DEVELOPMENT AND EVALUATION OF HERBAL ANTI-ODOUR FOOT SPRAY

Lemon fresh foot spray

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Abstract: These days, foot odor is a problem that affects everyone, which is a highly unbalanced feeling for humanity. So, a herbal anti-odor foot spray is created to solve that issue. Problem is caused by wearing shoes continuously throughout the day, which leads to foot perspiration. Most often, this issue arises in the summer. In addition, some microbial development might be visible while walking during the monsoon. Therefore, an experiment is conducted on this topic taking into account these two issues, and three formulations of 0.5%, 1%, and 1.5% are created. Because of its effective antibacterial properties and high compatibility to be used as cosmetic, ethanolic extract of betel leaf is utilized in that formulation. Lemongrass oil is also included to help remove any foul odors. The deodorant formulation is in spray form since it can be applied quickly. It is simple to apply to shocks or the foot as well. After application, this solution left a thin, non-greasy layer on the skin's surface that dried quickly. The foot spray has a pH between 5.10 and 5.92, which is in the range of skin pH and is not irritating to the skin. The highest bacterial inhibition zone in formula 3 (F3) with a concentration of 1.5% Betel ethanol extract and lemongrass oil has an average inhibition zone diameter of 3.5 cm.

Index Terms – Lemongrass, betel leaf, foot spray, antibacterial, anti-odour

I. INTRODUCTION

The foot is a part of body and, nowadays widespread problem is regarding foot odour. Foot odour come from foot due to some growth of micro-organism that happens due to excessive sweating and during the monsoon, as moisture amount is high. Individual belonging from all age group suffers from this problem. Due to that, the anti-odor foot spray has been launched in market and is manufactured by multiple companies. Gram positive bacteria are the main ones that are causing foot odour, so antibacterial foot spray is there along with additional property of fragrance. Nowadays, people are moving towards using an herbal product as they have fewer side-effects. Considering this factor, herbal anti-odour foot spray is formulated where a betel leaf (antibacterial) and lemongrass (fragrance) are active ingredients.

Herbs are natural products found in treatment of all diseases and skin problems owing to their high medicinal value, cost-effective, availability, compatibility. Betel leaf has strong aromatic flavor. Apart from that, good antibacterial property and other uses are also there. Lemongrass oil has good fragrance and energizes tired feet. Stimulating agent menthol which will add spring to your feet. These three are the main ingredients in formulation of foot spray.
II. MATERIALS

2.1 Betel leaf

2.1.1 Scientific classification:
- Kingdom: plantae
- Order: piperales
- Family: piperaceae
- Genus: piper
- Species: Piper betel

2.1.2 Geographical source: assam, Andhra Pradesh, Bihar, Odissa, …

2.1.3 Chemical constituents:

[Table 1: chemical constituent of betel leaf]

<table>
<thead>
<tr>
<th>CHEMICAL FAMILY</th>
<th>COMPONENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MONOTERPENES</td>
<td>TRANS-SABINENE HYDRATE</td>
</tr>
<tr>
<td>SESQUITERPENES</td>
<td>CARYOPHYLLENE, CADINENE, HUMULENE, murolene</td>
</tr>
<tr>
<td>ALCOHOLS</td>
<td>Cadinol,</td>
</tr>
<tr>
<td>ESTER</td>
<td>Methyl salicylate, chavibetol acetate</td>
</tr>
<tr>
<td>ALDEHYDES</td>
<td>n-decanal</td>
</tr>
<tr>
<td>PHENOLS</td>
<td>Chavicol, eugenol, methyl eugenol</td>
</tr>
</tbody>
</table>

2.1.4 Benefits:
- Betel leaves contain polyphenols, particularly chavicol which contains antibacterial properties that provide protection from pathogens.
- Anti-inflammatory properties – Reduce the join pain and Discomfort, common symptoms of common diseases known as RA and osteoporosis.
- Boost metabolism
- Having a good smell, used as fragrance ingredient in many cosmetics
- Manages blood sugar level – used in antidiabetic foot spray
- Boost mental health – used in depression
- Natural mouth freshener
- Relieves headache
- Lower cholesterol level

