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Formulation and evaluation of herbal face wash for antimicrobial activity

Jilsha .G*, Anusha N D*, Jasna Varghese*,Sreeja K *,Sruthy S.P*

* (Department of Pharmaceutics, Sanjo College of Pharmaceutical Studies, Vellapara, Palakkad, Kerala 678702, India.)

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Abstract:

This research employed an antimicrobial herbal facewash. It's simpler to believe that natural remedies are safer with synthetic subjects than with fewer side effects. *Myristica fragrans* is an annual spice belonging to the family *Myristicaceae*. It is grown worldwide for flavouring, essential oil, and traditional medicine. Nutmeg (Myristica fragrans) extracts may be antifungal and antibacterial. Acetone extract in Myristica fragrans seed extract was tested against Staphylococcus aureus and Escherichia coli. These bacterial species were tested against Myristica fragrans seed extracts, ciprofloxacin, and acetone. Antimicrobial evaluation was done using Muller Hinton agar well plate diffusion. Results showed that acetone extracts only had antibacterial activity against gram-positive bacteria: the diameter of the inhibition zone against Staph. aureus was 22mm, which is comparable to the standard inhibition zone (25mm), while formulation (Myristica fragrans facewash) showed 20mm. S.aureus colonises one-third to one-half of acne patients, even though propionic bacterium acnes is a major cause of acne. Nutmeg (Myristica fragrans) facewash is anti-acne. The formulation was tested for colour, appearance, stability, Ph, spreadability, foamability, washability, greasiness, and homogenicity. We suggest making pure herbal formulations with a harmless artificial ingredient.

Keywords — Mysritica frangrans, acetone extract, antibacterial activity, physical evaluation

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I. INTRODUCTION

To avoid over-drying the skin on your face, use a face wash. Cleanser is another name for face wash. All skin types benefited similarly from using the same face wash. Facial cleansers are great for restoring moisture to dry skin and washing away excess oil and dirt. To remove grime, oil, pollution,

etc., from your face, you can use either a face wash or a facial cleanser. Use a cleanser to remove dirt, oil, and makeup from your face. These contaminants are oil-soluble. A face wash can help get rid of them, but it may not be completely effective. Facial skin is the most sensitive, and regular soaps can strip it of moisture. A face wash is a gentle cleanser that effectively removes dirt and oil from the skin without irritating the delicate horny layer beneath. So that the skin always appears vibrant and fresh. Face washes can serve many functions, including cleansing, reducing the appearance of fine lines and wrinkles, combating acne, hydrating dry skin, and even evening out skin tone. It is hypothesised that skin whitening agents work by reducing the amount of melanin in the skin by inhibiting melanin production in melanocytes. Propanediol, Evodia arbutin, kojic acid, vitamin C, and its derivatives are just some of the agents used in whitening cosmetics because of their ability to inhibit melanin production without being too toxic to melanocytes.

In general, a face wash is suitable for all skin types; however, different products are now available on the market that are formulated to suit different skin types. For instance, an oily skin face wash is designed for people with oily skin and does not contain oils and leaves a thin oily film on the skin. The various types of face washes available on the market are as follows.

1. Oily skin face wash
2. Dry skin face wash
3. Normal skin face wash

A gel is a jelly-like solid material with properties ranging from soft and weak to hard and tough. Gels are defined as a significantly dilute cross-linked system with no flow in the steady-state. Gels are mostly liquid by weight, but they behave like solids due to a three-dimensional cross-linked network within the liquid. The cross linking within the fluid gives a gel its structure (hardness) and helps the adhesive stick (tack). Gels are thus a dispersion of liquid molecules within a solid, where the solid is the continuous phase and the liquid is the discontinuous phase. By clipping from gelatin, 19th century Scottish chemist Thomas Graham coined the term gel.

PLANT PROFILE

Biological source

Nutmeg (Myristica fragrans) is an evergreen tree belonging to family Myristicaceae. It is occasionally called the nutmeg family, due to its well-known member, Myristica fragrans, the source of the spices nutmeg and mace. The genus Myristica consists of about 150 species spread in the western Pacific and Asia.

