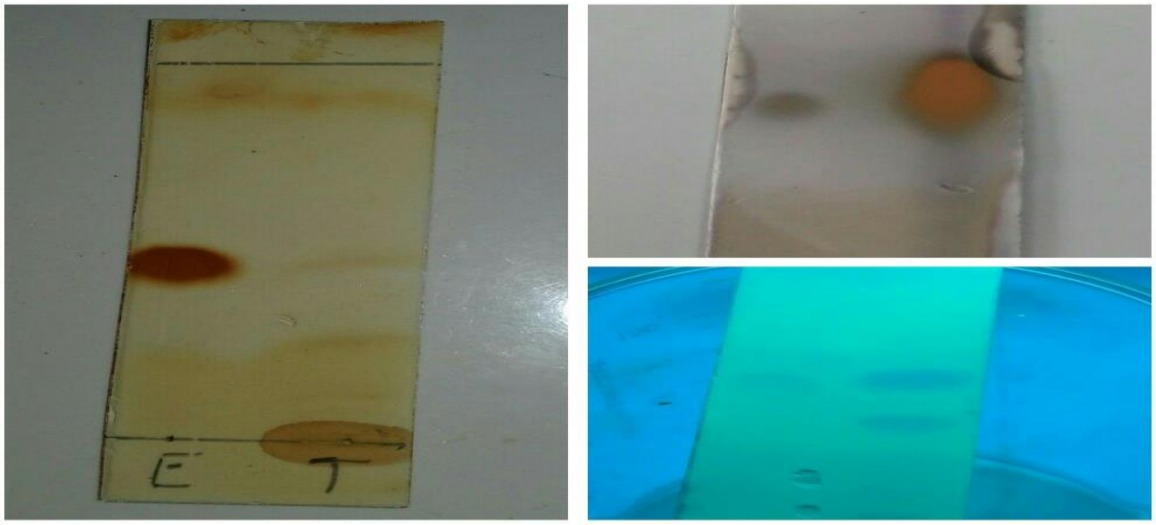


Fig. 3 Thin layer chromatography of methanolic extract of Tulsi

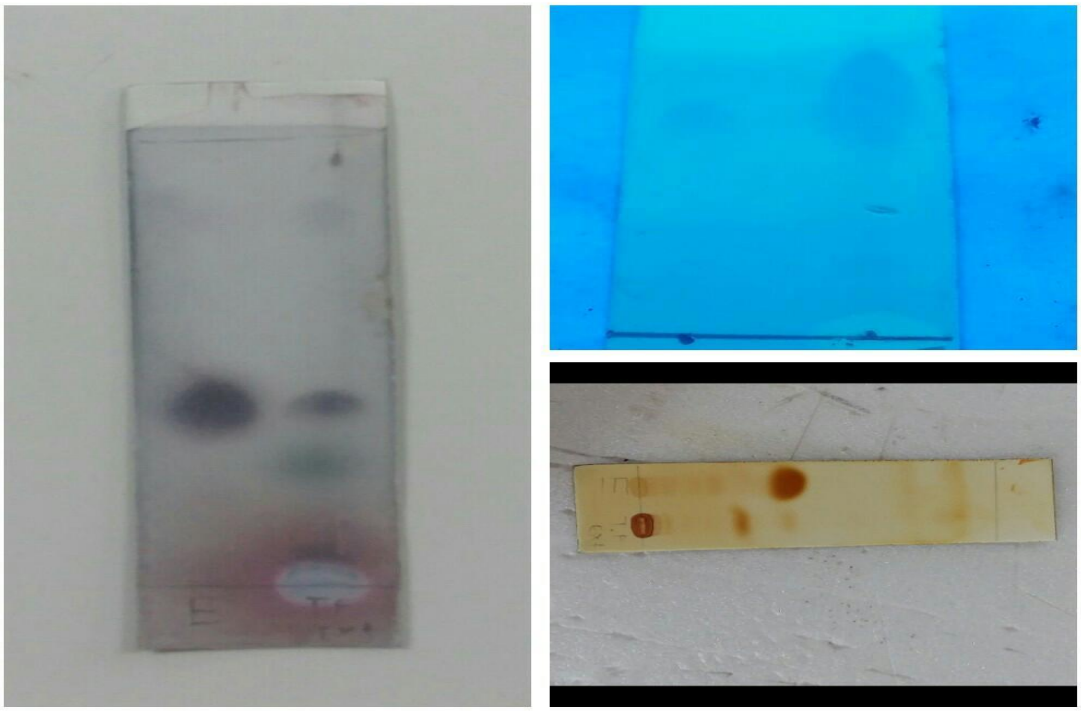


Fig. 4 Thin layer chromatography of methanolic extract of Jolly 51 tulsi drops formulation.

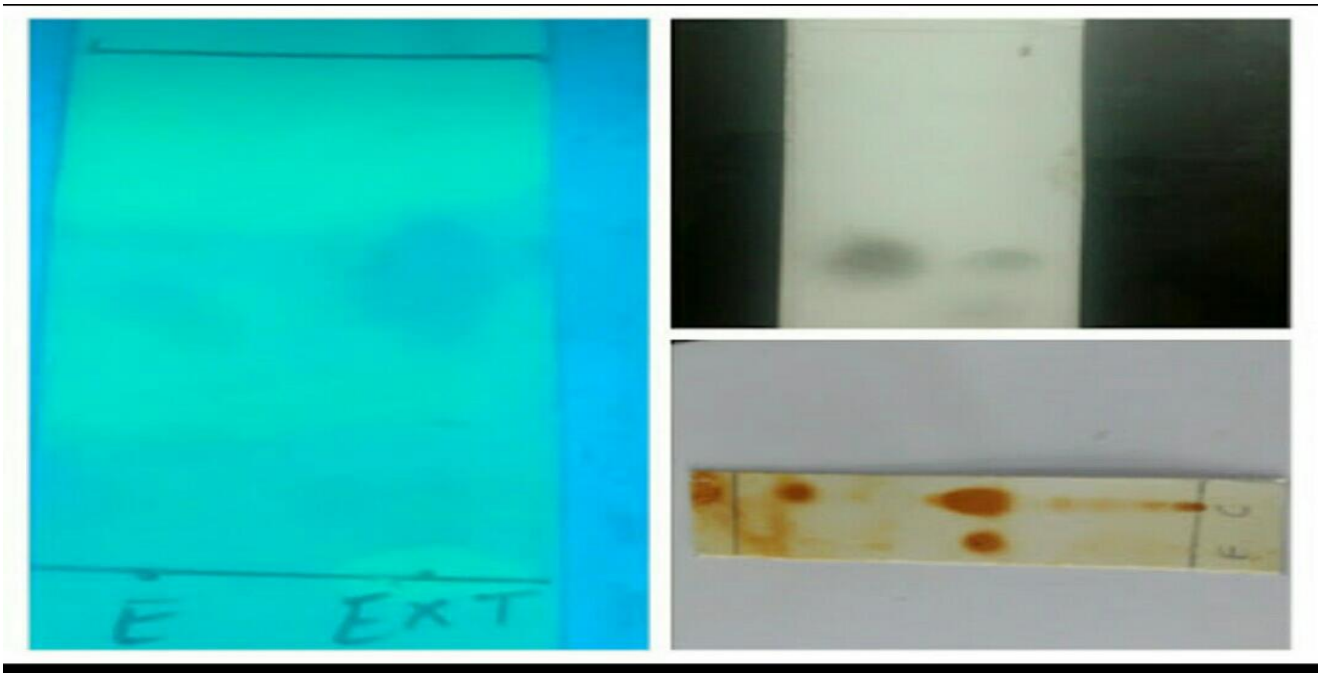


Fig 5. Thin layer chromatography of methanolic extract of clove.

Characteristic feature of drugs used in research

Name of drug	Family	Part used	Biological source
Clove	Myrtaceae	Flower bud	*Syzygium aromaticium*
Cinnamon	Lauraceae	Bark	*Cinnamomum zeylanicum*
Tulsi	Labiateae	Whole herb	*Ocumum sanctum*

CONCULSION

In present research work eugenol was successfully detected and identified
In methanol extract of clove, tulsi, cinnamon and in two formulation (Colgate pain out dental gel and jolly tulsi 51 drops).

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QUALITATIVE ASSESSMENT OF PROSTATE CANCER AWARENESS

*Swapnali Mahadik, Pranali Manjrekar and C.S. Ramaa**

ABSTRACT:
To appraise the awareness of prostate cancer and associated risk factors among men from population in Mumbai, India. A questionnaire-based survey was done. The questions were based on, general awareness about prostate cancer, diet and lifestyle habits, medical history, problems associated with prostate cancer and information about prostate screening. The survey was completed using 16 questions. We could get hold of 213 respondents. The age range was between 18 to 80 years. 36.15% (n=77) were aware about prostate cancer and 16.43% (n =35) had discussed prostate health with their family physician. None of them had ever undergone Prostate Specific Antigen testing and Digital Rectal Examination. The current study highlights the need for outreach programs to educate men about prostate cancer and inform them about prognosis and evaluation methods for diagnosing the disease. In addition strategies to improve the compliance of prostate screening programs are needed. Provision of proper guidance and motivation for the men to speak up.
KEY WORDS: Prostate cancer; Awareness; India; Response; Epidemiology; Screening; Risk factors; Population based cancer registries India; Future perspectives.

1. INTRODUCTION

1.1 What is Cancer?

Cancer is a disease of the cells, which are the body's basic building blocks. The body constantly makes new cells to help us grow, replace worn-out tissue and heal injuries. Normally, cells multiply and die in an orderly way. Sometimes cells don't grow, divide and die in the usual way. This may cause blood or lymph fluid in the body to become abnormal, or form a lump called a tumour.

1.2 The Prostate

The prostate is a small gland that sits below the bladder. The gland, which is about the size of a walnut, is part of the reproductive system. Only men have a prostate gland.

1.3 What is Prostate Cancer?

Prostate cancer develops when abnormal cells in the prostate gland start to grow more rapidly than normal cells, and in an uncontrolled way. Most prostate cancers grow more slowly than other types of cancer, although this is not always the case. Early (or localised) prostate cancer means cancer cells have grown but, as far as it is possible to tell, have not spread beyond the prostate. There are two stages of advanced prostate cancer. If the cancer grows and spreads outside the prostate gland into the seminal vesicles or nearby parts of the body, such as the bladder or rectum, it is called locally advanced prostate cancer. Metastatic prostate cancer is when the cancer has spread to distant parts of the body such as the lymph glands or bones.

1.4 Epidemiology of prostate cancer in India

We have reviewed the epidemiology (incidence, survival, and mortality) of prostate cancer across different PBCRs in India and highlighted certain important facts. Prostate is the second leading site of cancer among males in large Indian cities like Delhi, Kolkatta, Pune and Thi'puram, third leading site of cancer in cities like Bangalore and Mumbai and it is among the top ten leading sites of cancers in the rest of the PBRCs of India. The data shows that almost all regions of India are equally affected by this cancer. The incidence rates of this cancer are constantly and rapidly increasing in all the PBRCs. The cancer projection data shows that the number of cases will become doubled by 2020.

Delhi Cancer registry shows cancer of the prostate is the second most frequently diagnosed cancer among men in Delhi accounting for about 6.78% of all malignancies (2008–2009). The annual age adjusted incidence rate of prostate cancer in Delhi was 10.66 (2008) per 100,000 which is higher than South-East Asia (8.3) and Northern Africa (8.1) but lower than Northern America (85.6), Southern Europe (50.0) and Eastern Europe (29.1) and it is comparable to Western Asia(13.8).

1.5 What are the symptoms?

BPH may cause the following Lower Urinary Tract Symptoms (LUTS):

- Weak urine flow
- Frequent urination especially at night
- An urgent need to urinate
- Difficulty starting to urinate
- Leakage after urinating
- Incomplete emptying of the bladder

These symptoms may also occur in locally advanced prostate cancer. If you are concerned and/or are experiencing any of these symptoms, speak to your doctor

1.6 What are the risk factors

While the causes of prostate cancer are unknown, your risk of developing prostate cancer increases:

- As you get older – prostate cancer is mainly diagnosed in men aged 60–79
- If your father or brother has had prostate cancer – your risk is twice that of other men
- If you have a strong family history of breast or ovarian cancer, particularly BRCA1 and BRCA2 gene mutations.

While prostate cancer is rare in men under 50, men aged 45–55 are at particular risk of developing significant prostate cancer later in life if their prostate specific antigen (PSA) test results are above the 95th percentile. This means that PSA levels are higher than 95% of men in the same age range. You may have an inherited gene that increases your risk of prostate cancer if you have:
multiple relatives on the same side of the family with prostate, breast and/or ovarian cancers
a male relative under the age of 50 with prostate cancer.
If you are concerned about your family history, your GP can advise you on the suitability of PSA testing for you and your family.

1.7 Diagnosis
There is no single, simple test to detect prostate cancer. Two commonly used tests are the PSA blood test and the DRE. However these tests used separately or in combination, can only indicate changes in the prostate gland. They are not diagnostic tests. If either test shows an abnormality, your GP will refer you to an urologist for further evaluation.

1.7.1Prostate specific antigen blood test
Prostate specific antigen is a protein made by both normal prostate cells and cancerous prostate cells. PSA levels are measured using a blood test. The PSA test does not specifically test for cancer. A PSA reading above the typical range for your age may indicate the possibility of prostate cancer. The amount of PSA in blood can be raised even when a man does not have cancer. Other factors can increase PSA levels, including benign prostate hyperplasia recent sexual activity or an infection in the prostate. In addition, some men with prostate cancer have normal PSA levels.
Because PSA levels can be variable, your doctor will often use results from more than one blood test, over time, to help determine your risk of prostate cancer. Your doctor will also compare your PSA result against other men the same age as you.

1.7.2 Digital rectal examination
In a digital rectal examination a doctor inserts a gloved finger into your rectum to feel the back of the prostate gland. The doctor feels the size of the prostate and checks for abnormalities. The DRE may be uncomfortable, but is rarely painful.
If your doctor feels a hardened area or an odd shape, further tests may be done. Abnormalities do not always indicate prostate cancer and a normal DRE does not rule out

prostate cancer, as the test is unlikely to pick up a small cancer or one the finger can't reach.
Digital rectal examination is no longer recommended as a routine test for men who do not have symptoms of prostate cancer. For men who wish to be tested for the presence of prostate cancer, the DRE is still useful. The test may help doctors assess the prostate prior to biopsy

1.7.3Biopsy
If the PSA test or DRE show an abnormality, a biopsy is often the next step. During a biopsy, small amounts of tissue are taken from different parts of the prostate using a special needle. The samples are sent to a lab where a pathologist examines the tissue to see whether cancer cells are present. Multiple tissue samples are taken so that the pathologist can indicate the extent of the tumour in the prostate.
A biopsy is usually done with the help of a transrectal ultrasound (TRUS) probe.

1.7.4 Further tests
If the biopsy shows you have prostate cancer, other tests may be done to show the stage of the cancer.

- Blood tests
- Bone scan
- CT scan
- MRI scan

1.7.5 Staging and grading
Your doctor will assign a staging and grading category to your cancer, which will help you and your health care team decide which treatment or management option is best for you.

1.7.6 Prognosis
Prognosis means the expected outcome of a disease. Generally, prognosis is better when prostate cancer is diagnosed while it is early stage, and at a lower grade.
You will need to discuss your prognosis with your doctor. However, it is not possible for any doctor to predict the exact course of the cancer. Test results, the extent of the spread of the cancer, and factors such as your age, level of fitness, medical and family history are important in assessing your prognosis. These factors will also help your doctor advice you on the best management or treatment options and tell you what to expect.

1.7.7 Which health profession will I see?
If your GP suspects you have prostate cancer, you may be referred to an urologist who can arrange further tests and advise you about your options. After a diagnosis of prostate cancer, you will be cared for by a range of health professionals who specialise in different aspects of your treatment. This multidisciplinary team may include the following:
General practitioner (GP),

Urologist

Radiation oncologist

Medical oncologist
Endocrinologist
Cancer care coordinator

Oncology nurses

Continence nurses

1.8 Making management or treatment decisions

Prostate cancer is typically slow-growing, giving men time to make decisions regarding their management or treatment options. Sometimes it is difficult to decide on the type of management or treatment that is right for you. You may feel that everything is happening too fast. Take as much time as you need. Making sure you understand enough about your diagnosis, the treatment options and their side effects will help you make an informed decision in consultation with your GP and/or urologist.

1.8.1 Talking with doctors

When your doctor first tells you that you have cancer, you may not remember the details about what you are told. Taking notes or recording the discussion may help. Many people like to have a family member or friend go with them to take part in the discussion, take notes or simply listen. If you are confused or want clarification, you can ask questions

1.8.2 A second opinion

You may want to get a second opinion from another specialist to confirm or clarify your doctor's recommendations or reassure you that you have explored all of your options. Specialists are used to people doing this.

Your doctor can refer you to another specialist and send your initial results to that person. You can get a second opinion even if you have started treatment or still want to be treated by your first doctor. You might decide you would prefer to be treated by the doctor who provided the second opinion.

1.9 Management or treatment

There are different options for managing and treating prostate cancer. For some men immediate treatment is not required or may not be appropriate. Your treating specialist will advise you of your options based on your age, general health, the stage and grade of the prostate cancer, the severity of symptoms and the likely side effects of treatment.

Treatment Options-
Radical prostatectomy
Transurethral resection
Radiotherapy
External beam radiotherapy
Androgen deprivation therapy

Advanced prostate cancer treatment-
Chemotherapy
Bone therapies
Palliative treatment

1.10 Managing side effects
Treatment for prostate cancer may damage nerves and muscles near the prostate, bladder and bowel. This may cause side effects including urinary incontinence, changes in bowel habits, erectile dysfunction and infertility. Lower testosterone levels as a result of ADT can also cause loss of interest in sex (libido).
Side effects will vary from person to person. Some men will not have any, while others may experience a few. Side effects may last for a few weeks or be permanent. Fortunately, there are many ways to reduce or manage side effects. Many go away in time and most men are able to continue to lead active lives after their treatment.

1.11 Looking after yourself
Cancer can cause physical and emotional strain. It's important to try to look after your wellbeing as much as possible including factors like Nutrition, Staying active and Complementary therapies

1.12 After treatment: follow-up
After treatment, you will need regular checkups to monitor your health and see whether the cancer has returned. This will involve testing your PSA level at regular intervals.

1.13 Seeking support
Cancer may cause you to experience a range of emotions, such as fear, sadness, anxiety, anger or frustration. It can also cause practical and financial problems.

2. Methods
A questionnaire was used to assess the knowledge of men from students to working professionals living in Mumbai, India. A questionnaire was developed in collaboration with the guide and health care professional. 16 questions were used for the survey. The questionnaire included demographic data as well as specific questions associated with prostate cancer and regular health updates. Questions assessed: (1) general awareness about prostate cancer, (2) diet and lifestyle habits such as alcohol, tobacco consumption and smoking, (3) significant medical history, (4) information about prostate screening.
The data was analysed using standard statistical tools.

3. Results
- Study population and socio-demographic characteristics
  16 questions were distributed. The respondent responded to almost all the questions. The age range was between 31 to 70 and also below 30 and above 70. There were total 213 respondents. Out of these 213 respondents, 155 (72.76%) were married and 58 (27.23%) were single.
- Knowledge and awareness about prostate cancer

Respondents were asked about their knowledge of prostate cancer as a disease and the associated symptoms. 36.15% (n = 77) of the respondents were generally aware of prostate cancer as a disease, whereas 63.84% (n =136) were unfamiliar with the subject. Thirty five (16.43%) had previously discussed prostate cancer with their family physician at some point in their lifetime. Interestingly, 75 respondents (52.27%) thought prostate cancer was infectious and only 7.5% (n = 16) understood prostate cancer as a hereditary disease.

The most widely endorsed barrier could be hesitation, lack of knowledge, emotional barrier or inability to make an appointment with the physician without any symptoms. Hardly few of them had understanding about PSA and DRE test

Table 1.Socio-demographics and other characteristics of the study population

		Number	%
Age group	Below 30	41	19.24
	31-40	29	13.61
	41-50	80	37.55
	51-70	57	26.76
	Above 70	6	2.81
Marital status	Single	58	27.23
	Married	155	72.76
Highest level of education	Schooling	76	35.68
	University	121	56.80
	No formal education	15	7.04
Dietary and lifestyle habits	Sedentary	27	12.67
	Moderate	163	76.52
	heavy	23	10.79
Consumes	Alcohol	18	8.45
	Tobacco	57	26.76
	Cigarette	13	6.10
	None of the above	125	58.68
Awareness of prostate cancer	Yes	77	36.15
	No	136	63.84
Discussed prostate cancer with professionals	Yes	35	16.43
	No	178	83.56

4. Discussion

This is a survey-based study that evaluated the knowledge of prostate cancer symptoms and risk factors in a male population in Mumbai, India. Assessment of respondent knowledge about cancer indicated that there is considerable potential for improvement in prostate cancer screening awareness. Increased awareness was more common in students

compared to other members of the study population. Perhaps the use of social and mass media awareness about various life-threatening diseases, such as breast cancer, are likely more, but that of prostate cancer is nil. Additionally, understanding and knowledge about prostate cancer appeared to be associated with level of formal education as well as interaction and information from family physicians. However, with improved level of education and standard of living this may be a good qualitative assessment for future policy planning.

5. Conclusion

The measures to improve knowledge and awareness about prostate cancer screening, early advice and involvement in healthcare programs may ultimately lead to increased rates of survival and, hence, reduced mortality from this life-threatening disease. The current study highlights the need to prioritise planning for cancer support services in a country, such as India, with diverse cultural and economic needs. Furthermore, it emphasises involvement of the healthcare system to actively increase awareness of prostate cancer symptoms, services available, and screening or detection methods. An understanding of the heterogeneity in the socio-economic and cultural habits of men is essential in directing healthcare awareness.

Coming from a diverse ethnic and conservative/orthodox background, subjects may be embarrassed to talk to a healthcare professional about the symptoms due to lack of awareness. This may be attributed to education, participation in men development and awareness/outreach programs. Greater awareness among students is likely attributed to social media. Due to the structure of the healthcare system in Mumbai, accessibility to prostate screening centres is limited, acting as an obstacle for early detection as there are very few healthcare centres that offer free screening facilities. Also, the services at these clinics are only paid for directly by the patient or private insurers. Formal comparative statistics were not possible due to lack of stability in matching the large cohort with many variables.

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SCREENING OF NOVEL PYRAZOLINE DERIVATIVE FOR ANTIANGIOGENIC ACTIVITY

*Priyanka Chavan, Rushali Chavan and Deepali Jagdale**

ABSTRACT

Angiogenesis is a physiological process in embryogenesis, organ development, endometrial vasculature in menstrual cycle and wound healing. Angiogenesis has also been associated with several pathological conditions such as cancer, arthritis, etc. VEGFR is an important growth factor and a positive regulator of angiogenesis with its distinct specificity for vascular endothelial cells. The Zebra fish model has been developed foe *in vivo* screening of anticancer agents having VEGFR inhibitory activity. It has the advantage of *in vivo* imaging, large progeny and rapid development, thus zebrafish provides an attractive model to promote novel cancer drug discovery and reduce the hurdles and cost of clinical trials. The ability of zebrafish to faithfully recapitulate a variety of human cancers provides a unique *in vivo* system for drug identification and validation. The current work is small effort to check the anti VEGFR activity of synthesized drug by using antiangiogenic activity in zebrafish model. The synthesized drug was found to have anti-VEGFR activity. Thus the synthesized molecule holds importance as a lead to develop further molecules having anti VEGFR activity and may be screened for its anti pancreatic activity in future.

KEYWORDS

VEGFR, antiangiogenic, zebra fish, pyrazoline derivative

INTRODUCTION

Cancer is the worldwide health problem and the most frightening disease of human[1]. It is a leading cause of death worldwide accounting for 7.6 million deaths (13% of the total deaths) in 2008 and the figure is expected to be around 13.1 million by 2030[2]. The pancreas is an organ that is about 6 inches long and 2 inches in wide. It's located deep in belly between stomach and Currently pancreatic cancer is the fourth leading cause of cancer death and is anticipated to become the second by 2020. Angiogenesis play an important role in formation of vascular system and it is a normal and most vital process for in growth and development of new blood vessels from pre-existing vessels as well as wound healing[3]. Angiogenesis is most complex process control by pro and anti-angiogenic growth factor.

Angiogenesis play important role in development of new blood vessels which fundamental requirement for development of new organ at embryo as well as adult. Supply of oxygen and nutrient to different organ takes place through blood vessels which help in growth small tumor cell to large tumor cell and continue to metastasize[4]. So now a day, VEGFR is an important target for treatment of different cancer e.g. breast cancer, metastasize thyroid cancer, colorectal cancer, pancreatic cancer, ovarian cancer, and renal cell carcinoma.

SELECTION OF SCAFFOLD (RATIONALE)

Thiosemicarbazide is an important structural motif that has the potential to display chemical functionality in biologically active molecules. Optimization of this structure can result in ground breaking discovery of new class of anticancer agents. From the literature survey, we found that methisazone show anticancer activity due to presence of thiosemicarbazone group. Thiosemicarbazone of N-heterocyclic compound have low π-electrondensity of the side chain and heterocyclic ring of nitrogen atom should be good electron pair donor to receptor. In addition, due to decreasing the size of thiosemicabazide it can easily fit within the receptor cavity. On other hand, chalcone also display good anticancer activity. From the above discussion, we attempted to create a pyrazoline derivative by reaction of chalcone with thiosemicabazide by green chemistry.
From the above result, we designed pyrazoline ring containing molecules followed by adding the amide side chain. Selection of representative compounds is shown in **Fig 1** that was looked into for selection of scaffold.

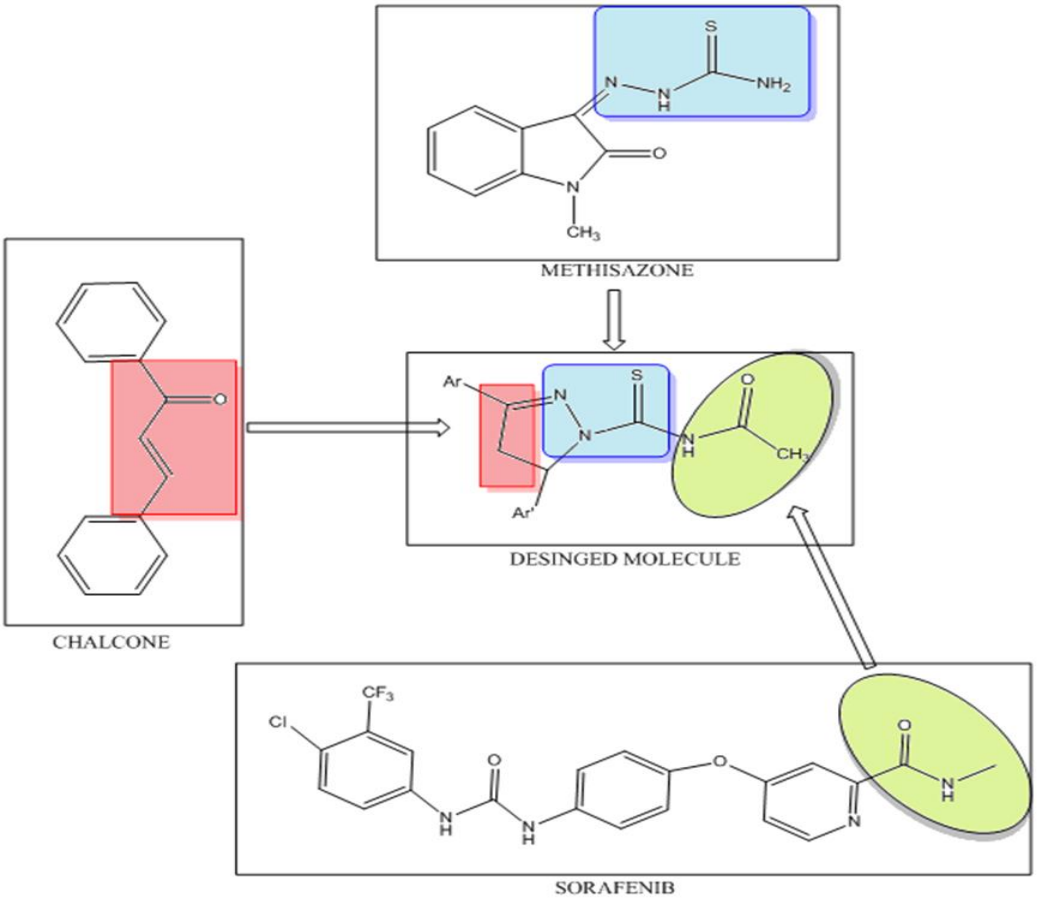

Fig 1. Designing of the pyrazoline derivative, Ar = phenyl; Ar' = phenyl.

MATERIALS AND METHOD

Materials

All animal care and experimental procedures complied with the OECD guideline and use of Animals for scientific purposes and was approved by Animal Ethics Committee. This study was done in accordance with OECD guidelines for experiments involving animals. A total of 12 animals were used in the experiments described here. Adult wild-type zebra fish were bought from color aquarium (khanda colony). The animals were kept in fish tank for acclimatization for 15 days before actual study. Fish were provided with an enriched environment.

Method

Toxicity study

Toxicity studies were performed according to OECD test guideline- 203 (Fish, Acute Toxicity Test) in order to determine maximum tolerable dose of test compound. According to the test, seven fishes were kept per group (1 gm of fish/ L of water) and the fishes were exposed to test substance for a period of 96 hours. The mortalities were recorded at 24, 48, 72 and 96 hours (**Table 1**) and the concentration which killed more than 50% of the fish (LC50) was determined. Based on the mortality, the dose was increased or decreased by a spacing factor of 2.2.

Fin regeneration method

The wild type adult zebra fish weighing between 0.5-1.5 gm were kept for acclimatization for about 15 days in laboratory conditions. The fishes were divided into 2 treatment groups, 6 fishes per group. The fishes were kept in glass beakers of the size of 250 ml containing about 150 ml of fish water and a single fish each beaker. Water had been added with sodium thiosulphate crystals as dechlorinating agent and rock salt as antibacterial agent. The beakers were kept in series to provide proper aeration. On every alternate day, drug was administrated to the fish till the 7th day. Images were captured frequently to observe the growth of caudal fin. Dose of the drug was decided based on the results of toxicity test. On the day of experiment, the fishes were anesthetized by using 2-Phenoxy ethanol (0.9ml/L of water). The image of whole fin was taken in order to compare it with regenerated fin. Their fin was cut up to 50% using sterile razor blade. The fish was then put back into recovery tank to recover from anesthesia and then transferred back to beaker.

Evaluation of anti cancer activity

Pre and post amputated images were collected and length was measured using scale. Each beaker containing fish was labeled according to the group based on which treatment it is receiving. Different doses of drug, isolated fraction and standard drug (Imatinib Mesylate) were given to each group. Dose of the drugs and standards were determined by toxicity test. The water in each beaker was changed and dose was

administered on every alternate day and up to 7 days. Images were taken on 3rd, 5th and 7th day post amputation (DPA) using Motic digital microscope (4X) on 3rd, 5th and 7th DPA. On 7th day, final images were taken. Length and area of the fin was measured by using Motic image plus 2.0 and Image J softwares. Percent of inhibition and percent of regeneration was calculated.

RESULTS AND DISCUSSION

Toxicity study

Toxic dose depend on the concentration at which 50% of fishes alive after 96 hours. From toxic study (**Table 1**) dose was decided as 0.4 mg/fish /L.

Table 1. The toxicity study data

Concentration	**Number of alive fishes**				
	Hours				
	0	24	48	72	96
1.94mg/fish/litre	6	6	6	0	0
0.88mg/fish/litre	6	6	6	1	0
0.4mg/fish/litre	6	6	6	5	4
Vehicle(control)	6	6	6	5	2

Antiangeogenic study

Three group (lower, middle, control) of drug were studied for anticancer activity according to toxic dose obtained from toxicity study. The factor of 2.2 was considered while calculation of the middle and lower concentrations. Both, lower and middle doses showed growth inhibition when compared with control group on day 3, 5 and 7. The results obtained are given in **Table 2**.

Table 2. Average growth of fin on day 3, 5 and 7 after treatment with the synthesized drug.

Compound	Study day	Group	Average growth(mm)
Control	3	A	1.1
		B	
		C	
	5	A	1.73
		B	
		C	
	7	A	5.8
		B	
		C	
Synthesized drugs	3	A	0.95
		B	
	5	A	1.45
		B	
	7	A	3.3
		B	

CONCLUSION

The synthesized derivative showed less growth of fin as compared with the control. This growth inhibition clearly indicates that the synthesized derivative inhibits the angiogenesis and hence has potency to inhibit VEGFR. Further the synthesized derivative has potency for development as a anticancer agent where VEGFR are over expressed.

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NOVEL GREEN SYNTHESIS OF COUMARIN-3-CARBOXYLIC ACID & ITS DERIVATIVES USING AQUEOUS FRUIT EXTRACT OF *Tamarindus indica* AS CATALYST

*Bhavya Shah, Nashit Ali Shaikh, Dhiraj Nikam**

Abstract: A simple, efficient and green procedure for the synthesis of 3-carboxycoumarins has been developed which involves the treating of 2-hydroxybenzaldehydes with Meldrum's acid (2,2-dimethyl-1,3-dioxan-4,6-dione) in presence of visible light using aqueous tamarind juice at room temperature. The protocol is much more efficient as the reactions are carried out at room temperature, yields are also quite high and the reactions go to completion within 2-7 min. The tamarind juice could be simply prepared from ripe fruit of *Tamarindus indica* in water. All the compounds were characterized by their melting points and compared with reference literature.
Keywords: Green synthesis; 3-carboxycoumarin; 2-hydroxybenzaldehyde; Meldrum's acid; Visible light; Aqueous tamarind extract.

Introduction: 3-Carboxycoumarins constitute an important class of compounds because of their enormous applications, as these are the required intermediates for the synthesis of number products with various biological activities. Generally, these compounds have been obtained by the condensation of substituted 2-hydroxybenzaldehydes with malonic acid, ethylcyanoacetate, malononitrile in the presence of piperdine, piperdine acetate, ammonium acetate, sulphuric acid adsorbed over silica, L-proline and ionic liquids. Use of Meldrum's acid was found to be much superior in terms of yields. Various condensation of 2-hydroxybenzaldehydes with Meldrum's acid in aqueous-ethanol medium using visible light, under the phase transfer catalysed condition using triethylbenzylammoniumchloride (TEBAC)and potassiumphosphate in ethanol. Some of the above-mentioned conditions possess shortcomings such as use of harsh and hazardous chemicals mainly organic solvents, longer reaction time, elevated temperature and poor yields. The reaction between Meldrum's acid and 2-hydroxybenzaldehyde is a Knoevenagel condensation reaction. The rate of the reaction increases in acidic medium. Aqueous fruit juice like Tamarind *(Tamarindus indica)* has been found to be a suitable replacement for various homogeneous acid catalysts. An aqueous extract of tamarind fruit juice is acidic due to presence of tartaric acid and ascorbic acid and acidity percentage is 50.3% and hence it will be work as an acid catalyst for condensation of aldehydes andactive methylene compounds.

Aim and Rationale: The research was carried out to discover a novel green method for synthesis of 3-carboxycoumarin and its derivatives to compare it with former existing green method. The grinding technique has been considered to be an efficient to carry out the synthesis of 3-Carboxycoumarin under solvent-free conditions with minimum cost and maximum yield but the only drawback of this method is long duration of the reaction,it takes about 20 minutes of grinding of Meldrum's acid and 2-hydroxybenzaldehyde in moist condition with additional 40 minutes waiting period.In order to increase the rate of reaction a novel method using aqueous extract of tamarind as a catalyst was discovered.

Materials and Method:

Synthesis of 3-carboxycoumarin & its derivatives via Grinding Method (Existing Method):A mixture of Salicyldehyde (3.75 ml) and Meldrum's acid (5 g) moisten with 10 drops of water was grounded in a glass mortar by a pestle at room temperature for 20 minutes and the reaction mixture was left at room temperature for 40 minutes. The reaction mixture was diluted with ice cold water. The solid that separated out was filtered at vacuum, washed with water and recrystallized from ethanol (50%) to get 3-carboxycoumarin,whose structure was confirmed by its spectral data (FTIR) and comparison of melting point with the literature value[20].

Preparation of aqueous extract of tamarind juice:

The ripe tamarind fruits were purchased from the local market. The upper shell of ripped fruit and its inner grain were removed. The brown material (pulp, 20 g) was boiled with water (100 mL), cooled and it was centrifuged. The clear portion of the aqueous extract (pH=1) of the tamarind fruits was used as catalyst for the reactions[31].

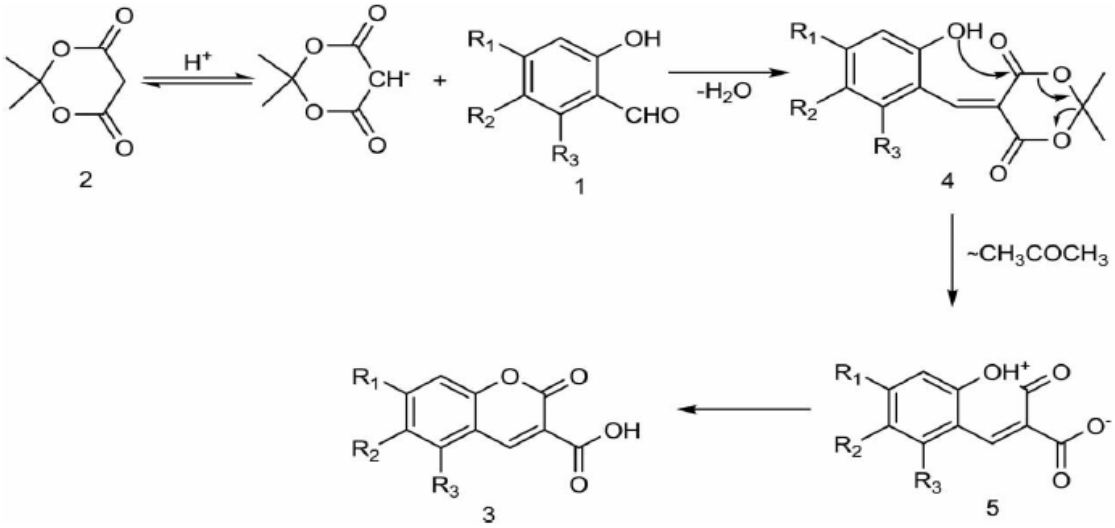
Synthesis of 3-carboxycoumarin & its derivatives by visible light induced Knoevenagel Condensation using Aqueous Tamarind Juice:

A mixture of Salicyldehyde (3.75 ml), Meldrum's acid (5 g) and Aq. Tamarind extract (pH=10), was stirred in daylight at room temperature for about 5-10 minutes. The mixture was then poured into an ice cold water and again stirred vigorously for 2-5 minutes. The solid that separated out was filtered using vacuum filteration, washed with ice cold water and recrystallize from ethanol (50%) to give 3-carboxycoumarin, whose structure was confirmed by its spectral data (FTIR) and comparison of melting point with the literature value.

Result and Discussion: In grinding method the dissociation of Meldrum's acid (2) takes place in presence of waterwhich generates the nucleophilic species that attack the aldehydic carbon directly with simultaneous protonation of carbonylic oxygen of salicyldehydeto give arylidene derivatives (4), which is further cyclized by nucleophilic attack of OHgroup on the carbonyl moiety of Meldrum's acid and gave the intermediate (5) and subsequent proton transfer gave 3-carboxycoumariun (3) (Scheme 2). The reaction is slow and requires trituration

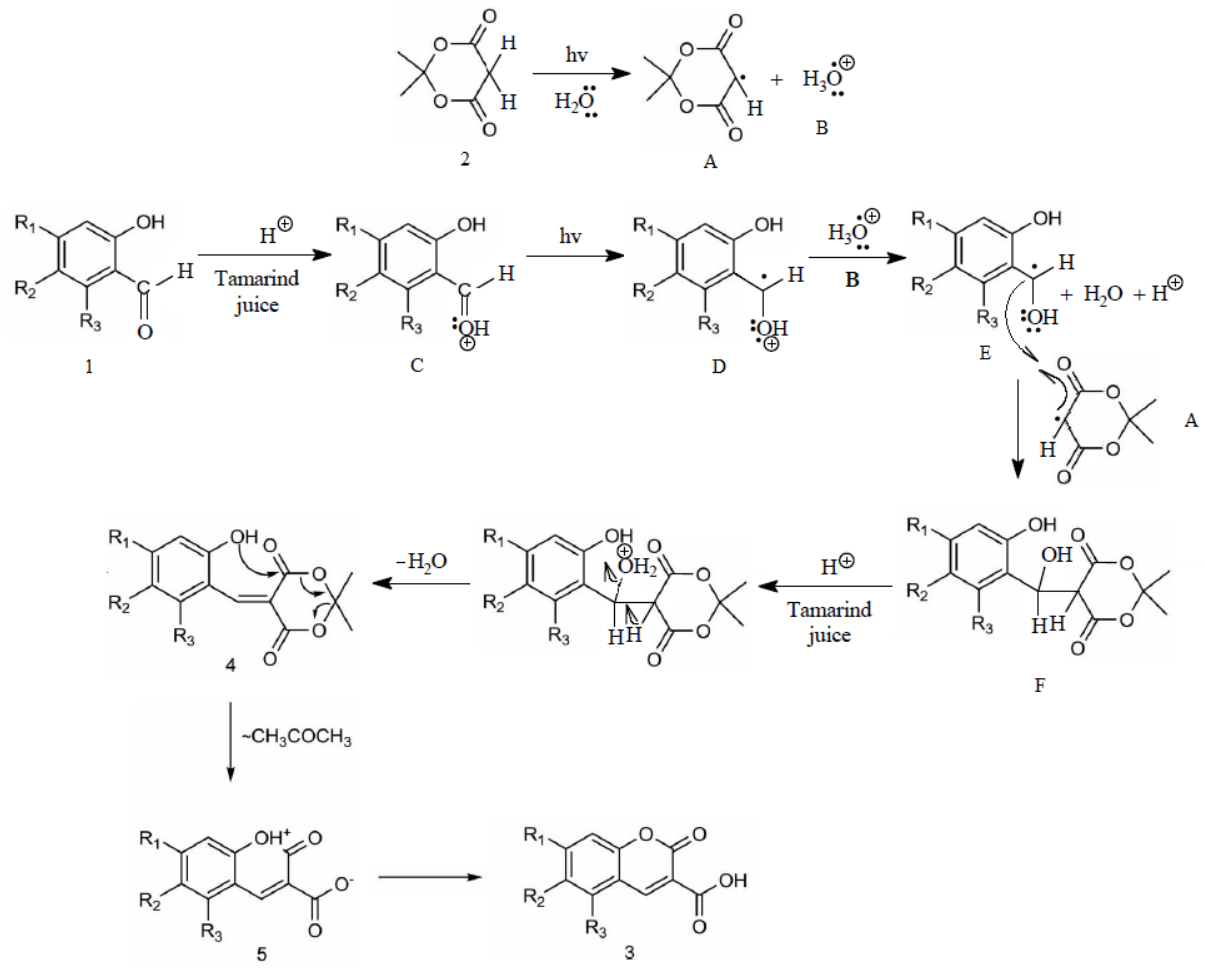
On the other hand on addition of aqueous tamarind extract (pH = 2) to a mixture of Meldrum's acid and salicyldehyde, the reaction proceeds without trituration just by visible light that to within 5-7 minutes of stirring the slurry is added to ice cold water and the separated solid is filtered with vacuum and recrystallized with ethanol (50%) giving 3-carboxycoumarin.

In the present instance, we speculate that the reaction may plausibly be initiated by homolytic C-H bond cleavage of Meldrum's acid (2) in the presence of visible light to produce a radical A and hydrogen radical which is trapped immediately by water molecule to form radical B. Salicyldehyde (1) becomes activated by protonation from tamarind juice (due to presence of tartaric and citric acid) to produce a protonated species C. One electron transfer from B to D produced a radical E, which couples with radical A to form F.



Protonation of F followed by dehydration from G to form arylidene derivative (Scheme 3). Further the reaction proceeds in the similar manner as that in former method.

Scheme 2: Plausible mechanism for the synthesis of 3-carboxycoumarins by Grinding Technique



Scheme 3: Plausible mechanism for the synthesis of 3-carboxycoumarins by Light Induced reaction using Aqueous tamarind extract

Conclusion: We have established a potentially efficient, absolutely clean, rapid and high yielding eco-friendly methodology, for the light induced Knoevenagel condensation of various aromatic aldehydes with Meldrum's acid using aqueous tamarind juice for synthesis of 3-carboxycoumarin and its derivatives devoid of any toxic catalyst/solvents, solid support or surfactant at any stage of the reaction and may be considered as an excellent improvement over the existing methods. Various derivatives of 3-carboxycoumarin can be synthesized by this method which may have potential biological activities.

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Figures and Tables:

	Grinding method		Visible Light Method	
Compounds / Factors	3-carboxycoumarin	6-bromo-3-carboxycoumarin	3-carboxycoumarin	6-bromo-3-carboxycoumarin
Substitution	R_1 = H R_2 = H R_3 = H	R_1 = H R_2 = Br R_3 = H	R_1 = H R_2 = H R_3 = H	R_1 = H R_2 = Br R_3 = H
Time (mins)	20 + 40	20 + 40	7	5
Yield (%)	82	85	75	79
Catalyst	Water	Water	Aqueous tamarind extract	Aqueous tamarind extract
Process	Trituration	Trituration	Visible light induced	Visible light induced
Melting point (^oC)	190	194	194	196
Lit. Melting Point (^oC)	191-192	199	191-192	199

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SOLVENTLESS GREEN APPROACH FOR SYNTHESIS OF OXIME USING GRINDSTONE CHEMISTRY

*Shamli Kamble, Nitesh Kanoujiya and Dhiraj Nikam**

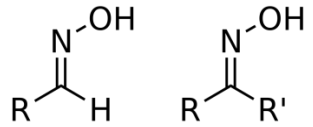
1) ABSTRACT :

A simple, efficient and eco - friendly method at ambient temperature was used to synthesize oxime derivatives. Various aldehydes and ketones were grinded with hydroxylamine hydrochloride at room temperature without using any solvent in presence of sodium hydroxide for few minutes in morter and pestle. All derivatives were purified by recrystallization using suitable solvents. The structure of synthesized compounds were confirmed by IR spectral data and authentication of compound was confirmed by physical constant.

Keywords : aldehydes, ketones, oxime derivatives, solventless, NaOH, eco – friendly.

2) INTRODUCTION :

An oxime is a chemicl compound belonging to the imines, with the general formula $R^1R^2C{=}NOH$, where R^1 is an organic side-chain and R^2 may be hydrogen, forming an aldoxime, or another organic group, forming a ketoxime. Oximes are usually generated by the reaction of hydroxylamine and aldehydes or ketones. The term oxime dates back to the 19th century, a combination of the words oxygen and imine.[1]



aldoxime ketoxime

Transformation of carbonyl functionalities into oximes has attracted intensive attention for several decades as an efficient method for characterization and purification of carbonyl compounds. Due to the nucleophilic character of oximes, they have been widely used for the preparation of a variety of nitrogen containing compounds such as amides, hydroximinoyl chlorides, nitrones and nitriles.

Oximes were usually prepared by the reaction of carbonyl compounds and hydroxylamine hydrochloride with adjustment of pH using a basic aqueous medium. Recently, some new techniques such as ultrasound irradiation , microwave irradiation and solvent free heating were applied to this reaction. Oxidation of amines or hydroxylamines was another usual method for the synthesis of oximes.

Conversion of carbonyl functionalities into oximes is an important reaction in organic chemistry. Conversions into nitriles, nitro compounds, nitrones, amines, and synthesis of azaheterocycles are some of the synthetic applications of oximes. They are also useful for selective a-activation and are extensively used as intermediates for the preparation of amides by the Beckmann rearrangement and fungicides and herbicides. In inorganic

chemistry, oximes act as a versatile ligand. Therefore, synthetic organic chemists are interested in the facilitation of oxime synthesis. Although alternative methods exist, reaction of carbonyl compounds with hydroxylamine hydrochloride remains still the most important route. Many improvements of this methodology have been convoluted. [2]

2.1 Chemical Reactions of Oximes :

1. Hydrolysis : Oximes proceeds to hydrolysis easily by heating in the presence of inorganic acids, leading to the formation of ketones/aldehydes and hydroxylamines.

