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Whites Science
A Scientific Erudition

Development, Standardization of Polyherbal Formulation of Anti-Fungal Cream of Plant *Curcuma longa, Eugenia aromatica, Foeniculam vulgare*

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ABSTRACT

Ayurveda is one of the world's oldest systems of medicine. It originated in India andhas evolved there over thousands of years. The term "Ayurveda" combines then Sanskrit words *ayur*(life) and*veda*(science or knowledge) [1]. Some herbal products, including many traditional medicine formulations, also include animal products and minerals [2]. Herbal products are sold as either raw plants or extracts of portions of the plant or in the form formulation i.e. tablet, capsule, syrup, cream and ointment etc. Present study deals with formulation [3], Standardization of cream made from alcoholic extract and essential oil of different plants [4]. The different parts of plants with anti- fungal activity were taken up for the present study and investigated for the phytochemical screening and used for the formulation of anti- fungal cream and Present study deals with formulation, Standardization, evaluation and efficacy studies of both cream made from alcoholic extract and essential oil of different plants [5].

Keywords: Anti-Fungal, Formulation, Standardization, Evaluation, Ointment

INTRODUCTION

Herbal Medicine [6]

Herbal medicine sometimes referred to as botanical medicine or herbalism it involves the use of plants or parts of plants to treat injuries or illnesses [7]. The parts of plants like seeds, leaves, stems, bark, roots, flowers etc and their extracts used in herbal medicine as teas and tinctures, topical applicators, pills, capsules and other formulation. Some of the pharmaceutical medications on the market are extracts of some of these traditional herbs. The lower cost, and often safer use, has attracted many medical professionals [8].

Herbal medicine: An overview [9]

Ayurveda, Chinese, Siddha, Unani, Homeopathic etc systems of medicine are based on plants for medication. There are many cultures in world use herbs in their healing rituals.

Advantages of herbal medicine

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- Herbal medicine is used because of patient tolerance and better acceptance.
- Rich agro-climatic, cultural and ethnic biodiversity in developing countries there is no problem of medicinal plants because their cultivation and processing is very environmental friendly.

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Limitations of herbal medicines [10]

- The prominent limitations of herbal medicines can be summarized as follow
- Ineffective in acute medical care.
- Inadequate standardization and lack of quality specifications.
- Lack of scientific data.

Standardization of Herbal Medicine [11]

The pharmaceutical manufacturing companies having much issues regarding availability of good quality raw material, authentication, standards value, standardization methodology of drugs and formulation and quality control parameters. The use of herbal medicine due to toxicity and side effects of allopathic medicines has led to sudden increase in the number of herbal drug manufactures

Standardization and quality control of crude drugs [12]

- For Identification of proper variety and adulterants Macro and Microscopic Examination are done.
- For removal of matter other than source plant to get pure drug Foreign Organic Matter are expelled out.
- For the identity and purity of crude drug Total ash, sulfated ash, water soluble ash and acid insoluble ash were calculated.
- For moisture content present in crude drugs that can degrade the product formed Moisture Content seen.
- For measure the chemical constituents of crude drug Extractive Values are calculated.
- Crude Fiber are determine excessive woody material Criteria for judging purity.
- Qualitative Chemical Evaluation indicates the identification and characterization of crude drug regarding phytochemical constituent.
- Chromatographic Examination done for major chemical constituent as marker.
- Quantitative Chemical Evaluation helps to estimate constituents.
- Toxicological Studies given pesticide residue, potentially toxic elements, and microbial count.

Analytical methods [11]

Chromatographic characterization: There are many chromatographic techniques which cover the large area of analytical results. Chromatographic techniques like Thin layer chromatography, High Performance Thin Layer Chromatography, Gas chromatography, Paper chromatography, and chromatography.

Purity determination [12]

Monograph having standards of purity and other qualitative assessments as foreign matter, ash, acid-insoluble ash, moisture content, loss of moisture on drying, and extractives and HPTLC can analyse the number of compounds both efficiently and cost effectively and more complete profile of the plant than is typically observed with more specific types of analyses.

Quantitative analysis:

PLANT DESCRIPTION

***Foeniculum vulgare* [13]**

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Scientific classification
Kingdom Plantae
Order Apiales
Family Apiaceae (Umbelliferae)
Genus *Foeniculum*
Species *Foeniculumvulgare*

Vernacular names: Hindi- Badi saunf, Saunf

Distribution: All over India up to 1830 m. and also sometimes found wild.

Part used: Seed and flower, fruits

Uses: On account of its carminative properties, fennel is chiefly used medicinally with purgatives to allay their side effects, for adults, fennel seeds or tea can relax the intestines and reduce bloating caused by digestive disorders.Ancient Romans regarded fennel as the herb of sight. Root extracts were often used in tonics to clear cloudy eyes. Extracts of fennel seed have been shown in animal studies to have a potential use in the treatment of glaucoma.Fennel may be an effective diuretic and a potential drug for treatment of hypertension.

Eugenia aromatic [14]

Scientific classification
Kingdom Plantae
Order Myrtales
Family Myrtaceae
Genus Syzygium
Species *Eugenia aromatica*

Vernacular names: Hindi- Laung

Distribution: A tree indigenous to the Moluccaislands , grown in India in certain parts of Tamilnadu (The Nilgiris and Kanniayakumari) and Kerala (Kottarakara and Chengannur).

Part used: Flower bud

Uses: Cloves oil is used as analgesic for dental problem and also decrease infection in the teeth because of its antiseptic properties. Cloves are used as a carminative and enhance hydrochloric acid secretion in stomach which results improvement in peristalsis and also act as natural anthelmintic.

Mentha piperita[15]

Scientific classification
Kingdom Plantae
Order Lamiales
Family Lamiaceae
Genus Mentha
Species *Mentha piperita*

Vernacular names: Hindi- Pudina

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Distribution: Originally the plant is a native of Europe and has been naturalized in many parts of India. The drug is almost entirely derived from cultivated source.

Part used: Leaves and stem

Uses: Peppermint helps to enhance memory. Volatile oil present in peppermint can act as natural pesticides. Peppermint also used as spices in home because of their pleasant flavour. Nowadays lots of cosmetics having it are as ingredient because of its cooling sensation.

MATERIALS AND METHODOLOGY

Collection of Plant Material

The seeds *Foeniculum vulgare*, buds of *Eugenia aromatica* and leaves of *Mentha piperita*, these all were received from a reputed supplier Herbal auto mission from Delhi and authenticated by Taxonomic Division of Maharishi Ayurveda Products Pvt. Ltd. NSEZ Noida.