Fig 1: Nutmeg (*Myristica fragrans*)

Myristica fragrans is the most profitable species in the genus Myristica. Other Myristica species grown in tropical regions besides M. fragrans include M. malabarica (Indian), M. argentea, and M. fatua. Although they have a similar appearance to M. fragrans, they have a less intense taste, aroma, and cost less.

Freshly ground nutmeg has an intensely perfumed aroma that is sweet, nutty, spicy, and slightly minty or eucalyptus-like, similar to cardamom or pine profiles. Nutmeg tastes best when freshly ground from whole seeds.

Scientific classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Magnoliales
Family: Myristicaceae
Genus: Myristica (N. Rehman et al)

Chemical Composition

Alkyl benzene derivatives (myristicin, elemicin, safrole), myristic acid, alpha-pinene, terpenes, beta-pinene, and trimyristin are the primary chemical components of Myristica fragrans. About ten percent of nutmeg is essential oil, which is made up of various terpene hydrocarbons like sabinene and pinene as well as myrcene, phellandrene, camphene, limonene, terpinene, myrcene, pcymene, and limonene (Jaiswal P et al). Nutmeg also produces nutmeg butter, a semisolid reddish-brown fat with a nutmeg aroma that contains 25 to 40% fixed oil. Trimyristin, oleic acid, linoleic acid, and resinous material are all components of nutmeg butter. The nutmeg butter fixed oil is applied topically for sprains and rheumatism, and it is also used in perfumes. The predominant glycoside with anxiogenic activity is trimyristin. Mace oil (up to 12% of the spice) has the same aroma components as mace itself, but its total fraction of terpenoids is nearly 90% higher. Non-volatile dimers of phenylpropanoid constituents of the essential oil, such as dehydrodi-isoeugenol, are found in both nutmeg and mace in the form of lignans (diarylpropanoids). Anxiogenic activity is provided primarily by the glycoside trimyristin.

Properties

Antimicrobial Activity

Essential oils from edible plants are an attractive alternative method for controlling food and feed fungi because, in hypotheses, they should not be toxic to humans and could potentially replace toxic synthetic fungicides. This makes the use of essential oils from edible plants an attractive method. The radial growth of Colletotrichum gloeosporoides, Colletotrichum musae, Fusarium oxysporum, Fusarium semitectum, Aspergillus niger, and Aspergillus glaucus was inhibited by nutmeg essential oil. Three antifungal lignans including erythroaustrobailignan-6 , meso-dihydroguaiaretic acid and nectandrin-B were isolated from the methanol extract of M. fragrans seeds and showed activities against fungal strains such as Alternaria alternata, Colletotrichum coccodes, Colletotrichum gloeosporioides, Magnaporthe grisea, Agrobacterium tumefaciens, Acidovorax konjaci and Burkholderia glumae . According to various reports, the methanol extract of M.fragrans arils (mace) possesses potent antifungal properties, and it is effective against Candida albicans as well as A. niger. Dihydroguaiaretic acid, which comes from mace, has also been shown to have anti-H. pylori activities. Malabaricone B and malabaricone C, two antimicrobial resorcinols found in M. fragransnas, also demonstrated potent antifungal and antibacterial activities (Naeem et al., 2016).

- Anti-inflammatory Activity

Nutmeg and mace oils relieve sprains, rheumatism, and paralysis. Petroleum ether extract resembled non-steroidal anti-inflammatory drugs. In rats, chloroform extract prevented carrageenan-induced oedema. Methanol extract had lasting anti-inflammatory activity. Myristicin in nutmeg appears to cause anti-inflammatory effects. Nutmeg essential oil contains small amounts of myristicin, a phenylpropene.

- Neuropharmacological Properties

Teenagers frequently substitute nutmeg for marijuana because it is less expensive. Nutmeg's analgesic properties have been used for centuries, making it a popular alternative to morphine and other narcotics.