2. Reduction : Oximes can be reduced by sodium metal, sodium amalgam, hydrogenation, or reaction with hydride reagents producing respective amines [3]

3. Amide : Oximes can also be altered into corresponding amide derivatives by treatment with various acids. Such type of reaction is termed as 'Beckmann rearrangement [4]. The derivatives, thus obtained, can be transformed into a carboxylic acid by means of acidic/basic hydrolysis. The main application of this reaction lies in the industrial synthesis of Caprolactam.

4. Neber Rearrangement : Oxime can be converted to alpha-aminoketone, in the presence of tosyl halide, a base and water [5].

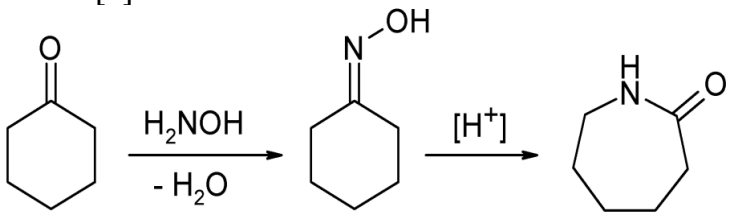
5. Tiemann Rearrangement : Amidoxime react with benzenesulfonyl chloride to form substituted ureas [6].

6. Cyclization: Phenethyl ketone O-2,4-dinitrophenyloximes can be encycled with NaH to form respective quinolines. Acetyloximes of γ,δ-unsaturated ketones can be cyclized through photochemical electron transfer or through hydroquinone-catalyzation and also with copper catalysts [7].

Other Transformations: Oximes can be transformed into esters, hydrazides, thioacids and hydroxamates, α-oximinokethones, which are also key intermediate in heterocyclic synthesis and in chemical transformation. Also they can be derivatize to Gem-Halo nitro compounds and also to α-halogen nitroso compounds by reacting them versatile halogenating agents like aqueous hypohalogenous acids, elemental halogens and N-halosuccinimide with oximes. By the oxidation with lead tetracetate or lead tetrabenzoate, we can prepare the important compound germinal nitrosoacetates

2.2 Applications of oximes :

1. In their largest application, an oxime is an intermediate in the industrial production of caprolactam, a precursor to Nylon 6. About half of the world's supply of cyclohexanone, more than a billion kilograms annually, is converted to the oxime. In the presence of sulfuric acid catalyst, the oxime undergoes the Beckmann rearrangement to give the cyclic amide caprolactam.[8]



2. Oximes are commonly used as ligands and sequestering agents for metal ions. Salicylaldoxime is a chelator and an extractant in hydrometallurgy.[9]

3. Oxime compounds are used as antidotes for nerve agents. A nerve agent inactivates acetylcholinesterase by phosphorylation. Oxime compounds can reactivate acetylcholinesterase by attaching to phosphorus, forming an oxime-phosphonate, which then splits away from the acetylcholinesterase molecule. Oxime nerve-agent antidotes are pralidoxime (also known as 2-PAM), obidoxime, methoxime.[10] The effectiveness of the oxime treatment depends on the particular nerve agent used. [11]
4. Perillartine, the oxime of perillaldehyde, is used as an artificial sweetener in Japan. It is 2000 times sweeter than sucrose.
5. Diaminoglyoxime is a key precursor to various compounds, containing the highly reactive furazan ring.
6. Methyl ethyl ketoxime is a skin-preventing additive in many oil-based paints.
7. Buccoxime and 5-methyl-3-heptanone oxime ("Stemone") are commercial fragrances[12]

2.3 What is green chemistry ?
Use of solvent for the processes in chemical industry defines a major part of the environmental performance and also has impact on cost, safety and health issues. The idea of “green” solvents expresses the goal to minimize adverse environmental impact resulting from the use of solvents. Despite the use of hazardous organic solvents, much of the efforts have been made to develop green methodologies with supercritical solvents[13], ionic liquids[14], etc. However, use of these solvents generally ploughed with high pressure conditions, use of expensive reagents and lengthy workouts. In tune with the search for green solvents, the use of water as a reaction medium has attracted notable interest and offers a clean, economical and environmentally safe protocol for many reactions.[15]

3) AIM

- To synthesize oxime derivatives in simple, effective and Solvent free approach.
- To characterise oxime derivatives by I.R. spectral analysis and their melting point.

RATIONALE :

Literature survey revealed that most of the reported procedures used for this purpose require the use of solid supports or other auxiliaries, solvents for the extraction from the solid supports, high temperatures, long reaction time, expensive and not readily available reagents and tedious work-up procedures. Thus, there is need for the protocol for the development of catalytic-free process with readily available reagents which operate under milder conditions.
Some of the methods like mild, convenient solvent free procedure for synthesis of oxime put forward by Christer B. Aakeröy et al their research showed the importance of green chemistry as the approach was environmentally friendly with no use of harmful solvent.

Encouraging from above method, we have made an attempt to prepare oxime derivatives by just simple mixing of hydroxylamine hydrochloride and mixture of aldehyde/ketone with methanol and sodium hydroxide mixture with further purification done by recrystallisation.

4) METHODOLOGY :

4.1 Experimental section :

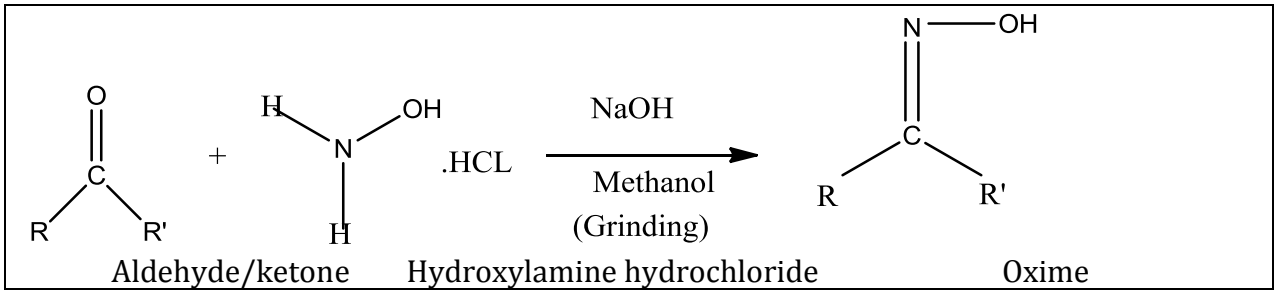
All chemical used were obtained from commercial sources. Melting points were measured in open capillary tube on veego (VMP-D) melting point apparatus. TLC was done using silica gel 60 F_{254} TLC plates. Infrared spectra were recorded on Schimadzu FTIR spectrometer.

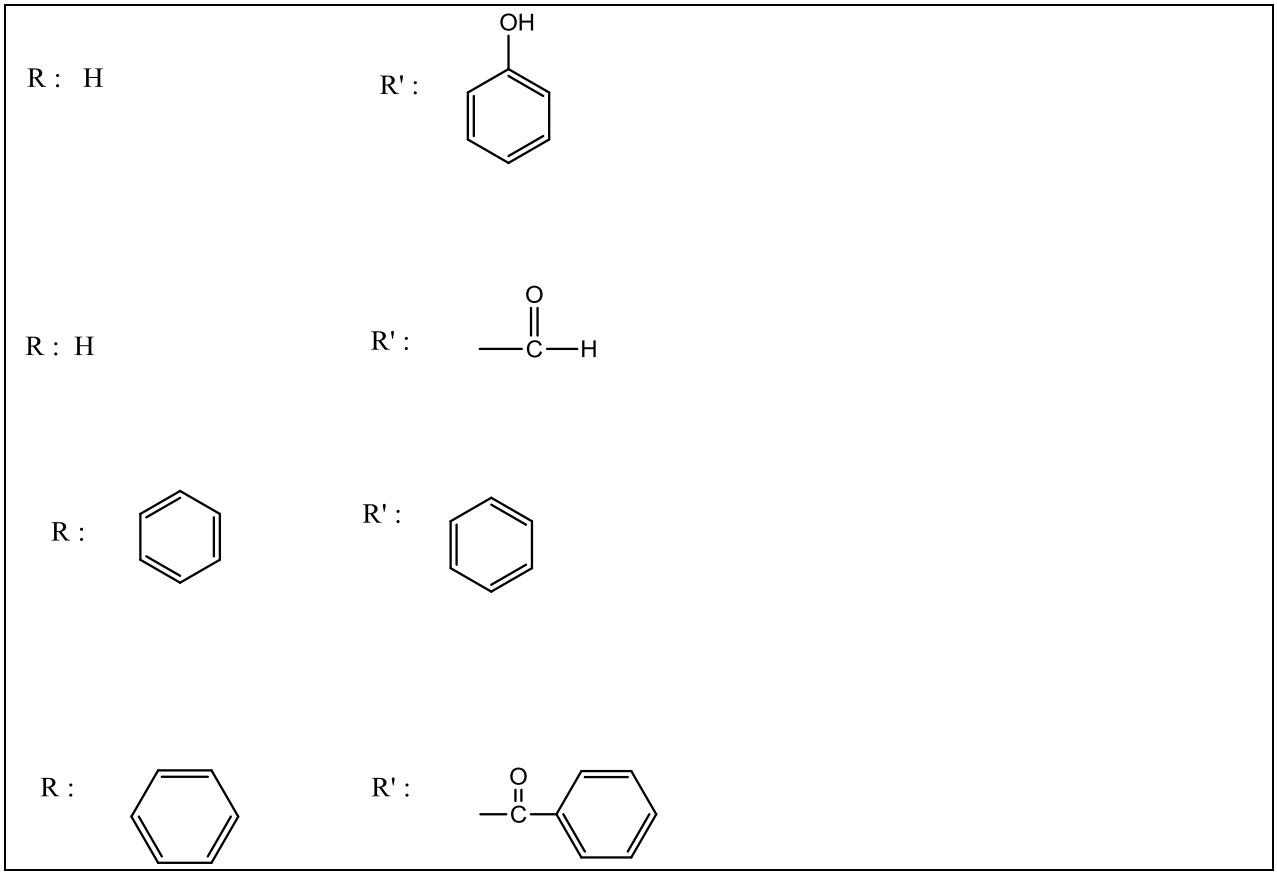
4.2 General procedure for preparation of oxime :

In a mortar, 1.0 mole of aldehyde and 1.2 moles (per aldehyde present) of hydroxylamine hydrochloride is ground together with a pestle. Then add 1.2 moles (per aldehyde present) of crushed sodium hydroxide. The mixture is grounded further with the addition of 2-4 drops of methanol, for 2 minutes at room temperature. The reaction mixture is left for 5 minutes. The mixture is grounded further for another 2 minutes with 2-4 drops of methanol to get the crude product. Reaction is monitored by TLC. The crude product is washed with cold water to get rid of any inorganic salts and it is air dried. Melting point is taken to confirm the formation of pure product.[26]

- Characterization of the derivatives were done by :
  1. IR spectrum using FTIR spectrometer.
  2. Physical constant.

4.3 General scheme for synthesis of oxime derivatives :

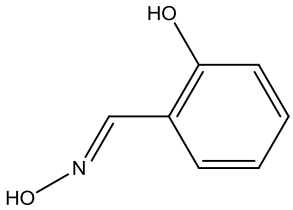




5) RESULT AND DISCUSSION :

5.1 SALICYLALDEHYDE OXIME :

- STRUCTURE :



- IUPAC NAME : 2- [(E)-(hydroxyimino)methyl]phenol
- MOLECULAR FORMULA:- $C_7H_7NO_2$
- MOLECULAR WEIGHT : 137.14 gm / mol
- MELTING POINT :61°C
- PERCENT YIELD :87.2 %
- IR SPECTRA :

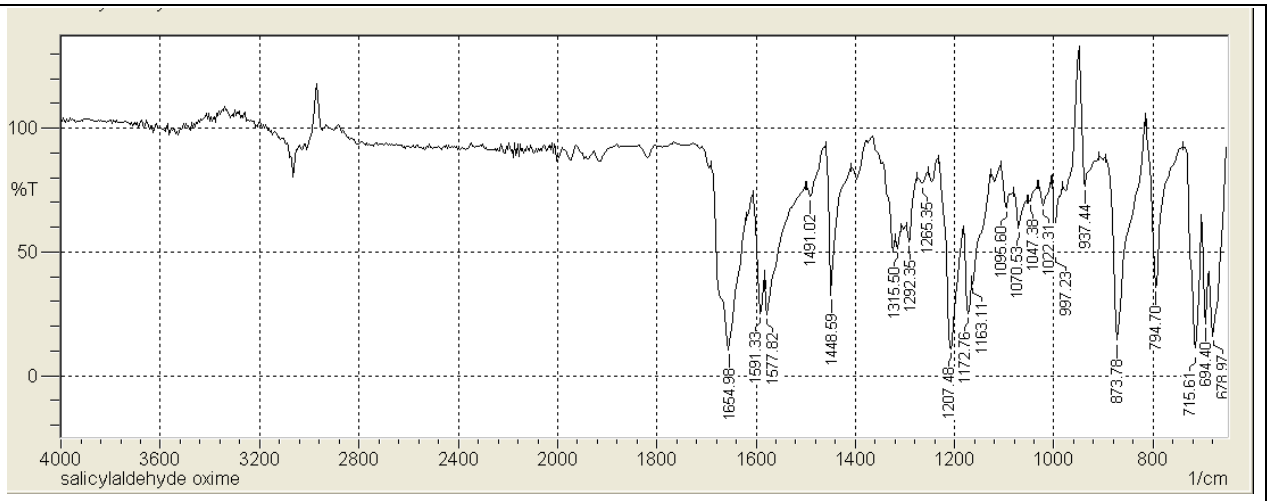
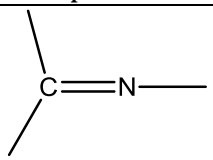
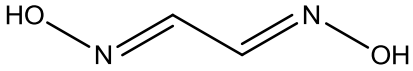


Fig no 1 : IR spectra of Salicylaldoxime

Group	Wave number (cm^{-1})
 (str) Oximes	1654.98
(str) phenol	3067.27

5.2 GLYOXIME :

- STRUCTURE :



- IUPAC NAME : N-[(E)-2-nitrosoethenyl] hydroxylamine
- MOLECULAR FORMULA:- $C_2H_4N_2O_2$
- MOLECULAR WEIGHT : 88.07 gm / mol
- MELTING POINT : 180 ° C
- PERCENT YIELD : 78.3 %
- IR SPECTRA :

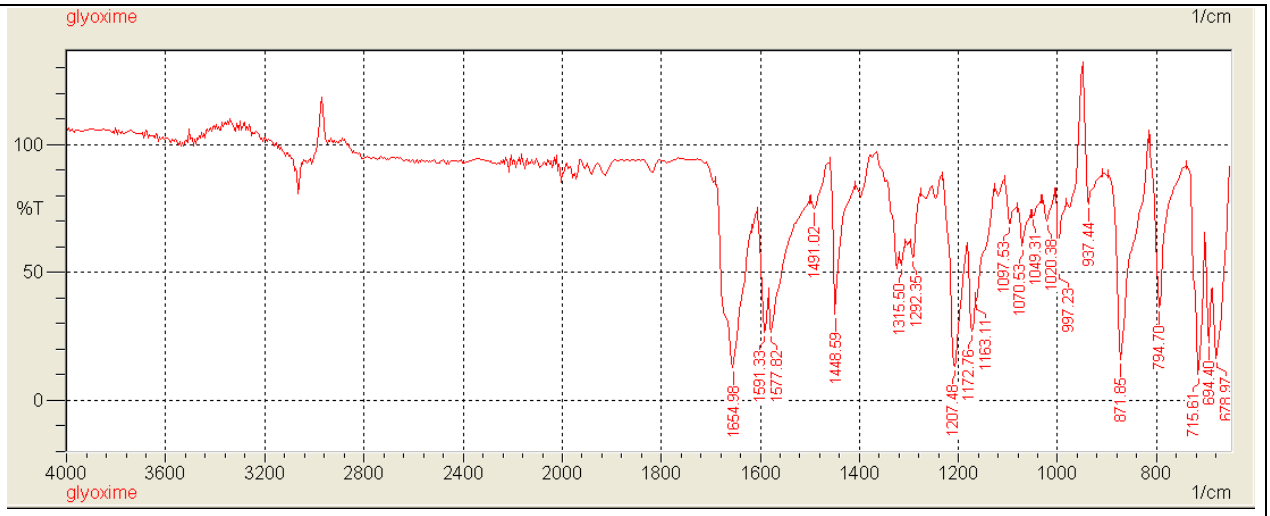
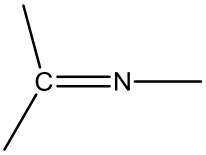
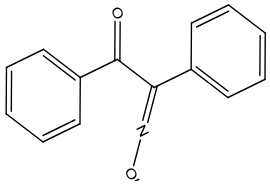


Fig no 2 : IR spectra of Glyoxime

Group	Wave number (cm^{-1})
 (str) Oximes	1654.98

5.3 BENZYL OXIME :

- STRUCTURE :



- IUPAC NAME : (2E)-2-hydroximino-1,2-diphenylethanone
- MOLECULAR FORMULA:-$C_{14}H_{12}N_2O_2$
- MOLECULAR WEIGHT :240.257 gm / mol
- MELTING POINT : 237°C
- PERCENT YIELD : 85.9%
- IR SPECTRA :

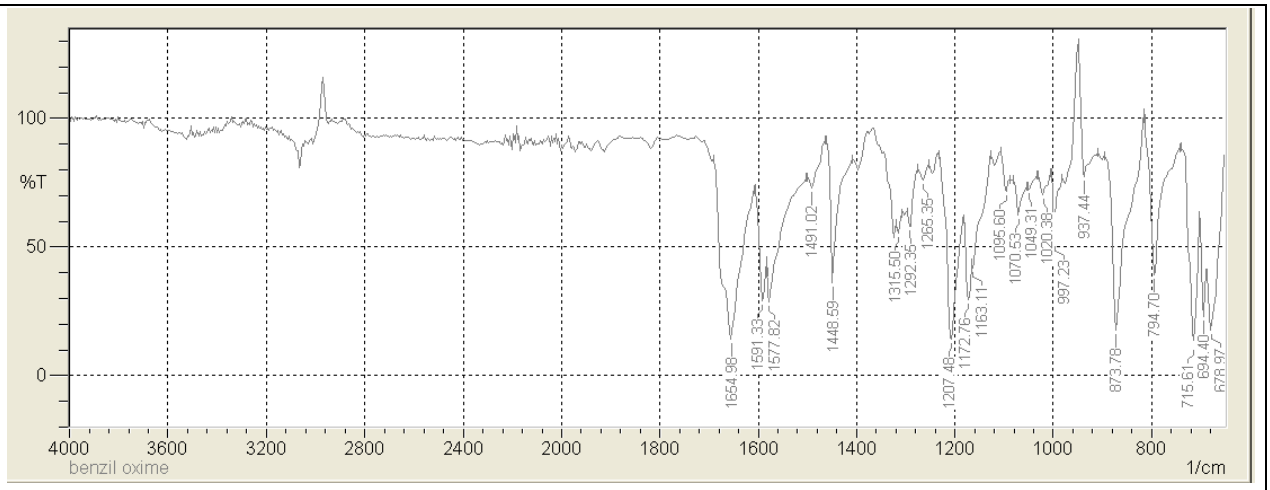
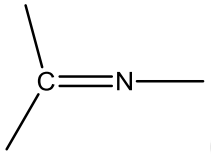
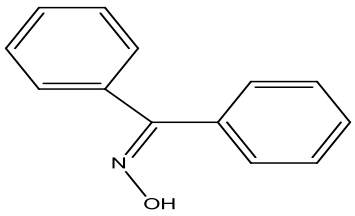


Fig no 3 : IR spectra of Benzyl oxime

Group	Wave number (cm^{-1})
 (str) Oximes	1649.19
 (str) aromatic	3067.27

5.4 BENZOPHENONE OXIME :

- STRUCTURE :



benzophenone oxime

- IUPAC NAME : N-benzhydrylidenehydroxylamine
- MOLECULAR FORMULA:- $C_{13}H_{11}N0$
- MOLECULAR WEIGHT : 197.237 gm / mol
- MELTING POINT : 142°C
- PERCENT YIELD : 82.7%
- IR SPECTRA :

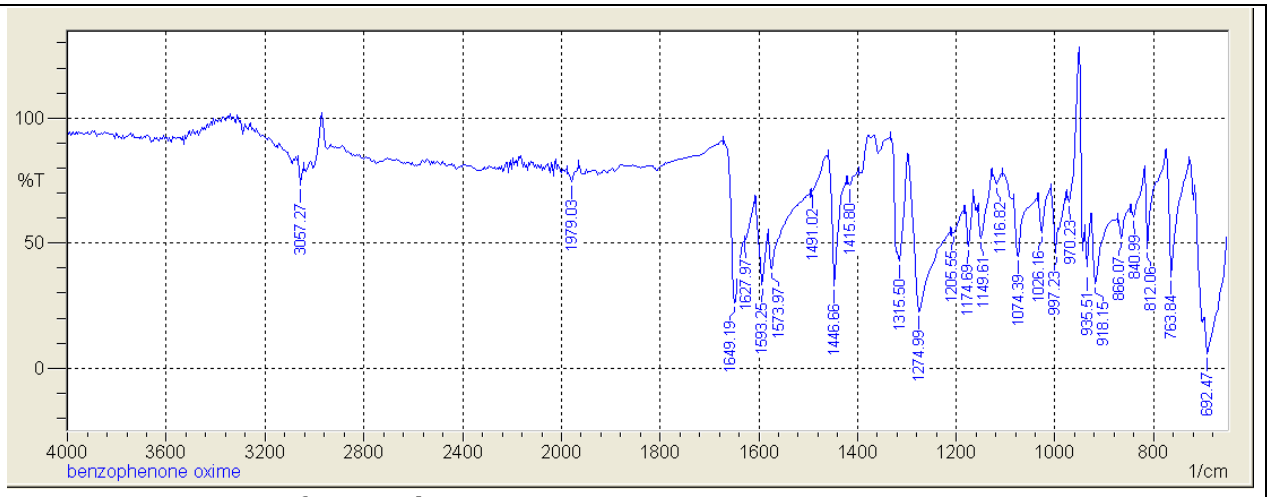
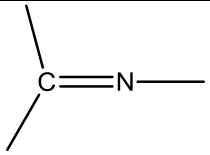


Fig no 4 : IR spectra of Benzophenone oxime

Group	Wave number (cm^{-1})
 (str) Oximes	1654.56
 (str) aromatic	3030

Sr.no	**Product**	**Time required (minutes)**	**Yield (%)**	**Melting Point(◦C)**
1.	Salicylaldoime	12	87.2 %	59 – 62◦C
2.	Glyoxime	14	78.3 %	177 -181 ◦C
3.	Benzyl oxime	10	85.9 %	235 – 238 ◦ C
4.	Benzophenone oxime	12	82.7 %	139-144 ◦C

1. Oxime derivatives were synthesized by condensation of hydroxylamine hydrochloride with salicylaldehyde/glyoxal/benzyl/benzophenone at ambient temperature under catalytic free conditions in shorter reaction times with excellent yields (85-95%).

2. % yield calculated based on the mass of product obtained after washing the crude mixture with water. Lower yield in some cases is due to loss of product during the washing procedure as some oximes are partially soluble in water.

3. Time required for every product was as follows :
   A) Salicylaldoxime : 12 minutes
   B) Glyoxime : 14 minutes
   C) Benzyloxime : 10 minutes
   D) Benzophenone oxime : 12 minutes
4. All these compounds were confirmed by their analytical and spectral data. Melting points were confirmed by comparing with reference standard from literature. The presence of peaks in the range of 1650-1685 cm-1 (C=O stretching) and 1555-1610 cm-1 (C=N stretching) from IR spectra confirmed the formation the product.
5. The method is superior to other introduced methods as it produces in most cases quantitative yields of the desired products with no waste, or any need of tedious purification procedures.

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6) Conclusion :

We developed a simple, green, and quick method for oxime derivatives. This techniqueis highly efficient. The important advantage of the present procedure, in addition to its simplicity just by grinding reactants together.Ability to obtain oxime derivatives in a short reaction time, in pureform, and with excellent yields.

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COMPARATIVE EVALUATION OF MARKETED PREPARATIONS OF ODT

*Shreya Adangale, Hemant Ahire and K.R.Jadhav**

ABSTRACT

Oral delivery is currently the golden standard in the pharmaceutical industry where it is regarded as the safest, most convenient and most economical method of drug delivery having the highest patient compliance. An oral dosage form in which a tablet is coated with a material to prevent or minimize dissolution in the stomach but allow dissolution in the small intestine. This type of formulation protects the stomach from the potentially irritating drug**.** Aspirin therapy is an essential part of the drug regimen for patients with acute myocardial infarction (MI), unstable angina, or after coronary angioplasty and coronary stenting. Aspirin, an acetylated salicylate (acetylsalicylic acid), is classified among the nonsteroidal anti inflammatory drugs (NSAIDs). The prototypical analgesic used in the treatment of mild to moderate pain. It has anti-inflammatory and antipyretic properties and acts as an inhibitor of cyclooxygenase which results in the inhibition of the biosynthesis of prostaglandins. Acetylsalicylic acid also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis. The purpose of this research work was to compare and evaluate quality standards in the various brands of enteric coated tablets of Aspirin. Three different brands of Aspirin were evaluated for various parameters like weight variation, hardness, disintegration time, and dissolution profile.

Keywords: enteric coated tablets, Aspirin, Hardness, Disintegration time

INTRODUCTION

Medical sector in the present world has changed into a therapeutic jungle. Pharmaceutical companies are competing among themselves to bring out new molecules for different ailments. A single generic drug is manufactured by different companies under different brand names. In India drugs are also manufactured by small scale sectors which may be widely distributed or locally marketed. Drugs especially for the common ailments are available as over the counter. It has found in several studies that the majority of the drugs are being consumed without proper prescription especially in rural areas. Many of the small scale sector pharma companies may or may not follow the international standards in the drug manufacturing process. So there is need for proper quality control of the drugs. The same can be done by qualitative and quantitative analysis of the drug formulation. Hypothetically speaking a single generic drug even though manufactured by different companies under different brand names, should match in the pharmaceutical parameters. Variation in the pharmaceutical parameter is mainly due to variation in the manufacturing process. These variations have a definite impact on the therapeutics of the drug, i.e. the drug may not provide the expected result.. Use of counterfeit and substandard drugs bear serious health implication; such as treatment failure and adverse reactions. A drug formulation (especially tablet, capsule etc) when consumed orally first disintegrate then gets dissolved in the secretions and finally absorbed into the systemic circulation. The

various processes which a drug undergoes inside the body are studied externally using various equipments.

Important parameters studied are weight variation, hardness, friability, disintegration, dissolution and pH of the drug. Hardness of the tablet is strength of the formulation it is assessed as the force or weight required to break the tablet, in simplest words it is the crushing strength. it is measured in kilograms and is the force required to break a tablet. Oral tablets have a hardness of 4 to 10 kgs, hypodermic and chewable tablets have hardness of 3 kgs and that of sustained release tablet is 10 to 20 kgs. Friability is defined as percentage of weight loss of the drug due to mechanical action. Tablets are constantly subjected to mechanical strokes and aberration during the process of manufacturing, packaging and transportation process. Dissolution studies help us to get an idea how effectively the drug will be released. Disintegration is defined as the state in which no residue of the tablet or capsule, except fragments of the undissolved coating or capsule shell, remains on the screen of the test apparatus or, if any other residue remains, it consists of soft mass having no palpably firm, unmoistened core .It is a useful guide to comparative bioavailability. Disintegration plays an important role in dissolution process. Hardness of the formulation has an effect on disintegration process. Each of the parameters is interrelated with each other hence the above parameters were considered in our study.

The analgesic, antipyretic, and anti-inflammatory effects of acetylsalicylic acid are due to actions by both the acetyl and the salicylate portions of the intact molecule as well as by the active salicylate metabolite. Acetylsalicylic acid directly and irreversibly inhibits the activity of both types of cyclooxygenase (COX-1 and COX-2) to decrease the formation of precursors of prostaglandins and thromboxanes from arachidonic acid. This makes acetylsalicylic acid different from other NSAIDS (such as diclofenac and ibuprofen) which are reversible inhibitors. Salicylate may competitively inhibit prostaglandin formation. Acetylsalicylic acid's antirheumatic (nonsteroidal anti-inflammatory) actions are a result of its analgesic and anti-inflammatory mechanisms; the therapeutic effects are not due to pituitary-adrenal stimulation. The platelet aggregation-inhibiting effect of acetylsalicylic acid specifically involves the compound's ability to act as an acetyl donor to cyclooxygenase; the nonacetylated salicylates have no clinically significant effect on platelet aggregation. Irreversible acetylation renders cyclooxygenase inactive, thereby preventing the formation of the aggregating agent thromboxane A2 in platelets. Since platelets lack the ability to synthesize new proteins, the effects persist for the life of the exposed platelets (7-10 days). Acetylsalicylic acid may also inhibit production of the platelet aggregation inhibitor, prostacyclin (prostaglandin I2), by blood vessel endothelial cells; however, inhibition prostacyclin production is not permanent as endothelial cells can produce more cyclooxygenase to replace the non-functional enzyme. The major drawback of aspirin is G.I. Mucosa ulceration can be avoided by providing the effective enteric coating.

Marketed preparation Aspirin of various brands in the form of entric coated tablets are available. Among these five brands like Ecospirin, loprin and Delispirin were selected and were evaluated for the above mentioned parameters.

The prime focus of this research work is though the above brands showed differences in the packaging and appearances there was a marked difference in their prices Rs 4.15 of

Loprin (Unichem laboratries ltd), Rs.4.26 of Ecospirin (Usv Ltd) and Rs. 3.83 of Delispirin (Aristo Pharmaceuticals Pvt Lmt) . These differences in their prices certainly pose a question on their quality. Thus evaluation of these marketed preparations makes it more clear about whether or not the brands available at a cheaper and economical price are as effective as that of their costlier counterparts.

The cost of various Aspirin enteric coated tablets is given below.

Brands	Price (in Rs)
Delispirin	**Rs 3.83**
Loprrin	Rs 4.15
Ecospirin	Rs 4.26

AIM AND RATIONALE

An important side effect is the gastrointestinal discomfort and bleeding hence different preparations of aspirin have been prepared commercially in an effort to diminish the gastrointestinal irritation and blood loss without causing a loss of its therapeutic benefits. There is a demand for Aspirin enteric coated mainly to reduce gastro irritation because this enteric coated tablet quality prevents stomach the bleeding and ulceration that can occur after frequent Aspirin use. Aspirin is used in the temporary relief of various forms of pain, inflammation associated with various conditions (including rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, osteoarthritis) and is also used to reduce the risk of death and/or nonfatal myocardial infarction in patients with a previous infarction or unstable angina pectoris. Aspirin tablets under various brands. Among these three brands Loprin (Unichem laboratries ltd), Ecospirin (Usv Ltd) and Delispirin (Aristo Pharmaceuticals Pvt Lmt) which has differences in their retail price. Order to check the quality of these the above marketed preparations were evaluated for various parameters like weight variation, hardness, wetting time, disintegration time and percent content using standard techniques and the results were compared with the standard values. A comparative study using the different evaluation parameters using the above five brands forms the basis of this project. Even the brand of the drug available at the cheaper price should not deviate much from the quality standards that has been prescribed as the standard value. Thus it would be easier to understand whether the brand and cost differences actually has an effect on the quality of the drug and why for a drug of a higher cost when the same drug is available at a more cheap and economical price.

MATERIALS AND METHOD

In the present study three different brands of Aspirin enteric coated tablets of were used namely Loprin (Unichem laboratries ltd), Ecospirin (Usv Ltd), Delispirin (Aristo Pharmaceuticals Pvt Lmt .Dose of the drug was kept constant i.e. 75mg. Pharmaceutical evaluation of the drug of the mentioned parameters by the following ways:

Weight variation:

The weight variation test was carried out in order to ensure uniformity in the weight of tablets in a batch. First the total weight of 20 tablets from each formulation was determined and the average is calculated. The individual weight of the each tablet was also determined to find out the weight variation and compare the individual tablet weight to average weight variation tolerance given as per I.P.

Dimensions:

1. Thickness: The thickness was measured by placing tablet between two arms of the vernier calipers. Five tablets were taken and their thickness were measured.

2.Diameter: : The diameter was measured by placing tablet between two arms of the vernier callipers. Five tablets were taken and their diameter was measured.

Tablet Hardness:

Hardness of tablet is defined as the force applied across the diameter of the tablet to break it. The hardness of tablet is an indication of its strength. Measuring the force required to break the tablet across tests it. The force is measured in kg and the hardness of about 1-2kg/cm^2 is considered to be satisfactory for orally disintegrating tablets. Hardness of three tablets from each formulation is determined by Monsanto hardness tester and their average value was calculated.

Friability test

The friability of tablets was determined by using Roche friabilator which subjects a number of pre- weighed tablets to determine the combined effects of abrasion and shock by utilizing a plastic chamber. It is expressed in percentage (%). Twenty tablets were initially weighed (Wt) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 10 revolutions by dropping the tablets at a distance of six inches with each revolution.The tablets are then dusted and re-weighed. Conventional compressed tablets that lose less than 0.5% to 1% of their weight are generally considered acceptable.

Disintegration test:

This test is provided to determine whether tablets or capsules disintegrate within the prescribed time when placed in a liquid medium under the experimental conditions. For the purposes of this test disintegration does not imply complete dissolution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the discs, if used, is a soft mass having no palpably firm core.

Aspirin tablets were taken in separate basket rackets, which were positioned in 1 litre beaker of 0.1N HCL for 2 hrs, which acts as a simulated gastric fluid at 37 degree Celsius without disks. Then same tablets were put in 1 litre beaker of PH 7.5 Phosphate buffer with disks and operated for 2hrs and 15 minutes.

Dissolution Test:
The dissolution was started with 0.1N HCL for 2hrs and samples were withdrawn at 15 min intervals. After 2 hours, the product was transferred to pH 6.8 buffer medium and the dissolution was out for 45 minutes and the aliquots were withdrawn after 5 minutess intervals. Bath volume was maintained at 1000 ml. The absorbance of each sample was observed in UV visible spectrophotometer at 276nm against blank solution. Electro lab Dissolution Tester with a rotating paddle at 50 rpm was used.

RESULTS AND DISCUSSION

1. The results of **weight variation** were as follows:

Brands	**Average Weight**	**±7.5% Permissible Limits**	**Inference**
1.Ecospirin	100 mg	92.5-107 mg	Pass
2.Loprin	97 mg	89.7-104mg	Pass
3.Delispirin	119 mg	110-128 mg	Pass

The **dimensions** of the tablets were measured and reported as follows:

Brands	**Thickness**	**Diameter**
1. Ecospirin	3.2mm	6.124mm
2.Loprin	2.14mm	6.136mm
3.Delispirin	3.06mm	6.06mm

The **hardness** of the tablets were found as follows:

Brands	**Hardness**	**Inference**
Ecospirin	2 kg/cm^2	Pass
Loprin	2 kg/cm^2	Pass
Delispirin	2 kg/cm^2	Pass

Limits: 1-2 kg/cm^2

The **friability** of the tablets was reported as follows:

Brands	**Inference**
1. Ecosprin	Pass
2.Loprin	Pass
3.Delispirin	Pass

Limits:NMT 1%

The **disintegration time** of the tablets was reported as follows:

Brands	**Disintegration Time**		**Inference**
	0.1 M HCL	**Phosphate buffer 6.8**	
Ecospirin	Does not disintegrate in 120 minutes	NMT 60 minutes	Pass
Delispirin	Does not disintegrate in 120 minutes	NMT 60 minutes	Pass
Loprin	Does not disintegrate in 120 minutes	NMT 60 minutes	Pass

Dissolution rate for the tablets were as follows:

Ecospirin		**Delispirin**		**Loprin**	
Time (in minuts)	% cumulative release	Time (In minutes)	% cumulative release	Time (In minutes)	% cumulative release
0	0	0	0	0	0
10	16.78	10	18.91	10	15.93
30	68.32	30	70.1	30	72.12
45	87.21	45	89.42	45	84.01
60	98.28	60	100.1	60	95.17
90	102	90	100.7	90	101

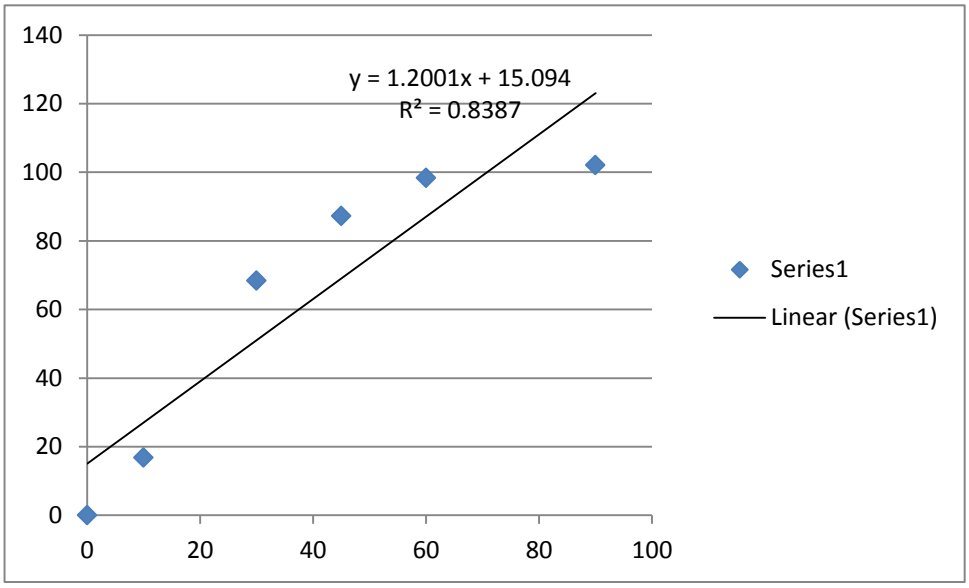

FIG: GRAPH FOR ECOSPIRIN

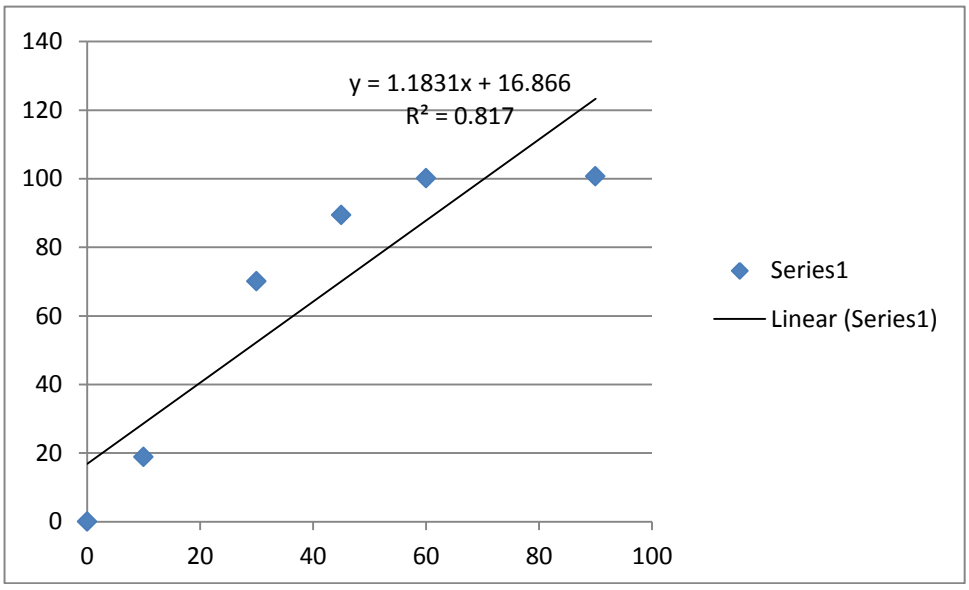

FIG: GRAPH FOR DELISPIRN

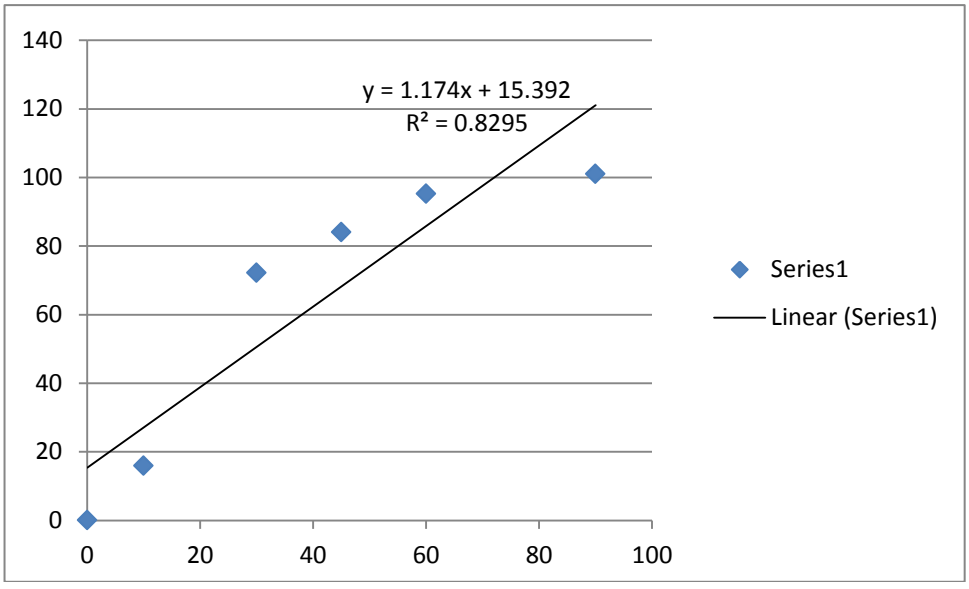

FIG: GRAPH FOR LOPRIN

Inference:

Enteric coated tablets passed the acid stage of the dissolution test, with no release of aspirin after 2 hours in 0.1N HCl and more than 75% of Aspirin was released in 90 minutes which says that the tablets pass the dissolution test.

CONCLUSION

The enteric coated tablets have a lot of advantage over uncoated tablets. Entric coating protects the inner linning of the stomach thus reducing the effects such as gastric irritation caused by drug

Thus the need of entric coated tablets is increasing. All three brands of the selected aspirin enteric coated tablets showed values are within the acceptable range for parameters such as hardness, weight variation, thickness measurement, disintegration time and dissolution studies. Though there was a cost difference in their respective marketed prices the quality of all the three brands of the mentioned tablets were found to be within the permissible limits. Finally we report that there was no much difference in their quality in accordance with their brands. Even the drug available at a cheaper price showed all the values as per the standard values. Further studies can be done in this regard with parameters like content uniformity and also by evaluating the excipients used in these marketed preparations.

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SYNTHESIS OF NOVEL PYRAZOLINE DERIVATIVE AND SCREENING OF ITS ANTI-ANGIOGENIC ACTIVITY

*Anagha Amrute, Osama Ansari and M.P.Toraskar**

ABSTRACT:
Chalcone is a central core for many important biological compounds**.** . Pyrazolines widely used as anti- tubercular, anti-bacterial and anti- cancer agents and these have broad spectrum anti-microbial activity. Pyrazolines are well known and important nitrogen containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. . The present review provides an insight view to pyrazolines synthesis and its biological activities along with the compilation of recent patents on pyrazolines. The process of vessel formation, called angiogenesis, is essential for tumor progression. Angiogenesis has also been associated with several pathological condition such as cancer, arthritis etc. Targeting of molecular pathways involved in such tumor angiogenetic processes by using specific drugs or inhibitors is important for developing new anticancer therapies.The ability of zebra fish to recapitulate a variety of human cancers provide a unique in vivo system for drug identification and validation. The current work is small effort to check the anti-angiogenic activity of synthesized drug using zebra fish model.

Keywords: Pyrazoline, Tumor angiogenesis, Zebra fish model.

1. INTRODUCTION:

Cancer: Cancer, in its most basic definition, is the abnormal uncontrolled growth of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed.

Difference between Normal Cells and Cancer Cells: Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. One important difference is that cancer cells are less specialized than normal cells. That is, whereas normal cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, unlike normal cells, cancer cells continue to divide without stopping. In addition, cancer cells are able to ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or apoptosis, which the body uses to get rid of unneeded cells. Cancer cells can induce nearby normal cells to form blood vessels that supply tumours with oxygen and nutrients, which they need to grow. These blood vessels also remove waste products from tumours.

Carcinogens: Carcinogens are a class of substances that are directly responsible for damaging DNA, promoting or aiding cancer. Certain pesticides, dyes, and chemicals used in metal refining are thought to be carcinogenic, increasing the risk of developing pancreatic cancer. When our bodies are exposed to carcinogens, free radicals are formed that try to steal electrons from other molecules in the body. Theses free radicals damage cells, affecting their ability to function normally, and the result can be cancerous growths.

Treatments for cancer: Cancer treatment depends on the type of cancer, the stage of the cancer (how much it has spread), age, health status, and additional personal characteristics. There is no single treatment for cancer, and pancreatic cancer is usually only curable when found in its earliest stages. Surgery, radiation, and chemotherapy are the most common treatment types. Treatments seek to remove the cancer and/or relieve painful symptoms that the cancer is causing.

Chalcone is a central core for many important biological compounds. A classical method for synthesis of chalcones is Claisen- Schmidt condensation or Aldol condensation reaction between substituted aryl ketone and aromatic aldehyde in the presence of aq. Alkaline bases $Ba(OH)_{2,}$ or LiOH as a catalyst. Chalcones also synthesized by using microwave irradiation, ultrasound irradiation and by Suzuki reaction. These undergo variety of chemical reaction to produce innumerable heterocyclic compound that are used as intermediates to prepare drugs with therapeutic value. Chalcone derivatives from natural and synthetic analogs exhibit diverse pharmacological activities such as anti-TB, anti-inflammatory, anti-neoplastic, anti- bacterial, anti-fungal, anti-malarial, anti- viral, anti- allergic and estrogenic. Pyrazolines widely used as anti- tubercular, anti-bacterial and anti- cancer agents and these have broad spectrum anti-microbial activity. Pyrazolines are well known and important nitrogen containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. They have several prominent effects, such as antimicrobial, anti-mycobacterial, anti-fungal, anti-amoebic, anti-inflammatory, analgesic, antidepressant and anticancer activities. They also possess some potent receptor selective biological activity like Nitric oxide synthase (NOS) inhibitor and Cannabinoid CB1 receptor antagonists activity. 4,5-dihydro-1H- pyrazolines seem to be the most frequently studied pyrazoline type compounds. As a result, a large number of such pyrazolines using different synthetic methods for their preparation have been described in the chemistry literature. The present review provides an insight view to pyrazolines synthesis (*E*)-5-phenyl-3-styryl-4,5-dihydro-1H-pyrazole-1-carboxamide and its biological activities along with the compilation of recent patents on pyrazolines.

2. Aim and rationale: "Synthesis of novel pyrazoline derivative and screening of its anti-angiogenic activity". The current work is effort to check the anti-angiogenic activity of synthesized drug using zebra fish model

3. Materials and methodology:

Reagents and Solvents: The reagents used were of analytical grade and were used without further purification. The solvents used were of commercial grade. They were used after purification.