2.1.5 Side-effects:
- Oral cancer
- Addictive for chewing one
- Allergic
- Discoloration of teeth and gums
2.1.6 Extraction procedure: (ethanolic extraction) [refer Fig.no.1.2, 1.3, 1.4]
10gm of dry powdered was extracted with the aid of using 150 ml of ethanol (70%). Potions of 1:15 of dried powdered as well as solvents were selected for Soxhlet extraction process. The process was run for 4-5 hours at 50 degrees Celsius.

2.1.7 Phytochemical screening:
- Definition: Phytochemical screening is the scientific process of analysing, examining, extracting, experimenting, and thus identifying the different classes of phytoconstituents present in various compounds.
- Chemical test is done for different phytoconstituent which are present in extracts are known as phytochemical screening.
- Importance:
  1) Helps in identifying (qualitative) the different chemical constituents present in compounds.
  2) Used for purification testing of synthetic drugs
  3) Gives information about medicinal importance of plant extract.
  4) Estimate the presence of various metabolites.
  5) Identifying the phytochemical

- Betel leaf contain many chemical components such as beta phenol, chavicol, and other phenolic compounds. It is main component of betel leaf having an antibacterial property.
- Phenolic test: a small amt of ethanolic extract was taken with 1ml of water in a test tube and 1 to 2 drops of iron3 chloride was added. A blue, green, red and purple colour is a positive test.
- Alkaloid: dragnetoff test, Mayer’s test
- Flavonoid: ferric chloride test

- Saponin: foam formation
  (Alcoholic extract is diluted separately with distilled water and shaken for 15 min in a graduated cylinder. If saponin is present foam is form.)
- Triterpenoid: Salkowski’s test
### Table 2: Phytochemical Screening

<table>
<thead>
<tr>
<th>SR NO.</th>
<th>Constituent</th>
<th>Chemical Test</th>
<th>Procedure</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenolic Compound</td>
<td>Phenolic test</td>
<td>Small amount of ethanolic extract + 1 ml of water in test tube + 1 to 2 drops of FeCl₃</td>
<td>Blue, green, red, purple colour are observed</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Dragendroff test</td>
<td>Few ml of filtrate + dragendroff reagent</td>
<td>Reddish brown precipitate</td>
</tr>
<tr>
<td>3</td>
<td>Alkaloid</td>
<td>Mayer’s test</td>
<td>Few ml of filtrate + 1-2 drops of Mayer’s reagent (along the sides of test tube)</td>
<td>Creamy white/yellow precipitate</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoid</td>
<td>Ferric chloride test</td>
<td>Extract + few drops of 10% FeCl₃ solution</td>
<td>Green precipitate</td>
</tr>
<tr>
<td>5</td>
<td>Saponin</td>
<td>Foam test</td>
<td>3 ml of extract + 10 ml of distilled water in test tube. Shake for 5 min and stand for 30 min</td>
<td>Honeycomb froth observed</td>
</tr>
<tr>
<td>6</td>
<td>Triterpenoids</td>
<td>Salkowski’s test</td>
<td>Filtrate + few drops of concentrated H₂SO₄</td>
<td>Golden yellow layer at bottom</td>
</tr>
</tbody>
</table>

### 2.2 Lemongrass:

#### 2.2.1 Scientific classification:
- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Liliopsida
- **Order:** Poales
- **Family:** Poaceae
- **Genus:** Cymbopogon
- **Species:** citrates