- Anticonvulsant Properties

Nutmeg essential oil showed significant anticonvulsant activity against electro shock-induced hind limb tonic extension. It inhibited pentylene-tetrazole-induced tonic seizures dose-dependently. It delayed strychnine-induced hind-limb tonic extensor jerks. At low doses, it was anticonvulsant, but at higher doses, pentylene-tetrazole and bicuculline caused clonic seizures.

- Antioxidant Activity

Nutmeg's antioxidant activity arises from β-caryophyllene and eugenol, which promote the activities of superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, and glutamine transferase enzymes. Antioxidants are catechol-like compounds like caffeic acid. Nutmeg seed antioxidants are ligan derivatives. After absorption, lignans and their glycosides are metabolised to produce catechol-structured biologically active compounds that give nutmeg seeds their antioxidant properties.

- For pimple and skin diseases

Pimples and acne scars are treated with nutmeg paste. Nutmeg powder mixed with sandalwood, Kumkumadi taila, olive oil, and others can be used on the face. Nutmeg evens out facial pigmentation and discoloration. Dark spots, pigmentation, and freckles are caused by sun exposure, hormonal changes, age, medications, and skin conditions.

- Antidiabetic activity

Macelignan in the seeds activated peroxisome proliferator receptor and reduced endoplasmic reticulum stress, improving insulin sensitivity and lipid metabolic disorders, suggesting it is a type 2 diabetes treatment.

- Dental Care

Nutmeg eugenol relieves toothache and prevents bad breath . This wonder spice prevents dental plaque by inhibiting mouth bacteria. Using nutmeg powder, brush teeth. Brush and rinse with water. Plaque buildup is prevented, whitening teeth.

- Spasmolytic

Nutmeg is helpful in clearing up the congestion resulting from cold and thus, is widely used in cough syrups. It's even helpful in aroma therapy.

- Other Uses

Nutmeg has culinary and medicinal uses. Cooking with nutmeg dates back millennia. In soups, meats, and vegetables. Nutmeg essential oil makes camphor, plasticizers, bases, solvents, perfume, and synthetic pine oil. Nutmeg has many antioxidant, health-promoting, and disease-preventing compounds. Traditional medicine uses nutmeg's active compounds as anti-depressants, anti-fungals, digestives, aphrodisiacs, and carminatives. Elemicin and myristicin in this spice stimulate and calm the brain. Eugenol relieves toothache in dentistry. Local massage with oil relieves rheumatic and joint pain. Gastritis, nausea, and digestion disorders have been treated with honey-prepared decoction. Mace lignin inhibits melanin biosynthesis and can whiten skin, according to recent research.

II. MATERIALS AND METHODS

1. Phytochemical screening of phytoconstituents

Plants contain many bioactive chemical substances that produce definite physiological and biochemical action in the human body. These bioactive constituents are alkaloids, tannin, flavonoids, phenolic compounds, etc. The acetone extract was considered suitable for all test. All tests were performed on the blank *(Mohammad Amzad Hossian 2013)*

(a) Test for flavonoids

1. **Alkaline reagent test -** To the different sample solutions, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour, which turned colourless after addition of few drops of dilute hydrochloric acid, indicated the presence of flavonoids.
2. **Shinoda test -** To the different sample solution, a few magnesium turnings were added followed by a few drops of concentrated hydrochloric acid. Appearance of a crimson red colour indicated the presence of flavonoids.

(b) Test for steroids

1. **Salkowski reaction -** To the different sample solution, chloroform was added followed by concentrated sulphuric acid along the side of the tube. A red-brown colouration indicated the presence of steroids.

(c) Test for alkaloids

The samples were dissolved separately in 1%HCL. One milliliter of the solution was taken and few drops of the following reagents were added and various tests performed.