Scheme of synthesis:

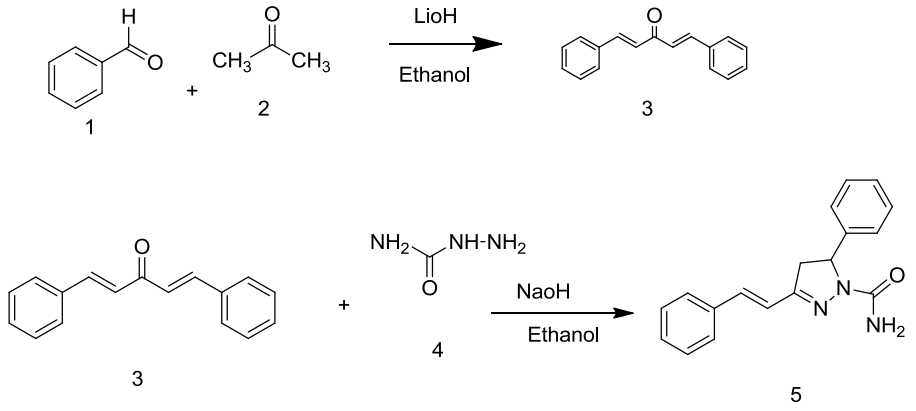
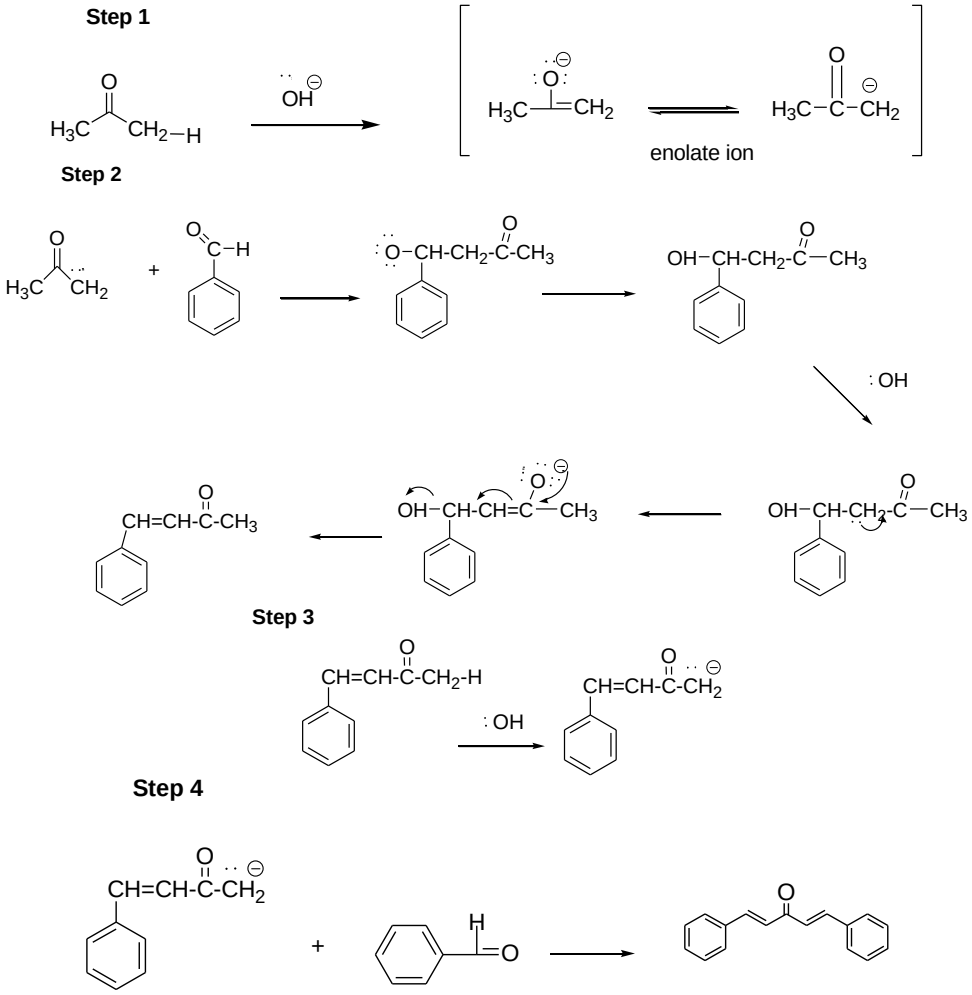


Figure 3: Scheme for synthesis of designed Pyrazoline

4. Reaction mechanism SCHEME OF SYNTHESIS:



5. PROCEDURE :

SYNTHESIS PYRAZOLINE:

Procedure for synthesis of Dibenzylidine acetone: Place 11.5ml benzaldehyde + 4ml acetone 1.26gm lithium hydroxide + 25ml ethanol in dry conical flask. Stirr mix using magnetic stirrer vigorously till yellow solid ppt out. Cool the flask by keeping in ice bath then filter. Then wash with ice cold water and ice cold ethanol. Recrystalise by using ether. Find melting point and yield. Do not add charcoal.

Dibenzylidene acetone react with semicarbazide to give Pyrazoline: A solution of NaOH (1gm, 0.025mol) and chalcone (0.001mol) in ethanol 25ml semicarbazide (0.92gm, 0.01mol) was added slowly under stirring. After addition, the reaction mixture was refluxed for one hour and the solution was washed with ether and cold water. Filtered crystals were washed with ice cold water, air dried and recrystallize from ethanol. The progress of the reaction and the purity of compounds is monitored by TLC using Silica gel G plates of size 3x8 cm (Sigma-Aldrich) and visualized under short and long wavelength of UV chamber. The compound was chemically characterised using FTIR. Yield was calculated and melting point was determined for the synthesised compound.

Procedure for biological evaluation:

Toxicity test: Toxicity studies were performed according to OECD test guideline- 203 (Fish, Acute Toxicity Test) in order to determine maximum tolerable dose of test compound. According to the test, seven fishes were kept per group (1 gm of fish/ litre of water) and the fishes were exposed to test substance for a period of 96 hours. The mortalities were recorded at 24, 48, 72 and 96 hours and the concentration which killed more than 50% of the fish (LC50) was determined. Based on the mortality, the dose was increased or decreased by a spacing factor of 2.2.

Fin regeneration method: The wild type adult zebra fish weighing between 0.5-1.5 gm were procured from a local supplier. The fishes were kept for acclimatization for about 15 days in laboratory conditions. The fishes were divided into 2 treatment groups, 3 fishes per group. The fishes were kept in glass beakers of the size of 250 ml containing about 150 ml of fish water and a single fish each beaker. Water had been added with sodium thiosulphate crystals as dechlorinating agent and rock salt as antibacterial agent. The beakers were kept in series to provide proper aeration. On every alternate day, drug was administered to the fish till the 7th day. Images were captured frequently to observe the growth of caudal fin. Dose of the drug was decided based on the results of toxicity test. On the day of experiment, the fishes were anesthetized by using 2-Phenoxy ethanol (0.9ml/1000 ml of water). The image of whole fin was taken in order to compare it with regenerated fin. Their fin was cut up to 50% using sterile razor blade. The fish was then put back into recovery tank to recover from anesthesia and then transferred back to beaker.

6. RESULT AND DISCUSSION:

Chemical Evaluation

- IUPAC name:(E)-5-phenyl-3-styryl-4,5-dihydro-1H-pyrazole-1-carboxamide
- Molecular weight: 219
- Molecular formula: $C_{18}H_{17}N_3O$
- Nature: Yellowish powder

- Melting point: 153°C
- Yield (% w/w): 72%
- FTIR spectra: An IR spectrum was recorded on a Quest ATR Diamond Accessory (Black) P31482 & Shimadzu 8100 infrared spectrophotometer.

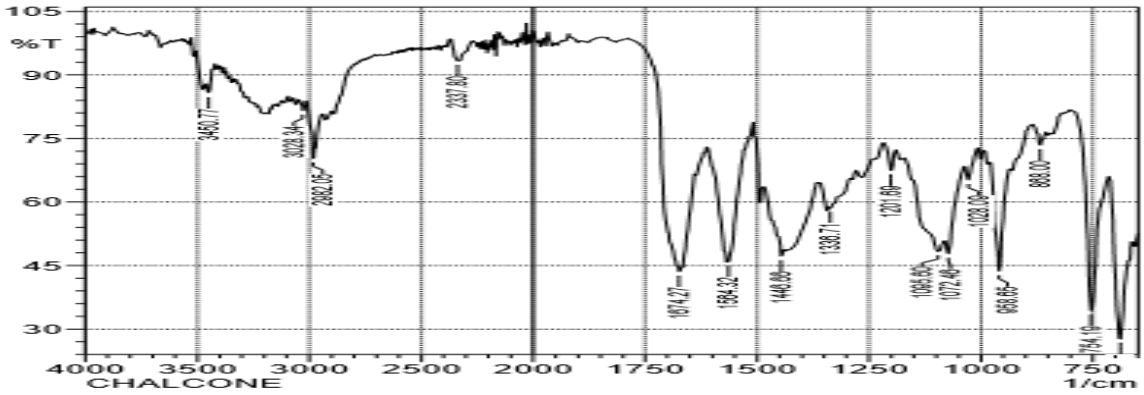


Figure 5: IR spectra of pyrazoline compound

Table 2: Interpretation of IR data of compound

Sr. no.	Characteristic absorbance peak (ν)	Wavelength (cm^{-1})
1.	NH_2	3450.77
2.	Ar-H	3028.34
3.	C=O	1674.27
4.	C-N	1072.46
5.	C=C	1446.66

Biological Evaluation:

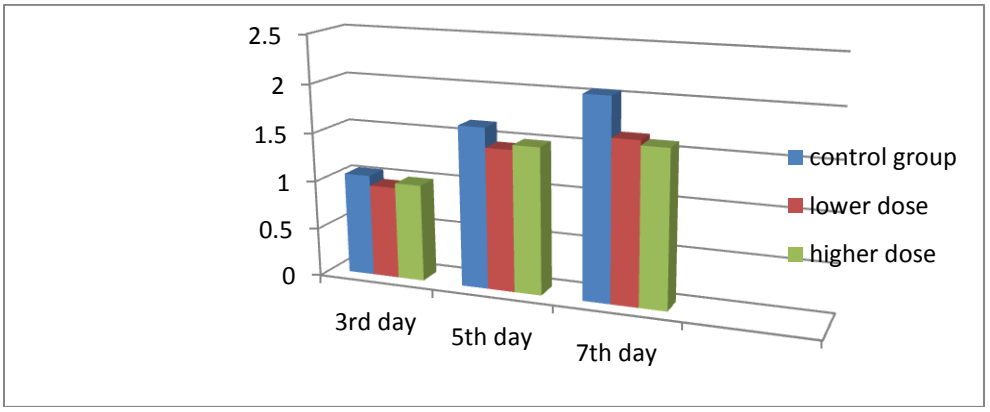
OBSERVATION TABLE:

CONCENTRATION	NUMBERS OF FISHES ALIVE				
	HOURS				
	0	24	48	72	96
0.1mg/fish/litre	6	6	6	6	6
0.22mg/fish/litre	6	6	6	6	6
0.48mg/fish/litre	6	6	6	6	6
1.05mg/fish/litre	6	6	6	6	6
Vehicle (control)	6	6	6	6	6

Observation: Toxic dose depends on the concentration at which 50% of fishes alive after 96 hours. From the observation, the conclusion drawn was that the fishes are stable upto 1.05mg/fish/litre dose.

Graphical result obtained for fin growth inhibition for the synthesized drug is as follow:

	3rd day length (mm)	5th day length (mm)	7th day length (mm)
Vehicle control	1.05	1.65	2.05
Lower dose	0.95	1.45	1.65
Higher dose	1	1.5	1.6



7. DISSCUSSION:

All the synthesized compounds were evaluated using spectroscopic technique like IR. Pyrazoline was derived by reacting Dibenzylidine acetone with semicarbazide. The IR spectra of these chalcones show the presence of C=O, C=C and two sharp bands of NH at 1674.27 cm^{-1}, 1446.66 cm^{-1} and 3450.77 cm^{-1} respectively. C=O and C=C peaks are characteristic peaks of chalcones, as these peaks denote the presence of α,β-unsaturated compounds**.** The growth of tail was observed lesser in fishes administration with drug than that of control. Hence it can be concluded that the drugs have antiangiogenesis property. The data helps in the preliminary idea of toxic and efficacious range of drugs. The *in-vivo* studies further support the claim for a better evaluation of the compounds. Testing can be done on higher animals.

8. CONCLUSION:

Compound showed good anti-angiogenic effect. As angiogenesis is one of the important factors required for cancer cell growth, inhibition of angiogenesis will lead to death of cancerous cells. Hence we can conclude that the present derivative can be effectively used as anti cancer agents after further studies.

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ANTI-ANGIOGENIC SCREENING OF NOVEL PYRAZOLINE DERIVATIVE

*Karishma Singh, Rohang Shukla and M.P.Toraskar**

ABSTRACT:

The process of vessel formation, called angiogenesis, is essential for tumor progression. Angiogenesis has also been associated with several pathological condition such as cancer. Targeting of molecular pathways involved in such tumor angiogenetic processes by using specific drugs or inhibitors is important for developing new anticancer therapies. Researchers need to use animal models that facilitate the elucidation of tumor angiogenesis mechanisms. VEGFR is an important growth factor and a positive regulator of angiogenesis with its distinct activity for vascular endothelial cells. Its been observed that the zebrafish emerged as a valid model organism to study developmental angiogenesis and more recently has been developed for in vivo screening of anticancer agents having VEGFR inhibitory activity.The ablility of zebra fish to recapitulate a variety of human cancers provide a unique in vivo system for drug identification and validation.The current work is small effort to check the anti-angiogenic activity of synthesized drug using zebra fish model.The synthesized drug was found to have VEGFR inhibitory action.Thus the synthesized molecule hold importance as a lead to develop further molecules having anti-VEGFR activity and may be screened for its anti-cancer activity in future.

Keywords-Cancer cells, Angiogenesis,Zebra fish model

1. INTRODUCTION

Difference between Normal Cells and Cancer Cells: Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. One important difference is that cancer cells are less specialized than normal cells. That is, whereas normal cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, unlike normal cells, cancer cells continue to divide without stopping. In addition, cancer cells are able to ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or apoptosis, which the body uses to get rid of unneeded cells.

Treatments for cancer: Cancer treatment depends on the type of cancer, the stage of the cancer, age, health status and additional personal characteristics. There is no single treatment for cancer. Surgery, radiation and chemotherapy are the most common treatment types.

Angiogenesis: Angiogenesis is the process of generating new capillary blood vessels. Tumor growth and metastasis are angiogenesis dependent . A growing tumor needs an extensive network of capillaries to provide nutrients and oxygen. In addition, the new intratumoral blood vessels provide a way for tumor cells to enter the circulation and to metastasize to distant organs.Hence, angiogenesis is one of the most important processes in tumor progression. In 1971 J. Folkman proposed that tumor growth and metastasis are angiogenesis dependent and blocking angiogenesis could be strategy to block the progress of the disease. Significance of VEGF-A and its receptors rules the proliferation of

endothelial cells in regeneration mechanism regulating angiogenesis. With this prospective, regenerative angiogenesis is studied using zebrafish as a model organism.

Following target was selected for the research study-

Vascular endothelial growth factor receptor (VEGFR): A prospective target

VEGFs signal through three tyrosine kinase receptors, known as Flt-1 (VEGFR-1), Flk-1/KDR (VEGFR-2) and VEGFR-3 predominantly expressed by endothelial cells. A degree of specificity has been shown for growth factor-receptor binding. . Recognition of the VEGF pathway as a key regulator of angiogenesis has led to the development of several VEGF-targeted agents, including agents that prevent VEGF-A binding to its receptors, antibodies that directly block VEGFR-2, and small molecules that inhibit the kinase activity of VEGFR-2 thereby block growth factor signalling. Some of them were approved by FDA to clinical applications. By blocking VEGFR, several anti-angiogenic effects are expected. Firstly, it will inhibit new vessel growth, perhaps accompanied by vessel regression and subsequent tumor cell death. VEGFR is a survival factor for endothelial cells and VEGF withdrawal can induce tumor endothelial cell death as well as prevent further angiogenesis. Blocking VEGFR will also induce endothelial cell apoptosis.

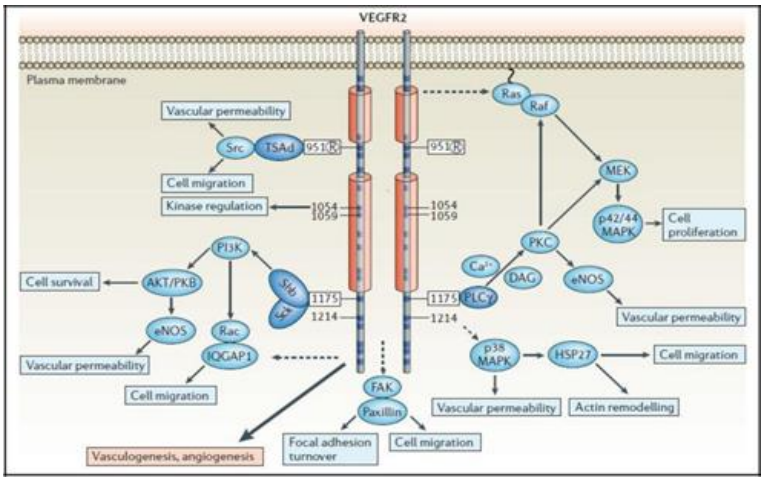


Figure 1: Mechanism of action of VEGFR signal transduction and phosphorylation site

VEGFR inhibitors: VEGFR inhibitors consist of two main classes.

a.Monoclonal antibodies

b.Small molecule tyrosine kinase inhibitors-Tyrosine kinase inhibitors (TKI) are targeted therapy drugs that block the signals needed for the tumor to grow. Small molecule tyrosine kinase inhibitors act on the kinase domain of the VEGFR receptor and prevent the phosphorylation of any substrate by the receptor thus preventing further signal transduction.

2. AIM AND RATIONALE: "Anti-angiogenic screening of novel pyrazoline derivative". The present work is an effort to evaluate the anti-angiogenic activity of previously synthesized compound using zebra fish model.

3. MATERIALS

Instruments:

1) Electronic weighing balance
2) 96 well sterile Microtitre plate
3) Motic Digital Microscope (4X, 10X)
4) Aerators.
5) Regulators

Chemicals:

1) 2-phenoxy ethanol (S. D. Fine Chemicals)
2) Sodium thiosulphate (S. D. Fine Chemicals)
3) Rock salt
4) Sodium chloride (Research Lab Fine Chem Industries)
5) Dimethyl sulfoxide (DMSO)

Softwares:

1. Motic Image Plus
2. ImageJ
3. Graph pad Prism 5.0.

Animal model**-** Zebrafish

Compound used- 3,5-bis(4-chlorophenyl)-N-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide

4. METHODOLOGY

Toxicity test: Toxicity studies were performed according to OECD test guideline- 203 (Fish, Acute Toxicity Test)in order to determine maximum tolerable dose of test compound. According to the test, seven fishes were kept per group (1 gm of fish/ litre of water) and the fishes were exposed to test substance for a period of 96 hours. The mortalities were recorded at 24, 48, 72 and 96 hours and the concentration which killed more than 50% of the fish (LC50) was determined. Based on the mortality, the dose was increased or decreased by a spacing factor of 2.2.

Fin regeneration method: The wild type adult zebrafish weighing between 0.5-1.5 gm were procured from a local supplier. The fishes were kept for acclimatization for about 15 days in laboratory conditions. The fishes were divided into 2 treatment groups, 3 fishes per group. The fishes were kept in glass beakers of the size of 250 ml containing about 150 ml of fish water and a single fish each beaker. Water had been added with sodium thiosulphate crystals as dechlorinating agent and rock salt as antibacterial agent. The beakers were kept in series to provide proper aeration. On every alternate day, drug was administered to the fish till the 7th day. Images were captured frequently to observe the growth of caudal fin. Dose of the drug was decided based on the results of toxicity test. On the day of experiment, the fishes were anesthetized by using 2-Phenoxy ethanol (0.9ml/1000 ml of water). The image of whole fin was taken in order to compare it with regenerated fin. Their fin was cut up to 50% using sterile razor blade. The fish was then put back into recovery tank to recover from anaesthesia and then transferred back to beaker.

Evaluation of anti cancer activity: Pre and post amputated images were collected and length was measured using scale. Each beaker containing fish was labelled according to the group based on which treatment it is receiving. Different doses of EEPB, isolated fraction

and standard drug (Imatinib Mesylate) were given to each group. Dose of the drugs and standards were determined by toxicity test. The water in each beaker was changed and dose was administered on every alternate day and up to 7 days. Images were taken on 3rd, 5th and 7th day post amputation (DPA) using Motic digital microscope (4X) on 3rd and 7th DPA . On 7th day, final images were taken. Length and area of the fin was measured by using Motic image plus 2.0 and ImageJ softwares. Percent of inhibition and percent of regeneration was calculated.

Biological evaluation:

Toxicity study observation table-

CONCENTRATION	NUMBER OF FISHES ALIVE				
	HOURS				
	0	24	48	72	96
0.1mg/fish/litre	6	6	6	6	6
0.22mg/fish/litre	6	6	6	6	6
0.48mg/fish/litre	6	6	6	6	6
1.05mg/fish/litre	6	6	6	6	6
Vehicle (control)	6	6	6	6	6

Observation: Toxic dose depends on the concentration at which 50% of fishes alive after 96 hours.From the observation,the conclusion drawn was that the fishes are stable upto 1.05mg/fish/litre dose.

5. RESULT & DISCUSSION

Dilutions of the compound was exhibited *in-vivo* activity. Inhibition of tail of zebrafish was observed and fin generation on 3rd and 4th day was measured which is shown below in tabular form and through graphical representation.Table no.1 shows the fin growth in (mm) on 3rd and 4th day in comparison to control group.In Table no.2 ,fin growth on 3rd and 4th day was recorded in cleft region.In Table no.3,fin growth on 3rd and 4th day was measured in ventral region.The graphs 1,2 and 3 below show the fin generation in mm in dorsal,cleft and ventral region on 3rd and 7th day of study.

Cleft	3rd day	7th day

Figure 4: Fin generation observed in dorsal region

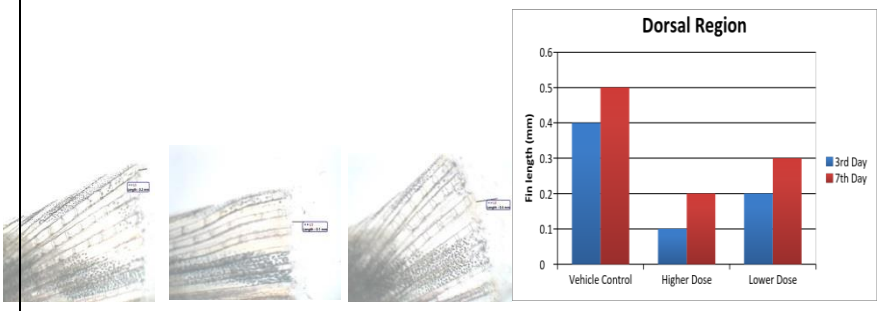
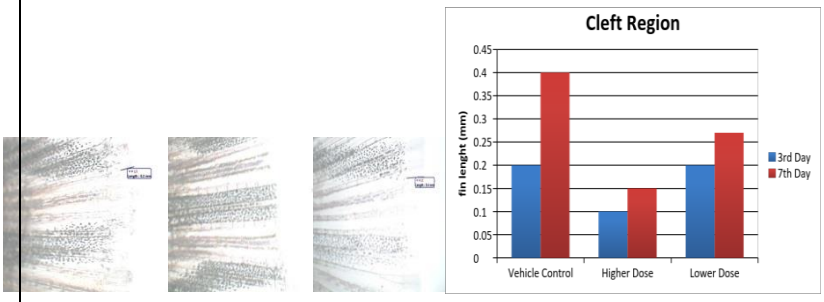


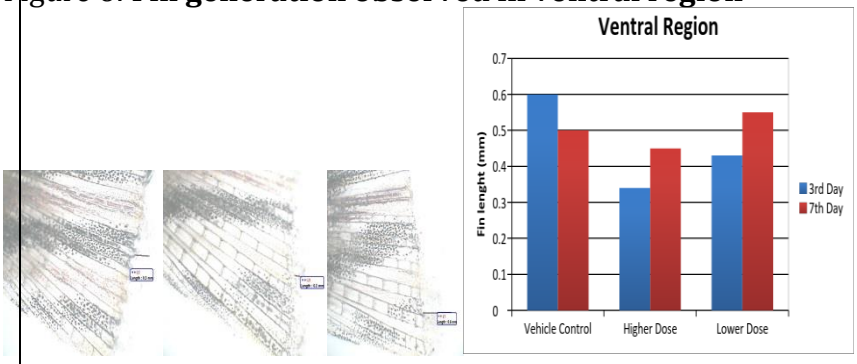
Figure 5: Fin generation observed in cleft region

Dorsal region	3rd day Length (mm)	7th day Length (mm)
Vehicle Control	0.4	0.5
Higher Dose	0.1	0.2
Lower Dose	0.2	0.3



Region	Length (mm)	Length (mm)
Vehicle Control	0.2	0.4
Higher Dose	0.1	0.15
Lower Dose	0.2	0.27

Figure 6: **Fin generation observed in ventral region**



Ventral Region	3rd day Length (mm)	7th day Length (mm)
Vehicle Control	0.6	0.5
Higher Dose	0.34	0.45
Lower Dose	0.43	0.55

Discussion: The main aim of the present work was to study the anti-VEGFR or anti-angiogenic activity of the synthesized compound.The toxicity study was performed for selection of study dose,whereas factor of 2.2 was used.On further study of synthesized molecule with respect to fin generation,the result obtained shows inhibition in zebrafish fin growth.After the observation of fin generation on 3rd & 7th day,we reported the generation of fin in length(mm) which is expressed in tabular form and compared to the control group in graphical form.

Table no1 ,2 &3 show the generation of fin in different regions.The graphs of dorsal,cleft and ventral region depict that in comparison to the control group(without drug) ,the fin growth observed on 3rd and 7th day at lower dose(containing drug) was less than that observed in control group.

Talking of higher dose(containing drug) it showed fin generation definitely less than that seen in control group but it was even lesser than that observed in lower dose which clearly states that drug has inhibitory action and at higher doses it's more effective.

6. CONCLUSION:

The growth of tail was observed lesser in fishes administered with pyrazoline derivative than that of control. Hence it can be concluded that the drugs have anti-angiogenesis effect. The data helps in the preliminary idea of toxic and efficacious range of drugs. Since the synthesized compound is seen to inhibit formation of vasculature in the tail region they could have a potential role as a VEGF inhibitor. The *in-vivo* studies further support the claim for a better evaluation of the compounds. Testing can be done on higher animals. The compound showed good anti-angiogenic effect. As angiogenesis is one of the important

factors required for cancer cell growth, inhibition of angiogenesis will led to death of cancer cells. Hence it can be concluded that the present synthesized compound possesses anti-angiogenic activity and this can be a lead for further research study of anti-cancer agents.

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PREPARATION AND EVALUATION OF GEL LOADED TURMERIC MICROSPONGE

*Shamika Shirke, Suniti Shinde and Manisha Karpe**

ABSTRACT:

A drug delivery system enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time and place of release of drug in the body .One of these includes topical preparations. Used for localized effect at the site of their application. A new method approached in TDDS is microsponge. Microsponge drug delivery system is a patented, higher cross linked porous, polymeric microsponge that can entrap wide range of activities and release them with desired rate. With lots of synthetic and inorganic drugs used in market for treatment of various diseases; natural drugs have also gained appreciation and still has high market demand even today. Natural drug helps by reducing toxicity and any adverse drug effects. Thus adding for better pharmacological effect. Turmeric active ingredient obtained from the powdered dry rhizomes of plant *Curcuma longa.* It is widely used as a colouring agent, medicinal agent, flatulence, jaundice, hepatitis, pains and haemorrhage. n approach is being made in combining the old tradition with new technology to gain more appreciation in the market. This leads to the formulation of "Gel Loaded turmeric Microsponge" as a Novel Drug Delivery.

Keywords: NDDS, Microsponge, Quasi Emulsion

INTRODUCTION

1.1 Drug Delivery System:

A drug delivery system enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time and place of release of drug in the body. An ideal drug delivery system should deliver the drug at the rate dictated by the needs of the body over the period of treatment.

The preparation and evaluation of gel containing turmeric microsponge which is a topical dosage form and it shows therapeutic activity as an anti-inflammatory.

1.2 Introduction to Turmeric:

[3] Turmeric is a product of *Curcuma longa*, a rhizomatous herbaceous perennial plant belonging to the ginger family Zingiberaceae, which is native to tropical South Asia. As many as 133 species of *Curcuma* have been identified worldwide. The main component of the root is a volatile oil, containing turmerone, and there are other coloring agents called curcuminoids in turmeric. Curcuminoids consist of curcumin demethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin, which are found to be natural antioxidants. Turmeric which is the active ingredient is obtained from the powdered dry rhizome of the plant *Curcuma longa*, commonly called turmeric is widely used as a colouring agent, medicinal agent and as spice in many food items. It contains wide variety of phytochemicals including curcumin, curcumol, eugenol, turmerones, zingiberene, etc.

1.3 Introduction to Topical Drug Delivery System:

Topical preparations are used for localized effect at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membrane. It includes two basic types:

External topical that are spread, sprayed or otherwise dispersed on to cutaneous tissues to cover the affected area.

Internal topical that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity.

Microsponge drug delivery system is a patented, highly cross linked, porous, polymeric microsponge that can entrap wide range of actives and release them with desired rate. This system is applicable for the improvement of performance of topically applied drugs. It is a unique technology for controlled release of topical agents and consists of micro porous beads.

Advantages of microsponge drug delivery over the drug delivery system:

- Microspheres cannot usually control the release rate of actives. Once the wall is ruptured the activity contained within the microspheres will be released
- The amount released from the microsponge is programmable.
- The rate of release mechanism depends on the pressure, volatility, temperature, solvent, partition coefficient.

MATERIALS AND METHODS:

2.1 Formulation development of microsponge

The drug was incorporated in a novel microparticulate microsponge system having a drug dispersed matrix. The selection of a particular encapsulation method is primarily determined by the solubility characteristics of the drug and polymer. The method should ideally produce (a) high yields of micro particles and free of extensive agglomeration, (b) appropriate encapsulation of the core material.

2.2 Methodology for formulating curcumin microsponge

Microsponges were prepared by Quasi Emulsion Solvent Diffusion Technique which requires two immiscible phases internal and external phase with a surfactant which aids formation of an emulsion by reducing the interfacial tension.

The method consists of two steps. In the first step inner phase was prepared and in second step outer phase was prepared.

Ingredients used:

Drug: turmeric
Polymer: ethyl cellulose
Solvent: dichloromethane
Glycerol: plasticity
External phase: polyvinyl alcohol

QUASI EMULSION TECHNIQUE

2.3: FORMULATION TABLE – MICROSPONGE

SR.NO	INGREDIENTS	QUANTITY TAKEN
1	Turmeric	1g
2	Ethyl cellulose	1g
3	Polyvinyl alcohol (5%)	20ml
4	Dichloromethane	10ml
5	Glycerol	1ml
6	Water	100ml

STEP 1: Preparation of inner phase

- Ethyl cellulose is used as polymer in this formulation.
- The polymer is allowed to soak in the solvent which is dichloromethane
- Drug turmeric is added to this solution leading to the formation of inner phase.

STEP 2: Preparation of external phase

- The external phase was prepared by adding 5% polyvinyl alcohol in water to produce 100ml.

STEP 3:

- The inner phase was poured drop wise by the help of syringe into outer phase at room temperature.
- Glycerol (1-2ml) was added at an adequate amount in order to facilitate plasticity
- After emulsification, the mixture was continuously stirred for 3 hours at 1000rpm.
- After the formation of microsponge the mixture was filtered to separated the microsponge.
- The product was air dried overnight
- DIAGRAM:

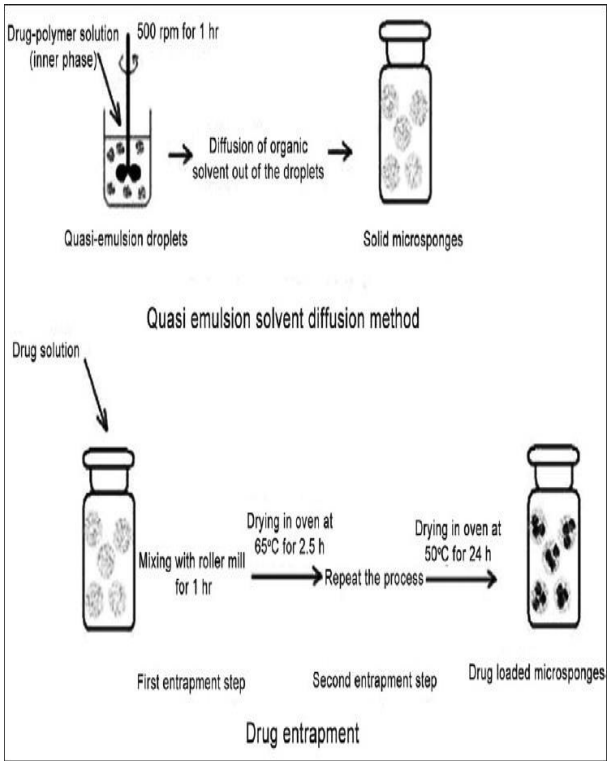

Fig1: Quasi Emulsion Diffusion Technique

2.4: FORMULATION TABLE – GEL

SR.NO	INGREDIENTS	QUANTITY
1.	Carbopol	Equivalent to microsponge
2.	Water	100 times that of microsponge
3.	Triethanolamine	Quantity sufficient
4.	Glycerine	Quantity sufficient

STEP 4: Preparation of Gel

- The amount of microsponge obtained was weighed.
- Amount of carbopol taken: quantity equal to that of microsponge. Carbopol was suspended in water
- Microsponge were soaked in glycerine.
- After the suspension of carbopol in water, the microsponges were added to it.
- Sufficient amount of triethanolamine was added.

DISCUSSION AND RESULT

An approach was being made in combining the old tradition with the new technology leading to the formulation of a novel drug delivery system –"gel loaded turmeric microsponges" as an anti –inflammatory.

Firstly, turmeric microsponges were prepared by quasi emulsion solvent diffusion technique. Various ratios of drug: Polymers were analyzed but 1:1 gave required type of

microsponge and was found to be stable. These microsponges were then checked for their porous nature, appearance, uniformity in size.
After the formulation for microsponges were complete, they were loaded into the gel. The gelling agents used here is carbopol. The gel was loaded with turmeric microsponges by the basic gel formulation process.
The gel formulation was then checked for the appearance, consistency of the gel
Following are the evaluations carried out:

- **Appearance**
- **Consistency-** semisolid
- **Odour**- pleasant
- **Colour**-white coloured gel loaded with yellow colour turmeric microsponges
- **Spreadibility**- easily spreadable
- **pH-** neural

UV spectroscopy – [2] For the determination of the percentage of turmeric entrapped in the microsponge, UV spectroscopy technique was used. The method consisted of obtaining the spectra of the pure turmeric and of gel loaded microsponge. The calibration curve obtained by these two were matched which, drew to a conclusion that the entrapment of turmeric was established in the microsponge.
The calibration curve obtained for turmeric is as follows:

Concentration of turmeric	Absorbance(at 421nm)
1ppm	0.043
2ppm	0.049
4pmm	0.057
6pmm	0.061
8ppm	0.071
10ppm	0.09

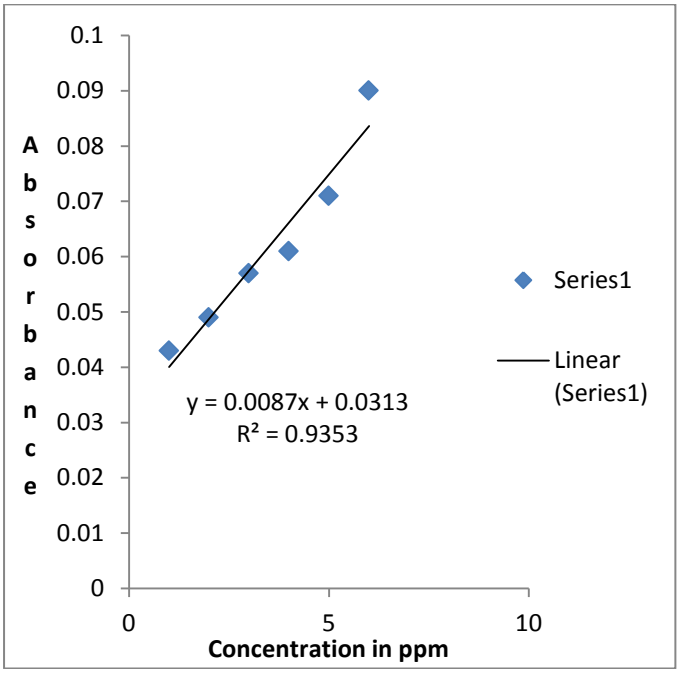

Fig 2: Calibration Curve of Turmeric

CALIBRATION CURVE OF GEL:

Concentration in ppm	Absorbance(at 421nm)
1	0.022
2	0.046
4	0.106
6	0.169
8	0.187
10	0.232

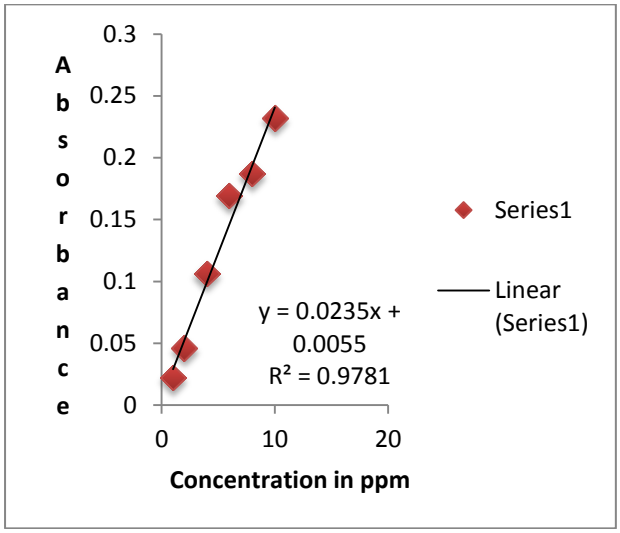

Fig 3: Calibration Curve of Gel loaded turmeric microsponge.

CONCLUSION

The purpose of present research work was to develop a topical turmeric loaded microsponge based anti inflammatory gel formulation. It has improved the efficacy and safety of turmeric that may be better administered through skin. A controlled release drug delivery system aims for a prolonged period so that can satisfy the goals of the reduction in inflammation.

The need of the hour is design a topical drug delivery system of turmeric that could not only increase the presence of the drug locally and for a prolonged period, but also reduce the risk of toxicity.

Microsponges were prepared by Quasi Emulsion Solvent Diffusion Technique. These microsponges are then loaded into the gel for getting gel loaded curcumin microsponge. Formed microsponges were evaluated for pH, particle size distribution, spreadablity, UV absorbance. Calibration curves of drug were constructed by UV Vis spectrophotometer.

The said formulation was found to be non irritant to the skin, providing cooling effect thus adding to the anti-inflammatory action. To conclude the formulation was found to be giving anti-inflammatory action along with sustain release of the drug.

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NEUROCOSMETICS: NEED AND FORMULATION

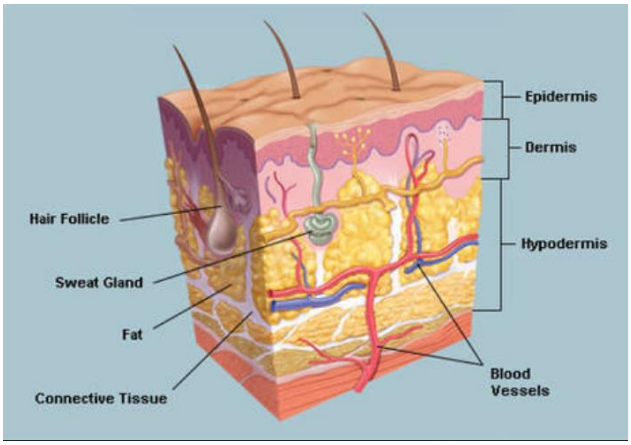
*Madhura Sawant, Nakiya Ratlamwala , Neha Dand**

ABSTRACT

The field of cosmetics is ever growing and highly competitive. With hundreds of brands available in the market, the real question arises that what is it that the consumer really wants? This research was aimed at understanding the needs of the average consumer by carrying out a public survey. The results of the survey pointed towards the attributes which are most desired in 3 types of cosmetic products, namely face wash, face cream and face pack. Based on the outcome of the survey a factor common to all 3 products was noted. The consumers based their choice on the "feel" of the product. This gave rise to the idea of using neurocosmetics. Neurocosmetic is holistic approach which physiologically acts on the mind via the skin. The ingredients used not only affected the skin to give their desired action but also affected the nerves by which they imparted a good "feel" to the products. A line of 3 products was formulated keeping in mind the conclusions drawn from the survey. Face wash, face cream and face pack were formulated keeping in mind the findings of the survey to make a product line in need with the demands of the consumer

INTRODUCTION

Diagram of Skin:



Function of Skin:-

- Skin is the most extensive sense organ in mammals. Skin has nerve ending for heat, hold, touch, texture and pressure.
- Thermo receptors in the skin will transmit signals to the brain to either constrict or dilate the dermal blood vessels
- Dilation of vessels produce heat loss(Cool you down)
- Constriction of vessels prevents heat loss(Warm you up)
- Skin also helps with nonverbal communication for example facial expression is the result of muscles pulling on the skin of the face

Main Function of Skin:-

- Sensation
- Heat Regulation
- Absorption
- Protection
- Excretion
- Secretion
- Vitamin D production

Neurocosmetic – Cosmetics of Neurotransmitters:-

- Neurocosmetic follows current cosmetic trends. More and more, cosmetic manufactures wish to treat the individual as a whole: the body and the mind. Neurocosmetic is holistic approach which physiologically acts on the mind via the skin.
- The Concept based on the knowledge of chemical vectors of nerve information, the neurotransmitters. These mediators are synthesized by every skin cell and interact between the nerve system and skin. Therefore neurocosmetic actives can play a significant role in balance by acting on these messengers, either by activating or inhibiting them.
- Neurocosmetic refer to topical ingredients that work on central nervous system.

The relationship between the skin and the central nervous system is ripe for exploration of keratinocytes, Langerhans cell, melanocytes etc. Endothelial cell of the skin are to some extent, modulated and controlled by nerves.The Emerging study of Neurocosmetics may lead to new ways of including a positive action on the skin nervous system that result in increased skin health.

Advantage of Neurocosmetics:-

- It is the latest generation of cosmetics.
- Neurocosmetic all the active ingredients used at optimal concentration and compatibility.
- Neurocosmetics provide the highest level of penetration.
- They provide protection from the damaging effect of free radicals and stimulate the production of collagen and elastic and smooth.

According to many experts, neurocosmetics is promising direction in cosmetology, which helps to solve many aesthetic problems due to influence on the nerve ending of the skin with specific substances. Since the nervous system is the controller of all other system, by acting directly on the nerve endings, we can correct various skin problems for example, to block the development of inflammation or activating the cell age related decline in their activities.

The action of Neurocosmetics can be directed to:-

- Contraction of facial muscles.
- Relaxation of facial muscles.
- Strengthening of the power cell (Stimulation, egeneration).
- Reduction of inflammatory reaction of the skin

AIM AND RATIONALE:

Aim: To formulate products that not only gives the desired action on skin but also impart a good "feel".

Rationale:

a) Prepare survey forms.
b) Conduct a survey.
c) Analyse their results and draw meaningful conclusions.
d) Develop a product line based on the outcome of the survey.

MATERIALS AND METHODS:

MATERIALS:

NAME OF INGREDIENT	BRAND NAME	NAME OF COMPANY
Menthol	MENTHOL LR	Research-lab fine chem industries
Charcoal	CHARCOAL LR	Research-lab fine chem industries
Carbopol	CARBOPOL 940 LR	SD fine chem limited
Sodium lauryl sulphate	SODIUM LAURYL SULPHATE LR	Research-lab fine chem industries
Propylene glycol	PROPYLENE GLYCOL LR	Research-lab fine chem industries
Methyl paraben	METHYL PARABEN LR	Research-lab fine chem industries
Propyl paraben	PROPYL PARABEN LR	Research-lab fine chem industries
Triethanolamine	TRIETHANOLAMINE LR	Research-lab fine chem industries
Kaolin	KAOLIN LR	Research-lab fine chem industries
Chicory	CHICORY POWDER	Om Shiv Shakti Stores,Belapur
Polyvinyl alcohol	POLYVINYL ALCOHOL	Research-lab fine chem industries
Sodium carboxymethylcellulose	CARBOXY METHYL CELLULOSE SODIUM SALT(HIGH VISCOSITY)	Research-lab fine chem industries
Ethanol	ABSOLUTE ALCOHOL	GOGIA AND COMPANY

Aloe vera gel	NATURE'S ESSENCE ALOEVERA GEL	Aromapathybeauty solutions
Cucumber extract	DR.JAIN'S CUCUMBER GEL	MANBROPHARMA PVT LTD.
Sulphur	SULPHUR POWDER LR	OZONE INTERNATIONAL
Lotus oil	ANCIENT HEALER LOTUS ESSENTIAL OIL	SURAJBALA EXPORTS PVT LTD.
Stearic acid	STEARIC ACID LR	Research-lab fine chem industries
Potassium hydroxide	POTASSIUM HYDROXIDE LR	Research-lab fine chem industries
Glycerine	GLYCERINE(PURIFIED) LR	Research-lab fine chem industries
Bentonite	BENTONITE POW DER(PURUFIED) LR	Research-lab fine chem industries

METHODOLOGY:
FORMULA FOR FACEWASH:

SR. NO.	INGREDIENTS	QUANTITY TAKEN	ACTIVITY
1	Menthol	2% w/w	Cooling agent. Provides a feeling of freshness
2	Charcoal	0.5% w/w	Adsorbent for removal of oil and pollution
3	Carbopol	2% w/w	Gelling agent
4	Sodium Lauryl Sulphate	5% w/w	Surfactant for foaming
5	Propylene Glycol	2% w/w	Humectant
6	Methyl Paraben	0.18% w/w	Preservative
7	Triethanolamine	q.s to pH 7	Neutralizer
8	Lotus oil	1% w/w	Perfume which gives a sense of cleanliness
9	Water	q.s to 100 gm	Vehicle

Procedure:
1.Soakcarbopol in ¾ th amount in water for 20 minutes.
2.In remaining water disperse menthol(powdered),Sodium lauryl sulphate,charcoal,propyleneglycol,methylparaben.
3.Add (2) in (1) with very slow stirring.
4.Addtriethanolaminedropwise till pH reaches 7 and gel is formed.
5. Add lotus oil.

FORMULA FOR FACEPACK:

SR. NO.	INGREDIENTS	QUANTITY TAKEN	ACTIVITY
1.	Kaolin	5%	Adsorbent clay for deep pore cleansing
2.	Bentonite	3%	Adsorbent clay for deep pore cleansing
3.	Chicory powder	2%	Skin tightening agent
4.	Polyvinyl Alcohol	15%	Film forming and binding agent
5.	Sodium Carboxymethylcellulose	5%	Viscosity modifier and Stabilizer
6.	Ethanol	10%	Solvent
7.	Titanium Dioxide	5%	Covering agent and opacifier
8.	Glycerine	5%	Humectant
9.	Methyl Paraben	0.18%	Preservative
10.	Lotus Oil	1%	Perfume
11.	Water	q.s to 100 gm	Vehicle

Procedure:
1.In ½ quantity of alcohol and water soak sodium carboxy methyl cellulose for 20 minutes.
2.In remaining water dissolve polyvinyl alcohol and methyl paraben at 70-80°C.
3.Add (2) and (1).
4.Mixkaolin,chicorypowder,bentonite,titanium dioxide and glycerine.
5.Levigate (4) with (3).
6.Add lotus oil.

FORMULA FOR FACE CREAM :

SR. NO.	INGREDIENTS	QUANTITY TAKEN	ACTIVITY
1	Aloevera gel	2.5%	Nourishing agent for removal of wrinkles and fine line
2	Cucumber extract	5%	Softener aiding in removal of fine lines
3	Sulphur	0.5%	Anti-acne agent
4	Lotus oil	1%	Perfume
5	Vanishing cream base	q.s to 100 gm	Non-oil base

Procedure:
Mix aloeveragel,cucumber extract and sulphur and levigate with vanishing cream base.