Pharmacognostic Studies

Macroscopical evaluation [16]

Organoleptic evaluation can be done by color, odour, size, shape, taste and touch and texture etc. Organoleptic evaluations are given below.

Colour: The sample was examined under sunlight or artificial light source.

Shape and size: The length, breadth and thickness are important parameter for evaluation of crude drug. A sheet of a calibrated paper used to calculate average length, breadth and thickness.

Odour and taste: when drug sample was crushed by applying pressure then the odour comes that can be weak, distinct, strong , and that was noted down and odour sensation like rancid, fruit, aromatic etc were also noted.

Microscopical evaluation: Microscopical evaluation was done for qualitative and quantitative parameters. The parameters observed were: tissues Arrangement, epidermal cells, testa and endosperm, and crystalline structures as Calcium oxalate, starch and presence of oil globules, aleurone grains and trichomes etc.

Powder microscopy

The seeds of *Foeniculum vulgare* were powdered and sieved, fine powder thus obtained was taken up for microscopical evaluation. By mounting drug on slide and spread out and observe the microscopical characters. Another small quantity was stained with staining reagent like phloroglucinol and HCl, ruthenium red, safranin, sudan red III etc, mounted and spread on slide and observed for microscopic characters.

Physicochemical studies [17]

Physicochemical parameters help to determine the inorganic and moisture content from which dry weight of the drug can be calculated (w/w).

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Determination of Ash values

a. Total ash value

Weighed accurately 2 g of air dried roots powder in a tarred platinum or silica dish and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed. Mix with hot water and collected the residue on an ash less filter paper, incinerated again the filter paper until the ash was white in appearance and add the filtrate, evaporated to dryness Calculate the ash value with reference drug.

b. Acid insoluble ash

Boiled the ash with 25 ml of 2M HCL for 5 min, collected and washed with hot water, ignited then cooled in desiccators and lastly weighed. Calculate the acid insoluble ash with reference drug.

Loss on drying

Take 2 g of coarse powder of sample in a dry and evaporating dish. Place in a hot air oven at 105±5°C for 5 hrs and allowed to cool at room temperature in a desiccator and then weigh it. Continue the process till difference between two successive weighings not more than 0.1% of sample weight.

Determination of extractive values

The water-soluble, alcohol soluble and ether soluble extractive values of air-dried sample were evaluated using the procedure given below.

Water soluble extractives

Macerate 5 g of air dried drug, coarsely powdered, with 100 ml of water in a closed flask for 24 hour, shaking frequently for 6 hour and allow standing for 18 hours after then shaking the flask and filter. Take 25 ml filtrate in porcelain dish and evaporated at 100^0C on water both to dryness and dry in oven at 105^0C, to constant weight, cool in a desiccator and weighed. Calculated the percentage of water soluble extractive value with reference to the air dried drug.

Preliminary Phytochemical Screening

Chemical tests [18]

Presence of types of constituents was determined by using following phytochemical tests as follows. The inference of which are summarized in result and discussion section.

Determination of pH

1g of drug was taken in a 100 ml volumetric flask and added in 100 ml of distilled water. The solution was put for about 4 hours and filtered. pH of filtrate was checked with the calibrated pH meter with standard of pH 4, 7, 9 (standard glass electrode).

Determination of swelling index

Take 1-1 gm of coarsely powdered drug in to the three 25ml of glass stoppered measuring cylinder. Add 25ml of water and determine the mean value of initial height of drug in the cylinder then shake the mixture thoroughly at interval of every 10 minutes for 1hour.Allow to stand for 3 hours at room temperature. Determine the mean value of final height of drug in the cylinder.

Calculation: Swelling index = Final mean - Initial mean

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Determination of foaming index [19]

Reduce about 1gm of the plant material to a coarse powder, weigh accurately and transfer to a 500 ml conical flask containing 100ml boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter in to a 100 ml volumetric flask and add a sufficient water through the filter to dilute the volume to 100 ml. Place the above decoction in to 10 stoppered test tubes in a series of successive portions of 1, 2, 3, 4 … up to 10 ml and adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, 2 frequencies per seconds. Allow to stand for 15 minutes and measure the height of thefoam.

- The foaming index is also less than 100 when height of foam is less than 1cm
- The foaming index is over 1000 when height of the foam is more than 1 cm.

Calculation

Foaming Index = 1000/a

Where a is the volume in ml. of the decoction used for preparing the dilution in the tube where foaming isobserved

Fluroscence analysis

Fluorescence analysis is done to determine the effect of the different reagent on the colour of the powdered drug in the normal day light, short wavelength(254nm) and long wavelength(366nm).Reagents used are Distill water, 1N NaOH in water, 1N NaOH in Methanol,50% Nitric acid,50% Hydrochloric acid, Sulphuric acid, Acetone, concentrate hydrochloric acid, Chloroform.

Extraction of Essential Oil and Analysis of Oil by Gas Chromatography [20]

The parameters of gas chromatography are given in Table 1 given below

Table 1: Oil analysis is done by the Gas chromatography given below

GC condition	
Instrument	GC NUCON-5700
Column	Stainless steel
Length	10feet
Inner diameter	2 mm
Outer diameter	3.175 mm
Mesh size	100-120 mesh
Column temperature	90-230 ^{0}C
Injector	230^{0}C
Detector	240^{0}C
Starting voltage	0.05mv

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Injection volume	0.2µl
Sensitivity	100
Attenuator	4
Height reject	0
Area reject	0
Threshold	0
End time	60 minutes
End value	70
Programming rate	4 ^{0} C/minute
Initial oven temperature	90^{0}C

Microbial Load Determination Microbial Contamination [21]

Procedure

Total aerobic count (T.A.C.) Add 1gm of the sample in case of powder in to flask containing 99 ml of the sodium creolite phosphate buffer solution (SCPS), shake the flask & keep in water bath at 37^{0}C – 45^0 C for 5 to10 minutes for dissolving & incubation (100 dilution). After this take 1ml from the flask and transfer it to tubes containing 9ml of the Sodium creolite phosphate buffer solution (SCPS) (1000 dilution). Transfer 1 ml. dilution to the sterile petri plate with the help of sterile pipet under laminar air flow. Now pouring 15 to 20 ml. of cooled (45^0 C.) Soyabean casein digest agar to plate & mix dilution with medium by rotating of plates. Allow the plates to solidify. After solidification incubate the plate at 37^0 C for 2 days in an inverted position in incubator.