1. **Dragendroff's test** - One milliliter of Dragendroff's reagent was added to the different sample solutions. Formation of reddish-brown precipitate indicated the presence of alkaloids.
2. **Mayer's test** - One milliliter of Mayer's reagent was added to the different sample solutions. Formation of cream colour precipitate indicated the presence of alkaloids.
3. **Hager's test** - One milliliter of Hager's reagent was added to the different sample solutions. Formation of yellow colour precipitate indicated the presence of alkaloids.
4. **Wagner's test** - One milliliter of Wagner's reagent was added to the different sample solutions. Formation of reddish-brown precipitate indicated the presence of alkaloids.

(d) Test for tannins

1. **Ferric chloride test** - Different sample solutions were treated with ferric chloride solution; appearance of blue and green colours indicated the presence of hydrolysable and condensed tannins.
2. **Lead acetate test** - Small quantity of the sample solution was dissolved in distilled water and 10% lead acetate solution was added to them, a white precipitate indicated the presence of phenolics and tannins.

(e) Test for carbohydrates

1. **Molisch's test** - The solution to be tested are mixed with a small amount of molisch's reagent (alpha-naphthol dissolved in ethanol) in a test tube and mixed well. A small amount of concentrated sulphuric acid was slowly added down the sides of the sloping test tube. Appearance of purple ring at the junction indicated the presence of carbohydrates.
2. **Felling's test (for reducing sugar)** - The sample solution are mixed with a small amount of Fehling's solution and heated. Appearance of a red precipitate indicated the presence of a reducing sugar.

(f) Test for saponin glycosides

1. **Froth formation test** - A small quantity of the samples was diluted with 20ml of distilled water and shaken vigorously; formation of 1cm layer of form which is stable for 10 min indicated the presence of saponins.

(g) Test for amino acid

1. **Ninhydrin test** - A solution of ninhydrin in ethanol is added to the sample solutions. Appearance of a purple colour indicated the presence of amino acids.

(h) Test for cardiac glycosides

1. **Keller-Killiani test** - Glacial acetic acid (0.4ml) and a few drops of 5% ferric chloride solution are added to the sample solutions. Concentrated sulphuric acid (0.5ml) is added

along the side of the test tube carefully. The formation of blue colour in the acetic acid layer confirmed the presence of cardiac glycosides.

(i) Test for Anthraquinone glycosides

1. **Hydroxyanthraquinone test -** To 1ml of the samples, a few drops of 10% potassium hydroxide solution were added. The formation of a red colour confirmed the presence of anthrequinone glycosides.

(j) Test for proteins

1. **Biuret test -** To 2ml of the sample solutions, 5 drops of 1%copper sulphate solution are added followed by 2ml of 10% NAOH. The content are mixed thoroughly. Formation of a purple or violet colour confirmed the presence of proteins

2. Preparation of herbal face wash

Nutmeg is the seed or ground spice of several species of the genus Myristica. Myristica fragrans (Myristicae), native to the Moluccas (or Spice Islands) of *Indonesia.*

Fig 2: Nutmeg seed and powder

Fig 3: Nutmeg seed and powder

(a) Collection of seed

The main ingredient, which is a natural material used in the present study i.e; nutmeg was purchased from local market (palakkad town, Kerala), in a form of fresh seed.

(b) Chemicals used

Name of ingredients	Property
Extract of nutmeg	Antibacterial
Carbopol 940	Gelling agent
Methyl paraben	Preservative
Propyl paraben	Preservative
Triethanolamine	Neutralizer
Propylene glycol	Humectant
Sodium lauryl sulphate	Foaming agent
Distilled water	Vehicle

Table 1: List of chemicals used and their property

(c) Instruments used

Instruments used for the whole process includes, PH meter, petri dish, magnetic stirrer, glass rod, glass slide, conical flask, beaker, iodine flask, funnel.

(d) Preparation of extract

Nutmeg seed extract was prepared with the help of acetone maceration and further evaporation of the solvent (acetone). Dried and powdered nutmeg seed (30gm) is allowed for maceration in acetone (250ml) in a stoppered iodine flask for 24 hours. It is then filtered into a conical flask with help of filter paper. The filtrate is collected and poured into a large petridish, and kept aside for effective separation of solvent without any contamination. The final residue (powder) is then collected.