FORMULA FOR VANISHING CREAM:

SR. NO.	INGREDIENTS	QUANTITY TAKEN	ACTIVITY
1	Stearic acid	17%	Reacts to form soap emulgent
2	Potassium hydroxide	0.7%	
3	Glycerine	5%	Humectant
4	Propyl paraben	0.18%	Preservative
5	Methyl Paraben	0.02%	Preservative
6	Water	q.s to 100 gm	Solvent, aqeous phase

Procedure:

1.Weigh stearic acid in a small beaker.
2.Heat it to 60-70°C on a water bath.
3.Dissolve KOH in water and heat.
4.Dissolve methyl paraben and propyl paraben into aqueous phase and oil phase respectively.
5.Add aqueous phase to oily phase with constant stirring.
6.Stop heating and stir,until the temperature reaches to 40°C.

METHODOLOGY – Survey Forms:

SURVEY ON PUBLIC PERCEPTION OF COMMONLY USED COSMETICS

Name:	
Age:	
Address:	
Mobile number:	
Email ID:	
Profession:	

We, the researchers of BharatiVidyapeeth's College of Pharmacy, Navi Mumbai are conducting a survey on commonly used cosmetics and what the consumer expects from them. This would help us in researching and developing cosmetic products. We request you to spend some of your valuable time for this.

1. Which cosmetic product do you use regularly?

□Facewash	□Day cream	□Night cream
□Face scrub	□Face pack	□Body lotion
□Make up	□Shampoo	□Conditioners

2. What factors attract you to buy a cosmetic product?
   - □ Advertisements
   - □ Packaging
   - □ Cost
   - □ A sense of feeling good
   - □ Someone's recommendation

3. Does your mood reflect the type of cosmetic you buy, whether it is its colour, fragrance, ingredients, packaging and texture?
   - □ Yes
   - □ No

4. When deciding to purchase a cosmetic product, how important is "feel"?
   - □ Not important
   - □ Somewhat important
   - □ No opinion
   - □ Important
   - □ Very important

5. Do you wish to know the results of our research?
   - □ Yes
   - □ No

   If yes, please select your preferred method to contact

- Email:
- SMS:
- Regular mail:

Signature:_____ Date: _____

We are grateful for your valuable time and inputs!

Survey on Facewash

1. What is your skin type?
   - ☐ Oily
   - ☐ Dry
   - ☐ Normal
   - ☐ Combination
   - ☐ Sensitive
   - ☐ Acne prone

2. While using a facewash , your main aim is
   - ☐ Oil control
   - ☐ Pollution control
   - ☐ Removal of dead skin
   - ☐ Anti-tightness
   - ☐ Medical reasons such as acne

3. What type of facewash would you prefer?
   - ☐ Foaming
   - ☐ Non foaming

4. What is the primary feeling that you want after using a facewash?
   - ☐ Glow
   - ☐ Flawless skin
   - ☐ Fairness
   - ☐ Freshness
   - ☐ Cleanliness

Thank you!

Survey on Face Cream

1. What is your skin concern?
   - Oily, acne prone
   - Dry, dehydrated
   - Dull, lack lustre
   - Fine lines
   - Wrinkles

2. What is more important to you when choosing a skincare product?
   - Contains agents for anti-ageing properties, whitening, hydration etc
   - Moisturizes and nourishes the skin
   - Must not contain preservative, emulsifier, perfume etc
   - Does not contain many chemicals but helps skin to build its own restorative strength
   - Should not be greasy and feels good on skin

3. What type of cream you prefer?
   - Day face cream
   - Night face cream
   - Anti-aging cream
   - Whitening cream
   - Greaseless gel

4. Which of the following type of ingredients would you prefer for a cream?
   - Oil-free ingredients
   - Natural ingredients
   - Dye free ingredients
   - Petroleum free ingredients

5. Natural products are better than synthetic products
   - Yes
   - No
   - I don't see a difference

Thank you!

Survey on Face Pack

1. Do you use a face pack? If yes give the type you use
   - ☐ Yes. If yes _____
   - ☐ No

2. What type of face pack do you like to use?
   - ☐ Mud
   - ☐ Clay
   - ☐ Heated clay
   - ☐ Peel Off
   - ☐ Scrub

3. What features do you look for, when purchasing a face pack?
   - ☐ Anti-aging
   - ☐ Moisturizing
   - ☐ Anti-acne
   - ☐ Deep pore cleansing
   - ☐ Firming
   - ☐ Fairness and glow

4. What factors would attract you to buy a face pack?
   - ☐ Packaging
   - ☐ Colour
   - ☐ Ingredients
   - ☐ Fragrance
   - ☐ Texture
   - ☐ Pack type
   - ☐ Skin benefits

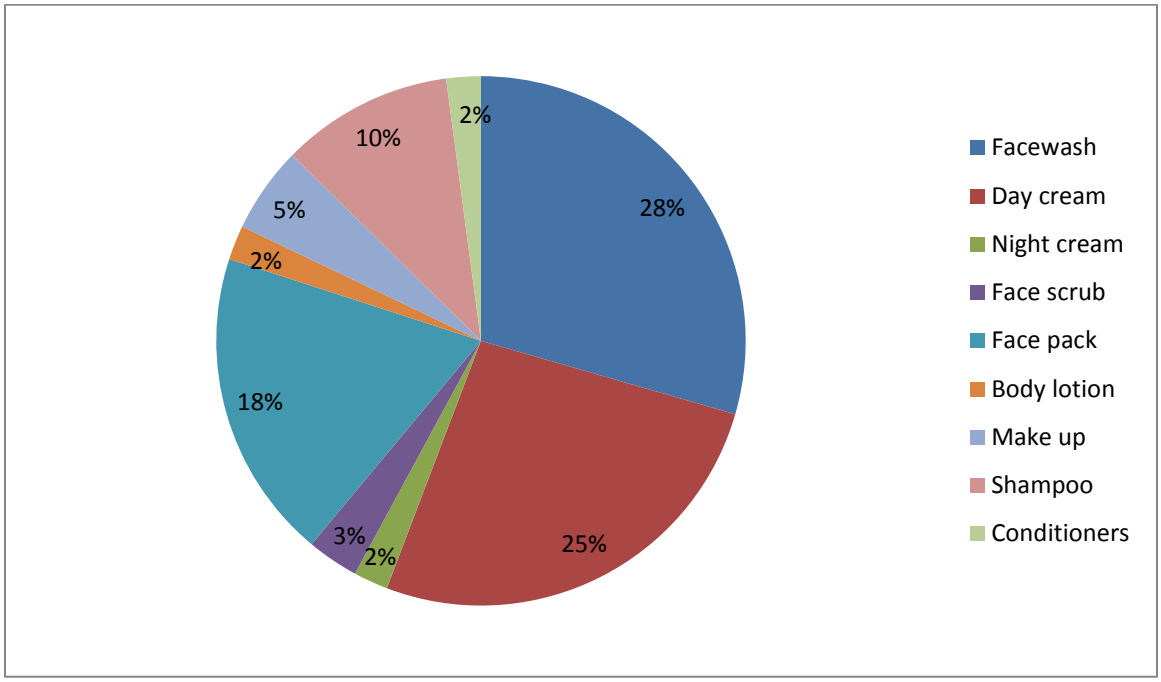
5. Do you have any complaints with your current face pack? If yes, kindly elaborate.
   _____

Thank you!

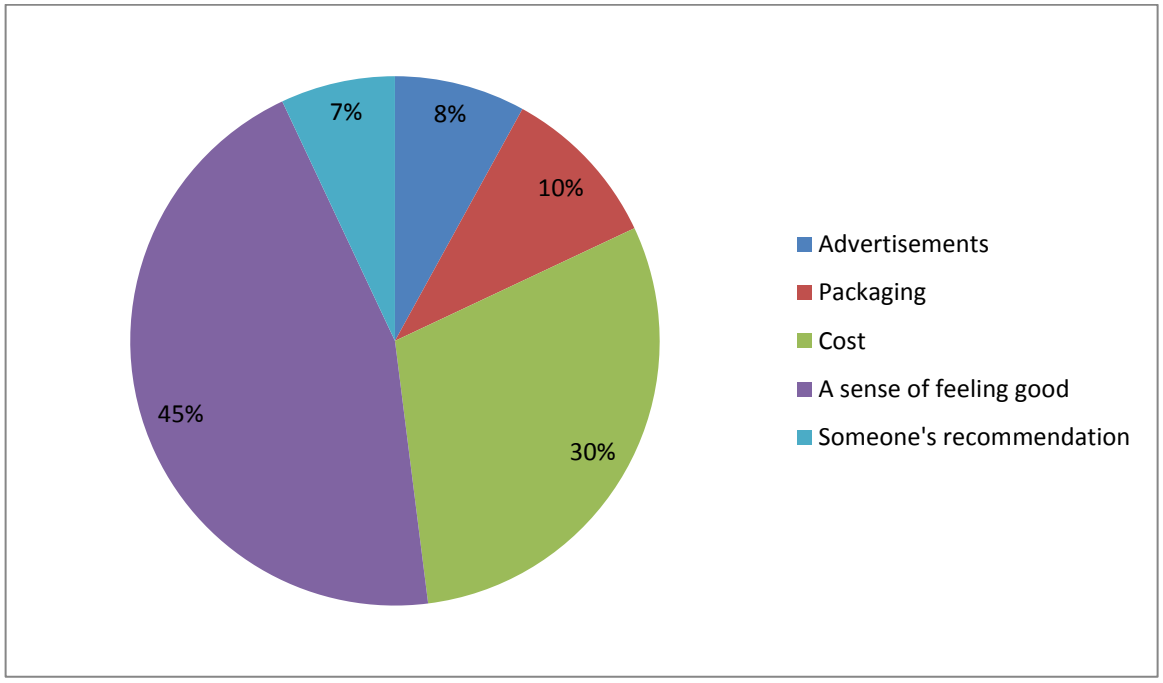
RESULTS OF SURVEY

GENERAL SURVEY: Following are the results for general survey

1. WHAT OCSMETICS DO YOU USE REGULARLY?



2. WHAT FACTORS ATTRACT YOU TO BUY A COSMETIC?



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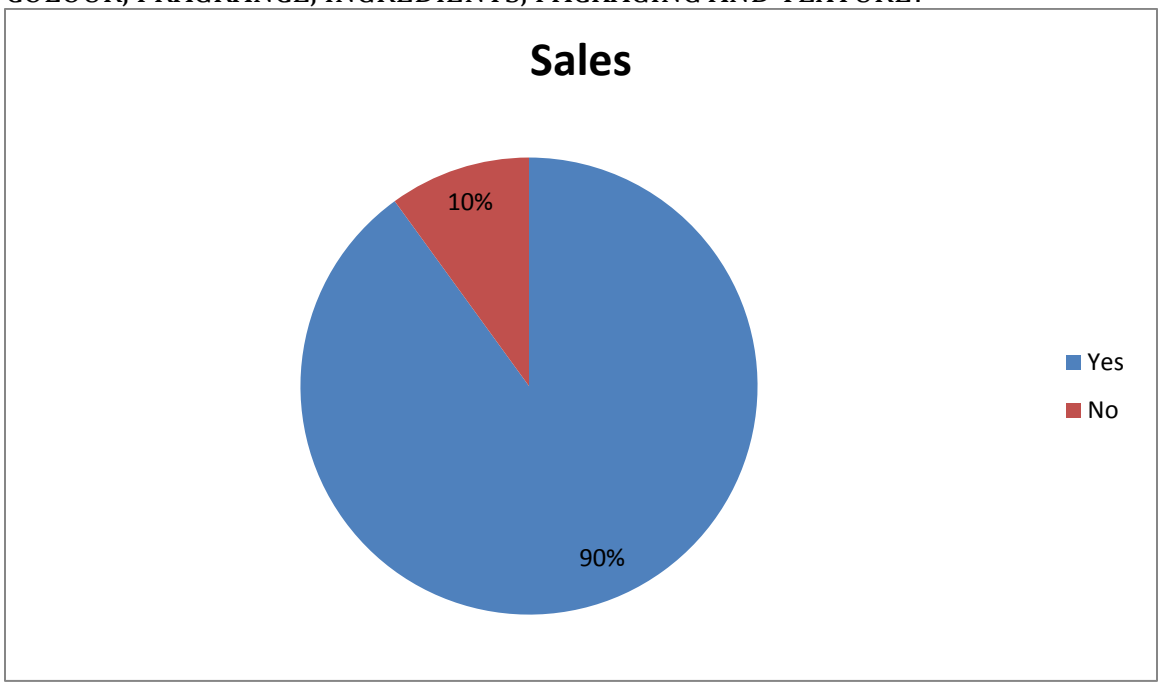
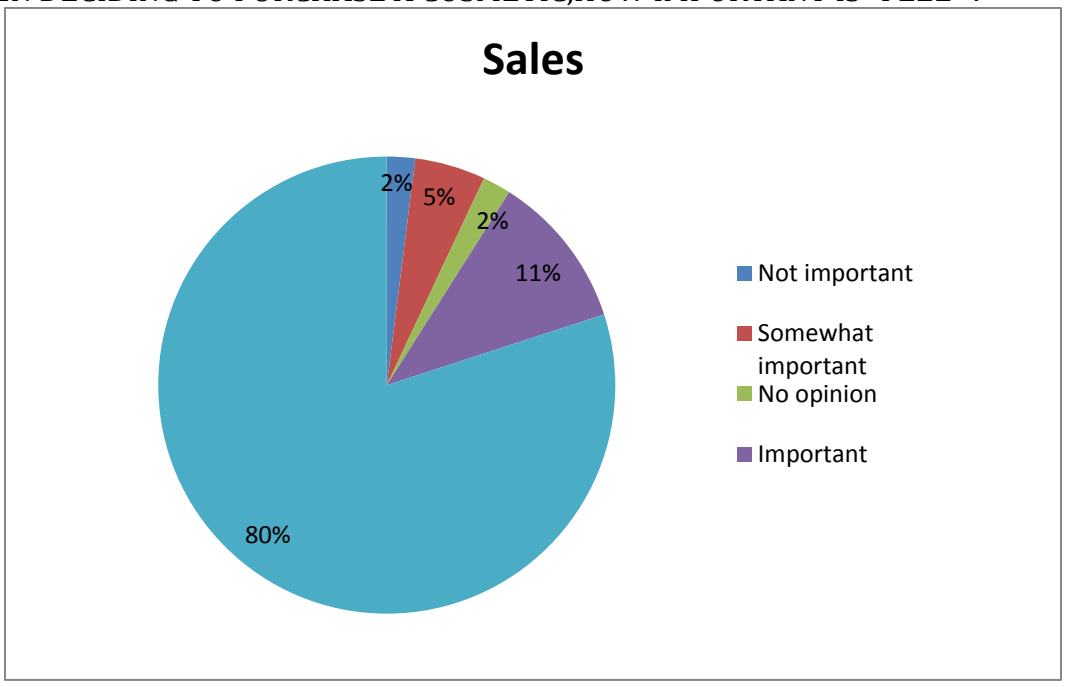
3. DOES YOUR MOOD REFLECT THE TYPE OF COSMETICS YOU BUY, WHETHER IT IS ITS COLOUR, FRAGRANCE, INGREDIENTS, PACKAGING AND TEXTURE?



4. WHEN DECIDING TO PURCHASE A COSMETIC,HOW IMPORTANT IS "FEEL" ?

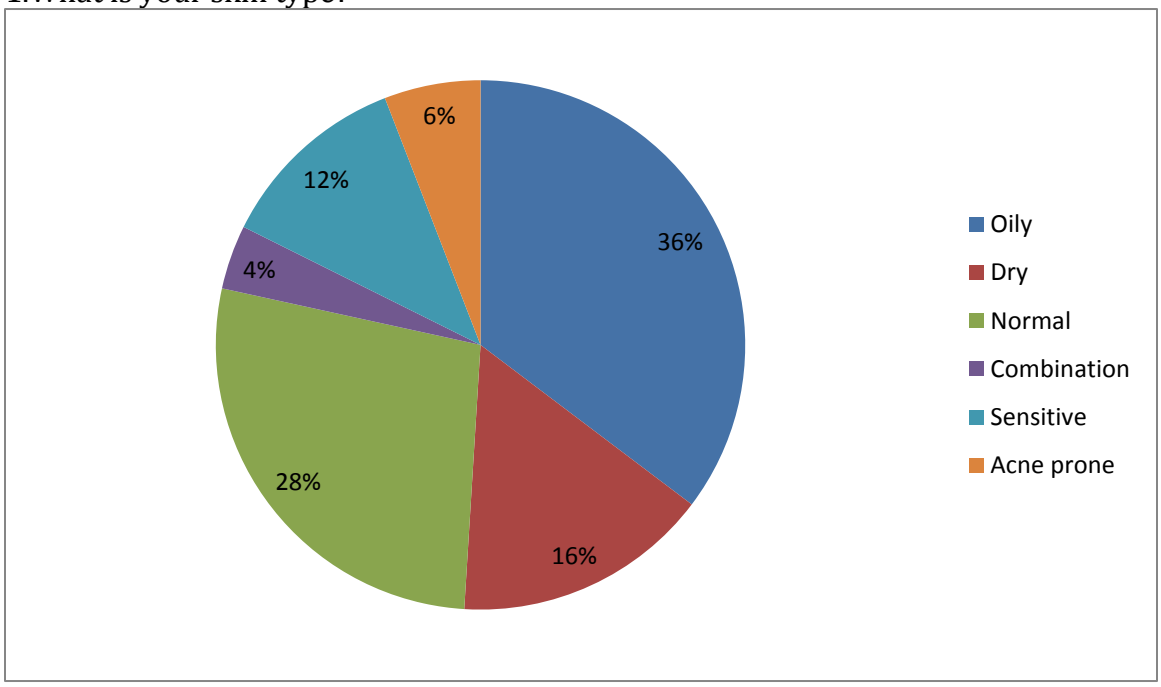


Conclusions drawn from the general survey on cosmetics usage and buying pattern

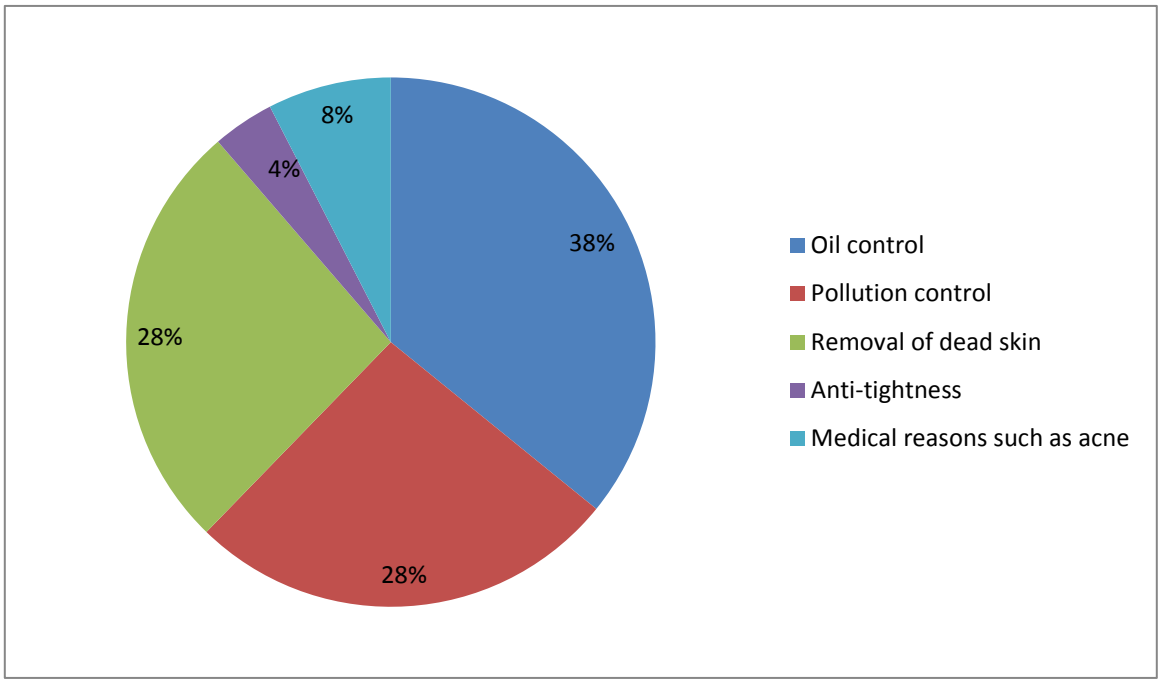
a) People normally use face wash, face pack and day cream.
b) Cost and sense of good feeling attract the consumers to buy the cosmetic product.
c) The fragrance ,colour ,ingredients ,packaging and texture of the cosmetic product reflects the mood of the consumers.
d) The feel of any cosmetic product is very important.

SURVEY OF FACEWASH:

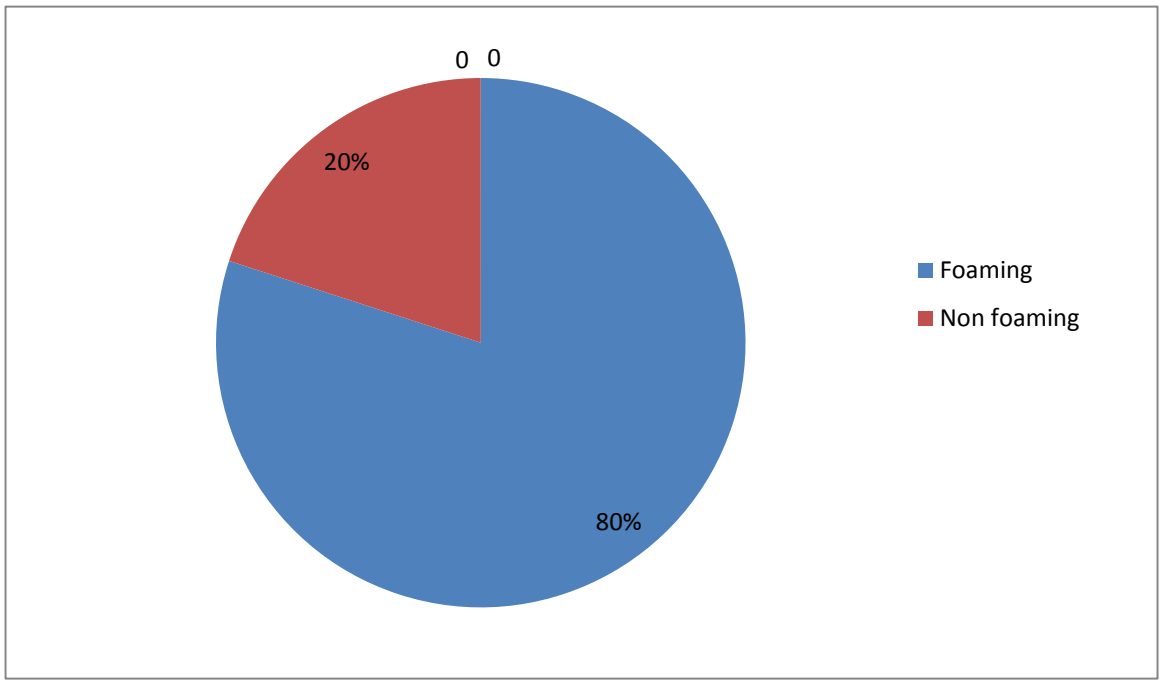
1.What is your skin type?



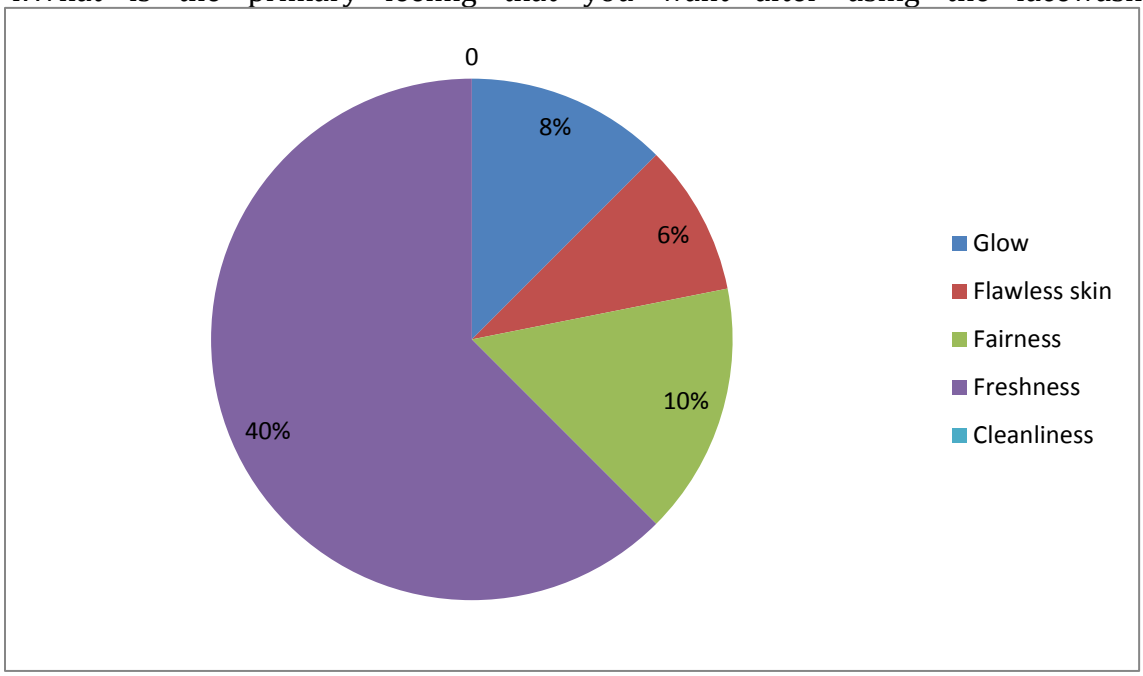
2. While using facewash your main aim is?



3.What type of facewash would you prefer?



4.What is the primary feeling that you want after using the facewash?

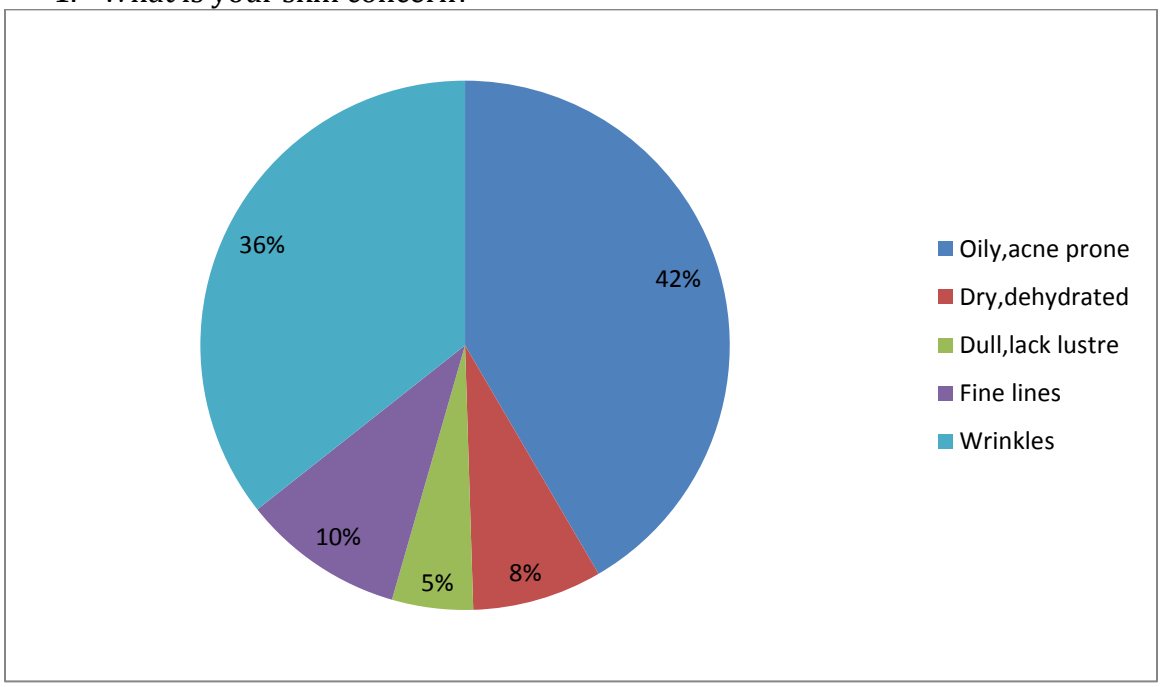


The survey points that an ideal face wash should incorporate the following characteristics:

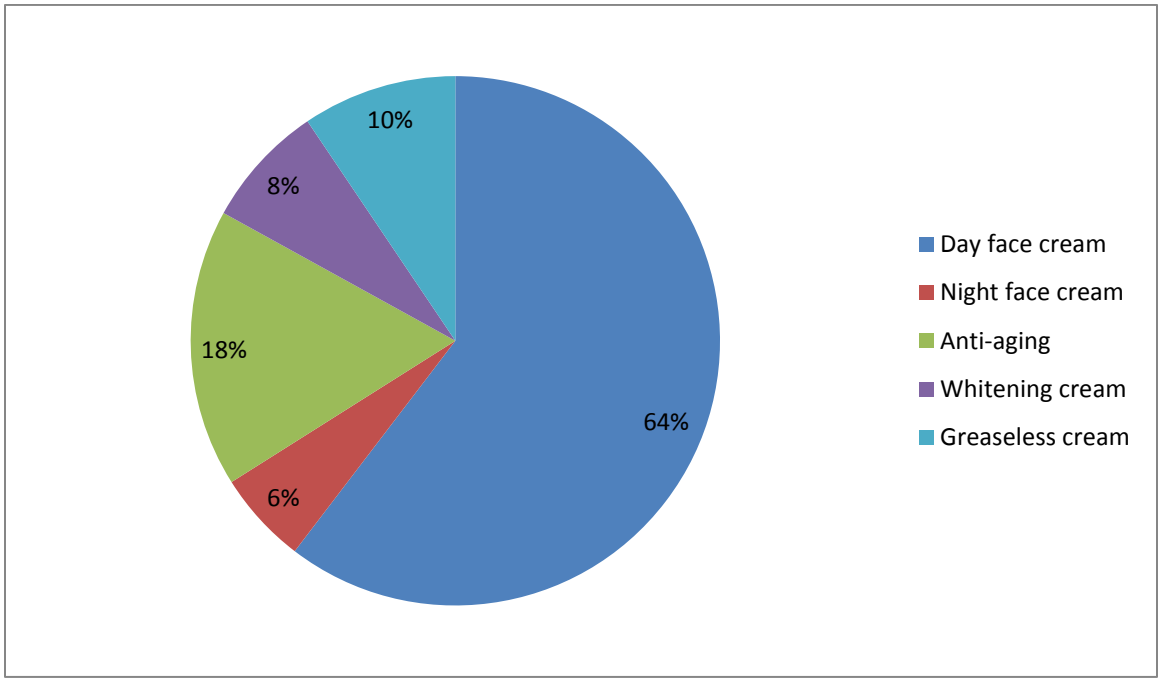
a) Majority of users had oily and normal skin type, so the face wash would need effectively remove oil from skin.
b) The face wash should provide oil and pollution control as well as removal of dead skin.
c) 80% of population preferred to have foaming type.
d) The primary "feel" desired was of cleanliness and freshness.

SURVEY FOR FACECREAM:

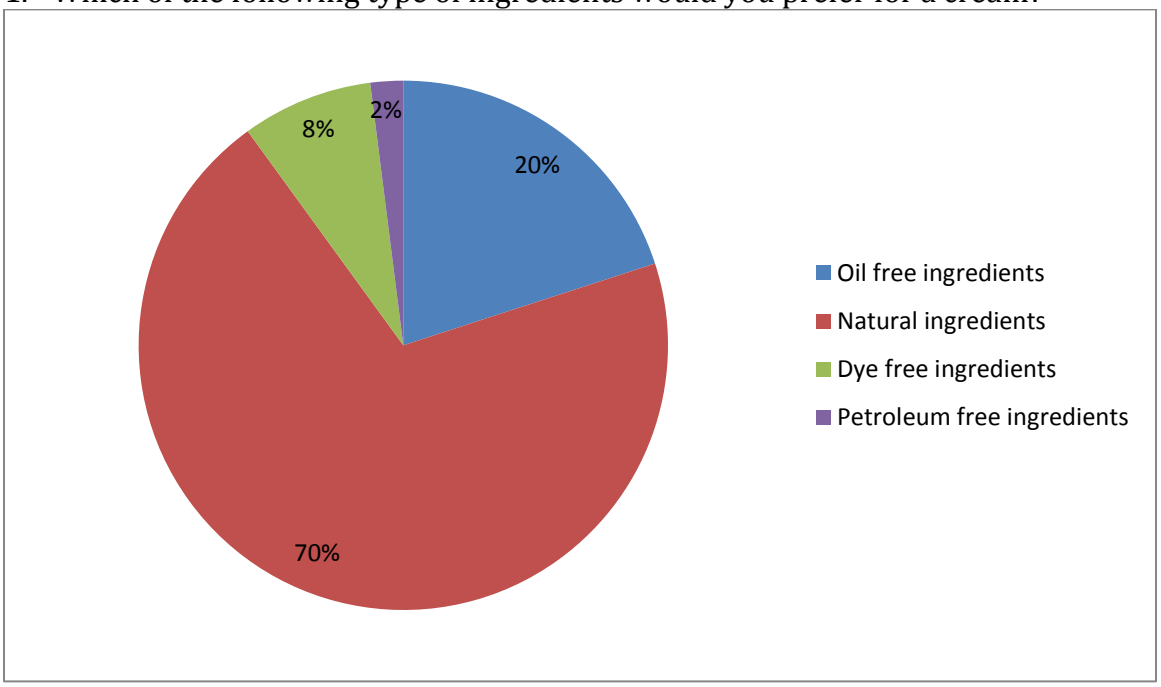
1. What is your skin concern?



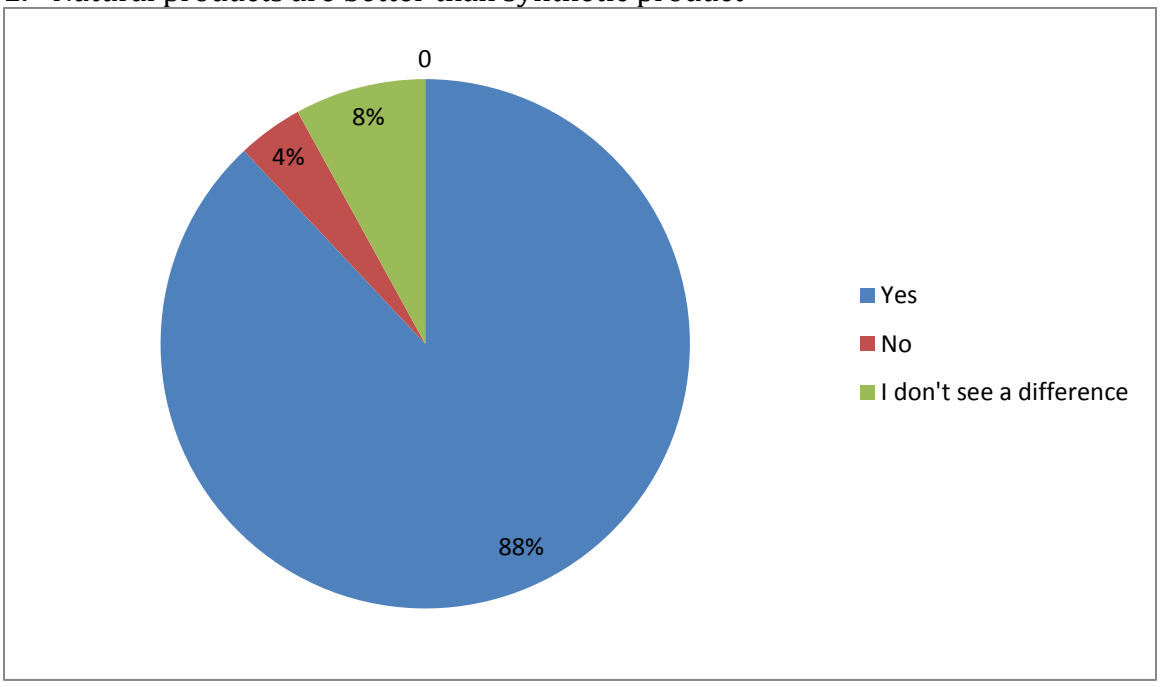
2. What type of cream you prefer?



1. Which of the following type of ingredients would you prefer for a cream?



2. Natural products are better than synthetic product

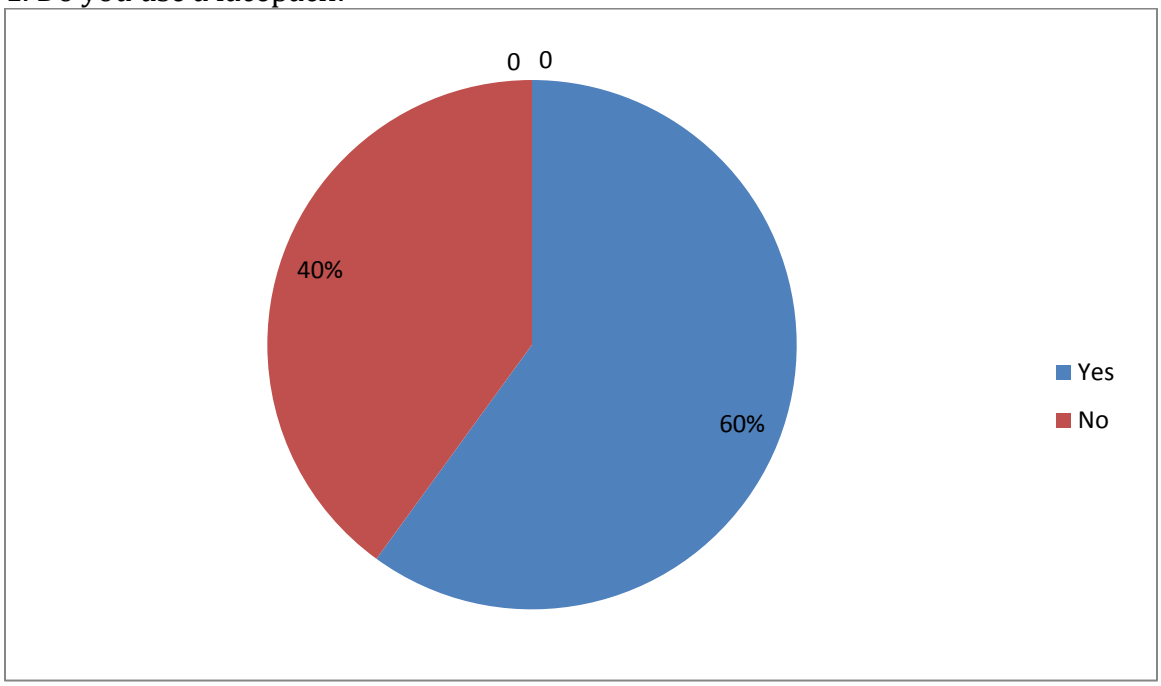


The survey shows that an ideal face cream should possess the following characteristics:

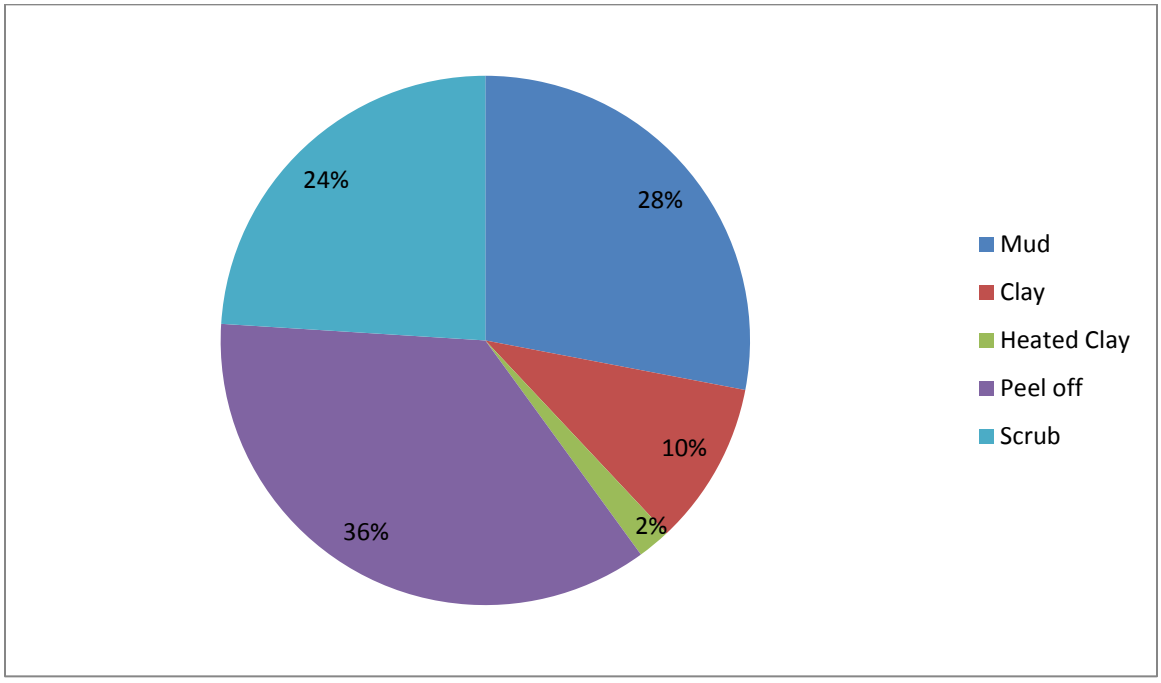
a) Primary skin concerns governing the choice of face cream were oily, acne prone skin and wrinkles.
b) People preferred to use a single day cream addressing the above concern rather than using multiple products.
c) The survey showed that a large majority of consumers preferred ingredients of natural origin.

SURVEY FOR FACEPACK:

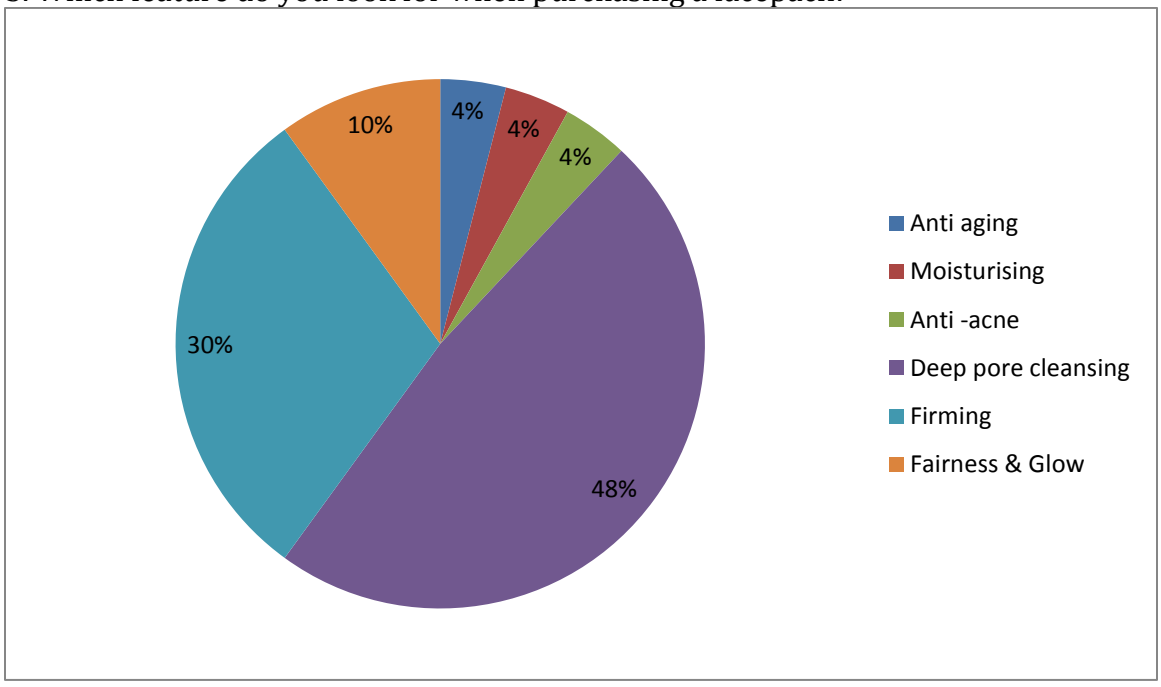
1. Do you use a facepack?



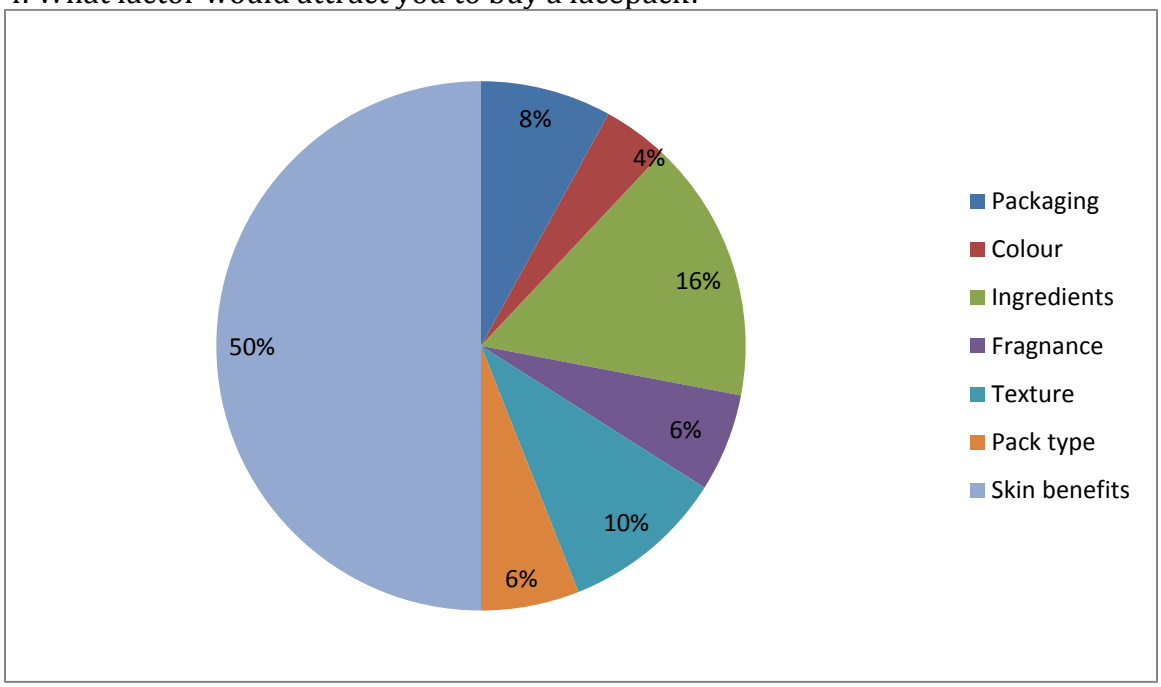
2. What type of facepack do you like?



3. Which feature do you look for when purchasing a facepack?



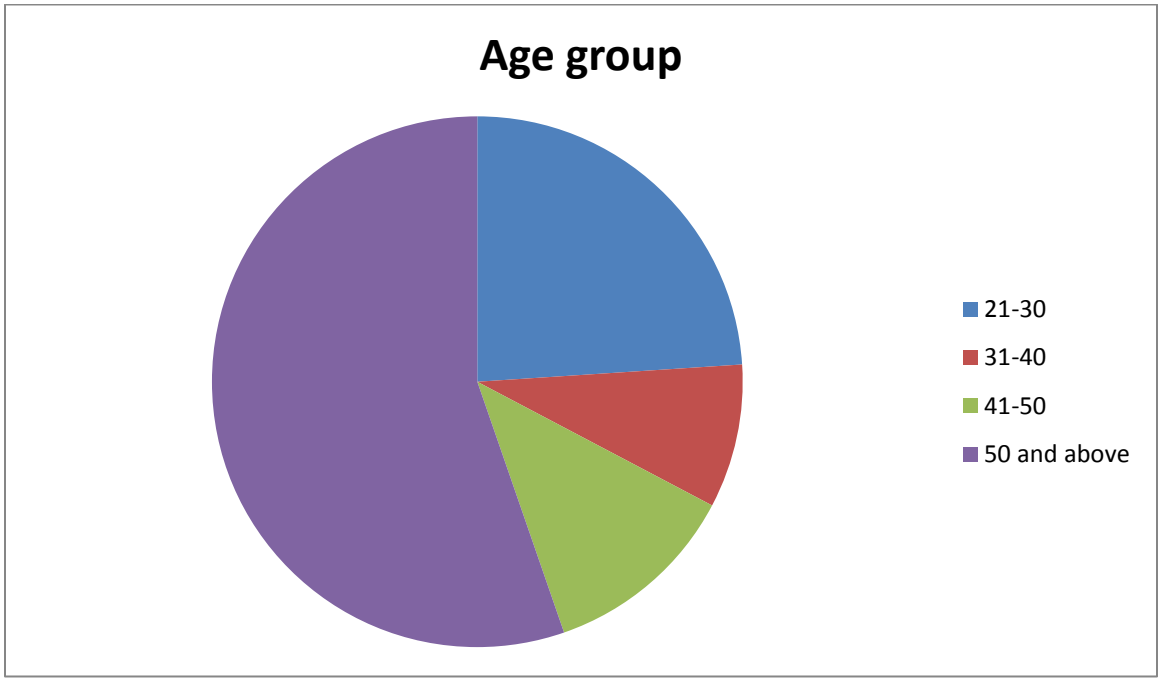
4. What factor would attract you to buy a facepack?