Calculation

$$\text{TAC Count} = \frac{\text{Number of colonies onplates}}{\text{Amount plated x Dilution factor}}$$

Coliform: Add 1gm of the sample in case of powder in to flask containing 99 ml of the Sodium creolite phosphate buffer solution (SCPS), Shake the flask & keep in water bath at 37^{0}C. – 45^{0}C for 5 to10 minutes for dissolving & incubation.(100 dilution). Transfer 1 ml. dilution to the sterile petri plate with the help of sterile pipet under laminar air flow. Now pouring 15 to 20 ml. of cooled (45^{0}C.) Mac konkey agar (MCA) to plate & mix dilution with medium by rotating of plates. Allow the plates to solidify. After solidification incubate the plate at 37^0 C for 2 days (48 hours) in an inverted position in incubator

Calculation

$$\text{TAC Count} = \frac{\text{Number of colonies onplates}}{\text{Amount plated x Dilution factor}}$$

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Bacillus: Add 1gm of the sample in case of powder in to flask containing 99 ml of the Sodium creolite phosphate buffer solution(SCPS), shake the flask & keep in water bath at 37^0C – 45^0C for 5 to10 minutes for dissolving & incubation(100 dilution). Transfer 1 ml. dilution to the sterile petri plate with the help of sterile pipet under laminar air flow. Now pouring 15 to 20 ml of cooled (45^0C.) MYPA media to plate & mix dilution with medium by rotating of plates. Allow the plates to solidify. After solidification incubate the plate at 30^0 C for 2 days (48 hours) in an inverted position in incubator.

Calculation

$$\text{TAC Count} = \frac{\text{Number of colonies onplates}}{\text{Amount plated x Dilution factor}}$$

Yeasts and Moulds : Add 1gm of the sample in case of powder in to flask containing 99 ml of the Sodium creolite phosphate buffer solution (SCPS), shake the flask & keep in water bath at 37^0C – 45^0C for 5 to10 minutes for dissolving & incubation (100 dilution). Transfer 1 ml. dilution to the sterile Petri plate with the help of sterile pipette. Under laminar air flow.Now pouring 15 to 20 ml. of cooled (45^0C.) Potato dextrose agar (PDA) Media to plate & mix dilution with medium by rotating of plates. Allow the plates to solidify. After solidification Incubate the plate at 25^0C for 3 days (Yeasts) to 5 days (Moulds) in an inverted position in BOD.

Calculation

$$\text{TACCount} = \frac{\text{Number of colonies on plates}}{\text{Amount plated x Dilution factor}}$$

E.coli: These are gram –ve, lactose fermenting bacillus, found in the intestinal tract of all warm blooded animals, including humans. Many Strains are encapsulated & most are motile possessing flagella, Sex pili& fimbriae may also be present. Optimum growth is seen at 37^0 C. Infections caused include urinary tract infection, diarrhea & meningitis. They readily ferment various sugars include lactose, and produce gas and form pinkish red colonies on Mac Conkey agar plates.

Primary test: Aseptically add 10 gm. of sample into 100 ml. Nutrient broth. Incubate the nutrient broth at 37^0C for 24 - 48 hours. After incubating examine the medium for growth. Now transfer 1 ml. of the incubated medium to the 9 ml. of Mac conkey broth (MCB) test tube that contain the inverted durham's tube. Incubate the tube at 37^0 C for 48 hours. After incubation streak a loopful of culture on Mac conkey agar (MCA) plate and incubate at 37^0C for 24 hours. Pick up the suspected colonies and prepare a gram stain slide.

Confirmatory tests H_2S production test: Inoculate the suspected colonies from MCA plates in Tripple sugar iron slant. Stab the strain and streak over slope. Incubate it at the 37^0 C for 7 days observe the blackening in butt. If blackening is obtained it means *E. coli* present in sample.

Indole production test: Inoculate a loopful of culture from MCA plates in the tube containing 5ml peptone water. Incubate it at 37^0C for 48 hours. Add 0.5ml of Kovac reagent, appearance of red colour confirms presence of Indole.

Salmonella species: These are gram (-ve) bacilli belonging to the enteric group of bacteria are facultative anaerobes and do not ferment lactose. No capsule is distinguishable in them. The temperature &ph range for growth are 20-46^0C & 4.1 – 9 respectively all salmonella species are obligate parasite & are found in no. of animal host including man infection caused to

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human are entrie fever (typhoid & parathyroid) and gastroenteritis these posses fimbriae that play imp role in adherence.

Primary test: Aseptically add 10 gm of sample into 100 ml of nutrient broth. Incubate the Nutrient broth at 37^0 C for 24 hours, Transfer 1ml of aliquots of enriched culture to 5ml of Selenite cystine broth (SCB) and incubate it at 37^0C for 48 hours, After incubation transfer a loopful of culture on Bismuth sulphite agar (BSA) and incubate at 37^0 C for 24 hours, pick up the suspected colonies and prepare grams stain slide.

Confirmatory test: Transfer suspected colonies on Tripple sugar iron (TSI) slants by inoculating the surface of the slope and then making a stab culture with the same incubating needle. Incubate at 37^0 C and observe daily for up to 7 days. The absence of acidity from the surface and blackening in the butt of TSI slant indicate presence of Salmonella.

***Pseudomonas aeruginosa*:** It is slender, grams negative bacillus, non-capsulated, non-sparing and actively motile by a polar flagellum most staring possess pill.

Primary test: Aseptically add 10 gm of sample into 100 ml. of Cetrimide broth (CB), Incubate it at the37^0 C. for 72 hours. After incubation transfer the loopful of culture on cetrimide agar plate and incubate at 37^0 C for 72 hours. Pick up of the suspected colonies and prepare grams stain slide.

Confirmatory test Oxidase test: Place 2-3 drops of freshly prepared 1 % (w/v) solution of N, N, N, N Tetramethyl-p- Phenylenediamine dihydro chloride on piece of filter paper (whatman No 1) and smear with suspected colony. If purple colour is produced with in 5 to 10 seconds the test is positive.

Staphylococcus aureus: These are gram (+ve), facultative anaerobic, non motile cocci non spore forming, having both an oxidative & fermentative type of metabolism. The temperature & pH range for growth are 7-50^0 C. & 4.5-9.3 respectively many specials are commensals, others pathogenic the major pathogenic species is Staphylococcus aurous which can cause boils, wound infection, toxic shock syndrome food poisoning acid thus forming yellow halo around colonies.