Fig 4: Nutmeg seed powder after extraction

(e) Formulation of Herbal facewash

Ingredients	**Amount**
Drug extract	0.4 gm
Carbapol-940	0.15 gm
Methyl paraben	0.01gm
Propyl paraben	0.1gm
Triethanolamine	0.2gm
Propylene glycol	0.25gm
Sodium Lauryl sulphate	0.25gm
Water	qs (ml)

Table 2: List of ingredients used in formulation of herbal anti–microbial facewash.

Procedure

1) Carbapol-940 was dispersed in distilled water and the beaker was kept aside to swell the carbapol-940 to form gel.

2) Take distilled water and required quantity of methyl paraben and propyl paraben. Then dissolved by heating on water bath, solution was cooled and propylene glycol 400 and sodium lauryl sulphate were added.

3) Further required quantity of extract was mixed to the above mixture and add this solution into the carbapol-940 gel with continuous stirring and triethanolamine was added dropwise to the formulation for adjustment of required skin pH and to obtain the gel at required consistency.

Fig 5: Final product

Nutmeg (*Myristica fragrans*) facewash

III. EVALUATION PARAMETER FOR HERBAL FACEWASH FORMULATION

The prepared formulation evaluated for following tests.

(a) PHYSICAL EVALUATION:

The physical appearance of the formulation was checked visually *(Koli et al*)

Colour:

The colour of the formulations was checked out against white background *(Koli et al).*

Odour:

The odour of the face washes were checked by manually.

Consistency:

The consistency was checked by applying on skin.

Washability:

Formulations were applied on the skin then easily remove by washing with water were checked manually *(Koli et al).*

pH:

pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant temperature. Instrument was calibrated before use with standard buffer solutions at pH 4 and 7. (*Mane P K.)*

Viscosity:

The viscosity of face wash was determined by using Brookfield Viscometer. The values obtained for sample is noted.

Greasiness:

The greasiness was assessed by the application onto the skin.

Homogenecity:

Homogenecity was tested by visual inspection after allowing them to set in a container. They are evaluated for their appearance and presence of aggregates *(Wira Noviana Suhery et al.,)*

Grittiness:

The product was checked for the presence of any gritty particles by applying it on the skin.

Extrudability:

Extrudability is defined as the weight in grams required for extruding 0.5 cm long ribbon of formulation in 10 seconds. The gel formulation was filled in a standard-capped collapsible aluminium tubes and sealed by crimping to the end. The tubes were placed between two slides and were clamped. 500 g weight was placed over the slides, and then the cap was removed. The length of the ribbon of the formulation that came out in 10 seconds was recorded.

Spreadability:

A total of 1 g of preparation is placed on a glass with size 20x20cm and covered with another glass. Then a weight is placed on it until it reaches a weight of 50g and its diameter is measured after 1 minute. This test aims to determine the speed at which the gels spreads on the skin on its application. The dispersion requirements are 5-7cm.

Foamability:

Small amount of gel was taken in a test tube containing water. Initial volume was noted, shaken for 10 times and the final volume was noted. Foamability was also analysed by applying onto skin with contact with water.

Stability testing of the formulation:

Stability Testing was done at various temperatures of 10˚C, 20˚C, 30˚C, 40˚C, 50˚C, 60˚C.The visual testing was done at each temperature. The formulation was found to be stable and good till 40˚C.The formulation was found to be unstable at 50˚C and 60˚C. (*Sweta V Kulkarni et al.,)*

(b) ANTIMICROBIAL EVALUATION

The minimum inhibition concentration determines the lowest concentration of an antimicrobial agent that prevents the visible growth of the microorganisms. Formulation was tested for antibacterial activity against test organism *Staphylococcus aureus* and *E. coli* using Agar well plate method. Staphylococcus aureus is an aerobic-bacteria and were obtained from microbial type culture collection centre.

Microbial Organisms: Gram-positive bacteria (*S.aureus*) and Gram-negative bacteria (*E. coli*) were used throughout this study. Both Gram–positive and Gram-negative strains were cultured using nutrient broth.