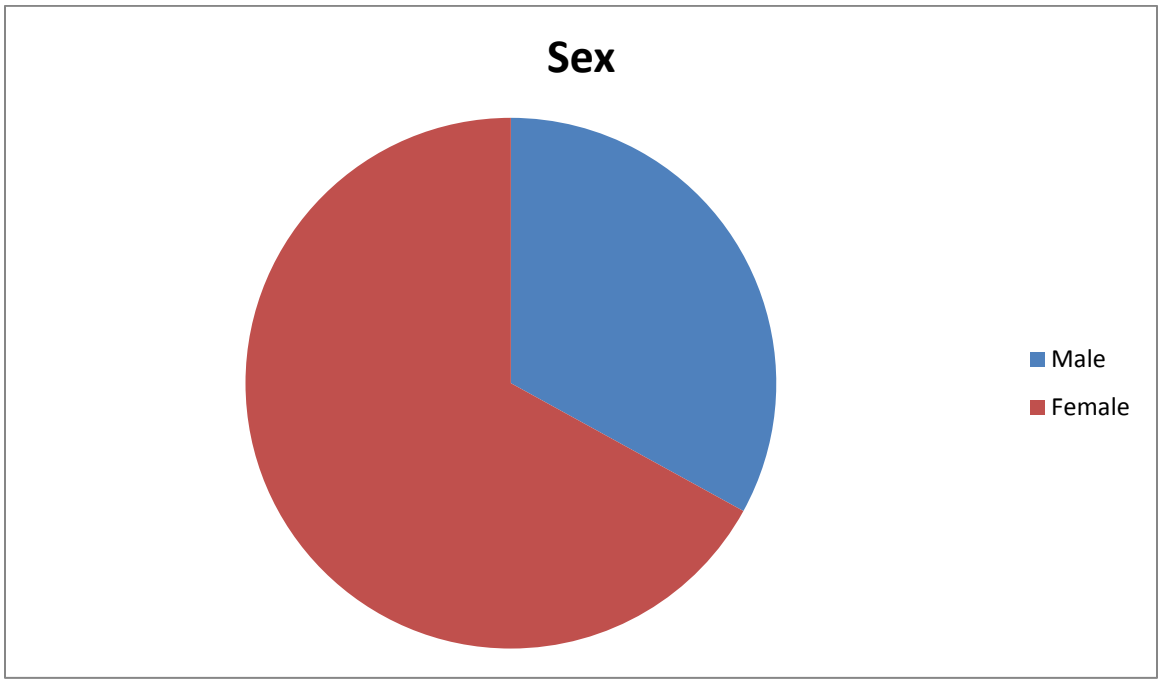


The survey shows that an ideal face pack should possess the following characteristics:

a) More than half the population uses a face pack.
b) Peel off face pack was most preferred followed by mud type and scrub type face pack.
c) The features most deciding the face pack were deep pore cleansing.

Annexure:





CONCLUSION:

Consumers are increasingly looking to 'balance' their busy lives; and at the same time 'an increasing number of consumers are looking at the chemicals they use as causes of inflammation and sensitivities. Survey was carried to know the perception of people about the type of cosmetic they would like to use. Neurcosmetics, beacause of the way they work, are best suited to sensitive skins by reducing the normal inflammatory response to elicit visible anti-ageing and skin improvement results.

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7. https://goo.gl/images/OPRWHP
8. https://goo.gl/images/LB4W4B

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HERBAL XEROGEL

*Kiritkumar Jain, Manali Jadhav and Neha Dand**

ABSTRACT

Herbal Medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition. *Cucurma longa*(turmeric), Leaves of *Ocimum sanctum* (Tulsi) and *Glycyrrhiza glabra* (liquorice) are an effective natural remedy for wound healing and treatment of mouth ulcers. The present paper deals with formulation and evaluation of xerogel prepared from turmeric and tulsi for treatment of mouth ulcers and wound healing. A xerogel is a solid formed from a gel by drying with unhindered shrinkage. A solid pharmaceutical dosage formulation using part of plant with excipients viz. Sodium CMC and Methyl paraben by Sol-Gel method was reported to be significantly stable. The present communication also deals with the evaluation of formulated xerogels (Time for gel formation, strength of the gel and particle size determination).

Keywords: Sol-gel process, xerogel, ulcers, wound healing, herbal

INTRODUCTION

WHAT IS XEROGEL?

Xerogel is an open network formed by the removal of all swelling agents from the gel. It usually retain high porosity (15%-50%) and enormous surface area (150-900 m^2/g), along with very small pore size (1-10nm).Examples of xerogels include silica gel and dried out, compact, macromolecular structures, such as gelatin or rubber. A gel is a solid jelly-like material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state. By weight, gels are mostly liquid, yet they behave like solids due to a three-dimensional cross-linked network within the liquid. It is the crosslinking within the fluid that gives a gel its structure (hardness) and contributes to the adhesive stick (tack). The present study relates to stable gel containing salts of carboxymethyl cellulose (sodium CMC) as a gelling agent which are useful for many purposes including preparation of medicines and cosmetics. The gel prepared by using sodium CMC as gelling agent is then dried to remove aqueous phase. The preparation obtained after removal of water is called as xerogel.

The advantage of xerogel when compared to normal gel is that it gives greater resistance to microbial contamination due to absence of aqueous phase and offer optimum shelf life to the product. Xerogel when comes in contact with the fluid it immediately converted into a thin film of gel in case of mouth ulcers and wound healing process and releases drug in a controlled manner. The method of application of xerogel is also simple as compared to normal gel.

TUMERIC AS ACTIVE AGENT

The question of whether medicines discovered today are safer, more efficacious, and more affordable than generic medicines (whose patents have expired) or medicines that are centuries old could be “No” for most of the modern medicine. If so, then it is logical to

revisit and revive these age-old medicines for the welfare of mankind. Turmeric derived from the rhizome of the plant *Curcuma longa* has been used by the people of the Indian subcontinent for centuries with no known side effects, not only as a component of food but also to treat a wide variety of ailment.

Turmeric is a spice of golden color that is used in cooking in the Indian sub-continent. Because of its color and taste, turmeric was named "Indian saffron" in Europe. Today, India is the primary exporter of turmeric. It was once considered a cure for jaundice, an appetite suppressant, and a digestive. In Indian and Chinese medicines, turmeric was used as an anti-inflammatory agents to treat gas, colic, toothaches, chest pains and menstrual difficulties. This spice was also used to help with stomach ache and liver problems, to heal wounds and lighten scars, and as a cosmetic. Turmeric contains a wide variety of phytochemicals, including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol,curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, tumerin, turmerones, and turmeronols.[1]

Uses of Turmeric:-The use of turmeric for health purposes is nothing new. As a folklore medicine, its use has been documented in both Indian and Chinese cultures. The long list of uses include antiseptic, analgesic, anti-inflammatory, antioxidant, antimalarial, insect repellent, and other activities associated to turmeric. Perhaps one of the most often prescribed uses is for wound healing.

TULSI AS ACTIVE AGENT

Ocimum sanctum (Sanskrit:Tulsi; family: Labiaceae), is found throughout the semitropical and tropical parts of India. Different parts of the plant are traditionally used in Ayurveda and Siddha systems for the treatment of diverse ailments like infections, skin diseases, hepatic disorders and as an antidote for snake bite and scorpion sting. A methanol extract and an aqueous suspension of O. sanctum leaves have anti-inflammatory, analgesic and immunostimulatory properties. Flavonoids isolated from O. sanctum scavenged free radicals in vitro and showed antilipoperoxidant activity in vivo at very low concentration. The free radical scavenging activity of plant flavonoids help in the healing of wounds. Low levels of antioxidants accompanied by raised levels of markers of free radical damage play a significant role in wound healing.

Tulsi enhances immunity and improves metabolic functions. Their extract has been found to reduce inflammation by inhibiting enzymes. It also lowers the stress and has antioxidant property. Tulsi (*Ocimum sanctum Linn*) 'basil' is a plant with many medicinal values. Ayurveda recommends tulsi in several formulations to enhance immunity and metabolic functions. It helps in reducing inflammation by inhibiting the inflammation-causing enzymes.

Tulsi leaf acts as an adaptogen, which makes it suitable for ulcers. An adaptogen is a substance that helps the body to adapt to the increasing levels of stress. The anti-bacterial properties in Tulsi make it an effective oral disinfectant that can destroy up to 99% of the bacteria and germs in the mouth and relieve the symptoms of an ulcer. Tulsi leaves also prevent bad breath, plaque, tartar formation and dental cavities.[2]

LIQUORICE AS ACTIVE AGENT

Glycyrrhiza glabra: The genus Glycyrrhiza includes about 20 species native to Europe, Asia, North and South America as well as Australia. The English name licorice is derived from "liquiritia" . Liquorice is a hardy herb or under shrub, erect grows to about 2m height. The

roots are long, cylindrical, thick and multi-branched, the used part of the plant is the root and rhizomes. A number of components have been isolated from liquorice, including a water-soluble, biologically active complex that accounts for 40-50 percent of total dry material weight.

This complex is composed of Triterpene, Saponins, flavonoids, polysaccharides, pectin, simple sugars, amino acids, mineral salts, and various other substances. Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of liquorice root. Among the natural Saponins, glycyrrhizic acid is a molecule composed of a hydrophilic part, two molecules of guluronic acid, and a hydrophobic fragment, glycyrrhetic acid. The yellow color of liquorice is due to the flavonoids content of the plant, which includes liquiritin, isoliquiritin (a chalcone), and other compounds. The isoflavones glabridin and hispaglabridins A and B have significant antioxidant activity, and both glabridin and glabrene possess estrogens-like activity1.Glycyrrhiza has the following, clinically proved Pharmacological activities such as anti ulcer activity, anti asthmatic activity, anti diuretic activity and antihepatotoxic activity. People with canker sores (mouth ulcer) who gargled 4 times per day with DGL (Deglycyrrhizinated liquorice) dissolved in warm water found pain relief.

Liquorice is effective in the reduction of pain and of the inflammatory halo and necrotic centre of apthous ulcers. Liquorice root (Glycyrrhiza extract) delivered in a small, oral patch serves to relieve both pain and accelerate healing of canker sores. *Glycyrrhiza glabra Linn* possesses antibacterial, antioxidant, antimalarial, antispasmodic, anti-inflammatory and anti hyperglycemic properties. Various others effects like antiulcer, antiviral, antihepatotoxic, antifungal and herpes simplex have also been studied.[3]

AIM:-To formulate and evaluate herbal xerogel meant for application on wounds and mouth ulcers.

RATIONALE

The present study focuses on formulation of xerogel with Tulsi, Turmeric and Liquorice as active ingredients. Xerogel is the latest formulation prepared by eliminating the moisture content of gel and are available as dry powder which in presence of fluid forms a film of gel over the respective area of oral cavity in case of mouth ulcers and on the damage area in case of wounds. There are numbers of formulation available in market for treating mouth ulcers and wound healing, each of the formulations have their own advantages and disadvantages. To overcome the detriment such as less shelf life, ease of microbial contamination and difficulty in application, xerogel is prepared. Using tulsi, turmeric and liquorice as active ingredient imparts leverages such as low cost, ease of availability, enhanced tolerance, More protection, fewer side-effects, Potency and efficiency is very high.

METHODOLOGY

PLANT MATERIALS

Plant material that Powder of *Ocimum sactum*(Tulsi), *Glycyrrhiza glabra* (Yastimadhu), *Curcuma longa (*turmeric) powder were obtained from Dr. Jain herbals.

Formulation table:- Obtained from SJ Carter, Dispensing for Pharmaceutical Students, pg no. 15- 20 and modified as per the need[4].

SR NO.	INGREDIENTS	QUANTITY FOR 100g of gel
1.	Tulsi	0.5g
2.	Turmeric	0.5g
3.	Liquorice	0.5g
4.	Sodium CMC	10g
5.	Methyl paraben	0.1g
6.	Purified water	q.s to 100g

Table no. 1: Formula table

PREPARATION OF XEROGELS

PROCEDURE: Obtained from ref.[5] and modified as per the need.

Sodium CMC was sprinkled on water in a evaporating dish with constant stirring and gentle Heating.

After complete addition of sodium CMC on water, Methyl paraben was added and the preparation was kept aside for 20 minutes.

After completion of 20 minutes Required quantity of powder of Tulsi, Turmeric and Liquorice is added in Gel formed with constant stirring.

The gel was dried in oven by forming a thin film of it on watch glass at 70 °**C** for 3-4 hours.

Thin dry film of gel was obtained which was grinded and sieve to form fine powder.

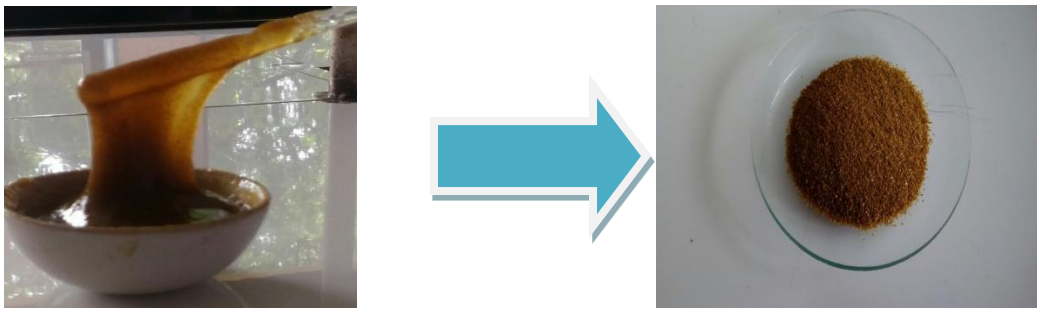

Figure no.: Xerogel (Humid and Dry forms)

EVALUATION OF XEROGELS

1. Particle size determination :-Particle size of the preparation was determined through microscopic evaluation method.

2. Time for gel formation :-2 ml of simulated salivary fluid was taken in watch glass and 50 mg of gel powder was sprinkled on it. The time taken by preparation to form gel was noted.

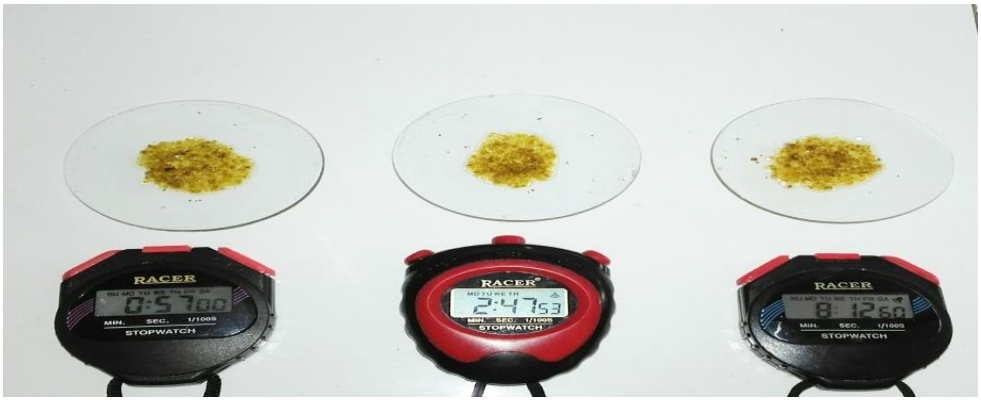

Figure no. 2: Swelling test of xerogels

Artificial salivary fluid composition

SR NO.	INGREDIENTS	QUANTITY IN %
1.	Sodium sulphate	0.03
2.	Urea	0.02
3.	Ammonium chloride	0.04
4.	Potassium chloride	0.03
5.	Lactic acid	0.3
6.	Sodium chloride	4.5
7.	Purified water	Up to 100ml

Make pH to 6.5-7 with 5M NaOH

Table no.2: Formula for artificial saliva

3. Strength of the gel formed :- 2 ml of simulated salivary fluid was taken in watch glass and 50 mg of gel powder was sprinkled on it. After formation of gel watch glass was inverted. The time taken by gel to fall was noted

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RESULT AND DISCUSSION

The preparation was sieved through different mesh size viz. 60 and 36. It was observed that powdered obtained through mesh size 60 forms gel within 1 minutes when it comes in contact with simulated salivary fluid while powder obtained through mesh size 36 takes 2-3 minutes for formation of gel. The powder which was not sieved took 8-9 minutes for formation of gel.

The particle size of the fine powdered preparation was found to be 15µm which makes it suitable for use in oral cavity and for application on wound. Smaller particle size also offers greater surface area for absorption of fluid and decreases the time taken by powder to form gel on application to mouth ulcers and wound. It also imparts lower irritability on application to affected area.

The strength of the gel was found excellent as none of the 3 samples of the gel fell out of the inverted watch glass. It indicates that the gel would stay on the affected area for longer period of time and release the drug in controlled manner. It also offer greater muco-adhesive property.

Tulsi , turmeric and liquorice shows synergistic action in case of mouth ulcer while combination of turmeric and tulsi aids in wound healing.

CONCLUSION:-

By evaluation studies, it was concluded that herbal xerogel possess good particle size, have optimum strength to form a film over the damage area and takes lesser time to form the film to treat mouth ulcers and wound healing.

The preparation since contains no liquid phase offer good shelf life and chances of microbial contamination are less. Being herbal formulation containing tulsi, turmeric and liquorice as active ingredient, the chances of having side effects are less.

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EXAMINATION OF SYNERGISTIC ACTIVITY OF HERBAL DRUGS

*Dhruvisha Pokar, Shwetali Rane and Nilkamal Waghmare**

ABSTRACT

The primary objective of this study was to observe the synergistic effect of herbal drug powder of *Curcuma longa* and *Ocimum sanctum* and also to formulate and evaluate the gel containing this herbal medicament to treat mouth ulcer which gives soothing and cooling effect without producing any irritation. Mouth ulcer is a break or depression in mucous membrane which lines inside the mouth cavity and usually have yellow or white color. The herbal drug in powdered form was individually and in combined form was observed for zone of inhibition by cup plate method. Zone of inhibition for plate containing combined drug was found to be more than zone of inhibition of plate containing individual drugs. The herbal drug possesses anti-inflammatory and antibacterial property which is substantially equal to marketed formulation.

Key words: Gel formulation, Herbal medicaments, Mouth ulcers.

INTRODUCTION

MOUTH ULCER

A mouth ulcer is an ulcer that occurs on the mucous membrane of the oral cavity.An ulcer is a break in the skin or mucous membrane with loss of surface tissue and the disintegration and necrosis of epithelial tissue. A mucosal ulcer is an ulcer which specifically occurs on a mucous membrane. These may form individually or multiple ulcers may appear at the same time (a "crop" of ulcers). Once formed, the ulcer may be maintained by inflammation and/or secondary infection.

Causes of mouth ulcers:

Traumatic ulceration: Most mouth ulcers that are not associated with recurrent aphthous stomatitis are caused by local trauma. The mucous membrane lining of the mouth is thinner than the skin, and easily damaged by mechanical, thermal, chemical, or electrical means, or by irradiation.

Mechanical: Common causes of oral ulceration include rubbing on sharp edges of teeth, fillings, crowns, false teeth (dentures), or braces.

Thermal and electrical burn: Thermal burns usually result from placing hot food or beverages in the mouth. This may occur in those who eat or drink before a local anesthetic has worn off.

Chemical injury: Caustic chemicals may cause ulceration of the oral mucosa if they are of strong-enough concentration and in contact for a sufficient length of time.

Irradiation: As a result of radiotherapy to the mouth, radiation-induced stomatitis may develop, which can be associated with mucosal erosions and ulceration

Aphthous stomatitis: Aphthous stomatitis (also termed recurrent aphthous stomatitis, RAS, and commonly called "canker sores") is a very common cause of oral ulceration. 10–25% of the general population suffers from this non-contagious condition.

Infection: Many infections can cause oral ulceration. The most common are herpes simplex virus, varicella zoster, and coxsackie A virus etc.

Drug-induced: Many drugs can cause mouth ulcers as a side effect. Common examples are alendronate, cytotoxicdrugs etc.

Allergy: allergic reactions of the mouth and lips may manifest as erosions.

Malignancy: Rarely, a persistent, non-healing mouth ulcer may be a cancerous lesion. Malignancies in the mouth are usually carcinomas, but lymphomas, sarcomas and others may also be possible

Others: A wide range of other diseases may cause mouth ulcers.eg:neutropenia, hypereosinophilic, leukemia, myelodysplastic syndromes, nutritional deficiencyetc.

CURRENT TREATMENTS FOR MOUTH ULCERS

Treatment of mouth ulcers may include soothing mouthwashes or antiseptic mouthwashes, such as chlorhexidine mouthwash or povidone iodine mouthwash. Paracetamol is useful to relieve pain due to viral infections causing mouth ulcers. Carbenoxolone gel or mouthwash can be used. These are synthetic drugs, which contain several adverse effects, so there are many herbal drugs, which are also used for treating mouth ulcers without any side effects. Polyherbal preparations are generally the mixtures of extracts, juices, pulps, secretions and exudations orpowders of medicinal herbs in solid, liquid or semisolid forms with or without a suitable base.

Examples of synthetic drugs
Benzocaine
Triamcinolone
Hydrocortisone
Benzydamine
Tannic acid

ADVANTAGES OF HERBAL PREPARATION

- Reduced risk of side effects.
- Effectives with chronic conditions.
- Lower cost.
- Widespread availability.
- The cultivation and processing is environment-friendly.
- Better patient tolerance as well as public acceptance.

AIM AND RATIONALE OF PRESENT STUDIES

AIM: The present study was aim towards the development of a polyherbal preparation. For this study, the drugs tulsi *(Ocium sanctum*) and turmeric (*Curcuma longa*) were used. Efforts were made towards studying the combined effect of this drugs towards the treatment of mouth ulcers.

Further trails were done to develop a semisolid gel formulation as a vehicle for these two drugs. Gels are semisolid system in which a liquid phase is constrained within a 3-D polymeric matrix having a high degree of physical and chemical cross- linking.

RATIONALE

- Idea behind to develop herbal formulation was to avoid the side effects seen with synthetic drug containing formulation.
- For example, salicylates containing formulation if applied in excess irritates the mucosa and itself causes ulceration. (present in smile gel).
- Another drug tannic acid if swallowed then it causes G.I irritation.
- The drug turmeric was selected as it as antibacterial and anti-inflammatory activity.
- Tulsi was selected for its antibacterial property.
- The rationale behind using this combination was to study the synergistic antibacterial activity.
- Second aim was to formulate a gel using combination of powder drugs.
- Gel as a formulation was selected due to its 3D matrix structure that has capability to hold any drug in the form of suspension.
- Gel also gives soothing and cooling effect necessary to relive the irritation caused in mouth ulcer.
- It also assists in drug release into mucous membrane.
- Due to these above factors it has patient compliance.

MATERIALS AND METHODS

To observe synergistic effect of herbal medicaments

Drugs: *Curcuma longa* and *ocimum scantum* powders.

Test organism: *Bacillus subtillis* and *E. coli.*

Media: Nutrient agar medium.

PROCEDURE

The antimicrobial activity of herbal drugs was evaluated using cup plate method. This method was used to determine the zone of inhibition of both drugs. The powder of *Curcuma longa* was weighed (3.5 gm) and suspended into propylene glycol and also powder of *Ocimum scantum* was weighed(0.5 gm) and suspended into propylene glycol. The three petri plates were filled with nutrient agar medium and inoculated with bacterial suspension. On each petri plate bores were made of around 8mm diameter. One bore on each petri plate. In next stage, petri dishes containing nutrient agar and test organism were impregnated with drug suspension.

On first petri plate: 3.5 gm of *Curcuma longa* suspended in propylene glycol was added.

On second petri plate: 0.5 gm of *Ocimum scantum* suspended in propylene glycol was added.

On third petri plate: 3.5+0.5 gm of both drugs were added.

The inoculated plates were incubated at 37 degrees Celsius for both microbial strains. After 24 hours, zone of inhibition was measured and compared.

FORMULATION

Two different formulations of gel were prepared using two different polymers i.e. Carbopol 940 and sodium carboxy methyl cellulose (sodium cmc). The excipients like propylene glycol was added to enhance softness of gel. Triethanolamine was added to adjust the pH. The formulation contains high amount of water, so a chance of microbial growth was high, hence sodium benzoate is used as a preservative.

FORMULATION TABLE 1:

INGREDIENTS	QUANTITY GIVEN(F1)	QUANTITY GIVEN(F2)
Curcuma longa	17.5 gm	17.5 gm
Ocimum scantum	2.5 gm	2.5 gm
Carbopol 940	2 gm	1 gm
Propylene glycol	4 gm	4 gm
Sodium benzoate	0.01 gm	0.01
Triethanolamine	q.s	q.s
Water	q.s to 100 gm	q.s to 100 gm

FORMULATION TABLE 2**:**

INGREDIENTS	QUANTITY GIVEN(F1)	QUANTITY GIVEN(F2)
Curcuma longa	17.5 gm	17.5 gm
Ocimum scantum	2.5 gm	2.5 gm
Sodium cmc	4 gm	5 gm
Propylene glycol	4 gm	4 gm
Sodium benzoate	0.01 gm	0.01 gm
Water	q.s to 100 gm	q.s to 100 gm

PROCEDURE OF FORMULATION OF GEL:

- All ingredients are weighed accurately.
- In one beaker required amount of water was taken and polymer i.e. gelling agent was suspended for 10 minutes.
- In porcelain dish required quantity of propylene glycol was weighed, both drug and preservative are added and mixed thoroughly.
- The propylene glycol suspension is added slowly with the help of stirring into beaker containing polymer.
- Mix properly.
- In case of Carbopol 940 triethanolamine was added to adjust pH to 7.

RESULTS AND DISCUSSION

The powders of the drugs tulsi (*Ocimum sanctum*) and turmeric (*Curcuma longa*) were evaluated for their antimicrobial activity using cup plate method. The drug turmeric was primarily selected as it is believed to have inherent anti- inflammatory activity which is also an important factor in treatment of mouth ulcers.

The in-vitro anti-microbial activity was calculated in terms of inhibition zone diameter (mm). The organisms *Bacillus subtillis* and *Escherichia coli* are used. The powder drugs were tested individually and in combination with one another. Different concentrations of the drugs were used in combination to find the minimum inhibitory concentration. These concentrations are given in the table below. The minimum concentration required of the herbal drugs to show a significant antimicrobial activity in terms of comparison with a marketed preparation containing choline salicylate solution B.P. and tannic acid (which is the major astringent used in mouth ulcer) was found to be 17.5% w/w of turmeric and 2.8% w/w of tulsi.

After the above findings, trails were done to formulate the powder drugs into a gel for ease of application. Propylene glycol was used to suspend the powdered drugs and Carbopol and sodium CMC as the gelling agent.
First the powders were formulated using Carbopol as the gelling agent. This formulation was then tested for antimicrobial activity using cup plate method. It was found that this gel was not able to release the drug and no zone of inhibition was seen.
On the next attempt sodium CMC was used as the gelling agent. This formulation was also tested for antimicrobial activity using cup plate method just as done for the first formulation. This formulation showed certain amount of inhibition but not as much as that of the combined powder drugs. Therefor CMC also proved to be an ineffective gelling agent for these drugs.
Due to time constrains further trails with other gelling agents was not possible. Hence this topic can further be taken up for developing a effective formulation for delivery of this drugs.

CONCLUSION

The present study demonstrates the antimicrobial activity of herbal powders of *Ocimum sanctum* and *Curcuma longa*. In the antimicrobial activity study of the drugs it is observed that, the powder drugs are active against organism, *Bacillus subtillis* and *Escherichia coli*. It was found that these two drugs when used in combination gives synergistic activity against the test organism. All drugs powders which are used in the dose range are safe for consumption and can be swallowed without any risk of systemic side effects.
The prepared formulation(gel) did not show any zone of inhibition hence more work needs to be done to formulate this powder drugs into a gel formulation. Other polymers can be used to formulate the gel. Therefore, we conclude that this topic can be taken up for further research to formulate a suitable gel that can efficiently release the drugs. This gel then can be a substitute over the other preparation available in the market in near future.

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ANTIFUNGAL ACTIVITY OF GARLIC.

*Jyotikumari Gupta, Rutuja Gole and Nilkamal Waghmare**

Abstract: Herbal drugs are promising to shows the wide spectrum of antactivity, antifungal activity. Garlic is a drug of herbal origin having the many active constituents like Alliin, Allicin, Ajoene, Allylpropl, diallyl trisulphate, sallylcysteriene, vinyl dithiines. Ajoene is the sulphur containing oil soluble active constituents which has antifungal activity and also known to have the platelets aggregation inhibitory activity. Antifungal activity of the garlic powder was check out by using the agar disk diffusion method were the garlic powder was mixed with vegetable oil which was used as solvent. This mixture was taken into different concentration as 0.1g/ml, 0.2g/ml ,0.3g/ml, 0.4g/ml. From which concentration of 0.2g/ml Shown to have the minimum zone of inhibition. So, this concentration will be taken for the further studies.

Keywords: Antifungal activity. Garlic- A drug from herbal origin, Fungus- Candida albicans.

INTRODUCTION.

Fungal diseases are often caused by fungi that are common in the environment. Most fungi are not dangerous, but some types can be harmful to health. Mild fungal skin diseases can look like a rash and are very common. Fungal diseases in the lungs are often similar to other illnesses such as the flu or tuberculosis. Some fungal diseases like fungal meningitis and bloodstream infections are less common than skin and lung infections but can be deadly.

AIM & RATIONALE.

Aim: To detect the antifungal activity on garlic.

Rationale: Though the market is flourish with lot of allopathy medicines, drug from herbal origin also promising to show antimicrobial activity. Also, herbal drugs have more patient compliance than that of conventional drugs since there is no problem regarding drug interaction and Adverse drug interaction. Garlic is chosen among all, to detect the antifungal activity as it is widely used by the population and economic as well.

MATERIALS AND METHOD.

Materials: Garlic powder, vegetable oil, Candida albicans culture, sabrouds chloramphenicol medium, saline solution, autoclave, petri plates, nichrome wire loop.

Method:

ZONE OF INHIBITION TEST FOR ANTIMICROBIAL ACTIVITY

Zone of Inhibition Test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of solids and textiles to inhibit microbial growth. Researchers who develop antimicrobial textiles, surfaces, and liquids use this test as a quick and easy way to measure and compare levels of inhibitory activity.

With this method, approximately one million cells from a single strain are spread over an agar plate using a sterile swab, then incubated in the presence of the antimicrobial object. If the bacterial or fungal strain is susceptible to the antimicrobial agent, then a zone of inhibition appears on the agar plate. If it is resistant to the antimicrobial agent, then no zone is evident.

- Standard concentration at which pure form of ajoene shows antimicrobial activity, more specifically antifungal activity is less than **20 microgram/ml**
- Ajoene is practically soluble in vegetable oil

- ➢ Since whole garlic (in powder form) as a drug is used for testing, therefore the amount of ajoene is present in 1 ml of solvent (i.e vegetable oil) is very less
- ➢ So, concentration to be taken for testing should be high in order to achieve required amount of main constituent to show its activity.
- ➢ Trial and error method is used by preparing some concentrations of garlic powder in vegetable oil such as 0.1g/ml, 0.2g/ml, 0.3g/ml, 0.4g/ml and 0.5g/ml.

Procedure:-

First step- preparation of slant

- Take 0.65g of sabrouds chloramphenicol is dissolved in 10 ml of distilled water in a test tube.
- Sterilization by using autoclave at 121 degrees temperature, 15 PSI pressure for time of 15 -20 minutes.
- Keep the test tube in slanting position and allow it to solidify.
- Using culture of candida albicans, make a streak on slant by with the help of sterile nichrome wire loop.
- Incubate for 24 hours.

Second step-preparation of nutrient broth

- Take 0.18g of sodium chloride in 20 ml of distilled water in a test tube
- Sterilization by using autoclave at 121 degrees temperature, 15 PSI pressure for time of 15 -20 minutes.
- Cool up to room temperature
- Take 2-3 loopful of slant and inoculate in broth under aseptic condition.
- Incubate for 24 hours.

Third step-preparation of plate

- To 9.75g of sabrouds chloramphenicol add 150 ml of distilled water and dissolve it completely by boiling the solution.
- Sterilization by using autoclave at 121 degrees temperature, 15 PSI pressure for time of 15 -20 minutes.
- Pour approximately 20 ml of this solution in 6 previously sterile plates and allow it to solidify.
- After solidification, pour around 0.2-0.25ml of broth on solidified plates and spread it using spreader.
- Using borer, make a well in a centre of a plate and inoculate the different concentrations (mentioned above) in 5 different plates and mark the plates accordingly
- Inoculate plain vegetable oil in one plate, to ensure oil itself is not showing any antimicrobial activity.
- Incubate for 24 hours.
- Remove and observe on black sheet.
  Observation table: Table no. (1)

RESULTS AND DISCUSSION:

Zone of inhibition starts at concentration of 0.2g/ml, whereas no inhibition is observed in case of plain vegetable oil.

Hence proved, the oil does not contribute in showing any antifungal activity and it is purely drug showing its activity.

CONCLUSION:

Ajoene, a Sulphur containing drug present in garlic which is practically soluble in vegetable oil shows antifungal activity at the concentration of 0.2g/ml using trial and error method by zone of inhibition technique.

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TABLE NO. (1)

SR.NO	CONCENTRATION (gm/ml)	INHIBITION (diameter in mm)
1	0.1	-
2	0.2	19
3	0.3	20
4	0.4	21
5	Plain vegetable oil	No inhibition.

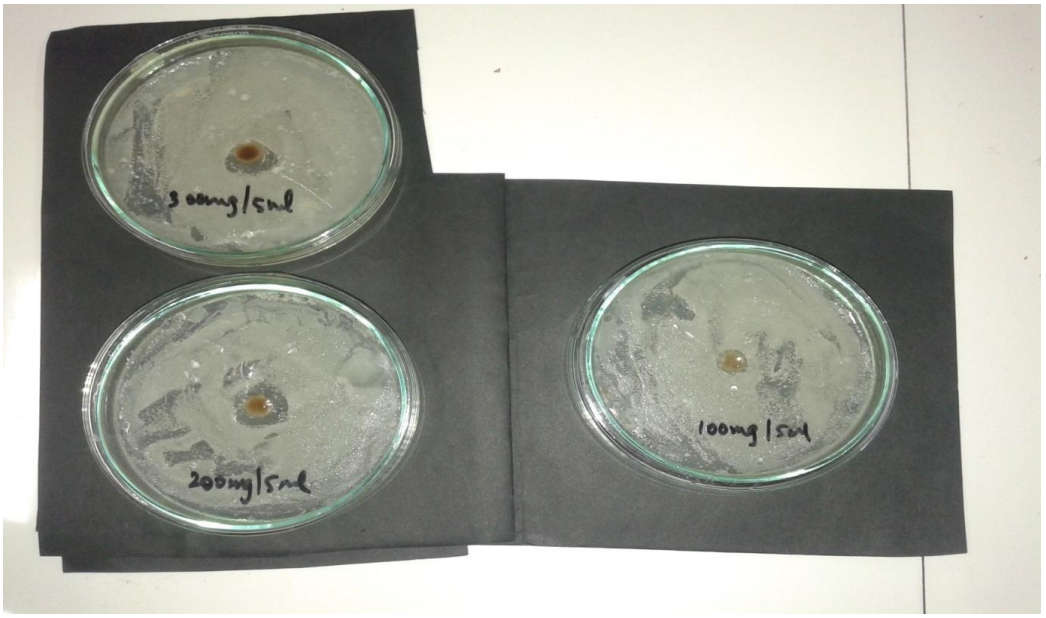

Fig. Plate no. 1,2 &3 i.e concentration of (0.1g,0.2g and 0.3g)/ml

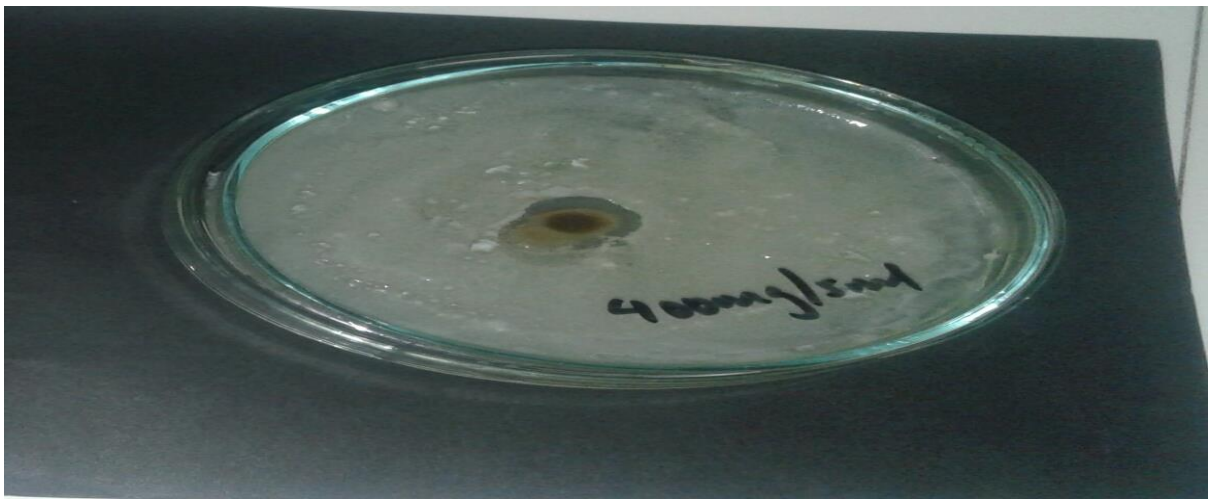

Fig. Plate no.4 i.e concentration of 0.4g/ml

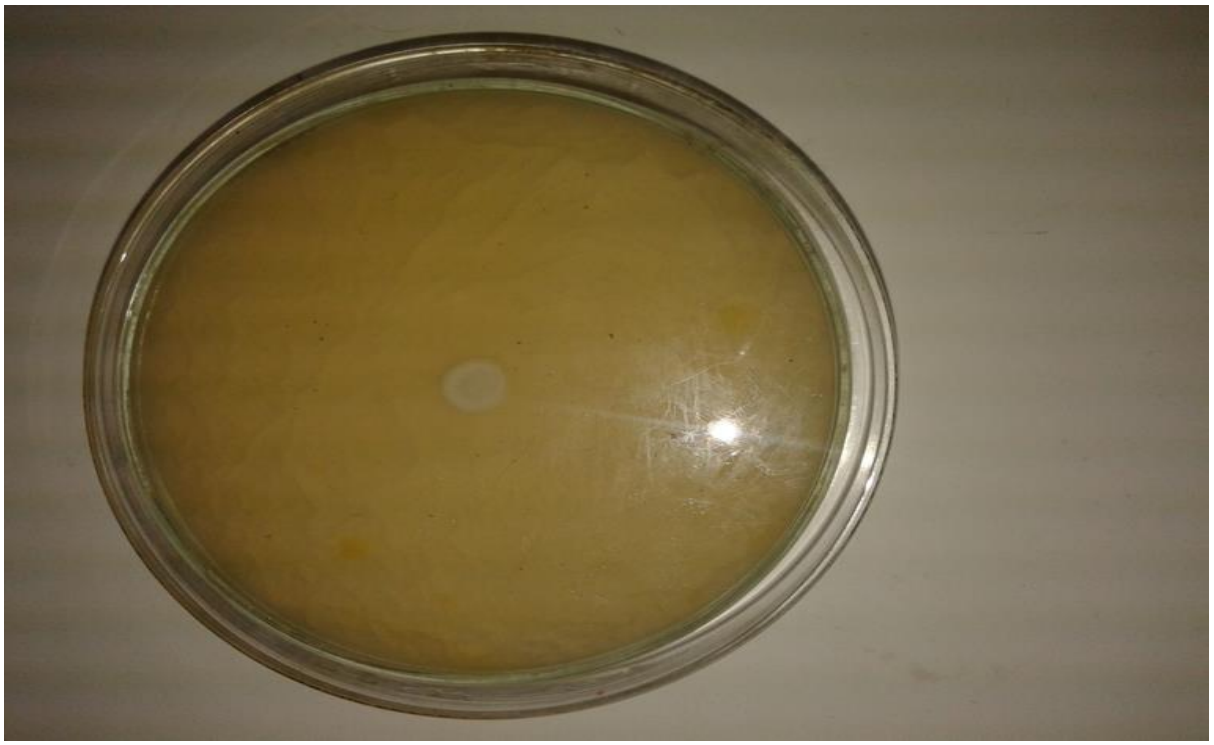

Fig. Plate of plain vegetable oil

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TOXICOLOGICAL EVALUATION OF *LEPIDIUM SATIVUM* USING BRINE SHRIMP LETHALITY TEST

*Vikas Mourya, Amruta Nandgawle and Pooja Pherwani**

Abstract

The main aim of the present study is to focus on the toxicity of the alcoholic extract of the **medicinal plant *Lepidium sativum* belonging to the family Brassicaceae (Crucifereae). Brine** shrimp lethality bioassay method was used for the present study and the toxicity was reported in terms of lethality concentration (LC_{50}). The shrimps were hatched, 10 active shrimps were added to the diluted test solutions and the surviving (larvae) shrimps were counted after 24 hrs and lethality concentration LC_{50} was calculated. The median lethal concentration (LC_{50}) of the test samples is obtained by a plot of % mortality against the logarithm of the sample concentration. LC_{50} values are calculated using a probit regression analysis and compared with either Meyer's or Clarkson's toxicity criteria. In the present study, alcoholic extract of *Lepidium sativum* exhibited potent brine shrimp lethality LC_{50} as 2.239 mg. The toxicity result of *L.sativum* extract showed that it is non-toxic to brine shrimp larvae.

Keywords: Alcoholic extract of *Lepidium sativum*; brine shrimp lethality assay; toxicity testing; LC_{50}

1. INTRODUCTION

The search for new drugs which are plant-derived has been receiving renewed interest among researchers throughout the world in view of discovering new drugs that possess potency to combat the menace of drug resistant pathogenic microorganisms, antitumor and anticancer agents.

Plants can be useful either in their crude or advanced forms, offering a source of drugs in their pure state. Recognized for their ability to produce a wealth of secondary metabolites, many of these natural products have been shown to present interesting biological and pharmacological activities, which could serve as the starting point in the development of modern medicines**.**

Well-known drugs which were developed from plant species are Vinblastine and Vincristine (first cures in human cancer) from *Catharanthus roseus*, Quinine (anti-malarial agent) from *Cinchona* species, Scopolamine (sedative) from *Datura metel* L., and many others which remained in use until present day [1].

Although many plants have valuable properties, some of them are known to carry toxicological properties as well. Recent studies indicate that although numerous plants are used as food sources, some of them may have mutagenic or genotoxic potential. Numerous research studies have recently focused on both pharmacology and toxicity of medicinal plants used by humans. This is of high importance in order to achieve a safe treatment with plant products.

The toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant. Various assays are used for the research of potential toxicity of herbal extracts based on different biological models, such as *in vivo* assays on laboratory animals. However, recent studies employed efforts for alternative biological assays that include species of *Artemia salina, Artemia franciscana, Artemia urmiana* and *Thamnocephalus platyurus*. These toxicity tests are considered a useful tool for preliminary assessment of toxicity [2].

During the past 30 years, the Brine Shrimp Assay has been widely used to test the toxicity of a great variety of plant products. Brine shrimp (*A. salina*) is most extensively studied of the *Artemia* species, estimated to represent over 90% of the studies in which *Artemia* is used as an experimental test organism [2].

Brine Shrimp Lethality Assay (BSLA) has been applied as an alternative bioassay technique to screen the toxicity of plant extracts, toxicity of heavy metals and metal ions, toxicity of cyanobacteria and algae, cytotoxicity of dental materials, toxicity of nanoparticles, as well as screening of marine natural products [2].
The technique is economic and utilizes small amount of test material. Since its introduction, this in vivo test has been successively employed for bioassay-guide fractionation of active cytotoxic and antitumor agents. Additionally, several studies demonstrated that there is a good correlation between the results for the lethal concentration that kills 50% of the exposed population (LC_{50}) obtained with the Brine Shrimp Lethality Assay using *A. salina* and the results of the Acute Oral Toxicity Assay in Mice.
Brassicaceae is one of the largest plant families consisting of about 300 genera and 1500 species, which include vegetable crops, medicinal plants and others. Several plants of this family are used as antidiabetic, antibacterial, antifungal, anticancer, antirheumatic and show potent insecticidal effects.
The genus *Lepidium* comprises several species growing in most temperature warm climate. Some species of *Lepidium* are used as salad and the pods are sometimes used as food.

2. RATIONALE

The search for new pharmacologically active agents obtained by screening natural resources such as plant extracts has lead to the discovery of many clinically useful drugs for the treatment of human diseases.
L. *Sativum* seeds have been used in different treatments. The seeds paste is applied to rheumatic joints to relieve pain and swelling. The seeds are chewed to treat sore throats, coughs, asthma and headaches. Seeds pounded in water are used to treat hiccoughs and stomach-aches. The seed oil is used as an illuminant and in soap manufacture. The seeds are used, fresh, dried or boiled consumed in drinks, either ground in honey or as an infusion in hot milk.. Its young leaves are eaten raw or cooked *Lepidium sativum* possesses several pharmacologique activities, leaves and seeds extracts were found to have Antihypertensive activity1, seed extracts has proved hepato protective, hypoglycemic and used in treating bronchial asthma [3].
Based on results obtained in the literature, *L. sativum* (Brassicaceae) was selected for this study.

3. METHODOLOGY

3.1. Plant Material

The seed *Lepidium Sativum* (Garden Cress Seeds- Family: Brassicaceae) is widely found throughout India. The seeds of *Lepidium Sativum* obtained from local shop.

3.2. Extraction

The ethanolic extraction of *Lepidium Sativum* (Garden cress Seeds) was carried out by maceration process. In this process the coarsely powdered sample (garden cress seeds) was placed in a stoppered container with the ethanolic solvent and allowed to stand at room temperature for a period of 5 days with frequent agitation until the soluble matter has dissolved. The mixture was drained, up to the mark (the damp solid material); pressed and combined liquids was clarified by filtration after standing and evaporated the solvent to get the dried extract.

3.3. Brine shrimp toxicity testing

The toxicity level of the *Lepidium sativum* extract was conducted [6]. Two grams of *Artemia salina* (Brine shrimps egg) were introduced into 2L of sea water in a hatching chamber and left under light for 48hours at room temperature to hatch. The nauplii (harvested shrimps) were attracted to one side of the chamber by the light source.

The extract (1g) was then dissolved in 10ml of dimethyl sulphoxide (DMSO) to give a stock solution containing 100 mg/ml of the extract.

Serial dilution were made from the stock solution into series of test tubes, and this was followed by the addition of sea water (up to 20 ml) and subsequently 10 larvae of brine shrimps to each tube (Table 1).

A concurrent control experiment set up was prepared, and had no extract. The toxicity of tested plant sample was determined by comparing their LC_{50} values with highly toxic substances suitable to be used as positive controls for this test was potassium dichromate [7].

The set up was left for 24 hours after which surviving shrimps in each test tube was counted and recorded. The concentration for killing fifty percent of the larvae (LC_{50}) was calculated using Finney table [6] (Table 2).