#### 2.2.2 Geographical source: found at South Asia, South-east Asia, Australia, Africa, ...

#### 2.2.3 Benefits:
- Fighting bacteria: antibacterial property - bacteria killer
- Reducing inflammation: treat skin inflammation
- Antioxidant
- Natural Deodorizer and Cleaner
- Skin healing properties.
- Hair health: strengthen your hair follicles, massage a few drops of lemongrass oil into your scalp for two minutes and then rinse. The soothing and bacteria-killing properties will leave your hair shiny, fresh, and odour-free.
- Low cholesterol level
- Massaging and relaxing
2.2.4 Side-effects:
- Lemongrass is likely safe for most people when used in food amounts.
- It is possibly safe when taken by mouth, applied to the skin, or inhaled as aromatherapy short-term for medicinal purposes.
- Rarely, lemongrass oil might cause a rash of skin irritation when applied to the skin.
- However, there have been some toxic side effects, such as lung problems after inhaling lemongrass and a fatal poisoning after a child swallowed a lemongrass oil-based insect repellent.

2.2.5 Extraction procedure:
1) Steam distillation:
- Put 150 grams of fresh lemongrass sample into 1 lighted round bottom flask with 250 ml of distilled water.
- The flask is equipped with a rubber stopper. Connect to the condenser and heat.
- 0-degree Celsius water Condensation through the condenser in the counter current to ensure steam.
- When it reaches 100 degree Celsius, it starts to boil Essential oil from lemongrass.
- When the lemongrass is heated and the essential oil is extracted from leaves mixed with water vapor.
- Through the condenser, steam Condensed into liquid. [refer Fig.no.2.2]
- With the use of ice cubes, cool as much as possible and avoid volatize.
- Use a 500ml beaker to collect directly, and then pour into the separatory funnel. [refer Fig.no.2.3]
- This forms two separate layers, Oil layer and water layer.
- Separated faucet Open the funnel to release water, and the oil Collect immediately 100ml stoppered bottle.
- The bottle is tightly closed to prevent the evaporation of essential oils.
- Oil is collected Weigh the volume of oil obtained.

2)Solvent extraction
3)Hydro distillation

2.2.6 phytochemical screening:
- Citral is main component of lemongrass oil.
- Chemical test for that is: alcoholic solution of Sudan red 3 is added into sample. Red colour is appeared which indicates presences of Citral.
2.3 Excipients:

[Table 3: excipients]

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>EXCIPIENTS</th>
<th>USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glycerine</td>
<td>Humectant and emollient</td>
</tr>
<tr>
<td>2</td>
<td>Alcohol (96%)</td>
<td>Antibacterial property, preservatives</td>
</tr>
<tr>
<td>3</td>
<td>Menthol</td>
<td>Cooling sensation</td>
</tr>
<tr>
<td>4</td>
<td>Tween 20</td>
<td>stabilizer</td>
</tr>
</tbody>
</table>

III. METHODOLOGY

1. Mix all the excipients (glycerine, menthol, polysorbate 20, alcohol) in a beaker of 100 ml.
2. Take specific quantity of extract (ethanolic extract of betel leaf) in another 100ml beaker
3. Add the mixture of excipient in extract beaker.
4. And make up the volume up to 100 ml with distilled water.
5. By using mechanical stirrer add some drops of lemongrass oil in final mixture. [refer Fig.no.3.1, 3.2]

[Table 4: Formulation table]

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>(F0) %</th>
<th>F1 (%)</th>
<th>F2 (%)</th>
<th>F3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic extract of betel leaf (antibacterial property)</td>
<td>-</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Alcohol (96%)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Glycerine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Menthol</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td>4.3</td>
<td>4.3</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Lemongrass oil (perfume)</td>
<td>-</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Distilled water (q.s.)</td>
<td>100ml</td>
<td>100ml</td>
<td>100ml</td>
<td>100ml</td>
</tr>
</tbody>
</table>

[Figure No.3.1]
[formulations]

[Figure No.3.2]
[f3 formulation]
IV. EVALUATION

4.1. Procedure for microbial testing:
(Inhibition zone diameter)

Preparation of agar plate:
1. Take 4.6 gram of nutrient powder and agar powder in conical flask and add 200 ml of distilled water and dissolve it.
2. After that autoclave the flask for sterility purpose for 15 mins at 121 0C and 15lbs
3. Sterile the petri plate along with flask, after wrapping it completely with newspaper.
4. Pour the nutrient agar in petri plate under laminar air flow. Add 25 ml of agar media in each plate.
5. Open the agar plate under UV light for 10 min and solidified the plate then close the plate and put it in inverted position in refrigerator for 24 hours. [refer Fig.no.4.1]