Primary test: Aseptically add 10 gm of sample into 100 ml of Nutrient broth. Incubate the nutrient broth at 37^0 C for 24 – 48 hours, after incubation transfer the 1ml of enriched culture in a tube contain 5 ml of Salt meat broth (SMB) and incubate it at the 37^0 C for 48 hours. After incubation streak a loopful of culture on Mannitol salt agar (MSA) medium and incubate it at 37^0 C for 48 hours, pick up the suspected colonies and prepare grams stain slide.

Confirmatory test

DNAase test: Pick up the suspected colonies from agar surface of Manital salt agar and streak on DNAase agar medium, and incubate it at 37^0 C for 24 hours. If growth is there DNA containing culture plates flooded with 3.6% HCL solution to ppt on hydrolysed DNA. DNAase positive culture surrounded by a clear zones.

Determination of residual organochlorine pesticides (Gc- Ms) [22]

Procedure: Crush the composite sample to coarse powder and homogenize. Mix 10 gm sample vigorously with 120 ml of acetonitrile-water mixture (2:1) using glass rod in a 250 ml beaker and keep overnight. Filter via suction using non-absorbent cotton pad pre-rinsed with Acetonitrile on Buchner funnel. Transfer the filtrate into a separating funnel. Add 120 ml

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sodium chloride solution, shake. Extract with 50 ml n-hexane thrice, shaking vigorously. Dry and combine organic phase over anhydrous sodium sulphate granules and concentrate it to 5ml using water bath at 50 to 60^{0}C. Clean the extract with 20-25 gm pre activated florisil (at 500 to 550^{0}C) and 5 gm anhydrous sodium sulphate column pre-rinsed with petroleum ether. Elute using 150 ml mixture comprising of n-hexane (141ml) and diethyl ether (9ml) at a flow rate of 1 drop per second. Concentrate the extract close to dryness on water bath and make up the volume up to 1 ml with n-hexane.

Calculation:-

$$\text{For ppb} = C \times V/W$$

Where:

C= concentration of compound in ppb or microgram/kilogram
V= final make up volume of the sample.
W= initial weight of sample which taken

Heavy Metal Content Determination

As per the latest guidelines issued by World Health Organization (WHO) no plant analysis is complete without the evaluation of certain parameters mentioned therein, heavy metal analysis being one of them. Out of twenty one types of heavy metals reported in medicinal plants, four of the most commonly reported ones i.e. Arsenic (As), Lead (Pb), Mercury (Hg) and Cadmium (Cd) content was determined in the experimental plant material (Baranowska*et al*., 2002; Ernst, 2002).

Heavy metal analysis (By Atomic Absorption Spectrometer) Preparation of sample

Weigh required amount of sample in a silica crucible and burn it on hot plate till organic matter is charred. Place in a muffle furnace at 500^{0}C for 5 hr. and cool. Add 20 ml nitric acid (65%) to the ash and heat on boiling water bath for one hour. Filter and wash the residue with water and make up volume to 100 ml with water.

Calculation:

$$\text{Trace Metal (ppm)} = C \times D/W \times 1000$$

Where: D=Dilution factor, W= weight of sample (gm), C= Concentration

HPTLC Analysis

Identification of Curcumin in *Curcuma longa* rhizome [23]

Curcuma longa TLC Identity test solution: 1 g of powdered drug was extracted by shaking with 5 ml methanol for 10 min. with slight warming. Filtered and used the filtrate.

Reference solution: 1 mg of curcumin was dissolved in 1 ml methanol

Solvent system: Chloroform: ethanol: glacial acetic acid (94 : 5 : 1)

Stationary phase: Precoated silica gel 60 F_{254} TLC Aluminum sheets plates of uniform thickness (0.2mm) is used as stationary phase

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Procedure: 5µl each of test solution and reference solution were applied on two different tracks on a precoated silica gel 60 plate (2.5 x 10 cm) of uniform thickness (0.2 mm). The plate was developed in the solvent system.

Scanning: Both reference and test solution tracks were scanned densito-metrically at 366 nm and recorded the fingerprint profile.

Visualization of spots (Post scanning): Under UV 366 nm light-Reference solution exhibits a bright yellow fluorescent spot (Rf.0.79). In the test solution track three bright yellow fluorescent (at Rf. 0.79, 0.60 and 0.43) spots one of which (Rf. 0.79) corresponds to curcumine. Also a narrow light yellow zone was visible just above the middle spot (Rf. 0.60) [observe as a valley peak in the densitometric scan].

Identification of Eugenol in *Eugenia aromatica* bud

***Eugenia aromatica* TLC identity test Test solution [24]**

1 g of powdered drug was extracted by shaking with 5 ml acetone for 10 min. with slight warming. Filtered and used the filtrate.

Reference solution: 20 mg of eugenol was dissolved in methanol and made up the volume to 25 ml with methanol in a volumetric flask. 5 mg of ursolic acid was dissolved in methanol and made up the volume to 25 ml with methanol in a volumetric flask. From this stock solution, pipette out 5 ml into a 10 ml volumetric flask and made up the volume with methanol.

Stationary phase: Pre-coated silica gel 60 F_{254} TLC (0.2mm) is used as stationary phase.

Solvent system: Toulene: ethyl acetate (9.9 : 0.1)

Procedure: Test solution and reference solution were applied on two different tracks TLC plate of uniform thickness and developed in their solvent system.

Visualization of spots (Post scanning): TLC plate spray with vanillin sulphuric acid reagent and in day light reddish brown spotseen at Rf 0.37 of eugenol and dark violet spot seen at Rf 0.94 of β-caryophyllene.

Development of Poly herbal Formulation for Fungal Disease

Anifungal Cream [25]

A cream is two types: oil-in-water (O/W) creams which are composed of small droplets of oil dispersed in a continuous phase, and water-in-oil (W/O) creams. Oil-in-water creams are more comfortable and Water-in-oil creams are more difficult to handle. Water-in- oil creams also acts as moisturizing agent. The cream containing medicinal substance is called medicated cream. This formulation should be stored in well dried completely filled and well stoppered tubes or other dispensing items in a cool dark place. The syrup should be stored at a temperature not exceeding 25^0C.