4. RESULT AND DISCUSSION

Depending on the conditions of the experiment, survivors were counted after 24 hours of exposure to the tested sample (Table 3). No deaths were observed to occur in the negative control after 24 hours.

After 24 hours of exposure, the median lethal concentration (LC_{50}) of the test samples has been obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration. LC_{50} values were estimated using a probit regression analysis [6] (Figure 1).

4.1. Toxicity testing criteria

The toxicity of herbal extracts expressed as LC_{50} values is commonly obtained either by comparison to Meyer's or to Clarkson's toxicity index.

According to Meyer's toxicity index, extracts with $LC_{50} < 1000$ µg/ml are considered as toxic, while extracts with $LC_{50} > 1000$ µg/ml are considered as non-toxic [8].

Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with LC_{50} above 1000 µg/ml are non-toxic, LC_{50} of 500 -

1000 µg/ml are low toxic, extracts with LC_{50} of 100 - 500 µg/ml are medium toxic, while extracts with LC_{50} of 0 - 100 µg/ml are highly toxic [8].
These calculations may not give the exact lethal concentration of the examined compound or extract that kills 50% of the population, but without doubt it represents a significant preliminary data for further toxicity testing assays (Figure 1).
The toxicity result of *L.sativum* extract showed that it is non-toxic to brine shrimp larvae. LC_{50} was found to be 2239µg/ml (i.e. 2.239 mg/ml) in treated larvae.

5. CONCLUSION

Lepidium sativum (Garden Cress) is an ancient herb that has been widely consumed and used in traditional medicine. The seeds of *Lepidium sativum*, Family Brassicaceae (cruciferae) were taken for the present study.
The seeds were selected and they were extracted. An alcoholic extract of seeds was prepared by maceration process.
Although, the brine shrimp lethality bioassay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts.
The LC_{50} was found to be 2239µg/ml (i.e. 2.239 mg/ml) in treated larvae.
The result suggest that the alcoholic extract of *Lepidium sativum* does not possess significant toxicity up to 2.239 mg/ml .

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7. TABLES AND FIGURES

Conc. µg/ml	No. of shrimps
500	10
1000	10
2000	10
3000	10
4000	10
5000	10

Table 1. Serial dilutions of the alcoholic extract of *Lepidium sativum*

%	0	1	2	3	4	5	6	7	8	9
0	__	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.25	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
__	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Table 2. Finney's table for transformation of percentage of mortality to probit values [8].

Conc. µg/ml	No. of shrimps	No. of affected shrimps	% Mortality	Log conc.	Probit value
500	10	1	10	2.69	3.72
1000	10	3	30	3	4.48
2000	10	4	40	3.3	4.75
3000	10	6	60	3.47	5.25
4000	10	7	70	3.6	5.52
5000	10	10	100	3.69	-

Table 3. Percent mortality by alcoholic extract of *Lepidium sativum.*

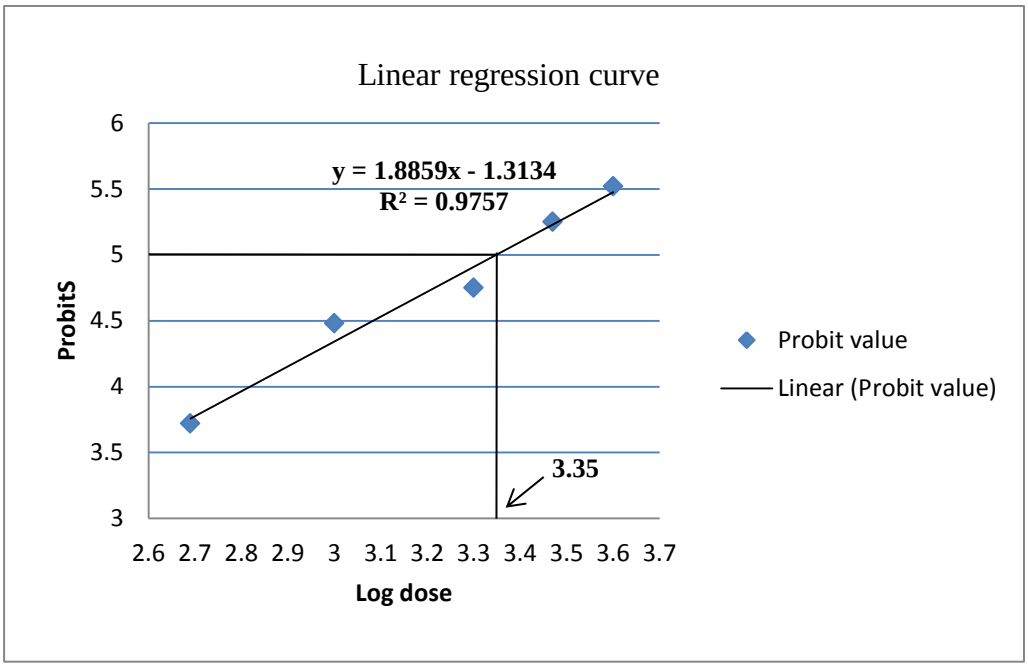

Figure 1. Obtaining the log value of the concentration by interpolation from the linear correlation between probit and log dose.

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CYTOTOXICITY (BRINE SHRIMP LETHALITY BIOASSAY) OF *HIBISCUS ROSA SINENSIS*

*Komal Shinde, Pranali Shelar and Pooja Pherwani**

ABSTRACT

We designed the present study to investigate the role of gentisic acid in the chemopreventive activity of Hibiscus rosa sinensis. Leaves of Hibiscus rosa sinensis also used as traditional medicine. Leaves are found to possess antioxidant, antifungal, anti-infectious, antimicrobial, anti-inflammatory, anti-diarrheic and antipyretic activity. The activity of the leaves is attributed to Gentisic acid. Glycosides (which contain Gentisic acid) were precipitated from the ethanolic extract of Hibiscus rosa sinensis. Is was used to determine the LC50 using the brine shrimp lethality test.

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Keywords: Gentisic acid, chemopreventive, brine shrimp lethality, cytotoxicity

INTRODUCTION

Plants contain secondary metabolites, which are organic compounds that are not directly involved in the normal growth, development, or reproductions of organisms but often play an important role in plant defence Examples include alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids and saponins[1]. Furthermore, there is growing interest in the chemical composition of plants towards discovery of more effective bio-therapeutic agents. The primary benefit of using plant-derived medicines is that they are readily affordable and accessible. Continuous exposure to chemicals and contaminants leads to increase the free radicals amount and causes irreversible oxidative damage including biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to ageing[6].

The Hibiscus genus (Malvaceae) contains several species, many of which have been used medicinally and is comprises of about 275 species in the tropics and sub-tropics and most Hibiscus species have a remarkable colour pattern with the base of corolla forming a deep-colour heart[2].

Natural products from plants are potent sources of potent anticancer agents. Previous studies had showed that Hibiscus rosa sinensis possesses antidiarrhetic and antiphologistic activity[3]. In vitro cytotoxicity screening offer a suitable strategy to select plant extracts with potential of antineoplastic properties. Cytotoxic assay is based on the evaluation of a rare characteristic of most cytotoxic agents, commonly drugs, hormones, nutrients and irradiation. Cytotoxic assays have been used to measure the amount of death resulting from treatment with compounds that can cause cancer[4].

The principal constituents of Hibiscus rosa sinensis Linn. are flavones. Flavones contain quercetin-3-diglucoside, quercetin-3,7-diglucoside, cyaniding-3,5-diglucoside quercetin-3-sophorotrioside, kaempferol-3xylosylglucoside, cyaniding-3-sophoroside-5-glucoside and other constituent are cyclopeptide alkaloid, cyanidin chloride, hentriacontane, riboflavin, ascorbic acid, thiamine, taraxeryl acetate, ß-sitosterol , cyclic acids sterculic and malvalic acids[5]. All the parts of Hibiscus rosa sinensis Linn and chemical constituents are used as anti-tumour, antifertility, antiovultory, antiimplantation, anti-inflammatory, analgesic, antiestrogenic, antipyretic, antispasmodic, antiviral, antifungal, antibacterial, hypoglycaemic, spasmolytic, CNS depressant, hypotensive and juvenoid activity[7].

Biological activity and chemical composition of Hibiscus rosa-sinensis

H. rosa-sinensis possess bioactive properties and is recommended to be used as an herbal alternative to cure many diseases. Phytochemicals like tannin, phlobatannins, cardiac glycosides, flavonoids, terpenoids, saponins and others are present in leaves, stem and

root of the plant. A number of previous studies reported that H. rosasinensis contains flavonoids, cyanidin, querecetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, ascorbic, citric, tartaric and oxalic acid. The leaf extract exhibited significant antioxidant and anticancer activities due to the increased flavonoids and terpenoids level and the phytochemical analyses indicated the constituents presented (flavonoids, terpenoids, saponins, tannins and glycosides) are responsible for pharmacological effects[8].

Gentisic acid (2,5-dihydroxybenzoic acid) has a widespread occurrence which has an analgesic, anti-inflammatory, antirheumatic, antiarthritic, and cytostatic agent, gentisic acid inhibits low-density lipoprotein oxidation in human plasma . It is believed that gentisic acid has an effective role in the anticarcinogenetic activity of China-rose hibiscus (Hibiscus rosa-sinensis) extract[9][10].

AIM: To study the leaf extract of *Hibiscus rosa sinensis* exhibiting cytotoxic activity against brine shrimp.

RATIONALE

The present study focuses on to investigate the role of gentisic acid in chemopreventive activity of Hibiscus rosa sinensis. The objective is to evaluate the potential of Hibiscus rosa sinensis. Cytotoxic studies were carried on various parts of Hibiscus rosa sinensis but efforts were taken to find out the lethal dose exhibited by leaf extract and successfully high toxic dose was evaluated for the brine shrimp. This method is very useful as a preliminary test which supports a more specific bioassay once active constituent is isolated.

METHODOLOGY

REQUIREMENTS

1. Artemia salina Leach. (Brine eggs), Sea salt (NaCl)
2. Small tank
3. Lamp to attract Shrimps
4. Pipettes (5, 10ml) and Micropipette
5. Glass vials, Magnifying glass

PLANT MATERIAL

Fresh Hibiscus rosa sinensis plants were collected randomly from different areas. These plants were selected because of their availability (common in wasteland and road side) in the area aside from their medicinal potentials. The plants were identified by comparing it with the herbarium specimens. The plants were washed with water and air dried in shade for about one to two days. Then tray-dried leaves were pounded and kept (at 20°C) in closed plastic containers (Figure no. 1).

PREPARATION OF EXTRACT

Twenty grams of the fine powder of each plant samples were weighed and added into a flask containing 250 ml of 95% ethanol. The solution was covered and allowed to stand for about 48 hours in room temperature. Then, it was shaken and filtered using Whatmann filter paper (No.1). After filtration, the solvent was removed by evaporation using a rotary evaporator under reduced pressure at temperature below 55°C.

EXTRACTION PROCEDURE

Gentisic acid in the chemopreventive activity of Hibiscus rosa sinensis extract. It is a phenolic Glycoside that has a play role as antioxidant anticancer activity. The dried plant

material is rendered into a moderately coarse powder. The powder is then extracted in a Soxhlet apparatus with aqueous ethanol. The non-glycosidal impurities which get extracted along with glycosides are removed by precipitating them with lead acetate solution, which is filtered out. The filtrate contains the glycosides.

TEST PERFORMED

Ferric chloride test 5% FeCl3 added to the sample solution blue colour is produced which changes with brownish green colour indicating phenolic glycosides.

PRINCIPLE

Brine Shrimp lethality bioassay is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. The method utilizes in vivo lethality in a simple zoological organism (Brine nauplii) as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. ED50 values for cytotoxicities are generally about one-tenth the LC50 values found in the Brine Shrimp test. Thus, it is possible to detect and then monitor the fractionation of cytotoxic, as well active extracts using the Brine lethality bioassay. The Brine Shrimp assay has advantages of being rapid (24 hours), inexpensive, and simple (e.g., no aseptic techniques are required). It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (2-20 mg or less).

PREPARATION OF SEA WATER

38 gm sea salt (without iodine) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution.

HATCHTING OF BRINE SHRIMP

Artemia salina leach (brine shrimp eggs) collected from shops was used as the test organism. Sea salt water was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The hatched shrimps are attracted to the light (phototaxis) and so nauplii free from egg shell was collected from the illuminated part of the tank. The nauplii was taken from the fish tank by a pipette and diluted in fresh clear sea water to increase visibility and 10 nauplii was taken carefully by micropipette.

An alternative dilution procedure were adopted in the preparation of the different dilutions of the plant extracts for Brine Shrimp Lethality Assay where 20 mg of each extract was dissolved in 2 mL of the solvent. There were three (3) replicates in each concentration.

BRINE SHRIMP LETHALITY ASSAY (BSLA)

Brine shrimp eggs were obtained from our guide, as a gift sample for the research work. Filtered, artificial sea salt water was prepared by dissolving 38 g of sea salt in 1 liter of distilled water for hatching the shrimp eggs. The sea saltwater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae are ready, 4 ml of the artificial sea salt water was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. Then the volume was adjusted with artificial sea salt water up to 5 ml per test tube. The test tubes were left

uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 hours.

PREPARATION OF TEST SOLUTIONS WITH SAMPLES OF EXPERIMENTAL PLANTS:

32 mg of each of the test samples were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) and finally the volume was made to 20 ml with sea water. Thus the concentration of the stock solution was 1600µg/ml. Then the solution was serial diluted to 800, 400, 200, 100, 50, 25, µg/ml with sea saltwater. Then 2.5 ml of plant extract solution was added to 2.5 ml of sea water containing 10 nauplii (Table No.1).

COUNTING OF NAUPLII:

After 24 hours, the test tube were inspected using a magnifying glass against a black background and the number of survived nauplii in each tube was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration (Table No. 2).

RESULT AND DISCUSSION

The lethality of a test sample in a simple zoological organism such as the shrimp (Artemia salina) has been utilized by Meyer et al. (1982) in the Brine Shrimp Cytotoxicity Test (BSCT). It is a very useful tool to screen a wide range of chemical compounds for their various bioactivities

Extract were subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, glycosides of the ethanolic extract of leaves was found to be the most toxic to Brine Shrimp nauplii, with LC50 of 100(µg/ml)

CONCLUSION

The glycosides of leaf extracts of Hibiscus Rosa sinensis exhibited cytotoxic activity against the brine shrimp and considered as containing active or potent components. The ethno-pharmacological activities of these plant species are due to the different bioactive compounds present in these plants. In this study, glycosides of the ethanolic extract of leaves was found to be the most toxic to Brine Shrimp nauplii, with LC50 of 100(µg/ml)

Although, BSLA is inadequate in determining the mechanism of action of the bioactive substances in the plant, it is very useful by providing a preliminary screen that can be supported by a more specific bioassay, once the active compound has been isolated. Thus, some useful drugs of therapeutic importance may develop out of the research work.

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FIGURES AND TABLES

Figure no. 1

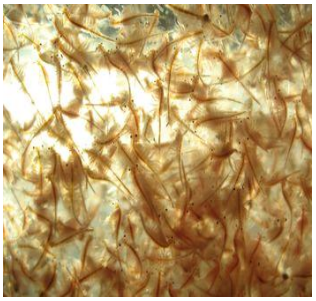

Figure no. 2

Sr. No.	Concentration (µg/ml)	Extract Solution	Sea water containing 10 napaulii	Final Volume
1.	800	2.5 ml (1600µg/ml)	2.5ml	5 ml
2.	400	2.5 ml (800µg/ml)	2.5ml	5 ml
3.	200	2.5 ml (400µg/ml)	2.5ml	5 ml
4.	100	2.5 ml (200µg/ml)	2.5ml	5 ml
5.	50	2.5 ml (100µg/ml)	2.5ml	5 ml
6.	25	2.5 ml (50µg/ml)	2.5ml	5 ml

Table No. 1

Sr. No.	Concentration (µg/ml)	T1	T2	T3	% Mortality	LC50
		No. of Surviving nauplii				
1.	25	7	7	7	30	100
2.	50	6	6	6	40	
3.	100	6	4	5	50	
4.	200	4	4	4	60	
5.	400	2	3	2	80	
6.	800	0	0	0	100	

Table No. 2

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FORMULATION OF HERBAL SOAP

*Prachi Pawar, Priyanka Pawar and Shrutika Patil**

ABSTRACT

Soap is a salt of fatty acid obtained by treating vegetable oils or animal fats with alkali. In current scenario herbal soap has generated considerable interest and enthusiasm amongst consumers due to ecofriendly nature of the product. The research work deals with the isolation of curcumin and embeline with soxhlet extractor from *Curcuma longa* and *Embelia ribes*, respectively. Both phytoconstituents are of therapeutic interest. Hence, these phytoconstituents incorporatedto formulate herbal soap. Further these soaps are evaluated for physiochemical properties like appearance, pH test, total fatty matter test, etc. The antimicrobial efficacy of the formulated soaps was checked by agar well diffusion method by using two bacterial strains *Bacillus subtilis* and *E. coli* and one fungal strain *Candida albicans* and thumb impression method. The result of studies showed that curcumin soap shows better activity than embeline soap.

Keywords: soxhlet, *Embelia ribes*, phytoconstituents, *Bacillus subtilis*

INTRODUCTION

Soap:

In chemistry, soap is a salt of a fatty acid. Soaps are obtained by treating vegetable or animal oils and fats with a strong base, such as sodium hydroxide or potassium hydroxide in an aqueous solution.

Herbal soap:

Products which are formulated using various permissible cosmetic ingredients to form the base in which one or more herbal ingredients are used to provide defined cosmetic benefits only, shall be called as “Herbal Cosmetics” Herbal soap is one of the herbal cosmetic

Types:

Beauty soaps:

Beauty soaps are produced to feature attractive fragrances, and ingredients for a variety of skin types. They can feature glycerin, or special oil blend.

Medicated soaps:

Medicated soaps and original soap are very similar. Unlike original soap, medicated soap has the addition of antiseptics and disinfectants.

Glycerin soaps:

Glycerin is a normally produced during the process of soap production. Soaps which include glycerin in them tend to make your skin feel moisture.

Transparent soap:

Transparent soap uses slightly different ingredients and usually some form of alcohol to alter the process which is also conducted at higher temperatures. Not all transparent soaps are glycerin soaps.

Liquid soaps:

Liquid soaps are actually very difficult to produce and many of the commercial liquid soaps are just in fact detergents.

AIM AND OBJECTIVE

1. Extraction of phytoconstituents from powdered plant material.
2. Formulation and evaluation of herbal soap.
3. To carry out antimicrobial activity of formulation.

MATERIALS AND METHODS

Plant material:

A. Curcumin:
   Biological source: *Curcuma longa*
   Family: Zingiberaceae
   Collecton: purchased from Patanjali Store, C.B.D. Belapur
B. Embeline:
   Biological source: *Embeliaribes*Burm.F.
   Family: Myrsinaceae
   Collection: purchased from SheetalAyurvedic Store, Chembur (East)

Extraction of active constituents: Embeline:
Material: Vidanga poweder-50gm
Petroleum ether (60-80)- 400ml
Soxhlet apparatus
Procedure:
1. Weigh about 50gm of vidang powder
2. Mount in soxhlet apparatus
Extraction:
1. Pour about 300ml of petroleum ether.
2. Start the soxhlet and allow the extraction to continue about 2 hrs.
3. After the completion of the process, the solvent was evaporated and ether was added for the precipitation of embeline.

Curcumin:
Material: Turmeric powder-50gm
Ethanol- 200ml
Soxhlet apparatus

Procedure:
1. Weigh about 50gm of turmeric powder
2. Mount in soxhlet apparatus.
Extraction:
1. Pour about 100ml of ethanol.
2. Start the soxhletamdallow the extraction to continue.
3. At the end, allow solvent to evaporate and concentrate the extract.

Microbial strains collection:The bacterial strains used for antibacterial activity were *Bacillus subtilis* and *E.coli*.The fungal strain used for antifungal activity was *Candida albicans.*

Control and standards:Streptomycin was used as standard for antibacterial activity while tioconazole was used as standard for antifungal activity.

Formulation:We have tried 4 formulations and selected the following formulation:

Formulation table: Table no.1

1. Weigh the oils (Castor oil, Coconut oil, Wool Fat, & Olive Oil) & glycerin into pot & melt.
2. Weigh the Lye (NaOH) & distilled water into 2 separate containers. Add the lye to the water while stirring to create a solution.
3. Don't have to let it cool down. Pour the lye solution into oils / glycerin & blend to trace.

4. Weigh out stearic acid and 10 extra grams. Melt using a double boiler on the stove.
5. Place pot onto scale & hit tare. Weigh stearic acid into pot. The extra stearic acid that measured out to melt will ensure you don't come up short if any sticks to your container while pouring .
6. Blend again. It will get quite thick because of stearic acid.
7. Weigh denatured alcohol & add to mixture. Stir quickly, breaking up the soap. It will start to dissolve a bit in the alcohol. Scrape the sides of pot to get all of it mixed together.
8. Immediately cover with press & seal the lid. Set to cook.
9. Let the soap cook for 2 hours. During this time the solvents will work on dissolving the soap crystals that form, creating soap. No need to stir.
10. After 2 hours, test soap for clarity.
11. Create sugar Solution by heating. If sugar doesn't dissolve, add a bit more water .
12. Add the sugar solution to pot & mix , cover & cook for 30 min.- 1 hour .
13. You can check the clearness again if you want. Then decide to add more alcohol or sugar solution.
14. Once it done, then add active ingredient.
15. Pour into the moulds and allow it to set for 3-4 hrs.

Physiochemical evaluation test**:** Following test were performed on formulated herbal soap:
1. Appearance: Embeline and curcumin soap were observed for the color, shape and particle size and air entrapment.
2. Primary skin irritation test: For this three human volunteers were selected and prepared soaps were given to them and checked for irritation.
3. Total fatty matter: Accurately weighed 5 gm of soap and transferred into 250 ml beaker. To completely dissolve the soap 100 ml hot water was added.40 ml of 0.5 N HNO3 was added to the mixture until contents were slightly acidic. The mixture was heated over water bath until the fatty acids were floating as a layer above the solution. Then the mixture is cooled suddenly in ice water in order to solidify the fatty acids and separate them. 50 ml of chloroform was added to the remaining solution and transferred into a separating funnel. The solution is shaken and allowed theto separate into 2 layers and the bottom layer was drained out. 50 ml of chloroform was added to the remaining solution in the separating funnel. The fatty acid dissolved chloroform is again separated as in the previous case and it is transferred to the collected fatty matter
4. pH test: Place water on the surface of the bar of the soap. Rub the water onto the soap until it lathers. Dip the pH strip in the bubbles. Check the pH strip against the pH chart.
5. Foam test: Compare foam forming ability of curcumin and embeline soap. Prepare 0.5% and 1% solution of SLS and 5%, 10%, 15% solution of each soap. Take 10 ml of each test solution in test tube, shake vigorously for 30 seconds and keep in stand. Note volume at 0min, 1min, 5min, 15min, 30min and 1hr.
6. Alkali content**:** 5gm of soap sample is dissolved in 100ml of hot water. About 40ml of 0.05 HNO_3 is added to make it acidic. The mixture is heated until fatty acids are floating as a layer above the solution. It is cooled under the ice water to solidify the fatty acids. The fatty acids were separated and the aqueous solution was treated with 50ml chloroform to remove the remaining fatty acids. The aqueous solution was measured and 10ml of it was titrated against 0.5N NaOH using methyl orange as indicator and from the titer value the total alkali content was calculated.

Antimicrobial assay:

A. Agar well diffusion method:

Antimicrobial activities of curcumin soap and embeline soap were evaluated using well diffusion method on nutrient agar and sabourd dextrose agar for bacteria and fungi, respectively. The inhibition zones were reported in millimeter (mm). *Bacillus subtilis, Staphylococcus aureus* and *E. coli* were used for the antibacterial assay and *Candida albicans* was used for antifungal assay. Nutrient agar plates and sabourd dextrose agar plates were inoculated with bacterial and fungal strains, respectively under aseptic conditions. The wells (diameter- 6mm) were formed with the help of borer and filled with 0.05ml of the test samples. The plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was reported in mm.

B. Thumb impression test:

Thumb impression of the hand exposed to the environment was placed on a sterile nutrient agar plate. Then, the thumb impression of the same hand was placed after washing with formulated embeline soap on the same plate without any overlaps of thumbprints. Same procedure was carried out for formulated curcumine soap with thumb impression of other hand. The pattern of microbial growth on the plates was observed after an incubation period of 24 hours at 37°C.

RESULT AND DISCUSSION:

It was accepted as all the properties of the formulation were satisfactory.

Hence, formulation was evaluated for physiochemical properties and antimicrobial activity.

Physicochemical Evaluation: Table-2

Antimicrobial activity:

1. Agar well diffusion method:

The antibacterial activity of selected formulation against bacterial strains *Bacillus subtilis* and *E.coli* and fungal strain *Candida albicans* was investigated by agar well diffusion method and by using streptomycin and tioconazole as standards for antibacterial and antifungal activity, respectively. The result indicates both curcumine soap and embeline soap shows antibacterial as well as antifungal activity.

Table-3, Fig-a,b,c

2. Thumb impression method:

The antibacterial activity of selected formulation was studied by thumb impression method. The result indicates that both embeline and curcumin soaps show significant decrease in microbial count after washing hands with individual soap.

Fig-d,e

CONCLUSION:

Physicochemical evaluation test (colour, total alkali content, etc.) showed that curcumin gives good colour to the product and antimicrobial activity test showed that curcumin shows greater activity for two microorganisms (E.coli and Candida albicans) than embeline.Hence, we can conclude that the curcumin soap had better activity than embeline soap.

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Figures and Tables:

Fig.a

Fig.b

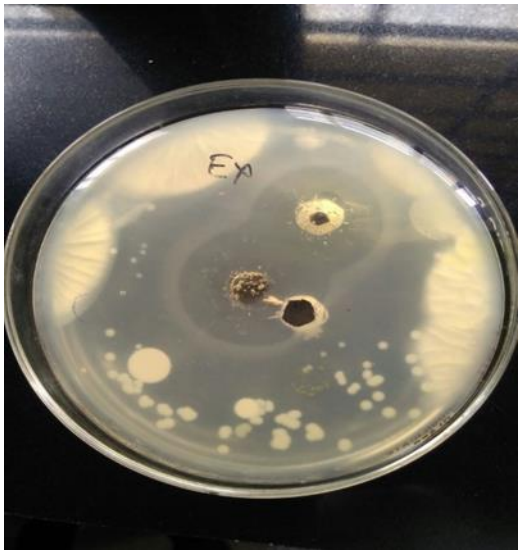

Fig.c

Fig. a: zone of inhibition for *E.coli*, Fig b: zone of inhibition for *Bacillus subtilis*, Fig. c: zone of inhibition for *Candida albicans*

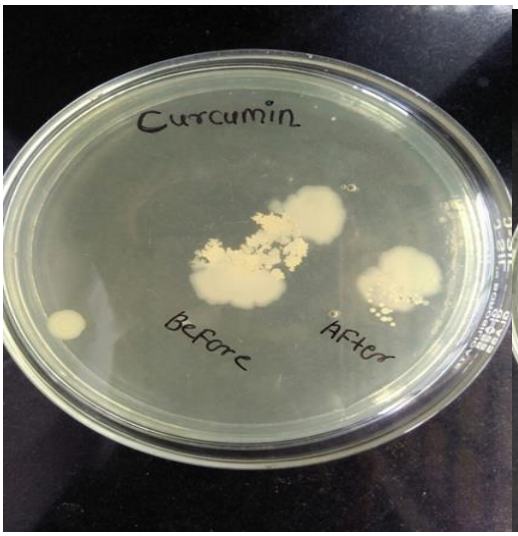

Fig. d **Fig. e**

Fig. d: Thumb impression before and after washing hands with curcumin
Fig. e: Thumb impression before and after washing hands with embeline

Table no.1:

SR. NO.	*INGREDIENTS*	*QUANTITY GIVEN (gm)*		*ACTIVITY*
		EMBELINE SOAP	*CURCUMINE SOAP*	
1.	*CASTOR OIL*	*7.62*	*7.62*	*SUPERFATTING AGENT*
2.	*COCONUT OIL*	*11.42*	*11.42*	*TO PRODUCE LATHER*
3.	*TALLOW*	*11.42*	*11.42*	*SUPERFATTING AGENT*
4.	*STEARIC ACID*	*4.57*	*4.57*	*HARDENING AGENT*
5.	*OLIVE OIL*	*3.05*	*3.05*	*TO RELIEVE DRYNESS*
6.	*ALKALI (NaOH)*	*5.80*	*5.80*	*SAPONIFY OILS*
7.	*DISTILLED WATER (FOR LYE SOLUTION)*	*11.58*	*11.58*	*TO DISSOLVE LYE*
8.	*GLYCERIN*	*9.52*	*9.52*	*HUMECTANT*
9.	*ETHANOL*	*19.05*	*19.05*	*TO MAKE SOAP TRANSPERENT AND CLEAR*
10.	*SUGAR*	*9.52*	*9.52*	*INCREASES THE LATHER*
11.	*ROSE WATER (FOR SUGAR SOLUTION)*	*83*	*83*	*TO DISSOLVE SUGAR, FOR FRAGRANCE*
12.	*DRUG*	*0.0016*	*0.017*	
	TOTAL	*100*	*100*	

Table no.2:

SR.NO.	TEST	EMBELINE SOAP	CURCUMINE SOAP
1	Appearance		
	I. Colour	Light yellow	Dark orange
	II. Shape	Disc shaped	Disc shaped
	III. Particle size	Uniform	Uniform
	IV. Air entrapment	No	No
2	Primary skin irritation test	No irritation	No irritation
3	Total fatty matter	65%	63%
4	pH test	8-9	8-9
5	Foam test	Good	Good
6	Alkali content	1.27	1.32

Table no.3:

SR.NO.	Solutions used	Average diameter of zone of inhibition (mm)		
		Bacillus subtilis	E.coli	Candida albcans
1.	Embeline	14.3	6.6	7.3
2.	Curcumin	5.3	7.3	8.6
3.	Streptomycin	10.18	9.10	NA
4.	Tioconazole	NA	NA	22.10

Table no. 1: Formulation table Table no. 2: Physicochemical Evaluation Table no. 3: Antimicrobial activity by Agar well diffusion method

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FORMULATION OF HERBAL HAND WASH WITH ANTIBACTERIAL ACTIVITY

*Preksha Chodankar, Nilam Dere and Shrutika Patil**

ABSTRACT

Hands are primary source of transmission of microbes and infection. Hand washing is very important preventive measure from infection in day to day life. Hand wash is a liquid soap formulation for washing hands. Nowadays herbal formulation has growing demand in the world market. Present study aim to formulate and evaluate the herbal hand washes using the phytoconstituents viz. Curcumin and Embeline. Both phytoconstituents are of biochemical, physiological and therapeutic intrest. In present investigation Curcumin and Embeline isolated from *Curcuma longa* and *Embelia ribes* respectively using Soxhlet apparatus, which was further used to formulate the hand washes. The efficacy of these hand washes is checked by using *Bacillus subtilis, Escherichia coli* and *Candida albicans.* The result of study reflect that Curcumin hand wash showed ood appeal and better antimicrobial activity as compare to Embeline hand wash.

Keywords: Hand wash, Curcumin, Embeline, Antimicrobial activity.

INTRODUCTION

Skin is one of the most exposed part of the body which requires protection from the pathogens. To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases handwashing is an absolutely important precaution. Handwashing is important way to help fight the disease. Harmful bacteria such as E-coli and Salmonella typhi can be carried by people, animal or equipment and transmitted to food. Synthetic hand wash have some adverse effect as follows:-

- Dryness
- Their frequent use can lead to skin irritation
- Also resistance among pathogen

So we formulated and evaluated Antibacterial efficiency herbal hand washes. A herbal hand wash formulation should give it's effect and at the same time it should not give any unwanted effect rather it should make hand soft and give strong antimicrobial action. Plant extracts and products have been used for centuries in traditional medicine, cosmetics, natural dyes, and in the treatment of diseases. The main advantage of using natural source is that they are easily available, cheap and harmless compared to chemical products. In present study we formulate herbal hand wash.

d wash using plant extracts with potential antibacterial activity and thereby establishing them as a potent antimicrobial agent in the formulation of herbal hand wash. *Embelia ribes*, commonly known as false black pepper and Vidanga, white flowered Embelia, is a species in the Primulaceae. It was originally described by Nicolaas Laurens Burman in his 1768 publication, Flora Indica.

AIM AND RATIONALE

Aim- To formulate and evaluate the herbal hand wash with potential antimicrobial activity.

Rationale- Develop a product with potential antimicrobial activity.

To analyse the result.

MATERIAL AND METHOD

COLLECTION OF PLANT MATERIAL:

1)Embeline:

Embelia ribes is a large, scandent struggling, medicinal climbing shrub with elongated branchlet shanging over the support.

Biological source: *Embelia ribes*

Collection: The Embeliaribes (seeds) was collected from "Sheetal Ayurvedic Store Chembur station, Chembur (East)

2) Curcumin:

Biological source: It consist of rhizomes of *Curcuma longa.*

Family: Zingiberaceae

Collection: Curcumin was obtained from our colleagues.

EXTRACTION OF ACTIVE CONSTITUENT:

Embeline:

Material: 50gm of Vidanga powder

Petroleum ether (60-80) – 400ml

Soxhlet apparatus

Procedure: 1. Weigh about 50gm of vidang powder.

2. Place it in a thimble of filter paper.

3. Mount it in Soxhlet apparatus.

Extraction: 1. Pour about 300ml of Petroleum ether(60-80)

2. Extraction is continued for 2 hrs.

3. The solvent is then evaporated.

4. Precipitation is done by addition of ether.

BACTERIAL STRAINS COLLECTION:

The microbes that were used for the antibacterial activity were *Escherichia coli*, *Bacillus subtilis*. The fungi that was used for assessment of anti fungal activity was *Candida albicans*. All the strains were procured from Bharti Vidyapeeth's College of Pharmacy, Navi Mumbai, Microbiology laboratory.

CONTROLS AND STANDARDS: Streptomycin was used as standard for anti-bacterial activity while Tioconazole was used as standard for antifungal activity.

ANTIMICROBIAL ACTIVITY TEST:

Antimicrobial activities of Curcumin handwash and Embelinehandwash were evaluated using Well Diffusion Method on Nutrient agar and Sabourd chloramphenicol for bacteria and fungi respectively. *Bacillus subtilis, Escherichia coli* was used for the antibacterial assay and *Candida albicans* was used for antifungal assay. Nutrient agar plates and Sabourd dextrose agar plates were inoculated with bacterial strains and fungal strains respectively under aseptic condition. The plate was punched with a sterile borer of 6mm size to prepare the well and it was filled with 0.05ml of the test sample. The plates were allowed to stand for 30 min. The plates were incubated at 37^0 C for 48 hrs. After incubation period, the diameter of the growth inhibition zones was measured. The diameter of the inhibition zones were reported in millimetre (mm).

FORMULATION TABLE: Table no. (1)

EVALUATION PARAMETERS

PHYSICAL EVALUATION-

- Appearance and Homogenicity:-
  The Herbal Hand washes were homogenous; Yellow and Pink in color and translucent in nature.

- Stability:-
  The stability studies were carried out by storing the formulation at different temperature conditions like 37^0C for 1 week.

- pH:-
  1gm of sample of Herbal Hand washes was taken and dissolved in 100 ml distilled water. The pH of solution was taken in previously standardized digital pH meter.

- Viscosity:-
  The viscosity of hand wash was determined by using digital Brookfield viscometer. 50ml of the herbal hand wash was taken into 100ml of beaker and the tip of viscometer was dipped into the beaker containing hand wash formulation and its viscosity was measured.

RESULT AND DISCUSSION.

- FORMULATION TABLE:
  From various formulation Table the table given Table no.(1) was accepted because the foam formed and the viscosity of the formulation was satisfactory.
- Curcumin and Embeline, both the phytoconstituents are good colouring agent. It gives good appeal to the hand wash formulations of each individual active.

- EVALUATION PARAMETERS: Table no. (2)

- ANTIMICROBIAL ACTIVITY: Table no.(3), fig(1), fig(2), fig(3).

The antimicrobial activity of selected formulation of Curcumin and Embeline against *Bacillus subtilis, E.coli,* and *Candida albicans* was investigated by agar well diffusion method. Streptomycin and Tioconazole was used as standard drug for antibacterial and antifungal activity respectively.

CONCLUSION.

After physicochemical evaluation (appearance, homogenity, pH, viscosity) and antimicrobial activity it showed that the Curcumin formulation had good appearance and better antimicrobial activity as compared to Embeline formulation.

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Sr. NO.	Ingredient	Quantity taken	Use
1	Carbopol-940	0.3	Jelling agent
2	Purified water	20ml	Vehicle
3	Triethanolamine	q.s	Neturalizer

Table No. (1) FORMULATION TABLE

Sr. NO.	Ingredient	Quantity taken	Use
4	Gel base	30ml	Base
5	Curcumin	0.0087gm	Antibacterial
6	Sodium Lauryl Sulphate	0.5gm	Surfactant
7	Methyl Paraben	0.1gm	Preservative
8	Rose Water	30ml	Vehicle

TABLE NO.(2) EVALUATION PARAMETER.

Sr. No.	Parameter	Observation
1	Appearance	Clear
2	Homogenity	Homogenous
3	P^H	6.9
4	Viscosity(Pascals)	45-120

TABLE NO.(3) ANTIMICROBIAL ACTIVITY.

Drug	*Bacillus subtilis* (mm)	*E.coli* (mm)	*Candida albicans* (mm)
Curcumin	5.3	7.3	8.6
Embeline	14.3	6.6	7.3
Streptomycin	9.1	10.3	NA
Tioconazole	NA	NA	22.1

Fig no. (1) Zone of Inhibition for *Candida albicans*

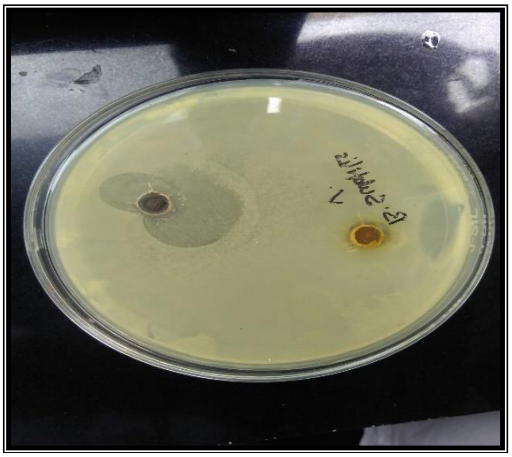

Fig no. (2) Zone of Inhibition for *Bacillus subtilis*

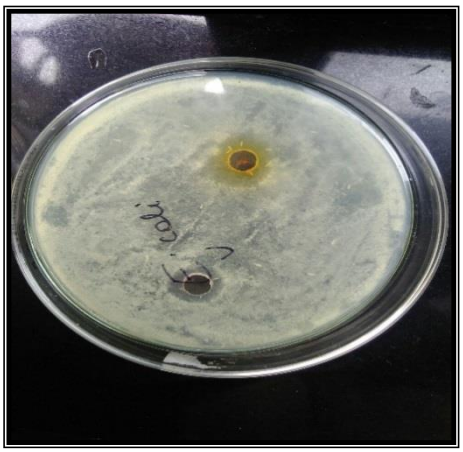

Fig no. (31) Zone of Inhibition for *E. coli*

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FORMULATION OF NATURAL MOSQUITO REPELLENT

*Nidhi Haldankar, Aniket Jadhav and Sneha Mundada**

1. ABSTRACT

Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of diseases and these are the primary source of synthetic and traditional herbal medicine. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses. The objective of the work was to formulate a mosquito repellent product containing Curcumin and Embelin obtained from *Curcuma longa* and *Embelia ribes* respectively. Isolated Curcumin and Embelin was confirmed by TLC technique. Bands were compared with standards by calculating Rf 0.74 and 0.60 respectively at visual detection and 254 nm. The formulation of cream was evaluated for various parameters like smooth texture and spreadability with a pH 7 which is non irritant and suitable for the skin. There is no phase separation during thermal stability. From the present work it was concluded that 1% Curcumin and 1.5% Embelin containing cream is safe, effective, usable for the skin and stable too.

KEYWORDS: Curcumin, Embelin, Natural mosquito repellent.

2. INTRODUCTION

Mosquitoes are among the most disturbing blood sucking insects afflicting human beings. Mosquito species belonging to Anopheles, Culex and Aedes genera are vectors for the pathogens of various diseases like Dengue fever, Malaria, Yellow fever, etc.[3] The mosquitoes eject their saliva into the blood of the host that creates an immune response due to the binding of the antibodies IgG and IgE to the antigens. The reactions result in irritations, itching, redness and sometime it develops into the bumps. It is the saliva of the mosquito that often causes an irritating rash that is a serious nuisance. In addition, mosquito bites can cause severe skin irritation through an allergic reaction to the mosquito's saliva by human mosquito contact. Mosquitoes bear set of sensors that have the capability to track their prey's presence, these includes:

A. Chemical Sensors: The studies have proved that the mosquitoes have the tendency to sense the lactic acid, carbon dioxide and the propen-3-ol upto many yards away. On breathing or perspiration, humans and the animals release these compounds. This is the reason that a person who sweats more becomes the target of the species and the one who sweats less don't get as many bites.

B. Heat Sensors: The mosquitoes also have the ability to detect heat and hence can target the warm blooded animals very fast once they get close enough.

C. Visual Sensors: Its been recorded that the mosquitoes are the intelligent insects as they can easily detect you by looking at your clothing if it contrasts to the background. You are easily detected by them as anything that moves is alive and hence full of blood.[4]

2.1. Control of mosquito borne diseases

Mosquito control and personal protection from mosquito bites are currently the most important measure to control this disease. Prevention of this type of disease involves protecting yourself against mosquito bites.[3] Mosquito control methods are habitat change, biological control, physical control and chemical control including individual safeguards from the mosquitoes. Among the approaches for control of these mosquito-borne diseases is the interruption of the disease transmission by killing or preventing mosquitoes from biting human being. This can be achieved by use of repellents.[5]

2.2. What is a mosquito repellent?

A mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages mosquitoes from landing on that surface. It is a substance that is synthesized in such a manner so that it makes the surface unpleasant and unattractive to mosquitoes so as to reduce the human mosquito contact. Mosquito repellents repel insects but do not kill them. Therefore, they are not technically insecticides nor pesticides. They help prevent and control the outbreak of mosquito borne diseases such as Dengue fever, Malaria, Yellow fever, Japanese Encephalitis, etc.[4]They contain active ingredient which is the only reason to repel mosquitoes by blocking their olfactory senses which detects the carbon dioxide and lactic acid that gets released when the human perspires. These products also contain some more ingredients which aids them with cosmetic finishing.[3]

2.3. Mosquito repellents of natural origin

Natural ingredients are included in some formulations of insect repellents. In the United States, citronella is a popular botanical ingredient in mosquito repellent formulations. The insecticidal properties of this oil were discovered in 1901, and it was used for a time as a hair dressing for the control of fleas and lice. Despite popular conception, citronella candles or incense were ineffective for reducing the biting pressure of mosquitoes. Neem oil, from *Azadirachta indica*, when formulated as 2% in coconut oil, provided complete protection for 12 hours from *Anopheles* mosquitoes.[6]

2.4. Mosquito repellent mode of action

In many cases, it has been found that behavior that can be labeled as repellency may be the result of any number of physiological or biochemical events. Mosquito repellency caused by DEET is thought to be due to the blocking of lactic acid receptors, abolishing upwind flight, resulting in the insect “losing” the host. Further evidence for the role of lactic acid in host seeking comes from studies examining mosquito physiology following a blood meal. Host-seeking behavior in *Aedes aegypti* stops after taking a blood meal. It has been found that following a blood meal, the sensitivity of lactic acid sensitive neurons drops, and this drop is co-incident with the cessation of host-seeking behavior. Lactic acid sensitivity returns to normal after oviposition.[6]

Turmeric is easily available in the Indian markets. It has been used traditionally in “ayurvedic medicine” as an antiseptic, wound healing, and anti inflammatory compound. One of the constituents of turmeric, curcumin has stated mosquito repellent activity. Also curcumin can be easily extracted from *Curcuma longa*. Embelin obtained from *Embelia ribes* is reported to have potential insect repellant activity. Synthetic mosquito repellent used for control of vectors are causing irreversible damage to ecosystem and also chemicals are non-degradable in nature. Synthetic repellents are expensive for everyday use and there are concerns about their toxicity and safety. Long term exposure of new born babies and children to pyrethroid based mosquito repellents is associated with clinical, biochemical and neurological effects. *N*-diethyl-3-methylbenzamide (DEET) dissolves synthetic fabrics and plastic on eyeglasses and watches. The other undesirable effects of DEET are undesirable odour, subchronic toxicity, mutagenicity, reproductive and neurological toxicity. To overcome problem, there is need for development of effective non DEET alternatives and prepare repellent by using biodegradable mosquito repellent. Compared to synthetic repellents, plant based repellents are simple, effective, inexpensive, environment friendly and readily available. They are widely accepted by the public even though very few of them have been evaluated for toxicity. The aim of this study was to develop a isolated phytoconstituent based mosquito repellent that is effective and safe to use.[5]

3. MATERIALS AND METHODS

3.1. Extraction & Isolation of Curcumin

Turmeric (*Curcuma longa*) powder was purchased from Patanjali Ayurved Limited, Mumbai, India. Curcumin, the active ingredient of turmeric was extracted from turmeric powder by using Soxhlet extractor. 50 g of dried powder was placed in a porous bag or "thimble" made of whatmann filter paper, which was placed in chamber of the Soxhlet apparatus. 225 ml ethanol was heated in a round bottom flask, which was attached to the Soxhlet extractor, and its vapors were condensed in condenser. The condensate then dripped into the thimble containing the turmeric powder, and extracted it by contact. When the level of solvent in chamber reached to the top of siphon tube, the liquid contents of chamber were flooded into flask and then the cycle began again. This process was carried out for a total of 21 hours. Yellow, solid crude curcumin was obtained. Embelin, was obtained from our college colleagues. Apparatus used: Soxhlet extractor, condensor, heating mantle, round bottom flask. Reagents used: Ethanol.

3.2. Preparation of mosquito repellent products

3.2.1. Mosquito repellent cream

An aqueous cream (oil in water type) was prepared by emulsifying the essential oils in water with an emulsifying wax. The formula for the mosquito repellent cream is as follows:[7]

INGREDIENTS	F1	F2	F3
Curcumin/Embelin	0.5%	1%	1.5%
Essential oils	5%	5%	5%
Cetyl alcohol	2%	2%	2%
Lanolin	1%	1%	1%
Mineral oil	2%	2%	2%
Stearic acid	15%	15%	15%
Glycerin	10%	10%	10%
Potassium Hydroxide	1%	1%	1%
Preservatives	Q.S.	Q.S.	Q.S.
Distilled water	Q.S. 100%	Q.S. 100%	Q.S. 100%

The oil in water type cream was prepared by incorporating lanolin, stearic acid, cetyl alcohol, mineral oil, propyl paraben, etc. in oil phase and Glycerin, potassium hydroxide, methyl paraben etc. in water phase respectively. Both oil and aqueous phases were heated to 75^0C. After heating, the oil phase was added into the aqueous phase with continuous stirring until homogenous cream was formed. After complete emulsification, essentials oils were added when the temperature dropped to 55°C± 60°C. Active ingredients-Curcumin & Embelin were then added by levigation method into separate formulations followed by addition of perfume.[1]

3.2.2. Mosquito repellent candle

The candle was made up of a mixture of hard paraffin and stearic acid as the hydrocarbon bases. The formula for the insect repellent candle is as follows:[8]

INGREDIENTS	F1	F2	F3
Curcumin/Embelin	0.5%	1%	1.5%
Lemon oil	10%	10%	10%
Eucalyptus oil	10%	10%	10%
Stearic acid	19%	19%	19%
Hard paraffin	Q.S. 100%	Q.S. 100%	Q.S. 100%

The candle was prepared by heating (70^0C) hard paraffin and stearic acid until they were melted. The essential oils were added when the temperature dropped to 55^0C± 60^0 C. Active ingredients- Curcumin & Embelin were then added into separate formulations. The wick was plunged repeatedly in liquid wax, in order to obtain the required diameter. It was introduced in a cup shaped mould, which was then filled with liquid wax. After cooling, solidified candle was removed from it.