Preparation of bacterial culture:
1. In this nutrient powder is taken in 200ml distilled water and dissolve it. (Agar is not present in this, so solidification is not possible of nutrient broth)
2. Sterile it by using autoclave and take some amount in test tube, cool it.
3. Add some microorganism in that nutrient media in sterile condition.

Bore plate method:
1. Spread the bacterial culture on agar plate with spreader. [refer Fig.no.4.2]
2. Make four holes on agar plate for different formulations.
3. Pour the different concentration of formulations in four wells under LAF.
4. Close the petri plate and put in incubator for growth of microorganism for 24 hours.
5. Measure the zone of inhibition by using scale. (Area of media where bacteria are unable to grow, due to presences of our formulations which is having a antibacterial property) [refer Fig.no.4.3]

Result of this test:
- F3 formulation is having a goof antibacterial property as in that percentage of ethanolic extract of betel leaf is more. Zone diameter of f3 formulation is maximum in comparison to other two formulations.
- Betel leaf extract are used because it can kill the gram-positive bacteria which is present on foot surface.
- Mechanism of action: blocking bacterial development by destroying the cell wall of bacteria.
- Gram-positive bacteria are having a simple cell wall structure. Therefore, it is easier for antibacterial compound to kill microorganism.
4.2. Effectiveness testing procedure:
1. Apply the formulation f2 and f3 on two different socks.
2. After that check the lemongrass fragrance at interval of 1 hour.
3. As a result, till 6-7 hours the fragrance is there, so, it is long lasting for 6-7 hours but, after that also bad odour of foot is overcome by citrus smell from that sock till 12 hours.
[refer Fig.no.4.4]

V. RESULTS AND DISCUSSION

[Table 5: Evaluation table]

<table>
<thead>
<tr>
<th>EVALUATION TEST</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLOUR</td>
<td>yellowish</td>
<td>yellowish</td>
<td>yellowish</td>
<td>Yellowish</td>
</tr>
<tr>
<td>ODOUR</td>
<td>Citrus smell</td>
<td>Citrus smell</td>
<td>Citrus smell</td>
<td>Citrus smell</td>
</tr>
<tr>
<td>APPEARANCE</td>
<td>Clear liquid</td>
<td>Clear liquid</td>
<td>Clear liquid</td>
<td>Clear liquid</td>
</tr>
<tr>
<td>pH</td>
<td>5.50</td>
<td>5.70</td>
<td>5.72</td>
<td>5.75</td>
</tr>
<tr>
<td>INHIBITION ZONE DIAMETER</td>
<td>1.5 cm</td>
<td>2.2 cm</td>
<td>3.5 cm</td>
<td>3.5 cm</td>
</tr>
<tr>
<td>EFFECTIVENESS</td>
<td>-</td>
<td>5hours</td>
<td>6hours</td>
<td>6.5hours</td>
</tr>
</tbody>
</table>

- If the concentration of betel leaf and lemongrass increased more than 1.5%, it causes irritation in nose and on skin. Due to this, the optimum concentration of formulation is 1.5%, and that formulation has excellent result in comparison with other formulations.

VI. CONCLUSION
The greatest solution for eradicating foot odour and keeping the skin on the feet bacteria-free is a combination of betel leaf and lemongrass oil. Based on an analysis of the evaluation criteria for various formulations, it can be said that the f3 formulation (1.5%) is outstanding and effective against gram-positive bacteria.

VII. ACKNOWLEDGMENT
We would also like to thanks to our Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda Gujarat for providing facility to perform project work on herbal anti-odour foot spray “LEMONFRESH”. We are also thankful to our faculty members who supported us in many steps.

REFERENCES