Formulation of Anti- fungal cream

The plant drug selection was done on the basis of ancient Ayurvedic text and modern clinical research data which shows that selected plat drug are very effective in controlling the symptom

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of fungal disease in patients with fewer side effect. *Eugenia aromatic* is the top essential oil which demonstrated marked inhibitory effect against hyphal growth and spore formation of *A. niger*(Pawar, Thaker). The antifungal activity of curcumin was evaluated against 23 fungi strains and it's in vitro inhibitory effect on the adhesion of Candida species to human buccal epithelial cells (BEC) was also investigated. The formula used in formation of anti-fungal cream given in Table 2

Table 2: Optimize formula for Anti- fungal cream

S.No	Ingredients	Quantity(100 gm)
1.	*Foeniculum vulgare* seed oil	**10.0 gm**
2.	*Eugenia aromatica* bud oil	**10.0 gm**
3.	Herbal extract (Curcuma longa)	**33.33 gm**
4.	Herbal extract (*Foeniculum vulgare*)	**33.33 gm**
5.	Herbal extract(*Eugenia aromatica*)	**33.33 gm**
i.	Glycoryl Monostearate SE	**30.0 gm**
ii.	Stearic acid	**40.0 gm**
iii.	Sesame oil	**100 gm**
iv.	Caprilic triglycerides	**30.0 gm**
v.	Lanolin anhydrous	**6 gm**
vi.	Coco caprylate	**30.0 gm**
vii.	Triethylamine	**2.0 gm**
viii.	BHT	**1.0 gm**
ix.	Glydant	**2.0 gm**
x.	**D.M water**	**639 gm**

Preparation of cream: Anti fungal creams are o/w emulsion based preparations containing aqueous phase and oil phase. Ingredients of oil phase were mixed together by melting in a china dish on constant stirring. Components of aqueous phase were mixed together and warmed to about same temperature (70-80 ^{0} C) oil phase. Aqueous phase was added to oil phase drop by drop on constant stirring. The preservative propylparaben and methyl paraben was added after cooling.

- Evaluation of Cream
- Determination of Ph value
- Determination of TFM
- Determination of moisture content (LOD)

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- Microbial analysis

RESULTS AND DISCUSSION

Authentication

The seeds of *Foeniculum vulgare*, bud of *Eugenia aromatica* and leaves of*Mentha piperita,*were authenticated by Taxonomic Division of Maharishi Ayurveda Products Pvt Ltd, NSEZ Noida.

PHARMACOGNOSTIC STUDIES

Morphological evaluation: The seeds of *Foeniculum vulgare*, bud of *Eugenia aromatica* and leaves of *Mentha piperita,* showed the following characteristic on morphological examination. The morphological characters of *Foeniculum vulgare* seed given in Table 3. The morphological characters of *Eugenia aromatica* bud given in Table 4.

Table 3: Morphological characters of *Foeniculum vulgare* seed

S. No.	Characters	Description
1.	Color	Greenish yellow
2.	Odour	Aromatic
3.	Taste	Sweet aromatic
4.	Size	Not specific
5.	Shape	Glabrous

Table 4: Morphological characters of *Eugenia aromatica* bud

S. No.	Characters	Description
1.	Color	Reddish brown
2.	Odour	Aromatic, spicy
3.	Taste	Agreeable ,warm
4.	Size	16-21 mm long
5.	Shape	Hypanthium flattened cylindrical

Microscopical evaluation: The microscopical evaluation of seed *Foeniculum vulgare given* in Figure 1 and bud of *Eugenia aromatica* in Figure 2.

Transverse section characters: The seeds of *Foeniculum vulgare*, bud of *Eugenia aromatica* on transverse section showed following characters.

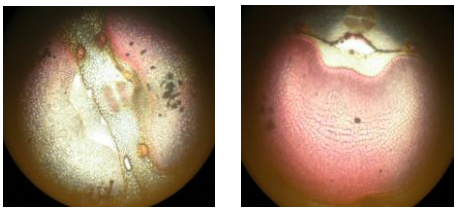

Figure 1: T.S. of seed of *Foeniculum vulgare* showing

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Figure 1 T.S.of seed of *Foeniculum*, *vulgare* showingreticulate parenchymatous cells, oil globules andtesta (seed coat), endosperm, epicarp, endocarp raphe carpophores ,vascular bundle.

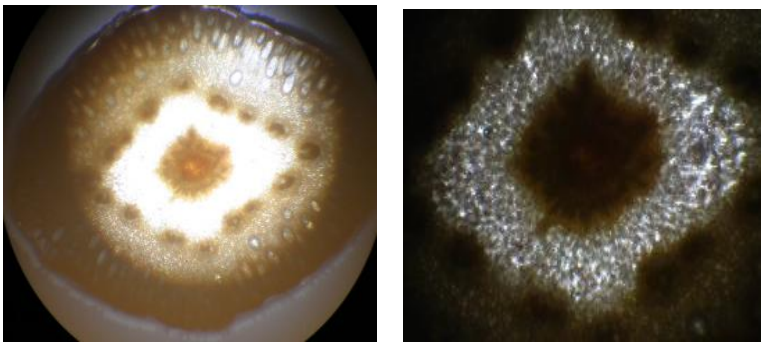

Figure 2: T.S. of bud of *Eugenia aromatica* showing

Figure 2 T.S. of bud of *Eugenia aromatica* showing oil globules, lacuna, columella, vascular bundle.

Quality Control Parameter & Analysis of Raw Materials

Physicochemical Studies

The seeds of *Foeniculum vulgare*, bud of *Eugenia aromatica* evaluated for determination of physiochemical parameters, namely ash value, moisture content and extractive values. The result of all these parameters given in Table 5 and Table 6 respectively.

Table 5: Result of physicochemical parameters of *Foeniculum vulgare* seed

S.No.	Parameter	Specification	Result
1.	Loss on drying at 105^0 C	NMT 9 % w/w	8.22 % w/w
2.	Water soluble extractives	NLT 16 % w/w	20.29 % w/w
3.	Total ash	NMT 9 % w/w	8.32 % w/w
4.	Acid insoluble ash	NMT 0.50 % w/w	0.05 % w/w

Table 6: Result of physicochemical parameters of *Eugenia aromatica* bud

S.No.	Parameter	Specification	Result
1.	Loss on drying at 105^0 C	NMT 25 % w/w	20.15% w/w
2.	Water soluble extractives	NLT 26 % w/w	27.41 % w/w
3.	Total ash	NMT 7 % w/w	6.98 % w/w
4.	Acid insoluble ash	NMT 0.50 % w/w	0.17 % w/w

Heavy Metal Analysis

The rhizome powder of ***Curcuma longa***, bud powder of ***Eugenia aromatica ,*** **seed powder of *Foeniculum vulgare*** and evaluated for determination of heavy metal content by using Atomic Absorption Spectroscopy (AAS). The result of heavy metal analysis given in Table 7 and Table 8 given below.