3.3. TLC of isolated drug and formulation. (Cream only)

Identification of curcumin and embelin in formulated cream was determined using TLC. Cream sample was dissolved in methanol so that cream was broken and filtered. Then, resulting solution was applied on TLC silica gel plate using capillary against that of standard and isolated samples of curcumin and embelin. It showed the presence of curcumin and embelin in the cream formulation at Rf 0.74 and 0.60 respectively at visual detection and 254 nm.[1]

Mobile phase for Curcumin

n-PROPANOL	WATER	GLACIAL ACETIC ACID
8 ml	1 ml	1 ml

Mobile phase for Embelin

n-PROPANOL	n-BUTANOL	AMMONIA
7 ml	1 ml	2 ml

3.4. Evaluation of Cream

The cream was evaluated for appearance, spreadability, irritancy test, viscosity, pH, thermal stability tests.

3.4.1. Appearance

The appearance of the cream was judged by its color, texture, roughness and its odour.

3.4.2. Spreadability

The Spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the Spreadability. Two sets of glass slides of standard dimensions were taken. The herbal cream formulation was placed over one of the slides. The other slide was placed on the top of the formulation, such that the cream was sandwiched between the two slides weight was placed upon the upper slides so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. The upper slide allowed slipping off freely by the force of weight tied to it. The time taken for the upper slide was noted.

3.4.3. Irritancy test

Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.

3.4.4. pH

The pH meter was calibrated using standard buffer solution. About 5 ±0.01 g of the cream was weighed in a 100 ml beaker and dissolved in 45.0 ml of distilled water and dispersed the cream in it. pH of cream was measured at room temperature using the pH meter.

3.4.5. Thermal Stability

The formulated cream was kept in petriplates inside the incubator at 45° ± 1° for 48 hrs. The sample passed the test, if on removal from the incubator shows no oil separation or any other phase separation.[1]

4. RESULTS AND DISCUSSION

The present study was conducted to determine the mosquito repellent activities of curcumin and embelin in order to obtain safe and efficient herbal mosquito repellent formulations. This was evaluated on the grounds of feedback obtained from volunteers. In the investigation, the creams formulated with curcumin and showed a significant and embelin showed moderate repellent activity.

Formulated cream was evaluated for the parameters like visual appearance, spreadability, irritancy test, pH, thermal stability. Appearance of cream was good having smooth texture and easily spreadable property. Irritancy test was performed and there was no sign of redness and itching which indicated that the cream is safe for topical application. pH of cream was found to be 7 which is nearer to the required pH of skin. Cream was thermally stable at 45°C ± 1°C and no phase separation was seen after 48 hours.

To confirm isolated phytoconstituent TLC was performed using n-propanol: water: glacial acetic acid (8: 1: 1) and n-propanol: n-butanol: ammonia (7: 1: 2) as mobile phase Along with that TLC showed the presence of Curcumin and Embelin respectively at Rf 0.74 and 0.60 respectively at visual detection and 254 nm in the formulated cream.

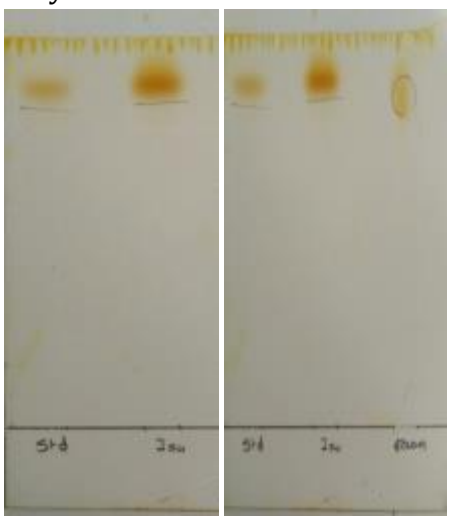


Image 1: TLC of Curcumin

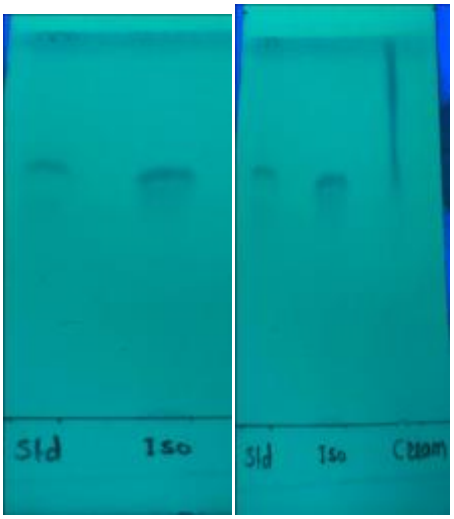


Image 1: TLC of Embelin

Mobile phase for Curcumin -			**Mobile phase for Embelin-**		
n-propanol :	**Water :**	**Glacial acetic acid**	**n-propanol :**	**n-butanol :**	**Ammonia**
(8 ml :	**1 ml :**	**1 ml)**	**(7ml :**	**1 ml :**	**2 ml)**

All the results are tabulated below:

Table 1: Results for Evaluation of mosquito repellant cream.[1]

SR. No.	PARAMETERS	CURCUMIN CREAM	EMBELIN CREAM
1	DESCRIPTION	Pale yellow colored, smooth cream having characteristic odour.	Pink colored, smooth cream having characteristic odour.
2	SPREADABILITY	6.1 g cm/sec	6.2 g cm/sec
3	IRRITANCY TEST	Non irritant	
4	pH	7	
5	THERMAL STABILITY	Stable at 45˚C ± 1˚C for 48 hours.	

Feedback from 60 volunteers was taken for evaluating the properties of formulations of three concentrations (0.5%, 1% & 1.5%) tabulated in the following tables.

Table 2: Feedback of volunteers: For Curcumin cream

FORMULATION	MOSQUITO REPELLENCY	SPREAD ABILITY	AESTHETIC APPEAL	PLEASANT ODOUR	WASHABLE
0.5% Curcumin	1.8	2	2.4	1.8	2.2
1% Curcumin	3	2.2	3	3	2.8
1.5% Curcumin	2.2	1.8	2.2	1.8	2.2

Table 3: Feedback of volunteers: For Embelin cream

FORMULATION	MOSQUITO REPELLENCY	SPREAD ABILITY	AESTHETIC APPEAL	PLEASANT ODOUR	WASHABLE
0.5% Embelin	1.8	1.8	2	1.8	2.4
1% Embelin	1.8	2	2.4	1.8	2.2
1.5% Embelin	1.6	2	2.2	2.2	2

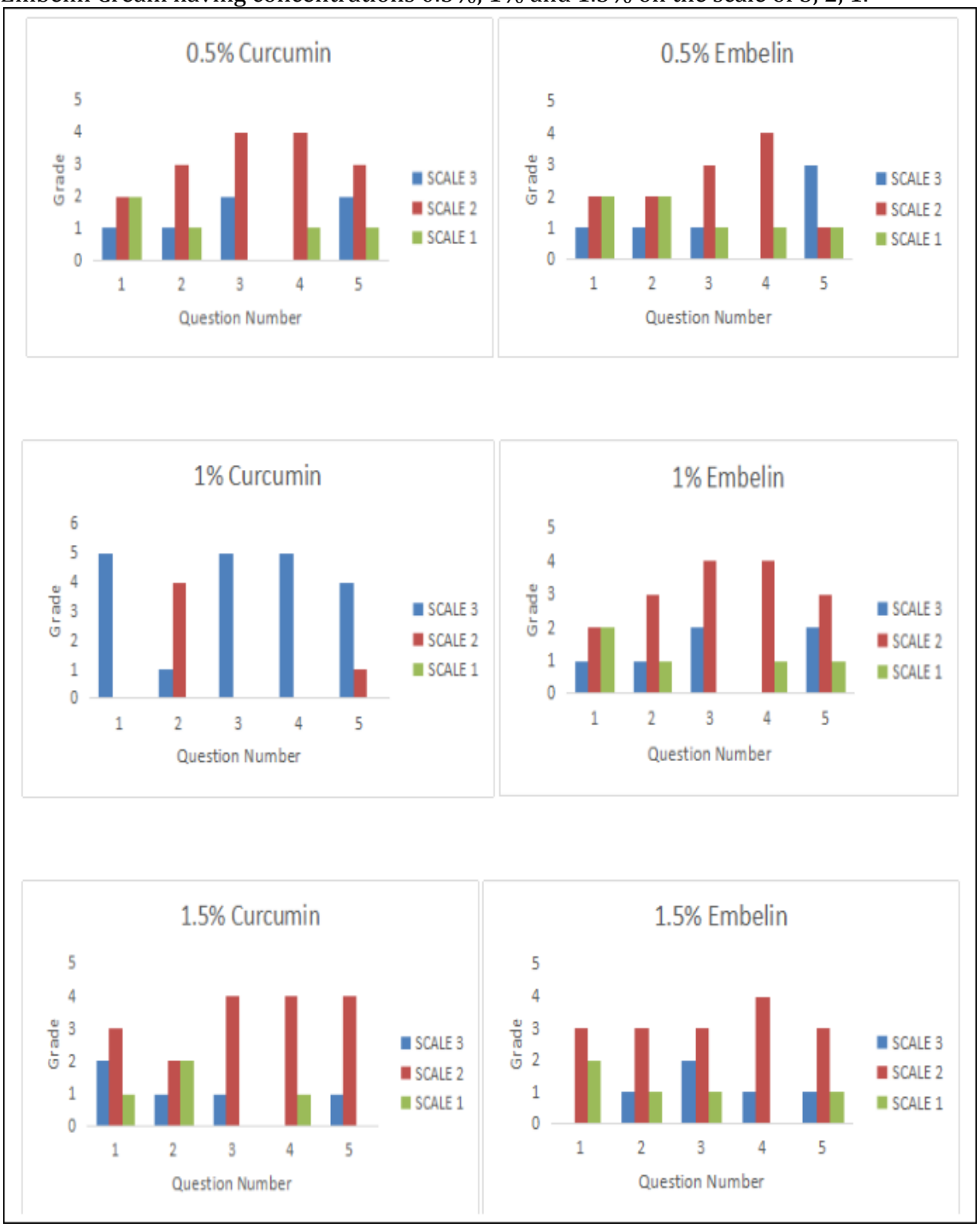
Table 4: Feedback of volunteers: For Curcumin candle

FORMULATION	MOSQUITO REPELLENCY	AESTHETIC APPEAL	PLEASANT ODOUR	CHARRING	SMOKE CREATION
0.5% Curcumin	1.8	2	2.4	1.8	2.2
1% Curcumin	3	2.2	3	3	2.8
1.5% Curcumin	2.2	1.8	2.2	1.8	2.2

Table 5: Feedback of volunteers: For Embelin candle

FORMULATION	MOSQUITO REPELLENCY	AESTHETIC APPEAL	PLEASANT ODOUR	CHARRING	SMOKE CREATION
0.5% Embelin	2.4	2.8	2.4	2.4	1.4
1% Embelin	1.8	1.8	2.2	1.6	2
1.5% Embelin	2	1.8	1.6	1.6	1.6

Graph 1: The graphs below illustrate the grades for various properties of Curcumin and Embelin Cream having concentrations 0.5%, 1% and 1.5% on the scale of 3, 2, 1.



Graph 2: The graphs below illustrate the grades for various properties of Curcumin and Embelin Candles having concentrations 0.5%, 1% and 1.5% on the scale of 3, 2, 1.

5. CONCLUSION

Thus in the present study both the formulations i.e. 1% Curcumin and 1.5% Embelin containing creams offer a remarkable mosquito repellency. Based on these results, both phytoconstituents find potential applications in the pharmaceutical use. Further studies are needed to explore the other allied activities of Curcumin and Embelin.

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FORMULATION OF HERBAL HAND SANITIZER

*Vidhi Rathod and Sneha Mundada**

ABSTRACT

Herbal hand sanitizer was prepared using extracts of Curcumin and Embelia. The antibiotic sensitivity test of the extract was checked by using Agar Diffusion Method and that of the sanitizer was checked by Thumb Impression Method. Results depicted that the extract gives large zone of inhibition against Bacillus subtilis, Escherischia coli and Candida albicans. The efficacy of the extract was evaluated using microorganism suspensions; which revealed that it is efficient in reducing higher number of microorganism from the hands. Thus, owing to higher antimicrobial activity and efficacy, these herbal extracts were used in the preparation of herbal hand sanitizers.

Keywords: Curcumin, Embelin, Herbal hand sanitizer

INTRODUCTION

Hygiene is defined as maintenance of cleanliness practice which carries utmost importance in maintenance of well-being. Keeping bodily hygiene and usage of cleanser are requisite of healthy living. These concepts highlight the need of maintaining hygiene in prevention of diseases. Thus, occurrence of nosocomial infections is alarmingly increasing and has emerged as a serious concern in hospital care outcome; resulting in prolonged hospitalization, ample disease and mortality, and excessive costs.

Escherichia coli, many Pseudomonas species and Staphylococcus aureus are commonly involved opportunistic microorganisms that primarily cause nosocomial infections. These pathogens also tend to become incorporated in to normal flora of health care workers.

Hand Sanitizer

In the scenario mechanized life style; a consumer will always prefer readymade formulation of alcohol hand rub rather than hand washing (application of a non-antimicrobial or antimicrobial soap; and mechanical friction is generated by rubbing the hands together for 1 minute, followed by rinsing with water and then drying thoroughly with a disposable towel). Hand sanitizer or hand antiseptic is a supplement or alternative to hand washing with soap and water. Various preparations are available including gel, foam and liquid solution. The active ingredient in hand sanitizer is mostly alcohol, inactive ingredients includes a thickening agent such a polyacrylic acid for alcohol gel, humectants such as glycerine for liquid rubs, polypropylene glycol and essential oils of plants. The level of alcohol varies between 60-85% (most commonly used level is 62%). Alcohol rub sanitizers kill most bacteria, fungi and stop some viruses too. Alcohol rub sanitizers containing at least 70% alcohol (mainly ethyl alcohol) kill 99.9% of the bacteria on hand 30 seconds after application and 99.999% in 1 minute.

Traditional healers have long used plant to prevent or cure infectious conditions. Considering this ultimatum; an attempt has been made to screen classical literature for the herbs with antimicrobial properties and it has been found that Curcuma longa (Turmeric) and Embelia ribes(Vidang) holds that antimicrobial potency. Thus, it was aimed to formulate and evaluate herbal hand sanitizer comprising of alcoholic extracts of these astonishing herbs using other suitable excipients; which can be used as ready to use herbal hand sanitizer.

Curcumin

Turmeric is the dried rhizome of Curcuma longa of the family Zingiberaceae. The main chemical constituent of turmeric is the colouring material known as curcumin. Since then, this polyphenol has been shown to possess anti-inflammatory, hypoglycemic, antioxidant, wound-healing, and antimicrobial activities.

Embelin

Vidang consists of dried ripe fruits of Embelia ribesof the family Myrisinaceae. It consists of a hydroquinone derivative embelin. It occurs in golden yellow colour, insolube in water but soluble in alcohol, benzene and petroleum ether. Embelin also reported to have anti-inflammatory, antibacterial, antitumor, antioxidant and free radical scavenging activities.

MATERIAL AND METHODS

Plant material

A. Curcumin:

Biological source: *Curcuma longa*
Family: Zingiberaceae
Collection: purchased from Patanjali Store, C.B.D Belapur

B. Embeline:

Biologial source: *Embelia ribes*
Family: Myrsinaceae
Collection: purchased from Sheetal Ayurvedic Store, Chembur (East)

Extraction of active constituents:
Embeline:

Material: Vidanga poweder-50gm
Petroleum ether (60-80)- 400ml
Soxhlet apparatus

Procedure:
1. Weigh about 50gm of vidang powder
2. Mount in soxhlet apparatus

Extraction:
1. Pour about 300ml of petroleum ether.
2. Start the soxhlet and allow the extraction to continue about 2 hrs.
3. After the completion of the process, the solvent was evaporated and ether was added for the precipitation of embeline.

Curcumin:

Material: Turmeric powder-50gm
Ethanol- 200ml

Soxhlet apparatus

Procedure:
1. Weigh about 50gm of turmeric powder
2. Mount in soxhlet apparatus.
Extraction:
1. Pour about 100ml of ethanol.
2. Start the soxhletamdallow the extraction to continue.
3. At the end, allow solvent to evaporate and concentrate the extract.

Microbial strains collection: The bacterial strains used for antibacterial activity were Bacillus subtilis and E.coli.The fungal strain used for antifungal activity was Candida albicans.

Control and standards: Streptomycin was used as standard for antibacterial activity while tioconazole was used as standard for antifungal activity.

FORMULATION

As per Table 1.

1. In a suitable formulation vessel, at room temperature and under steady mixing weigh in items 1 and 3 then add item 2 slowly to prevent large clumps.
2. Mix until the mixture is uniform.
3. Add item 4 slowly followed by item 6.
4. Finally add item 5 to thicken the formulation.
5. The resulting formulation is a viscous water-clear gel. If necessary adjust the pH of the formulation with item 5 to pH 7-7.5.
6. All ingredient amounts are given assuming the ingredients were supplied 100% pure.
7. Formulators are advised to measure the total active mass of their ingredients and adjust the composition with water.

Physiochemical evaluation test:

1. Appearance: Embeline and curcumin soap were observed for the color and consistency

2. Primary skin irritation test: For this three human volunteers were selected and prepared soaps were given to them and checked for irritation.

3. pH test: For this the formulation was placed on a moist pH paper and the observation was noted.

Antimicrobial assay:

A. Agar well diffusion method:

Antimicrobial activities of curcumin soap and embeline soap were evaluated using well diffusion method on nutrient agar and sabourd dextrose agar for bacteria and fungi, respectively. The inhibition zones were reported in millimeter (mm). Bacillus subtilis, Staphylococcus aureus and E. coli were used for the antibacterial assay and Candida albicans was used for antifungal assay. Nutrient agar plates and sabourd dextrose agar plates were inoculated with bacterial and fungal strains, respectively under aseptic conditions. The wells (diameter- 6mm) were formed with the help of borer and filled with 0.05ml of the test samples. The plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was reported in mm.

B. Thumb impression test:

Thumb impression of the hand exposed to the environment was placed on a sterile nutrient agar plate. Then, the thumb impression of the same hand was placed after washing with formulated embeline soap on the same plate without any overlaps of thumbprints. Same procedure was carried out for formulated curcumine soap with thumb impression of other hand. The pattern of microbial growth on the plates was observed after an incubation period of 24 hours at 37°C.

RESULT AND DISCUSSION:

It was accepted as all the properties of the formulation were satisfactory. Hence, formulation was evaluated for physiochemical properties and antimicrobial activity.

Physicochemical Evaluation: Table-2

Antimicrobial activity:

1. Agar well diffusion method:

The antibacterial activity of selected formulation against bacterial strains Bacillus subtilis and E.coli and fungal strain Candida albicans was investigated by agar well diffusion method and by using streptomycin and tioconazole as standards for antibacterial and antifungal activity, respectively. The result indicates both curcumine sanitizer and embeline sanitizer shows antibacterial as well as antifungal activity.

Table-3, Fig-a,b,c

2. Thumb impression method:

The antibacterial activity of selected formulation was studied by thumb impression method. The result indicates that both embeline and curcumin sanitizers show significant decrease in microbial count after cleaning hands with individual sanitizers

Fig-d,e

Figures and Tables:

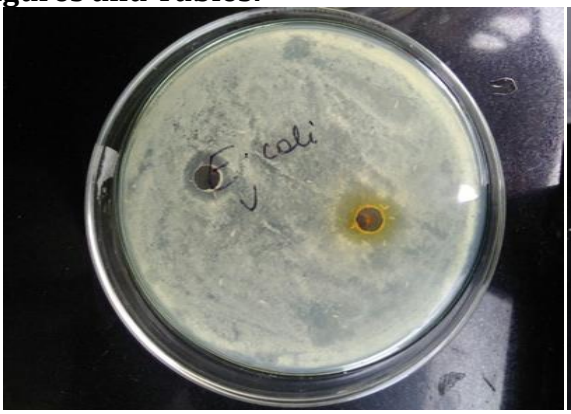

Fig.a

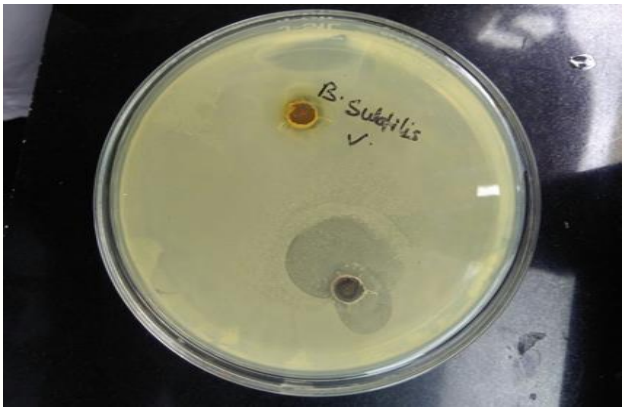

Fig.b

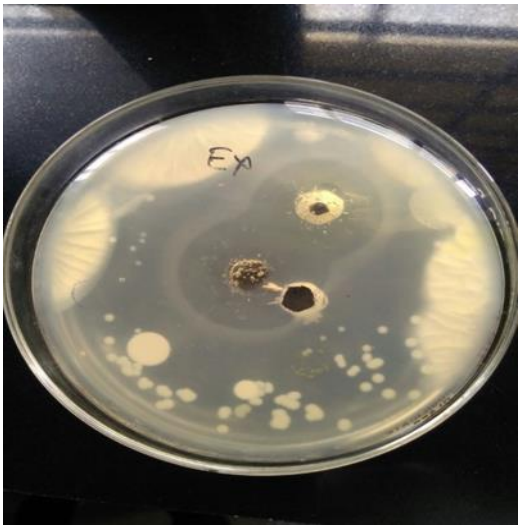

Fig.c

Fig. a: zone of inhibition for *E.coli*, Fig b: zone of inhibition for *Bacillus subtilis*, Fig. c: zone of inhibition for *Candida albicans*

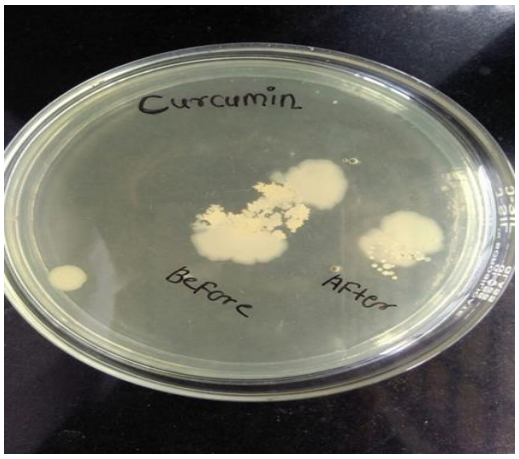

Fig. d **Fig. e**

Fig. d: Thumb impression before and after washing hands with curcumin
Fig. e: Thumb impression before and after washing hands with embeline

Table no.1:

Sr. No.	Ingredients	Quantity (% wt)	Activity
1.	Curcumin/Embeline	175µg/ml	Antibacterial, Antifungal
2.	Deionised water	26	Base liquid
3.	Carbopol 940	0.24	Thickner
4.	Glycerine	0.70	Humectant
5.	Ethanol	70	Biocide
6.	Triethanolamine	0.26	Neutralizer
7.	Perfume	qs	Fragrance

Table no.2:

SR. No.	PARAMETERS	CURCUMIN SANITIZER	EMBELIN SANITIZER
1	DESCRIPTION	Pale yellow colour	Pink colour,
2	CONSISTANCY	Semisolid and homogenous	
3	IRRITANCY TEST	Non irritant	
4	pH	6.5-7	

Table no.3:

Sanitizer	Average diameter of zone of inhibition (mm)		
	Bacillus subtilis	*Escherichia coli*	*Candida albicans*
Curcumin	20.3	12.6	13.3
Embelin	11.3	13.3	14.6

CONCLUSION

Hands are the most common mode of transmission of pathogens to patients and proper hand hygiene can prevent health care-associated infections and the spread of antimicrobial resistance. Scientific evidence and ease of use support of alcohol-based hand sanitizers during patient care. It may be concluded that Herbal Hand Sanitizer can be formulated and evaluated further following this line on working. Thus, there is immense potential in establishing the use of antimicrobial herbal hand sanitisation products using curcumin and embelin, as a measure to control the multidrug resistant microbes as well as check their spread through hands to self and from person to person

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IN VITRO EVALUATION OF ANTIHYPERGLYCEMIC POTENTIAL OF *Withania coagulans*

*Farhin Motlani, Yugandar More and Sandeep Patankar**

Abstract:-In Ayurvedic system and folk medicine, many plants have been reported to cure various health problems and diseases. *Withania coagulans* belonging to the family Solanaceae are considered to have antimicrobial, anti-inflammatory, antitumor, anti-hyperglycemic, cardiovascular etc also its active constituent. Withanolides is reported to show antidiabetic activity .Hence studies on antihyperglycemic activity of aqueous extract of *Withania coagulans* were undertaken in present investigation.

Key-words:- *Withania coagulans*, withanolide, anti-hyperglycemic ,glucose uptake.

INTRODUCTION:-

Diabetes mellitus (**DM**), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar level levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications.

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus

Type 1 Diabetes mellitus results from the pancreas's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown.

Type 2 Diabetes mellitus begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The most common cause is excessive body weight and not enough exercise.

In ancient system of medicine, many plants have been reported to cure various health problems and diseases.CharakaSamhita and SushrushaSamhita give extensive description on various medicinal plants. Medicinal plants play an important role in the development of new herbal drugs. India has a rich history of using various potent herbs and herbal components for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reported in numerous scientific journals[1].

W. coagulans is commonly known as 'Indian cheese maker' or 'vegetable rennet' because fruits and leaves of this plant are used as a coagulant.

The fruits of the plant are sweet and are reported to be sedative, emetic, alterative and diuretic. This plant has been reported to possess antimicrobial, anti-inflammatory, antitumor, anti-hyperglycemic, cardiovascular, immuno-suppressive[1,2].

Botanical description *W. coagulans* is a rigid, gray-whitish small shrub, about 60-120 cm tall. The leaves are about 2.5-7.5 cm long and 1.5 cm broad, usually lanceolate oblong, sometimes ovate, obtuse, narrow at the base and very short stalked. The flowers are about 7-12 mm across, yellowish, and are dioecious and polygamous in nature. The flowers are found in axillary cymose clusters.

The berries are about 7-12 mm in diameter, red, smooth and enclosed in leathery calyx. The seeds are dark brown, ear shaped, glabrous with sharp fruity smell.

Taxonomy:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnolipsida
Order: Solanales
Family: Solanaceae
Genus: Withania
Species: *W. coagulans*

AIM:-The study is aimed at evaluating the antihyperglycemic activity of *Withania coagulans.*

RATIONALE*:*-As diabetes is one of the most trending disease and the allopathy medicine used to cure this disease has various adverse side effects. So, to avoid this side effects medicinal plant have been introduced which play an important role in the development of new herbal drugs. The antihyperglycemic activity of extract of *Withaniacoagulans*was determined using organic solvent like chloroform; methanol & hexane and aqueous extract even *in vivo* testing were carried out using male albino rats. The present aim is to investigate antihyperglycemic potential drugs by *in vitro* testing which serve as a rapid screening model.

MATERIALS AND METHODS:-Dried fruits of *Withaniacoagulans* belonging to family Solanaceae were purchased from the local market of Mumbai, India and identified by organoleptic description which are given in Indian journal. The whole fruits were soaked in distilled water and kept at room temperature upto 48hr. The extract was filtered and concentrated in rotatory evaporator [2,3].
The extracts were scrutinized for their antidiabetic potential (glucose uptake by yeast cells) and the percent inhibition was calculated by using absorbance which was detected by UV spectroscopy.significance inhibition of glucose was observe in aqueous extract[4].

Evaluation of antihyperglycemic potential of *W.coagulans*

Glucose uptake by Yeast cell

Yeast cells were prepared according to the method of Cirillo, 1962. Briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water[5,6]. Various concentrations of extracts (1500, 1750, 2000 and 2250microlitre) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min [7].After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. The absorbance was determined by UV spectroscopy at 573nm
The percentage increase in glucose uptake by yeast cells was calculated using the following formula
Inhibition = [(Abs sample − Abs control) / Abs sample] × 100
Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

Table 1: Absorbance of the samples with different glucose conc at 575nm

Srno.	Concentration of extract	5mMoles	10mMoles	25mMoles
1	1500µL	0.113	0.114	0.125
2	1750µL	0.130	0.137	0.133
3	2000 µL	0.145	0.151	0.150
4	2250 µL	0.152	0.154	0.156

Table 2: Percent Inhibition of glucose due to plant extract

Srno.	Conc of extract (µl)	5mMoles	10mMoles	25mMoles
1	1500	15.9%	13%	10.4%
2	1750	26.9%	27%	15.7%
3	2000	34.4%	34.4%	25.35%
4	2250	37.5%	35%	28.20%

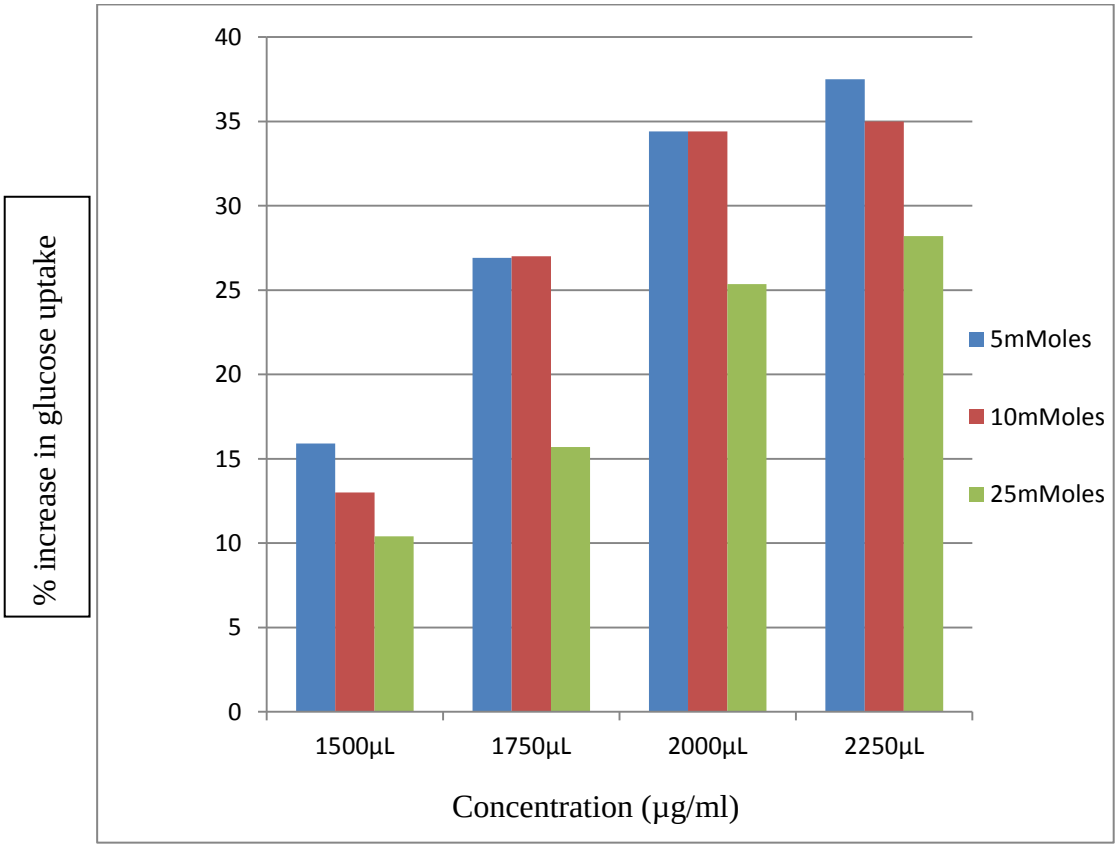

Fig.4: Histogram showing concentration of plant extract vs % increase in glucose uptake

The rate of glucose transport across cell membrane in yeast cells system was explored and the results are given in fig.1. The amount of glucose lingering in the medium after a specific time serves as a marker of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in all the 3 glucose concentrations. The distinctiveness of the sugar-transport system in yeast has been getting rehabilitated concentration in several laboratories Recent studies on the transport of non metabolizable

sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereo specific membrane carriers It is reported that in yeast cells *(W.coagulan*) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient[6,7]. This means that effective transport is only attained if there is removal of intracellular glucose.

RESULTS AND DISCUSSION:-The results of glucose uptake by yeast cell revealed that the activity of aqueous extract was significant to confirm the theory of glucose transport and diffusion. It differs with the sample and glucose concentration. The study found that the glucose uptake rate increases with increasing concentration of plant extract decreased with increasing extracellular glucose.
Glucose transports occur due to facilitated diffusion down the concentration gradient. This means that effective transport is attained due to plant extract.It was found that glucose transport occur only if the intracellular glucose is effectively reduced.The data obtained suggest that the plant extract is capable of effectively enhancing glucose uptake and hence it is capable of enhancing effective glucose utilization thereby controlling blood glucose level.

CONCLUSION:-The results of the work consequently signify the potential of the extract to manage hyperglycemia. However, these effects need to be confirmed using *in vivo* models and clinical trials for its effective utilization as therapeutic agents. *Withaniacoagulan*posses various biological activity. It is an important medicinal herb as it contain many phytochemicals which can cure various diseases. Withanolides are steroidal lactones having significant pharmacological activities. In various studies it has been seen that the *withaniacoagulans*posses several medicinal properties such as Anti inflammatory, Anti hyperglycaemic, Anti microbialetc

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ANTI-BACTERIAL ACTIVITY OF NATURAL PLANTS AND FORMULATION AND EVALUATION OF HERBAL OINTMENT

*Mounika Bharath, Shruti Bhagat and Sandeep Nikam**

ABSTRACT

Even in areas where modern medicine is available, the interest on herbal medicines and their utilization have been increasing rapidly in recent years. . Plant derived substances and herbal medicines have recently attracted the great interest towards their versatile application, as medicinal plants are the richest source of bioactive compounds used in traditional and modern medicine. The present work is to formulate and evaluate the ointment of Neem (Azadirachta indica) and Turmeric (Curcuma longa) extract. The ethanolic extracts were prepared by using maceration method. The preliminary in vitro antibacterial activity of the methanolic extracts of sun-dried leaves of Azadirachta indica and Curcuma longa leaves was determined against Staphylococcus aureus and Bacillus subtilis, using the Agar well diffusion method. The ointment base was prepared and formulation of ointment was done by incorporating the extract in the base by levigation method. After completion of formulation it was evaluated for its physicochemical parameters like colour, odour, pH, spreadability, extrudability, consistency, diffusion study, solubility, washability.

Keywords: Maceration, Levigation, Extrudability, Spreadability

INTRODUCTION

Certain European and oriental countries have been exploring the use of herbs and has been in practice since the centuries. Great work has been done which eluded the common man's reach and knowledge. With the techno-savvy lifestyle in 21st century human sufferings are coming out with different names. The basic herbs have the answer with no side effects and effective remedies and the golden fact is use of herbal treatment is independent of any age group. When two or more herbs are used in the formulation they are known as polyherbal formulations.[1] Numerous studies have been conducted with the extracts of Neem leaves (*Azadirachta indica* Family-Meliaceae) and extract of turmeric rhizomes (*Curcuma longa* Family-Zingiberaceae) with the combination of many other herbal drugs.[2,3] Along with other dosage forms herbal drugs are also available in the form of ointment which is semisolid preparation used topically for several purposes e.g. as protectants, antiseptics, emollients, antipruritics, keratolytics and astringents. Neem is consists of leaves and other aerial parts of Azadirachta indica Family- Meliaceae. Neem leaves and neem oil has many properties like antiseptics, insecticides also attributed antifertility and antiviral properties. [4]

Turmeric consists of dried as well as fresh rhizomes of plant known as Curcuma longa. Family- Zingiberaceae. It is used as antiseptic, expectorant, condiment or spice. Neem when mixed with Turmeric makes an excellent cure for acne, psoriasis, Eczema, Scabies, Scars and other skin infections and disorders. [4].The delivery of drugs through the skin has long been a promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and non- invasive nature of the treatment. Along with other dosage forms, herbal drugs are also formulated in the form of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. These include the skin and the mucus membranes of the eye, vagina, anus, and nose. An ointment may or may not be medicated. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base. Ointments are used topically

for several purposes, e.g. as protectants, antiseptics, emollients, antipruritic, keratolytics and astringents. Ointment bases are mainly anhydrous and generally contain one or more medicaments in suspension or solution. This present study was carried out to evaluate the antibacterial properties of the methanolic extract of Azadirachta indica and Curcuma longa leaves in formulated ointments.

2. MATERIALS AND METHODS

Plant material:-The leaves of Azadirachta indica was obtained from local areas of taloja (raigad district) and the rhizomes of Curcuma Longa were obtained from Pali district sudhagad. The collected leaves were dusted and washed for removing unwanted foreign materials, sun-dried for a week then grinded to obtained coarse powder.

2.1. Test microorganisms:-The microorganisms used for the study were Bacillus subtilis and E.coli. In this study, the bacterial cultures were obtained from agar slants which was already stored in microbiology lab and incubated for 24hrs.
Agar is jellylike substance derived from purifying the cell walls of red algae. It is added to microbiological media for solidification purposes. It has no nutritional value, so when it is used in microbiology to culture microorganisms, various nutrients are added to increase bacteria growth in Petri dishes or test tubes. When a test tube is used for storing the bacteria, it is referred to as an agar slant since the liquid culture solidifies while the tube is in a tilted position. A screw-cap top on the slants prevents the agar from drying out.

Medium preparation
The medium is prepared differently for slants than Petri dishes. Sterilization is done with the agar in the tubes; Petri dishes are pre-sterilized before sterilized agar is poured into them. Measure the amount of water needed and put it in a pot. Heat it on a stove until it is almost boiling. Add dry ingredients and stir the mixture slowly until they dissolve. Before adding agar, mix it with a small amount of cold water to prevent lumping. Use caution when adding agar to the hot liquid since it can foam and overflow the pot. Add small amounts of agar at a time and stir to evenly distribute the agar. Turn off the heat after bringing the agar to boil.

Sterilizing tubes
Place test tubes without the caps on a test tube rack. Fill the test tubes by transferring about 5 milliliters -- about .17 ounce or 1 teaspoon -- of the molten agar from the pot using a pipette, a small funnel or a syringe. Place all the caps loosely on the test tubes -- the agar won't be sterilized if they are sealed tight -- and sterilize all the tubes for about 25 minutes at 250 degrees Fahrenheit.

Slanting
When the agar is still hot, tilt the rack holding the test tubes on a solid surface or a thick book, making sure the medium inside the tubes is at a slanted position. Allow the medium to cool and solidify at this angle, which increases the surface area of the agar.

Storage
Tighten the caps of the test tubes after the agar has cooled. The slants are ready for use once the agar has solidified. They can be stored at room temperature or in the refrigerator for future use.

Inoculation

Inoculate the slant by transferring cells with an inoculating loop from a single-colony microorganism on a plate to the slant's surface. Move the loop across the surface of the slant and cap the tubes. Incubate the slant until there is evidence of growth, then put the tube in a refrigerator. [5]

PREPARATION OF BACTERIAL CELL CULTURE

Nutrient broth is prepared and filled in test tubes .A well isolated colony is picked up from the agar slants containing growth of bacteria with the help of sterile nichrome wire loop and inoculated in the respective nutrient broths and incubated for 24 hours for the growth of the bacteria.

2.2. Microbiological media, chemicals: - Nutrient Agar and Nutrient broth was obtained from the microbiology lab.

COMPOSITION AND PREPARATION OF NUTRIENT AGAR

Composition of Nutrient Agar

Sr no.	INGREDIENT	QUANTITY
1.	PEPTONE	0.5%
2.	BEEF EXTRACT	0.3%
3.	Nacl	0.5%
4.	AGAR	1.5%
5.	DISTILLED WATER	
6.	PH(at 25^0C)	7.4

Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 litre (1000ml) of distilled water.we have prepared for 250 ml so, 7 gms of nutrient agar was suspended in 250 ml of distilled water.
2. Heat this mixture while stirring to fully dissolve all components.
3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.
6. Replace the lid of each Petri dish and store the plates in a refrigerator till the use. [7]

Composition and preparation of nutrient broth

Composition:-

Peptone	5gm
Beef extract	3gm
Distilled water	1000ml
pH	7.0

Preparation:-Suspend 8 grams of powder in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2-8°C. The color is amber, slightly opalescent. The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium. [8]

2.3. Preparation of methanolic extract of Azadirachta indica and Curcuma longa leaves: - The sun-dried leaves of Azadirachta indica and Curcuma longa was powdered using a home mixer. 100g of milled leaves each of Azadirachta indica and Curcuma longa was extracted with methanol by maceration for 24 hr. The extract was filtered and concentrated using an electric water bath to obtain semisolid extract. The extract was stored in a refrigerator. A stock concentration of 500mg/ml was prepared from which working concentrations of 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml were prepared.

2.4. Evaluation of antibacterial activity of Extract The antibacterial activity of the methanolic extract of the leaves of Azadirachta indica and Curcuma longa at concentrations of 200mg/ml and 300mg/ml were determined using agar well diffusion method. A molten agar was seeded with 0.1 ml of a 24 h-48 h broth culture of the test organism (B. subtilis, E. coli, P. aeruginosa and S. aureus) containing approximately in a sterile petri dish and allowed to set. Wells of appropriate diameter were created with a sterile cork borer and filled to about three-quarters full with solutions of the methanolic extract of the leaves of Cassia Azadirachta indica and Curcuma longa. The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured. The in vitro bacterial response to the extract was evaluated using the diameter of the zones of inhibition as follows.

OBSERVATION TABLE:-

SR NO.	NAME OF PLANT EXTRACT	CONCENTRATION (mg/ml)	ZONE OF INHIBITION	
			E.COLI	**B.SUBTILIS**
1.	Turmeric extract	200	1.2cm	0.5cm
		300	2.6cm	1cm
2.	Neem extract	200	2cm	0.4cm
		300	2.4cm	4.8cm
3.	Combination(synergistic effect	200	2.4cm	2cm
		300	3.8cm	2.5cm

TABLE NO.1

2.5. Preparation of Ointments:-Topical ointment bases namely simple ointment BP were prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment was done by incorporating 10 g of the semisolid methanolic extract of Azadirachta indica and Curcuma longa into the various bases by triturating in a mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w of Azadirachta indica and Curcuma longa extract. The prepared herbal ointments were put in ointment jars, labelled and were stored at room temperature pending the evaluation. [9]

Formulation table:-

Sr no.	INGREDIENTS	QUANTITY
1.	Prepared neem and turmeric extract	0.06g
2.	Wool fat	0.5g
3.	Cetostearyl alcohol	0.5g
4.	Hard parafin	0.5g
5.	White soft parafin	8.5g

2.6. Physical evaluation of formulated Ointments:-Physical assessments were carried out on the ointments over a period of 5 days using the following parameters: Appearance, Odour, Texture and Colour. The pH of formulation was determined by using pH paper. 0.5g of the weighed formulation was dispersed in 50 ml of distilled water and the PH was measured

Homogeneity: All the developed ointments were tested for homogeneity by visual inspection. They were tested for their appearance with no lumps.

- **Colour and Odour**

Physical parameters like colour and odour were examined by visual examination.

- Consistency

Smooth and no greediness is observed.

- **PH**

P^H of prepared herbal ointment was measured by using digital P^H meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. P^H was determined in triplicate for the solution and average value was calculated.

- Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability. Spreadability was calculated by following formula

$$S=M\times L/T$$

Where,

S= Spreadability
M= Weight tide to the upper slide
L= Length of glass slide
T= Time taken to separate the slides

- Extrudability

The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

- Diffusion study

The diffusion study was carried out by preparing agar nutrient medium. A whole board at the center of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted.

- LOD

LOD was determined by placing the formulation in petri-dish on water bath and dried for the temperature 105^{o}C.

- Solubility

Soluble in boiling water, miscible with alcohol, ether, chloroform.

- Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

- Non irritancy Test

Herbal ointment prepared was applied to the skin of human being and observed for the effect.

- Stability study

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 29°C and 15°C. The herbal ointment was found to be physically stable at different temperature i.e. 29°C, 15°C within four weeks.

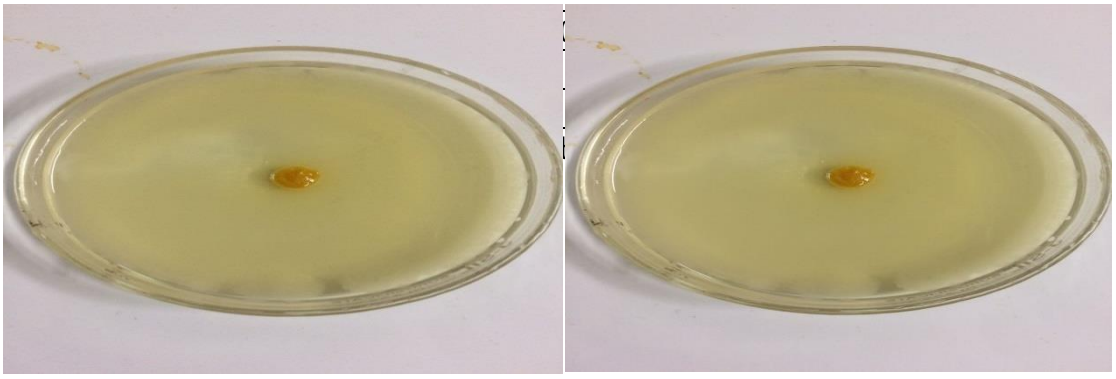
- Physicochemical evaluation of formulated ointment

Table 3: Physicochemical evaluation of formulated ointment

Physicochemical parameters	**Observation**
Colour	Yellow
Odour	Characteristic
Consistency	Smooth
PH	5.4
Spread ability(seconds)	7
Extrudability	0.2 gm.
Diffusion study	0. 3 cm
Loss on drying	30%
Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform
Washability	Good
Non irritancy	Non irritant
Stability study (29°C, 15°C)	Stable

3. RESULTS

The preliminary in vitro antimicrobial activity of the methanolic extract of Azadirachta indica and Curcuma longa leaves presented in Table 1 showed excellent activity against E.coli and Bacillus subtilis. The in vitro antimicrobial activity of the methanolic extract of Azadirachta indica and Curcuma longa leaves -based herbal ointments presented in Table 2. They have not shown excellent antibacterial activity.



Growth of inhibition for E coli

Growth of inhibition for b.subtilis

4. DISCUSSION

In the preliminary antimicrobial sensitivity screening, the methanolic extract of Azadirachta indica and Curcuma longa showed excellent activity against E.coli and Bacillus subtilis (Table 1). Most of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membranes infections of man. The activity of the extract was concentration-dependent as revealed by the zone of inhibition. The Azadirachta indica and Curcuma longa -based herbal ointments demonstrated excellent antibacterial activity. The results also revealed that the extracts incorporated into the ointment bases showed better activity than that of the crude extract of Azadirachta indica and Curcuma longa. This implied that there might have been better diffusion of drug for the herbal ointments than for the crude extract. The activity against E .coli and B. SUBTILIS is of significant interest because it is commonly found on the hands, face and in deep layers of the skin and is perhaps the most widely encountered and very undesirable. It is implicated as the commonest etiologic agent of boils, carbuncles, breast abscess and infantile-impetigo. Azadirachta indica and Curcuma longa herbal ointment did not show any activity against Pseudomonas. The prepared formulations show a smooth and homogeneous appearance. The pH values of all the prepared formulations ranged from 6.1 to 7.3, which are considered acceptable to avoid the risk of irritation upon application to the skin. The pH values of the formulations are within the normal pH range of the human skin (6.8 ± 1). From the study, the ointments showed no changes in pH, consistency and phase separation after keeping for 10 days.