Medium: Muller-Hinton agar

Method: Agar well diffusion method

Standard: Ciprofloxacin

Control: Acetone

Concentration of extract: 200 microgram/ml

PROCEDURE

Agar well diffusion method

1. The bacteria *Staphylococcus aureus* and *E. coli* was inoculated by swabbing on the surface of Muller Hinton agar media plate.
2. Wells of 6 -8mm in diameter was performed in the MHA media and each well filled with 100 microlitre of extract, formulation, control, standard respectively.
3. The plates were kept in laminar air flow for 30 minutes for proper diffusion of the gel and thereafter incubated for 24 hrs. The radius for the zone of inhibition was measured and compared against standard (ciprofloxacin) and recorded.

IV. REPORT AND DISCUSSIONS

1 PHYTOCHEMICAL SCREENING

All results of the phytochemical investigations are shown in table.

CHEMICAL TEST	ACETONE EXTRACT
Test for flavonoids	
Alkaline reagent test	+
Shinoda test	+
Test for steroids	
Salkowski reaction	-
Test for alkaloids	
Dragendroffs test	+
Mayer's test	+

Hager's test	+
Wagner's test	+
Test for tannins	
Ferric chloride test	-
Lead acetate test	-
Test for carbohydrates	
Molisch's test	+
Test for saponin glycosides	
Froth formation test	+
Test for amino acid	+
Test for cardiac glycosides	
Keller-Killiani test	+
Test for Anthraquinone glycosides	
Hydroxyanthraquinone test	+
Test for proteins	
Biuret test	+

Table 3: Results of phytochemical screening

. **Fig 11: Phytochemical screening**

II. EVALUATION OF FORMULATION

a) Physical evaluation

PARAMETER	FORMULATION
Colour	Orange colour
Consistency	Semisolid
Wash ability	Washable
Foamability	Foamable
pH	6.5
Stability test	Stable until 40°C
Spreadability	d = 2.9 cm S = 6.6cm^2
Irritation test	Non irritant
Grittiness	Nil

Table 4:Results of physical evaluation

Fig 6: Colour- orange

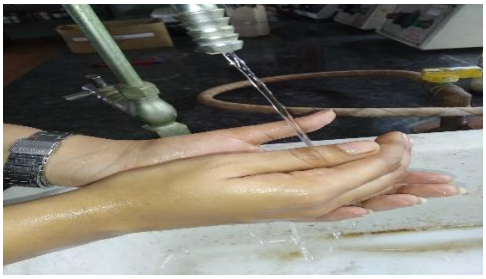

Fig 7: Washability - washable

Fig 8: Fombability-foamable Ph – 6.5

Fig 9: Spreadability-6.6cm

(b) Antimicrobial evaluation

SAMPLES	ZONE OF INHIBITION (mm)	
	Staphylococcus aureus	E. coli
Ciprofloxacin	25	25
Herbal extract	22	10
Formulation	20	8
Control	0	0

Table 5: Results of antimicrobial evaluation

Fig 10: Antibacterial activity of nutmeg extract against S. aureus and E. coli

Fig 11: Antibacterial activity of nutmegfacewash against S. aureus and E. coli

V. CONCLUSION

Natural remedies are preferred over synthetic ones because they have fewer side effects. Herbal formulations are in demand worldwide. The herbal gel face wash with nutmeg acetone extract is a great starting point (Myristica fragrans). This study found that the single herbal formulation with acetone extract of nutmeg had better antibacterial, anti-hyperpigmentation, antioxidant, anti-aging, and other properties when used on skin.

Nutmeg facewash has the most antibacterial activity against S. aureus compared to E. coli in our study. Nutmeg facewash had good appearance, spreadability, washability, foamability, stability, consistency, and Ph.

Nutmeg is a potent natural antimicrobial. Nutmeg is natural and less likely to cause pathogenic microorganism resistance. Gel facewash stays on until washed off. Thus, gel and nutmeg extract make the formulation more effective than synthetic. Even though formulation needs invitro-studies for efficacy.

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