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Table 7: Result of heavy metal analysis of *Foeniculum vulgare* seed

S. No.	Parameter	Specification	Result
1.	Lead	NMT 3 mg/kg	0.11mg/kg
2.	Arsenic	NMT 1 mg/kg	0.19 mg/kg
3.	Cadmium	NMT 0.5 mg/kg	0.0006 mg/kg
4.	Mercury	NMT 0.1 mg/kg	0.00568mg/kg

Table 8: Result of heavy metal analysis of *Eugenia aromatica* bud

S. No.	Parameter	Specification	Result
1.	Lead	NMT 3 mg/kg	0.21 mg/kg
2.	Arsenic	NMT 1 mg/kg	0.58 mg/kg
3.	Cadmium	NMT 0.5 mg/kg	0.004 mg/kg
4.	Mercury	NMT 0.1 mg/kg	0.00324mg/kg

Pesticide Analysis

The rhizome powder of *Curcuma longa*, bud powder of *Eugenia aromatica,* seed powder of *Foeniculum vulgare* were evaluated for determination of pesticide content by using GC-MS. The result of pesticide analysis given in Table 9 and Table 10 given below:

Table 9: Result of pesticide analysis of *Foeniculum vulgare* seed

S. No.	Parameter	Specification	Result
1.	Alpha and Beta HCH	NMT 10 mcg/kg	.028mcg/kg
2.	Gamma HCH	NMT 10 mcg/kg	.035 mcg/kg
3.	DDT &Metabolites	NMT 50 mcg/kg	.499 mcg/kg

Table 10: Result of pesticide analysis of *Eugenia aromatica* flower bud

S. No.	Parameter	Specification	Result
1.	Alpha and Beta HCH	NMT 10 mcg/kg	0.18 mcg/kg
2.	Gama HCH	NMT 10 mcg/kg	0.056 mcg/kg
3.	DDT &Metabolites	NMT 50 mcg/kg	.112 mcg/kg

Phytochemical Screening

Aqueous extracts over screened for various classes of the phyto constituents. The result of phytochemical screening of aqueous extract given in Table 11 and Table 12.

Table 11: Result of phytochemical screening of aqueous extract of *Foeniculum vulgare* seed

S. No.	Phytoconstituents	Aqueous extract
1.	Volatile oils	+ve
2.	Protein/Amino acid	-ve

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3.	Steroids/ Terpenoids	-ve
4.	Tannin and Phenolic compounds	+ve
5.	Alkaloids	-ve
6.	Glycosides	-ve
7.	Saponins	+ve
8.	Flavonoids	-ve

(+) = Present; (-) = Absent

Table 12: Result of phytochemical screening of aqueous extract of *Eugenia aromatica* bud

S.No.	Phytoconstituents	Aqueous extract
1.	Volatile oils	+ve
2.	Protein/Amino acid	-ve
3.	Steroids/ Terpenoids	-ve
4.	Tannin and Phenolic compounds	+ve
5.	Alkaloids	-ve
6.	Glycosides	+ve
7.	Saponins	-ve
8.	Flavonoids	-ve

(+) = Present; (-) = Absent

Determination of Swelling Index

The seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica* and *rhizome powder of Curcuma longa* were evaluated for determination of swelling index. The result of determination of swelling index given in Table 13.

Table 13: Result of determination swelling index

S.No.	Drug	Swelling index
1.	***Foeniculum vulgare***	1.0
2.	***Eugenia aromatica***	0.5

Determination of Foaming Index

The seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica* were evaluated for determination of foaming index. The result of foaming index given in Table 14 and Table 15.

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Table 14: Result of foaming index of *Foeniculum vulgare* seed

Decoction	1	2	3	4	5	6	7	8	9	10
Water	9	8	7	6	5	4	3	2	1	0
Height	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm
Foaming index	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100

Table 15: Result of foaming index of *Eugenia aromatica* bud

Decoction	1	2	3	4	5	6	7	8	9	10
Water	9	8	7	6	5	4	3	2	1	0
Height	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm	<1cm	1.0cm	1.1cm
Foaming index	<100	<100	<100	<100	<100	<100	<100	<100	>1000	>1000

Fluroscence Analysis

Fluroscence analysis is done to determine the effect of the different reagent on the colour of the powdered drug in the normal day light, short wavelength (254nm) and long wavelength(366nm).Reagents used are distilled water, 1N NaOH in water, 1N NaOH in methanol, 50% nitric acid, 50% hydrochloric acid, sulphuric acid, acetone, concentrate hydrochloric acid, chloroform. The result of fluroscence analysis given in Table 16 and Table 17.

Table 16: Result of fluroscence analysis of *Foeniculum vulgare* (Seed)

S.No.	Reagents	Day light	λ (254nm)	λ (366nm)
1.	Crude powder	Greenish	Greenish	Greenish
2.	Distilled water	Light green	Light green	Greenish
3.	1N NaOH in water	Greenish yellow	Yellow	Green
4.	1N NaOH in methanol	Light yellow	Yellow	Colourless
5.	50% Nitric acid	Light green	Colourless	Green
6.	50% HCl	Light yellow	Black	Green
7.	Sulphuric acid	Dirty brown	Yellow	Black
8.	Acetone	Whitish	Yellow	Yellow
9.	Conc. HCl	Brown	Brownish black	Black
10.	Chloroform	Light yellow	Yellow	Colourless

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Table 17: Result of fluorescence analysis of *Eugenia aromatica*(Flower bud)

S. No.	Reagents	Day light	λ (254nm)	λ (366nm)
1.	Crude powder	Dark brown	Colourless	Black
2.	Distilled water	Light brown	Blackish	Dark greenish
3.	1N NaOH in water	Brown	Black	Green
4.	1N NaOH in methanol	Blackish brown	Black	Black
5.	50% Nitric acid	Black	Brown	Black
6.	50% HCl	Brown	Black	Dark green
7.	Sulphuric acid	Black	Black	Black
8.	Acetone	Dark brown	Black	Black
9.	Conc. HCl	Dirty brown	Black	Black
10.	Chloroform	Brown	Black	Black

Gas Chromatography Analysis of Essential Oils

There are four essential oil used in formulations named like oil of *Foeniculum vulgare* seed*, Eugenia aromatica* bud*,*. The most common method of separating essential oil is distillation. GC analysis of essential oil of *Foeniculum vulgare* seed gives 8 peaks and total area is 2360.308 mv-secs , GC analysis of essential oil of *Eugenia aromatica* bud gives 11 peak and total area is 2507.885 mv-secs .