5. CONCLUSION

From the ancient time Neem and Turmeric is used for their various medicinal properties like antibacterial, antifungal, anti-inflammatory etc. Thus this ointment could become a media to use these medicinal properties effectively and easily as a simple dosage form.This study shows that Azadirachta indica and Curcuma longa has antibacterial activity and has high potential as antibacterial agent when formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. The potency of the Azadirachta indica and Curcuma longa herbal ointment against E.COLI AND B. SUBTILIS could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile impetigo and wounds. This investigation suggests the use of both hydrophilic and hydrophobic ointments containing Azadirachta indica and Curcuma longa as antibacterial ointment preparation. A, a water-miscible topical base, was possibly a better vehicle for the release of the antibacterial compounds present in Azadirachta indica and Curcuma longa extract.

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ESTIMATION OF PROTIEN CONTENTS FROM DIFFERENT SEEDS

*Beena Gaikwad, Ajay Chauhan and Varsha Jadhav**

ABSTRACT

This article describes the use of the Biuret method of protein assay, to verify the protein concentrations of unknown solutions. To calculate the protein concentrations of the unknowns' samples were passed through a spectrophotometer which had a known protein concentration and there absorbance levels were found. Then a calibration graph was plot that determines the concentration levels of the unknown samples

Keywords:

1. Biuret method.
2.Protein content of seeds.

INTRODUCTION

The quantitation of protein content is important and has many applications in food industry practices and in research especially in the field of biochemistry. The exact monitoring of protein content in samples is a critical step in protein analysis .The different protein assay techniques have been developed for the assessment of the protein concentration in a sample .Modern instrumental methods such as mass spectrometry, absorption spectroscopy, chromatography etc. are expensive, difficult for manipulation and time challenging . Traditional spectrophotometric methods are cheap, fast, easy-working and the most common way to quantitate protein concentrations . Spectrophotometric protein quantitation assays are methods that use UV and visible spectroscopy to rapidly determine the concentration of protein, relative to a standard or using an assigned extinction coefficient. Methods are described to provide information on how to analyse protein concentration using UV protein spectroscopy measurements, traditional and common dye-based absorbance measurements: Biuret, Lowry and Bradford assays and the fluorescent dye-based assays: amine derivatization and detergent partition assays. The Biuret method is based on the reaction Cu2+ with functional groups in the protein's peptide bonds. The formation of a Cu2+ protein complex requires two peptide bonds and produces a violet coloured chelate product. It is based on a biuret reaction that includes the use of Folin-Ciocalteu reagent for enhanced colour development. Proteins are firstly treated with alkaline copper sulphate in the presence of tartrate. This step is then followed by addition of the Folin-Ciocalteu reagent. Reduction of the Folin-Ciocalteu reagent is measured as a blue colour at 750 nm.). Colour is caused by electronic transitions involving the valence electrons to another.Many of the dye-based assays have unique chemical mechanisms that are prone to interference from chemicals prevalent in many biological buffer preparations. It is also good to know which particular range of protein concentration an assay is sensitive to . In the ideal test, the most preferred calibration curve generates a linear response to the standard solutions thatcovers the range of the concentration of the unknown. As the linearity range for the calibration curve is known, it will give the assay more accurate, time efficient and cost effective.

AIM & RATIONAL

The quantitation of protein content is important and has many applications in food industry practices and in research especially in the field of biochemistry. The exact monitoring of protein content in samples is a critical step in protein analysis .The different protein assay techniques have been developed for the assessment of the protein concentration in a sample.

As we know for poor citizens it's very it's difficult to have dry fruits like almond as they can't afford to add to their daily diet so instead almonds we would suggest to eat Groundnut in diet so that they get the required amount of protein in their daily diet and due to which there will be no health issues or disorder like malnutrition.

METHODOLOGY

Material:

1. Protein sample of unknown concentration
2. Standard BSA (4mg/mL)
3. Distilled water
4. Biuret reagent
5. Test tubes
6. Label
7. Test tube rack
8. Pipette
9. Pipette bulb
10. Spectrophotometer
11. Cuvettes
12. Tissue paper

Method:

1. Take 10 gm of different types of seeds & Soak it in water for 24 hours.
2. Crush the seeds in glass mortal & pestle.
3. With the help of 100ml water extract proteins.
4. Take 1ml of extract of each seeds in test tube & label it.
5. Make 2 sets of test tube for standard & unknown protein sample :
6. 1st set : Take 6 test tubes with the numbers 1, 2, 3, 4, 5, and 6 for preparation of a standard curve.
7. 2nd set : Take another 6 test tube for unknown concentration of seeds extract.
8. Add 1ml Working Biuret Reagent to each tube.
9. Incubate the tubes for 20 minute at 37^0C temperature.
10. In the meantime, turn on the spectrophotometer to allow it to warm up. Adjust the wavelength to 540nm.
11. Add 1ml distilled water to a cuvette to zero the absorbance of the spectrophotometer. Measure the absorbance of each tube and record the value in the results section & determine the concentration of Unknown Protein.

Result

NAME OF SEED	CONTENT (mg/ml)	CONTENT (gm/10gm)
Pisum sativum(chickpea)	5	0.5
Phaseolus vulgaris (Bean)	6	0.6
Cicero arietium (Rajma)	4.5	0.44
Morning Oleifera (Drumstick)	7	0.7
Glycine Max (Soya)	18	1.8
Arachis hypogaea (Groundnut)	16.14	1.64

Discussion

The subunits which make up proteins are amino acids. The amino acids are joined together by dehydration synthesis to forms chains, which are hundreds of amino acids long which is called proteins. Proteins function as enzymes or as structural units in cells. They do most of the work in a cell. Almost all of the exciting stuff such as metabolism, memory, hormone action, and movement involves proteins. In this lab, we have learnt method of measuring protein concentration, biuret assay.

The biuret reaction is a method that can be used to determine the amount of soluble protein in a solution. The biuret reagent (copper sulfate in a strong base) reacts with peptide bonds (which join amino acids to form proteins) and changes colour when it does so. The spectrophotometer has been used to measure the intensity of the colour produced. The more protein present the darker the colour.

In order to quantitatively determine how much protein is represented by a particular absorbance reading it is necessary to construct a standard curve. This is done by performing the biuret reaction on a series of prepared solutions of gelatin at 1,2,3,4,5 and 6 mg/ml in water. The absorbance readings obtained from these solutions are used to construct a graph of absorbance as a function of protein concentration. This graph is called the standard curve for assay, and can be used to convert the absorbance readings for the experimental samples into a protein amount or concentration.

Based on the graph that has been constructed, it shows that the standard protein concentration for the samples as shown in the result. From the graph we can see that, the highest concentration of protein is Arachis hypogaea (groundnuts) and the lowest is Pisum sativum (chickpea).

Conclution

There are two ways of measuring protein concentration in a solution. It is either by using biuret assay or Lowry assay. Both can be used to measure the protein concentration in a solution but the most accurate way to measure the concentration is by using biuret assay. This is due to the fact that biuret assay can measure the protein from as low as 1 µg/mL to 1500 µg/mL which can measure the protein from 5 to 160 mg/mL. in this experiment, we had tested the protein concentration in six different types of seeds, which are,Phaseolus vulgaris (Beans),Pisum sativum (chickpea), Cicero arietium (Rajma), Moringa Oleifera (Drumstick),Glycine Max(Soybeans), Arachis hypogaea Arachis hypogaea (Groundnut).From the graph plotted, Groundnut contain the most protein while Pisum sativum (Chickpea) contain the least.

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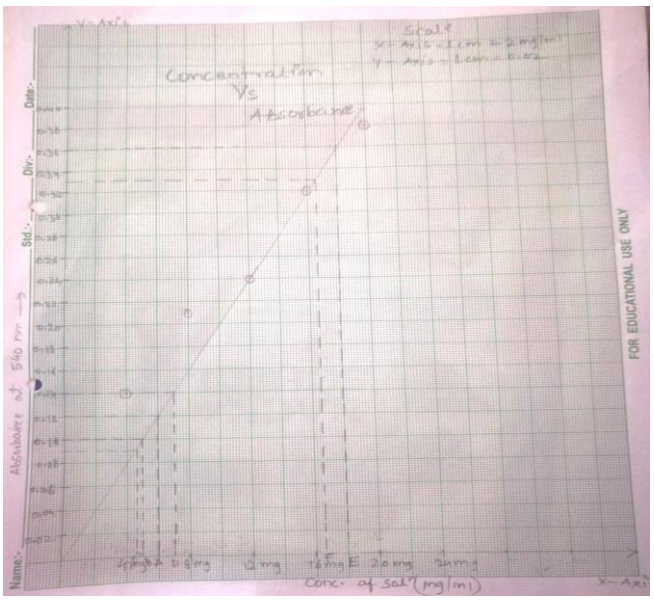
Observation Table

Sr.No.	Standard solution concentration(4mg/ml)	Biuret reagent (ml)		Absorbance (540 nm)
1.	1ml	3		0.08
2.	2ml	3	Keep all the test tubes at 37^0C in a water bath for 10 minutes. Cool & make up the volume 10ml by addition of distilled water.	0.21
3.	3ml	3		0.24
4.	4ml	3		0.32
5.	5ml	3		0.38
6.	(Unknown A) 1ml	3		0.12
7.	(Unknown B) 1ml	3		0.10
8.	(Unknown C)1ml	3		0.09
9.	(Unknown D)1ml	3		0.14
10.	(Unknown E)1ml	3		0.36
11.	(Unknown F)1ml	3		0.33
12.	Blank	3		0

NOTE:

Unknown A = Phaseolus vulgaris (Bean)
Unknown B = Pisum sativum (Chickpea)
Unknown C = Cicero arietium (Rajma)
Unknown D = Moringa Oleifera (Drumstick)
Unknown E = Glycine Max (Soybeans)
Unknown F = Arachis hypogaea (Groundnut)

Graph



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EVALUATION AND COMPARISON OF BINDING PROPERTIES OF GUMS AND MUCILAGES

*Shivani Modhave, Nita More and Varsha Jadhav**

ABSTRACT

Gums and mucilages are widely used natural materials for conventional and novel dosage forms. These natural materials have advantages over synthetic ones since they are chemically inert, nontoxic, less expensive, biodegradable and widely available. They can also be modified in different ways to obtain tailor-made materials for drug delivery systems and thus can compete with the available synthetic excipients. In this review, we describe the use of natural gums and mucilages as binding agents in pharmaceutical formulations.

The conclusion was made based on the observations obtained from the flow properties of granules prepared by using natural gums and mucilages such as aloevera, acacia ,tragacanth , okra and guar gum etc. Flow properties such as percent compressibility, angle of repose, and flow rate were determined and studied. Depending upon the flow properties the conclusion was made.

Keywords: Gums and Mucilages, Binding agents, Flow properties.

INTRODUCTION

Gums are considered to be pathological products formed following injury to the plant or owing to unfavorable conditions, such as drought, by a breakdown of cell walls (extra cellular formation; gummosis) while,mucilages are generally normal products of metabolism, formed within the cell (intracellular formation) and/or are produced without injury to the plant.[3] Gums readily dissolve in water, whereas, mucilage form slimy masses. Gums are pathological products, whereas mucilages are physiological products. Acacia, tragacanth, and guar gum are examples of gums while mucilages are often found in different parts of plants. For example, in the epidermal cells of leaves (senna), in seed coats (linseed, psyllium), roots (marshmallow), barks (slippery elm) and middle lamella (aloe) . Gums and mucilages have certain similarities—both are plant hydrocolloids. They are also translucent amorphous substances and polymers of a monosaccharide or mixed monosaccharides and many of them are combined with uronic acids. Gums and mucilages have similar constituents and on hydrolysis yield a mixture of sugars and uronic acids. Gums and mucilages contain hydrophilic molecules, which can combine with water to form viscous solutions or gels. The nature of the compounds involved influences the properties of different gums. Linear polysaccharides occupy more space and are more viscous than highly branched compounds of the same molecular weight. The branched compounds form gels more easily and are more stable because extensive interaction along the chains is not possible.[3]

Advantages of natural gums and mucilages in pharmaceutical sciences:

The following are a number of the advantages of natural plant–based materials. Biodegradable—Naturally available biodegradable polymers are produced by all living organisms. They represent truly renewable source and they have noadverse impact on humans or environmental health (*e.g.*, skin and eye irritation). Biocompatible and non-toxic— Chemically, nearly all of these plant materials are carbohydrates composed of repeating sugar (monosaccharides) units. Hence, they are non- toxic.
Low cost—it is always cheaper to use natural sources. The production cost is also much lower compared with that for ynthetic material. India and many developing countries are dependent on agriculture.
Environmental-friendly processing—Gums and mucilages from different sources are easily collected in different seasons in large quantities due to the simple production processes involved.
Local availability (especially in developing countries) —In developing countries, governments promote the production of plant like guar gum and tragacanth because of the wide applications in a variety of industries

Disadvantages of synthetic polymers in pharmaceutical sciences :
The synthetic polymers have certain disadvantages such as high cost, toxicity, environmental pollution during synthesis, non-renewable sources, side effects, and poor patient compliance. Acute and chronic adverse effects (skin and eye irritation) have been observed in workers handling the related substances methyl methacrylate and poly- (methyl methacrylate) (PMMA).
Reports of adverse reactions to povidone primarily concern the formation of subcutaneous granulomas at the injection site produced by povidone. There is also evidence that povidone may accumulate in organs following intramuscular injections. Acute oral toxicity studies in animals have indicated that carbomer-934P has a low oral toxicity at a dose of up to 8 g/kg. Carbomer dust is irritating to the eyes, mucous membranes and respiratory tract. So, gloves,eye protection and dust respirator are recommended during handling.

Applications of gums and mucilages:
Gums and mucilages of different sources and their derivatives represent a group of polymers widely used in pharmaceutical dosage forms. Various kinds of gums are used in the food industry and are regarded as safe for human consumption.
However, there is growing concern about the safety of pharmaceutical excipients derived from natural sources. Plant gums and exudates are now screened for their use as pharmaceutical adjuvants. Mucilages of different origins are also used in conventional dosage forms of various drugs for their binding, thickening, stabilizing and humidifying properties in medicine.

Newer uses of different gums and mucilages in cosmetics and textiles has increased the demand and screening of gums has become an important pharmaceutical area. However, different gums and mucilages used as pharmaceutical adjuvants have stringent specifications, which few natural agents can fulfill.

AIM AND RATIONALE

There are synthetic binders which are used in tablet formulation. Even the concentrations used are not so high. The aim behind using natural binders and studying their efficiency was if results found to be satisfactory they can be used as an alternative to synthetic binders.

Being obtained from natural source they will be economical in use and also these natural binders exert some therapeutic effect. The side effects if any due to synthetic binders will be avoided.

METHODOLOGY

Materials- Aloevera mucilage ,Acacia (powder), Tragacanth (powder), Guar gum and Okra mucilage, Lactose , Starch

Methods

1) Preparation of Alovera mucilage granules -

The granules were prepared using wet granulation method. Lactose and starch powder were weighed according to required quantity. Lactose and starch were mixed properly in mortar. Aloevera mucilage was added as required to bind powder properlyto form a mass which was passed through #8 to form coarse granules.

2) Preparation of Acacia and Tragacanth mucilage –

Wet granulation method was followed for preparation of granules. Acacia and tragacanth were suspended and solubilised in water respectively and that suspension and solution was used for the binding . Lactose and starch were mixed properly and then suspension or solution was added as required to form the mass to be formed into granules by sieving through #8.

3) Preparation of Guar gum mucilage -

Lactose, starch powder and guar gum were mixed properly and then water was added as required to form a mass which was passed through #8 to form granules.

4) Preparation of mucilage

The okra was cut into pieces and its mucilage was removed . The mucilage was added in water and heated to boiling, then the solution was cooled and the alcohol was added to the solution to precipitate the mucilage. The precipitate was collected and dried in oven at 60°C till it reaches the constant weight. This powder was added to lactose and starch mixture and then water was added required to form a mass which is sieved to form granules (#8).

The granules obtained were dried at 60°C and then were passed though sieve no. 22 and then the collected granules were passed through seive no. 36 (#36) so that fines were removed.

The collected granules were weighed and fines of its 10% were weighed and mix The flow properties of collected granules were further studied.

RESULTS AND DISCUSSION

Observation table Table No. 1

(Observations of flow properties of granules)

Sr No.	Binders	Quantity required	% Compressibility	Angle of repose
1)	Aloevera	0.5gm	5.94% (Excellent)	27.37 (Good)
2)	Acacia	0.5 gm	3.6% (Excellent)	23.74 (Good)
3)	Tragacanth	0.5gm	8.9% (excellent)	12.95 (excellent)
4)	Guar gum	0.25gm	14% (excellent)	16.75 (excellent)
5)	okra	1 gm	5.07% (excellent)	34.40 (passable)

Percent compressibility was found out by initially measuring the tapped and bulk volume and further calculating the tapped and bulk density. Angle of repose was calculated by using a formula:

Angle of repose = [1/tan] (h/r)

The % compressibility of granules made from aloe vera was found to be 5.94% (5-15; Excellent) and angle of repose was found to be 27.37(20-30; Good).
The% compressibility of granules made from acacia was found to be 3.6% (< 20; Excellent).
The % compressibility of granules made from okra was found to be 5.07% (5-15; Excellent) and angle of repose was found to be 34.40(30-40; Passable).

CONCLUSION

Depending upon the observations obtained on evaluation of flow properties we conclude that granules obtained from Tragacanth shows good flowproperties as compared to other binders used. The % compressibility and angle of repose of granules of Tragacanth and Guar gum were lying in excellent range but the values of guar gum were greater as compared to Tragacanth. Aloevera and Acacia showed similar flow properties. Angle of repose of Okra granules lied in the passable range . So amongst all, Okra shows least binding ability, while Tragacanth shows the highest. Increasing order of flow properties of granules Tragacanth> Guar gum> Acacia> Aloe vera> Okra

Different quantities of gums and mucilage were used to prepare granules. On the basis of quantities required , guar gum showed the maximum binding ability at lowest quantity(0.25gm). The maximum quantity was required for Tragacanth and Okra i.e 1gm. Equal quantities of Acacia and Aloevera were required.

Therefore , taking into consideration all aspects / parameters, Guar gum is observed to better binding agent amongst the five selected i.e Aloevera , Acacia, Tragacanth, Guar gum and Okra.

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STANDARDISATION OF MARKETED HERBAL PREPARATIONS OF ALOE VERA

*Kriti Jain, Sneha Kadam and Vaibhavi Garge**

ABSTRACT

The presence of various life improving constituents in plants made scientists to investigate on the plants for their uses. Medicinal plants constitute a source of raw material for both traditional and modern system of medicine.
As herbal medicinal products are complex mixtures that originate from biological sources, great efforts are necessary to guarantee a constant and adequate quality. Botanical parameters such as sensory or organoleptic evaluation, histological observations and assay methodology are discussed.
The given research article consists of various tests carried out on the products of Aloe vera. The aloe vera gel and juice have cosmeceutical and nutraceutical properties respectively. The objective of the work is to standardize the marketed herbal preparations.
The formulation of the gel and the juice were evaluated for their organoleptic and analytical parameters. The assay methodology was carried out by the process of distillation and using colorimetric techniques to find the absorbance at wavelength of 500 nm and 440 nm to obtain a ratio.
The ratios obtained were in accordance with results mentioned in the IP and therefore mini-research results were positive.

KEYWORDS

Indian pharmacopoeia – Herbal preparations – Aloe vera – Analytical evaluation

INTRODUCTION

ALOE VERA

Scientific classification	
Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Liliaceae
Genus:	*Aloe*
Species:	***A. vera***
Binomial name	
Aloe vera (L.) Burm.f.	
Synonyms	
• *Aloe barbadensis* Mill. • *Aloe barbadensis* var. *chinensis* Haw. • *Aloe chinensis* (Haw.) Bake	

Aloe vera is a plant species of the genus Aloe. The preparations are obtained from the dried juice of leaves of Aloe barbadensis, known in commerce as Curacao Aloes and hybrid of this species known as Cape Aloes. Indian aloe is obtained from Aloe barbadensis
Aloe vera can be used in various dosage forms.
Cosmeceutically it may be present as a preparation of shampoo, moisturising lotion, face pack, soap, skin nourishing cream, sunscreen, face wash.
Nutraceutically it may exist as a juice.
The research articles has made use of Aloe vera gel and juice for research purposes.

USES OF ALOE VERA GEL
MEDICINAL USE
Traditional uses include the external treatment of minor wounds and inflammatory skin disorders.
The gel may be used in treatment of minor skin irritations, including burns, bruise and abrasions.
In recent times, the oral consumption of *Aloe vera* has been promoted as prophylaxis and therapy for a variety of unrelated systemic conditions.
Aloe vera may be used in veterinary medicine as laxative or in topical applications.

COSMETIC USE

The gel may be used as emollient and moisturizer in cosmetics and personal care products.
The gel is used in the cosmetic industry as a hydrating ingredient in liquids, creams, lip balms, healing ointment and face packs.
Other product containing *Aloe vera* include after shave gel, mouthwash, hair tonic, shampoo, and skin moistening gel

USES OF ALOE VERA JUICE
Aloe vera juice has digestive and immunity-boosting benefits. It helps in treatment of intestinal issues and builds immunity.
It can also stimulate the uterus and improve menstrual conditions. In fact, aloe vera juice has been mentioned in Ayurvedic texts too. As per Ayurveda, aloe vera juice can benefit the eyes and help prevent various eye disorders. It is also useful in treating jaundice and bronchitis.
Aloe vera juice can cleanse the digestive tract, which is vital for the treatment of irritable bowel syndrome.
Aloe vera juice also contains aloin (Refer image 1), which is a potent laxative.
Preliminary research suggests aloe vera juice intake can help improve blood glucose levels.
Aloe vera juice also contains chromium, magnesium, zinc, and manganese – all of which improve the effectiveness of insulin. However, as seen, further research is required.

AIM

To standardize marketed herbal preparations namely Aloe vera gel and Aloe vera juice.

RATIONALE

A herb is a plant or a part of a plant which is used for its scent, flavour or therapeutic properties. Traditional herbal medicine and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs.

Herbal preparations are more affordable and easier to obtain than conventional medications. They hold healing properties and more often than not one herb (like aloe) can be used for two or more completely different purposes (like nutraceutical and cosmetic).

India is a diverse country and there is a wide variety of flora and fauna available which therefore, lead to easy availability of herbal medications.

However, one of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae, is extracted with boiling water during the decoction process. This may be the main reason why quality control of oriental herbal drugs is more difficult than that of western drug. Herbal medicines don't go through the stringent testing conditions that normal drugs do.

Many consumers believe that herbal medications are natural and therefore, safe. This is a dangerous simplification. Some herbal medications are associated with toxicity. The often under regulated quality of herbal medicines amounts to another safety issue.

In order to conduct a risk benefit analysis of specific herbal medicine for a specific indication, we require definitive efficacy and safety data. This is currently the case for only few such preparations.

REQUIREMENTS

Colorimeter
Conical flask
Beaker
Glass rod
Measuring cylinder
Distillation apparatus

REAGENTS

Methanol
Ferric chloride hexahydrate
Hydrochloric acid
Sodium hydroxide
Carbon tetrachloride

METHODOLGY

ASSAY OF ALOE VERA GEL AND JUICE

Assay: Aloe vera Gel and Juice were used equivalent to 0.2gm of aloe vera fine powder and moistened with 2ml of methanol and add 5ml of water at about 60°C, mix and add a further 75ml of water at about 60°C, shake for about 30 minutes, cool, filter through a filter paper, washing the flask with 20ml of water and add sufficient water to the combined filtrate and washings to produce 1000ml. Transfer 10ml of the solution to a flask containing 1ml 0f 60% w/v solution of ferric chloride hexahydrate and 6ml of hydrochloric acid, heat in a water bath under a reflux condenser for 4 hours so that the water level is always above that of liquid in the flask, cool, transfer the solution to a separating funnel, rinsing the flask successively with 4ml of 1M sodium hydroxide and 4mk of water and adding the three quantities, each of 20ml, of carbon tetrachloride and wash the combined carbon tetrachloride layers with two quantities, each of 100ml, of water, discarding the washings. Dilute the organic phase to 100ml with carbon tetrachloride, evaporate 20ml carefully to dryness on a water bath and dissolve the residue in 10ml of 1M sodium hydroxide. Immediately measure the absorbance at about 440nm and at about 500nm. Calculate the content of anhydrous barbaloin, taking 200 as the specific absorbance at 500nm. The result of the assay is valid if the ratio of the absorbance at about 500nm to that of 440nm is not less than 1.9.

Refer image 2

RESULT AND DISCUSSION

The organoleptic properties of the gel and the juice were in accordance with the ones mentioned in the IP 2014

ALOE VERA GEL

TEST	OBSERVATION	INFERENCE
COLOUR	LIGHT GREEN COLOUR	PASS
ODOUR	PLEASANT	PASS
TEXTURE	SMOOTH	PASS
CONSISTENCY	SEMI SOLID	PASS

ALOE VERA JUICE

TEST	OBSERVATION	INFERENCE
COLOUR	DARK GREEN COLOUR	PASS
ODOUR	UNPLEASANT	PASS
TEXTURE	SMOOTH	PASS
CONSISTENCY	LIQUID	PASS

(Refer images 3 and image 4)

The result of the preparation is valid if the ratio of the absorbance of the preparation at 500 nm to that at about 440 nm is not less than 1.9 as per IP 2014

PREPARATION	**RATIO**
ALOE VERA GEL	**3.4**
ALOE VERA JUICE	**2.1**

The ratio of absorbance of ALOE VERA GEL was found out to be 3.4 which passes in accordance with the one stated in IP2014; hence the claims made have passed.

The ratio of absorbance of ALOE VERA JUICE was found out to be 2.1 which passes in accordance with the one stated in IP2014; hence the claims made have passed.

CONCLUSION

Plants and herbs have been used since early days of the humankind and are still used throughout the world for health promotion and treatment of disease. Plant and natural sources form the basis of today's modern medicine and contribute largely to the commercial drug preparations manufactured today.
Regardless of the reason, those using herbal medicines should be assured that the product they are buying are safe and contain what they are supposed to, whether this is a particular herb or a particular amount of a specific herbal component.
Consumer should also be given science based information on dosage, contraindication and efficacy. To achieve this, global harmonisation of legislation is needed to guide the responsible production and marketing of herbal medicine. If sufficient scientific evidence of benefit is available for a herb, then such legislation should allow for this to be used appropriately to promote the use of that herb so that these benefits can be realised for the promotion of public health and the treatment of disease
We understand that the quality control and quality assurance tests on herbal preparations are not carried out as strictly as required or at par with conventional medicines.
The main idea of the above research was to perform organoleptic or sensory tests and standardize the given marketed preparation of Aloe vera (Aloe vera gel and Aloe vera juice).
The methodology according to IP was followed and the results were obtained.
The organoleptic properties were in accordance with the result stated in IP.
The assay of both the preparations were also valid as the ratios obtained were as per the IP.
Therefore, the preparations pass.

According to us the preparations (Aloe vera gel and Aloe vera juice) used were up to the mark and the results were satisfactory. We would also like to say that the use of herbal preparations needs to be increased since they are cheaper, obtained from natural resources and are less toxic as compared to conventional medicines.

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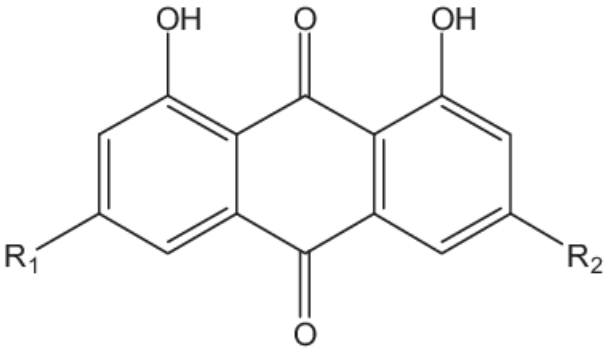
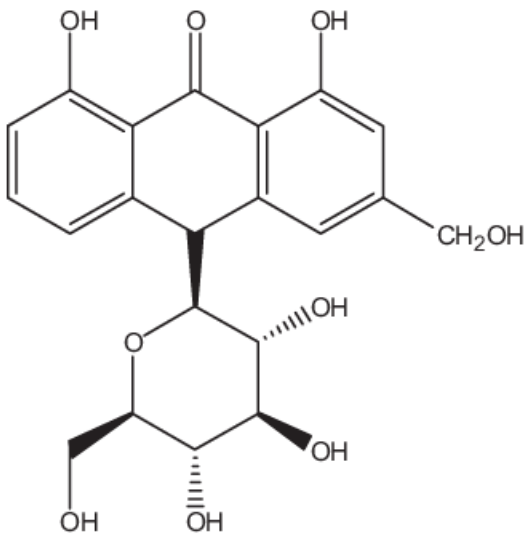


emodin	R_1=OH	R_2=CH_3
aloe-emodin	R_1=H	R_2=CH_2OH
rhein	R_1=H	R_2=COOH
chrysophanol	R_1=CH_3	R_2=H
physcion	R_1=CH_3	R_2=OCH_3



Aloin

Image 1

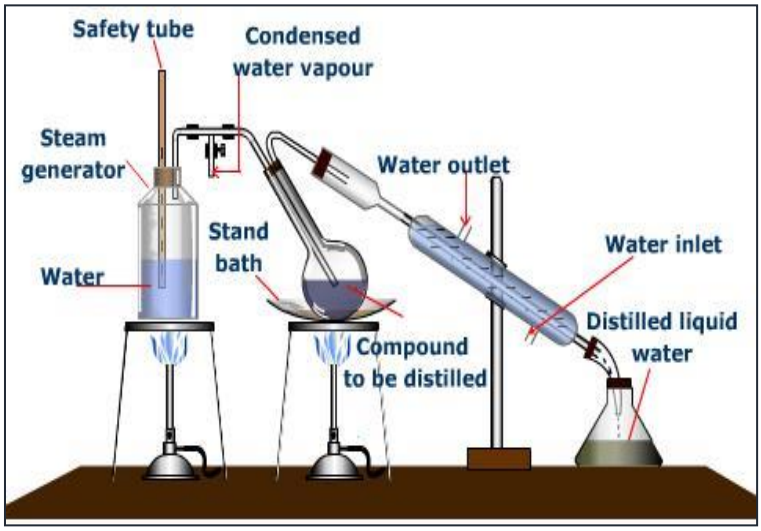


Image 2

Image 3

Image 4

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ECO-FRIENDLY SOLUBILIZATION TECHNIQUES FOR SPECTROPHOTOMETRIC ANALYSIS OF SOLID DOSAGE FORMS.

*Minal Suryawanshi, Ummehani Tinwala and Vineeta Khanvilkar**

1. ABSTRACT

Spectrophotometric analysis of majority of water insoluble drugs includes use of organic solvents like methanol, ethanol, chloroform, benzene etc. The primary disadvantage of organic solvents is the toxicity associated with them. Other disadvantages include numerous adverse effects caused by single exposure like dermatitis, headache, drowsiness, nausea, eye irritation. Their long term exposure causes serious effects such as neurological disorders, chronic renal failure and liver damage. Therefore there is need for the development of alternative eco-friendly methods for solubilisation of such drugs. Different eco-friendly techniques namely hydrotropy, mixed-solvency and use of solid as solvent are available in the literature for solubilization of drug. An attempt was made to develop an eco-friendly solubilization method using different easily available solids as solvents to extract the drug from its solid dosage form. The extracted solutions were then analyzed by UV spectrophotometric specific absorbance method. The results were then compared to evaluate the utility of the solubilization method.

KEYWORDS: Spectrophotometry, Hydrotropy, Solubilization.

2. INTRODUCTION

Spectrophotometric method of analysis are based on measurement of absorbance by the solution of drug. For this analysis of any dosage form, the drug needs to be in solution. Thus the complete extraction of drug from the dosage form into the solution is an important step. Extraction of water soluble drug is not a problem but the extraction of water insoluble drug is a challenge for accurate determination of drug content. In most of the cases, organic solvents are used for solubilization of such drugs. However the organic solvent suffer from the disadvantage of high cost, toxicity and pollution. Hence there is need for methods which are eco-friendly.

Hydrotropy is a solubilization technique in which addition of large amount of a second solute results in an increase in the aqueous solubility of another solute. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds.

The literature survey[2] reveals that the UV-spectrophotometric estimation of poorly water soluble drugs could be conducted successfully by using the approach of hydrotropic solubilization technique. The aqueous solubility of many drugs like nalidixic acid, norfloxacin ,tinidazole, metronidazole, metformin hydrochloride, etc were found to be enhanced by the incorporation of sodium citrate, sodium salicylate, sodium ascorbate, potassium acetate as hydrotropic solubilizers. We have tried to apply the same approach for extraction of propranalol hydrochloride from its formulation.

4.AIM AND RATIONALE

The standard IP procedure follows the use of methanol as a solvent for the assay of Propranolol HCl tablets. Methanol comes under class 2 solvents which includes the solvents to be limited for use. Class 2 includes non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. PDE value of methanol is 30mg/day. Methanol has various drawbacks such as high cost, toxicity and pollution.

5. MATERIALS AND METHODS

Instruments used: **Magnetic stirrer**(REMI 1MLH), **Sonicator**(PCi Analytics), **UV Spectrophotometer**(SHIMADZU).

Three different brands of Propranolol hydrochloride tablets were procured from the local market namely **Ciplar** (10mg), **Inderal** (10mg), and **BetaCap** (20mg).

Methanol, Sodium citrate, Sodium acetate were of AR grade procured from Research-Lab Fine Chem Industries(An ISO 9001, 14001 and OHSAS 18001 Certified Company).

Preparation of solutions of solids to be employed as solvents:

1. Sodium Acetate 1M solution: 82.03g dissolved in 1000ml Distilled Water.
2. Sodium Acetate 0.5M solution: 41.01g dissolved in 1000ml Distilled Water.
3. Sodium Acetate 0.1M solution: 8.203g dissolved in 1000ml Distilled Water.

The solvents were scanned in the UV-visible range to make sure they do not interfere with the absorbing capacity of the Drug.

The Procedure followed was as follows:

1. Twenty tablets were weighed and crushed to get a fine powder.
2. Tablet powder containing a quantity equivalent to 20mg of Propranolol hydrochloride was weighed accurately and shaken with 20ml of water for 10 minutes.
3. 50ml of the solvent was then added, the flask was again shaken for 10 minutes, sufficient solvent was added to produce 100ml. The solution was filtered.
4. 2ml of the filtrate was then diluted to 10ml with the solvent.
5. Absorbance of the resulting solution was recorded at the wavelength maximum of 290nm.
6. The content of Propranolol Hydrochloride was calculated taking 206 as the specific absorbance at 290nm.

6. RESULTS AND DISCUSSION

1. 0.1M and 1M solution of Sodium Acetate were scanned in the UV range from 200-400nm. The spectra obtained did not show any peak after 240nm. As the wavelength maxima for Propranolol HCl is 290nm and sodium actetate solutions were not absorbing at 290nm, it was decided to proceed further.
2. The tablet powders were extracted with 0.1M, 0.5M and 1M sodium acetate solutions. The absorbance of solutions after proper dilutions were recorded at 290nm. $A^{1\%}_{1cm}$ value of Propranolol 206, was used for calculation of Propranolol content.

The results obtained for the assay are tabulated in Table no. 1:

Name of tablets	**Label Claim**	**% Drug Estimated (% Tablet Content)**		
		0.5M Sodium acetate	**1M Sodium acetate**	**IP Method**
Ciplar	**10mg**	**99.8%w/w**	**102.3%w/w**	**98.5%w/w**
BetaCap	**20mg**	**93.75%w/w**	**97.9%w/w**	**98.9%w/w**
Inderal	**10mg**	**103.7%w/w**	**105%w/w**	**100.2%w/w**

7. CONCLUSION

The UV Spectrophotometric Assay method mentioned in the research work is new, simple, environment friendly. This method has advantage over the conventional method which uses methanol for extraction of drug from the tablets. Apart from this if this method is validated, it may prove boon to analysts as it will reduce the cost of analysis along with reduction in health related problems associated with continued use of organic solvents.

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3. ICH Guidelines for residual solvents.

Acknowledgement:
Dr. R. K. Maheshwari, Professor of Department of Pharmacy, Shri G.S. Institute of Technology and Science, Indore.

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ECO-FRIENDLY SOLUBILIZATION TECHNIQUES FOR SPECTROPHOTOMETRIC ANALYSIS OF SOLID DOSAGE FORMS.

*Shubham Auti and Vineeta Khanvilkar**

1. ABSTRACT

Spectrophotometric analysis of majority of water insoluble drugs includes use of organic solvents like methanol, ethanol, chloroform, benzene etc. The primary disadvantage of organic solvents is the toxicity associated with them. Other disadvantages include numerous adverse effects caused by single exposure like dermatitis, headache, drowsiness, nausea, eye irritation. Their long term exposure causes serious effects such as neurological disorders, chronic renal failure and liver damage. Therefore there is need for the development of alternative eco-friendly methods for solubilisation of such drugs. Different eco-friendly techniques namely hydrotropy, mixed-solvency and use of solid as solvent are available in the literature for solubilization of drug. An attempt was made to develop an eco-friendly solubilization method using different easily available solids as solvents to extract the drug from its solid dosage form. The extracted solutions were then analyzed by UV spectrophotometric specific absorbance method. The results were then compared to evaluate the utility of the solubilization method.

KEYWORDS: Spectrophotometry, Hydrotropy, Solubilization.

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Hydrotropy is a solubilization technique in which addition of large amount of a second solute results in an increase in the aqueous solubility of another solute. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds.

The literature survey[2] reveals that the UV-spectrophotometric estimation of poorly water soluble drugs could be conducted successfully by using the approach of hydrotropic solubilization technique. The aqueous solubility of many drugs like nalidixic acid, norfloxacin ,tinidazole, metronidazole, metformin hydrochloride, etc were found to be enhanced by the incorporation of sodium citrate, sodium salicylate, sodium ascorbate, potassium acetate as hydrotropic solubilizers. We have tried to apply the same approach for extraction of propranalol hydrochloride from its formulation.

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5. MATERIALS AND METHODS

Instruments used: **Magnetic stirrer** (REMI 1MLH), **Sonicator**(PCi Analytics), **UV Spectrophotometer**(SHIMADZU).

Three different brands of Propranolol hydrochloride tablets were procured from the local market namely **Ciplar** (10mg), **Inderal** (10mg), and **BetaCap** (20mg).

Methanol, Sodium citrate were of AR grade procured from Research-Lab Fine Chem Industries (An ISO 9001, 14001 and OHSAS 18001 Certified Company).

Preparation of solutions of solids to be employed as solvents:

- Sodium Citrate 1M solution : 258.06g dissolved in 1000 ml distilled water
- Sodium Citrate 0.1M solution : 25.806g dissolved in 1000 ml distilled water
- Sodium Citrate 0.5M solution : 129.03g dissolved in 1000 ml distilled water

The solvents were scanned in the UV-visible range to make sure they do not interfere with the absorbing capacity of the Drug.

The Procedure followed was as follows:

1. Twenty tablets were weighed and crushed to get a fine powder.
2. Tablet powder containing a quantity equivalent to 20mg of Propranolol hydrochloride was weighed accurately and shaken with 20ml of water for 10 minutes.
3. 50ml of the solvent was then added, the flask was again shaken for 10 minutes, and sufficient solvent was added to produce 100ml. The solution was filtered.
4. 2ml of the filtrate was then diluted to 10ml with the solvent.
5. Absorbance of the resulting solution was recorded at the wavelength maximum of 290nm.
6. The content of Propranolol Hydrochloride was calculated taking 206 as the specific absorbance at 290nm.

6. RESULTS AND DISCUSSION

1. 0.1M and 1M solution of Sodium Citrate were scanned in the UV range from 200-400nm. The spectra obtained did not show any peak after 240nm. As the wavelength maxima for Propranolol HCl is 290nm and sodium actetate solutions were not absorbing at 290nm, it was decided to proceed further.
2. The tablet powders were extracted with 0.1M, 0.5M and 1M sodium citrate solutions. The absorbances of solutions after proper dilutions were recorded at 290nm. $A^{1\%}_{1cm}$ value of Propranolol 206, was used for calculation of Propranolol content.

The results obtained for the assay are tabulated in Table no. 1:

Name of tablets	***Label Claim***	***% Drug Estimated (% Tablet Content)***		
		0.1M Sodium Citrate	***0.5M Sodium Citrate***	***IP Method***
Ciplar	***10mg***	***96.11%w/w***	***93.44%w/w***	***99.5%w/w***
BetaCap	***20mg***	***93.75%w/w***	***98.65%w/w***	***102.5%w/w***
Inderal	***10mg***	***105%w/w***	***102.4%w/w***	***99.2%w/w***

7. CONCLUSION

The UV Spectrophotometric Assay method mentioned in the research work is new, simple, environment friendly. This method has advantage over the conventional method which uses methanol for extraction of drug from the tablets. Apart from this if this method is validated, it may prove boon to analysts as it will reduce the cost of analysis along with reduction in health related problems associated with continued use of organic solvents.

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Acknowledgement:
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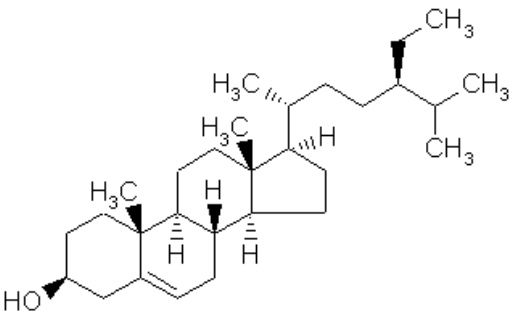
STUDIES ON β-SITOSTEROL

*Sayali Nangare, Mrunalini Narvekar and A. P. Jadhav**

ABSTRACT:
The present study was performed to detect and identify *β*-sitosterol from Air plant (*Bryophyllum pinnatum*), Linseed (*Linum usitatissimum*) and Olive oil (*Olivia europaea*). Thin layer chromatography (TLC) was used for detection of *β*-sitosterol in the extracts of drugs. The retention factors of extracts and standard *β*-sitosterol were compared to confirm its presence. Development of green color spots after devitization using Liebermann-Burchard reagent on the TLC plate confirmed the presence of *β*-sitosterol.
Key words: *β*-sitosterol, Air plant, Linseed, Olive oil, Liebermann-Burchard reagent.

INTRODUCTION:
β-sitosterol is one of the most common dietary phytosterols found in and made exclusively by plants. Other phytosterols include campesterol and stigmasterol. The structure of *β*-sitosterol is similar to that of cholesterol. It differs from cholesterol by the presence of an extra ethyl group.



β-sitosterol

Air Plant
The common names in English are Couelus, Miracle Leaf, Katakataka
Botanical name- *Bryophyllum pinnatum*
Family- Crassulaceae

- *Bryophyllum pinnatum* is given for the treatment of a cough, asthma, cold with candy sugar.
- It is used against dysentery.
- The plant root is used to treat high blood pressure.
- It is also used to prevent any kind of cardiac problem.
- *Bryophyllum pinnatum* is used for the treatment of fever.
- Rhizome powdered is used for the treatment of constipation.
- *Bryophyllum pinnatum* paste is used for the treatment of boil, wound, soar or cuts.
- Using juice of this as eardrop heals the ear pain. It is a natural remedy for ear pain.
- The paste of Pashan Bheda mixed with honey is used for the beginners teething trouble.
- Because of anti-diabetic property, it is used for the treatment of diabetics.

- The root of this plant is believed to protect the liver and proved useful for the treatment of hepatitis.

Linseed
Botanical name: *Linum usitatissimum*
Family: Linaceae

- Lower cholesterol, protects against heart disease and controls high blood pressure.
- Counter irritation associated with gout, lupus and fibrocystic breasts.
- Controls constipation, haemorrhoids and diverticular disorders.
- Treats acne, psoriasis, sunburn.
- Promotes healthy hair.

Olive oil
Botanical name: *Olivia europaea*
Family: Oleaceae

- Colorectal cancer
- High cholesterol
- High blood pressure

AIM AND RATIONAL:

The purpose of study is to identify and characterize the bioactive compounds from the plants. In this paper, we report the extraction and characterization of know compounds from the plant namely *β*-sitosterol.

I. A revolution in chemical technology has occurred in the last 50 years. New technologies have enabled the isolation identification and subsequent synthesis of biological compounds. Although some chemical compounds found in plants cannot be synthesized today because of technical or economic constraints, an increasing number of chemical compounds are being produced in the laboratory. Despite these capabilities, renewed interest has developed in using naturally produced chemicals from plants as sources of new food proteins, medicines, biocides and other materials.
II. Standardization of these herbal drugs is a challenge to the entire scientific fraternity.
III. Due to re-emergence of new virulent resistant strains it is necessary to seek new anti-microbial. One of the possible basic approaches to cure and control infections caused by multiple drug resistant (MDR) strains of bacteria is to explore the medicinal properties of herbs and higher plants.
IV. In the pharmaceutical industry *β*-sitosterol can be used as such for medical use or as a raw material in manufacture of steroid intermediates.
V. *β*-sitosterol being phytoconstituent is related with less side effects and toxicity. Moreover it can give synergistic activity with many drugs like antidiabetics, antibiotics, anticancer agents etc.

MATERIAL AND METHODS:

Standard Prepartion: 1gm/10 ml of *β*-sitosterol was dissolved in ethyl acetate to prepare a solution.

Sample preparation:

Air Plant: Leaves of *Bryophyllum pinnatum* were collected from Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Navi Mumbai and were cleaned thoroughly after separation. These leaves were then subjected to maceration with ethyl acetate as solvent. The resulting solution was concentrated by heating on an electric water bath at 70°C and this extracted was used in present study.

Linseed: Powdered form of linseed (*Linum usitatissimum*), 2 gm was subjected to maceration using 10 ml methanol as a solvent. The solution was heated on an electric water bath at 70°C and methanol was evaporated to obtain concentrated extract which is used in following study.

Olive oil: 3 ml of olive oil was extracted with 3 ml of methanol. Two layers are separated. *β*-sitosterol is soluble in alcohol. Therefore, alcoholic solution is used to perform the present the present investigation.

THIN LAYER CHROMATOGRAPHY

Preparation of mobile phase:

The mobile phase used in the present research work was prepared by mixing Toluene, Ethyl acetate and Glacial acetic acid in the volume ratio 8:2:0.2. During development of each plate a fresh mobile phase was prepared.

Preparation of post derivatization reagent:

Liebermann-Burchard reagent was used as post derivatizing reagent. 5 ml of acetic anhydride and 5 ml of sulphuric acid are added carefully to 50 ml of absolute ethanol while cooling in ice.

Procedure:

Silica gel plate $60F_{254}$ was used for TLC. A line was drawn with a pencil 1cm at the bottom from both ends of the plate. The plate was allowed to activate in oven at 105 °Cfor 30 minutes. The extract was spotted on the line drawn on the plate using a capillary and then allowed to dry. The dry plates were then placed into a glass beaker containing (8:2:0.2) ratio of mobile phase and the beaker was covered with a petriplate. The solvent rose up on the plate by capillary action to the line that was drawn to mark the position of the solvent front. The plates were allowed to dry and the spots were developed by dipping in Liebermann-Burchard reagent. The R_f value of the spots were measured using meter ruler.

CHROMATOGRAPHIC CONDITIONS:

1. Stationary phase: Precoated silica gel plate $60F_{254}$
2. Mobile phase: Toluene: Ethyl-acetate: Glacial acetic acid (8: 2: 0.2 v/v)
3. Chamber saturation: 15 minutes
4. Seperation technique: Linear ascending technique
5. Quantity of mobile phase: 5 ml

Fig 1. TLC plates after derivatization

RESULT:

In the present work, *β*-sitosterol is extracted with the help of methanol from linseed & olive oil; and with ethyl actate from air plant. TLC analysis of extract showed characteristic spots after post derivatization with Liebermann-Burchard reagent. The Rf value for *β*-sitosterol was found to be 0.38 and it was compared with standard *β*-sitosterol.

CONCLUSION:

In the present work, detection and identification of *β*-sitosterol in *Bryophyllum pinnatum, Linum usitatissimum* and *Olivia europoea* was carried out. Spot of extract developed on TLC was comparable with the standard *β*-sitosterol which confirmed its presence. The spots developed indicated that all sample constituents were clearly separated without any tailing and diffuseness.

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