Microbial Load Determination

Aerobic bacteria and fungi are normally present in plants material and may be increased due to faulty growth, harvesting, storage or processing. Pathogenic organism including *Enterobactor, Enterococcous, Clostridium, Pseudomonas, Shigella and Streptococcus* has been shown to contaminate herbal ingredients. The result of microbial analysis given in Table 18 and Table 19

Table 18: Result of microbial analysis of *Foeniculum vulgare* seed

S. No.	Parameters	Specification	Results
1.	Total aerobic count	NMT 1250000 CFU per gm	5×10^4
2.	*Enterobacteriaceae*(Coliform)	NMT 1000 CFU per gm	NAD
3.	*E.coli*	NMT 10 CFU per gm	NAD
4.	*Salmonella sp.*	Absent	Absent
5.	*Staphylococcus aureus*	NMT 100 CFU per gm	NAD
6.	Yeasts	NMT 100 CFU per gm	NAD
7.	Moulds	NMT 10000 CFU per gm	600
8.	*Bacillus cereus*	NMT 1000 CFU per gm	NAD
9.	*Pseudomonas aeruginosa*	NMT 100 CFU per gm	NAD

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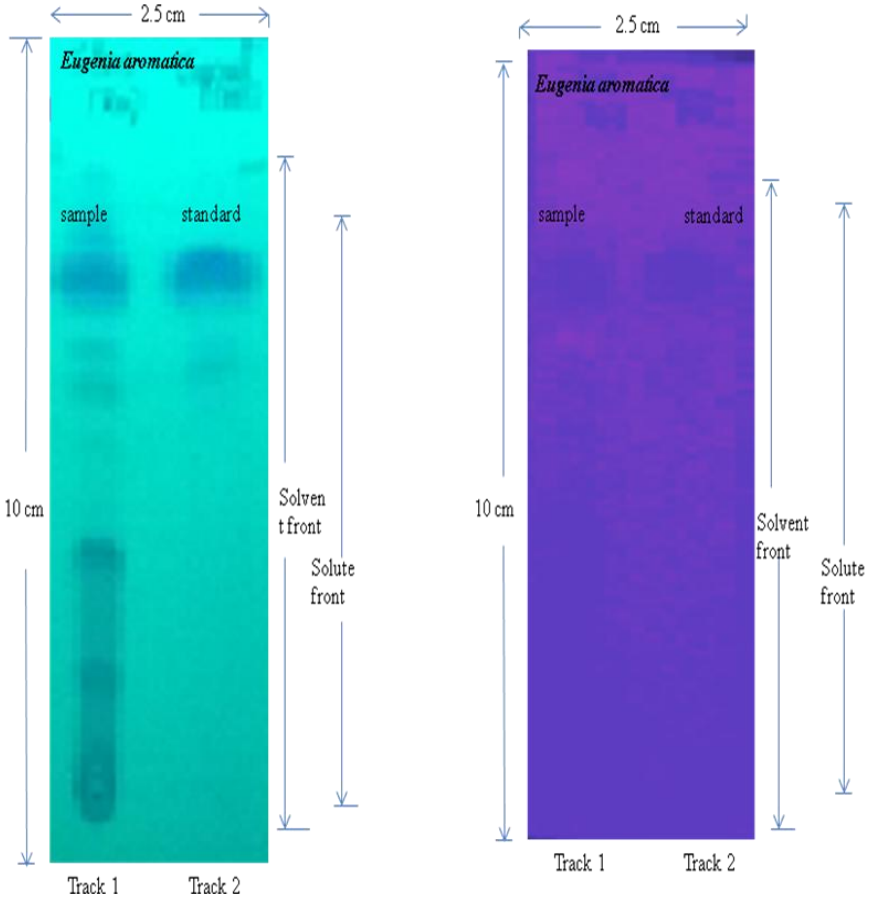
Table 19: Result of microbial analysis of *Eugenia aromatica* flower bud

S.No.	Parameters	Specification	Results
1.	Total aerobic count	NMT 1250000 CFU per gm	60000
2.	*Enterobacteriaceae*(Coliform)	NMT 1000 CFU per gm	NAD
3.	*E.coli*	NMT 10 CFU per gm	NAD
4.	*Salmonella sp.*	Absent	Absent
5.	*Staphylococcus aureus*	NMT 100 CFU per gm	NAD
6.	Yeasts	NMT 100 CFU per gm	NAD
7.	Moulds	NMT 10000 CFU per gm	1000
8.	*Bacillus cereus*	NMT 1000 CFU per gm	NAD
9.	*Pseudomonas aeruginosa*	NMT 100 CFU per gm	NAD

TLC and HPTLC analysis: TLC of *Eugenia aromatica* bud given in figure 3.

Identification of Eugenol in *Eugenia aromatica* bud



Track 1 = Test solution

Track 2 = Standard solution of Eugenol

Figure 3: Thin layer chromatography of flower bud extracts of *Eugenia aromatica*.

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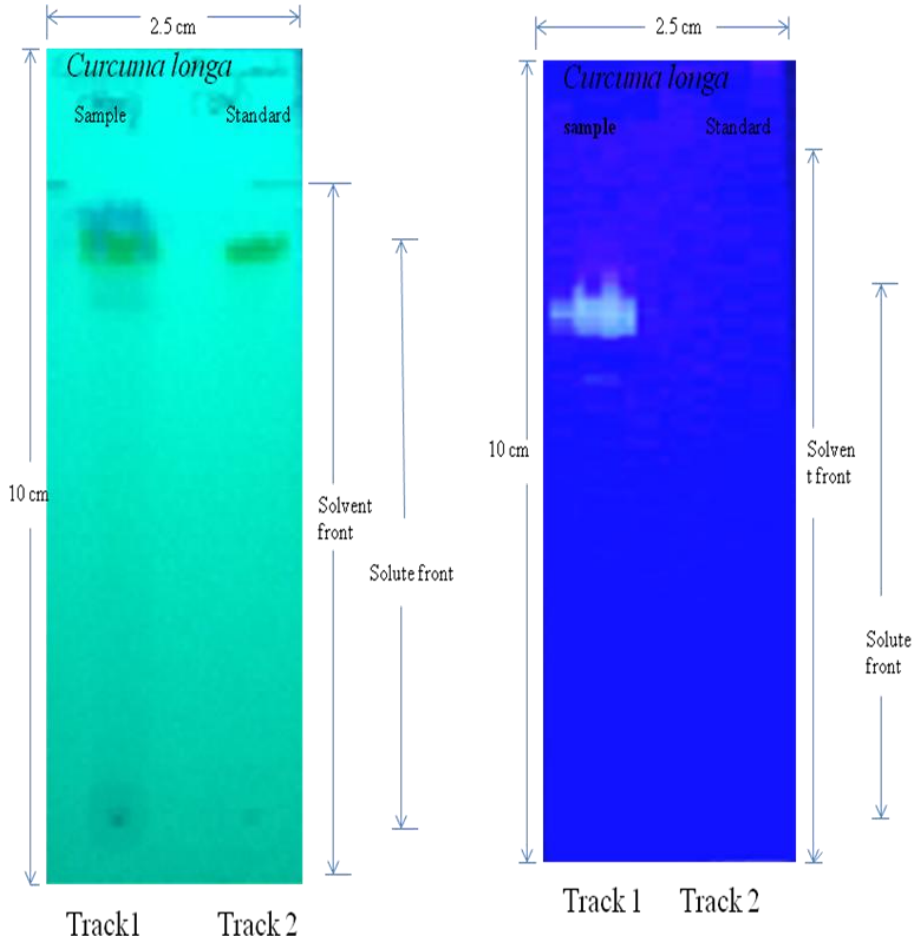
For identification of eugenol in flower bud extracts of *Eugenia aromatic* samples TLC analysis was performed by using standard solution of Eugenol. The solvent systems consisting Toulene: ethyl acetate (9.9 : 0.1). Track 1 showed four spots and Track 2 showed two spots, Rf values of Track T_1 was 0.41, 0.58, 0.64 ,0.82, and Track T_2 was 0.64, 0.82.

Identification of Curcumin in *Curcuma longa* rhizome

For identification of curcumin in rhizome extracts of ***Curcuma longa***samples TLC & HPTLC analysis was performed by using standard solution of curcumin. The solvent systems consisting Chloroform : ethanol : glacial acetic acid (94 : 5 : 1)Track 1 showed two spot and Track 2 showed one spots, Rf values of Track T_1 was 0.80, 0.87 and Track T_2 was 0.80. The detailed HPTLC graph was given in annexure. The result of TLC of Curcuma longa given in figure 4.



Track 1 = Test solution

Track 2 = Standard solution of Curcumin

Figure 4: Thin layer chromatography of rhizome extracts of *Curcuma longa*

Evaluation of Cream: The organoleptic evaluation of Cream given in Table 20 and physicochemical evaluation of Cream given in Table 21.

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Table 20: Organoleptic evaluation of Cream

S.No.	Parameter	Cream	Ointment
1.	Colour	Off white	Light green
2.	Odour	Aromatic	Aromatic
3.	Appearance	Semi solid	Semi solid

Table 21: Physicochemical evaluation of Cream

S.No.	Parameter	Cream	Ointment
1.	Total fatty matter	23.39 %w/w	22.85%w/w
2.	pH	7.21	4.74
3.	Total solid	26.49 %w/w	24.38%w/w

DISCUSSIONS

A detailed pharmacognostic studies including morphology and microscopy (Fresh sample and powder) were carried out. Microscopic studies (T.S.) of ***Foeniculum vulgare*** seed showed the presence of testa (seed coat), endosperm, epicarp, reticulate parenchymatous cells, oil globules and raphe, carpophores ,vascular bundle, endocarp. Microscopic studies (T.S.) of ***Eugenia aromatica*** bud showed the presence of oil globules, lacuna, columella, vascular bundle. it was found that seeds of ***Foeniculum vulgare*** showed total ash value 8.32%, acid in soluble ash 0.05%,water soluble extractives 20.29% and loss on drying 8.22%.Bud of ***Eugenia aromatica*** showed total ash value 6.98%, acid insoluble ash 0.17%,water soluble extractives 27.41% andloss on drying 20.15%. The water extraction was carried andwas subjected to preliminary phytochemical tests to detect the presence of various classes of chemical constituents. From these qualitative chemical tests the seeds of ***Foeniculum vulgare*** was found to possess tannins and phenolic compounds, saponins mainly , the buds of ***Eugenia aromatica*** was found to have tannins and phenolic compounds, glycosides The seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica* were evaluated for determination of swelling index and swelling index of seed powder of *Foeniculum vulgare* was 1.0, bud powder of *Eugenia aromatica* was 0. The seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica*, foaming index of the seed powder of *Foeniculum vulgare* of dilution from 1 to 10 was found less than 100 , bud powder of *Eugenia aromatica* of dilution from 1 to 8 was found less than 100 and the dilution 9 to 10 was found greater than 1000. The fluroscence analysis of seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica* .Herbal ingredients particularly those with high starch content may be prone to increase microbial growth. It is not uncommon for herbal ingredients to have aerobic bacteria present at 10^2 - 10^8 colony forming unit per grams. Pathogenic organism including *Enterobactor, Enterococcous, Clostridium, Pseudomonas, Shigella and Streptococcous* has been shown to contaminate herbal ingredients. The microbial load in seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica.* The levels of pesticide residues α, β and γ HCH, DDT and DDE were analyzed in seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica.* The levels of heavy metals Pb, As, Cd and Hg were analyzed in the seed powder of *Foeniculum vulgare*, bud powder of *Eugenia aromatica* using Atomic absorption spectroscopy. Essential oil is extracted and GC analysis of essential oil is done, number of peak obtained from *Foeniculum vulgare* seed is 8 peaks and total area is 2360.308 mv-secs , *Eugenia aromatica* bud gives 11 peak. Anti-fungal cream was prepared by using different ingredient like *Foeniculum vulgare*, *Eugenia aromatica* and *Curcuma longa.* Quality analysis like physicochemical parameters, heavy metal, pesticide and microbial load analysis were carried out for all crude herbs used in the formulation and almost the all parameters were

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found to be within the permissible limits. The Anti-fungal cream was evaluated for their organoleptic properties (colour, odour, and appearance), physicochemical evaluation (total ash, loss on drying, pH, total fatty matter (TFM)), was carried out and were found to be within permissible limits. The pharmacological studies of extract of plants show activity i.e. Anti–fungal during investigation compared with standard of both.

CONCLUSION

The herbal crude drugs obtained from natural sources having lots of benefits in human's life because it can be used as single herb or either in combinations of some drugs with synergistic effects. These drugs are used from ancient time for betterment of health of humans and animal too. There are lots of traditional formulations used till. Herbal products are used as either raw or in formulation form like tablet, capsule, syrup, cream and ointment etc. The different parts of plants anti- fungal activity were taken up for the present study and investigated for the phytochemical screening and used for the formulation of anti- fungal cream. Present study deals with formulation, Standardization of cream made from alcoholic extract and essential oil of different plants.

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