Formulation and Evaluation of Herbal Antiperspirant and Deodorant Foot Powder from Mulberry Leaves Extract

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Abstract

Background: Sweating is a normal physiological process occurs in the body, but excessive sweating (hyperhidrosis) indicates a significant adverse impact on the quality of life for individuals. Sweating can be considered to have stressful experience, reduces self-confidence and social acceptance as it interferes with daily activities. Excessive sweating on the foot provides optimum conditions for bacterial growth and subsequently leads to smelly foot. Objective: The objective of the current study was to formulate and evaluate antiperspirant and deodorant foot powder from mulberry leaves extract. Methods: The formulated powder was tested for its antiperspirant and deodorant activity against Staphylococcus epidermidis by measuring its zone of inhibition using disk diffusion method. Furthermore, the formulated powder was tested for its physiochemical parameters as per standard procedures. Results: The study results proved that mulberry leaf extract showed considerable antibacterial activity against Staphylococcus epidermidis bacteria in concentration of 200 mg/ml and 400 mg/ml. Phytochemical screening on the extract confirmed the presence of alkaloids, reducing sugars, phenols, flavanoids, carbohydrates, proteins, etc. The invitro evaluation of the formulated herbal foot powder was tested on the micrometric test parameters like angle of repose, bulk density, tapped density, hausner ratio, carrs compressibility index and sterility test. The result on the micrometric properties was found to have poor flow property. However, the formulated herbal foot powder from mulberry leaves extract can be used as a potential herbal antiperspirant and deodorant foot powder with further studies on improving the flow property of the powder and toxicity testing using suitable models.

Keywords: Mulberry leaves extract, Staphylococcus epidermis, Antiperspirant, Deodorant, Foot powder.

Introduction

Hyperhidrosis is a most unpleasant experience to most of the people, which reduces self-confidence and social acceptance as it interferes with daily activities. This excessive sweating is further worsened by the production of unpleasant odour which was resulted due to bacterial growth at the site of sweating [1]. Excessive sweating affects one or more body areas, such as palms, soles (palmar), armpits (axilla) and face that leads to unpleasant odour. However, there are various topical, systemic, surgical and nonsurgical therapies available for the treatment of excessive sweating hyperhidrosis [2]. Antiperspirant is a preparation used to reduce perspiration especially for people who suffer with hyperhidrosis whereas deodorant is a formulation that removes or conceals unpleasant smells, especially bodily odours. Many of the antiperspirants available in market was formulated using special fragrances to deodorize the body odour caused by sweating and effective against odour-producing bacteria. The odour causing bacteria tends to grow faster and proliferate mostly in the warm areas of the body that stays moist for longer time. The effectiveness of antiperspirants was brought out by forming complexes with water, small scales of skin and lipids to create a protein mass which causes blockage in sweat glands and thus reducing the volume of sweat secretion [3].
Body odour can also occur in our feet (palmar), groin, armpits, genital area etc. People describe the quality of foot odour as a thick, cheese-like smell, as malt vinegar and also as ammonia like odour. Even though sweat was considered odorless, but it creates a beneficial environment for certain bacteria to proliferate and produces odour on the foot. The other condition that can trigger more sweating in foot was wearing closed toe shoes for many hours. Socks used generally do not cause foot odour, but it promotes to trap the hair on the feet and may contribute to odour's intensity by increasing surface area thereby facilitates bacteria proliferation and causes unpleasant odour [4].

Excessive production of sweat were also influenced by variety of factors like hereditary, diet, exercise, dermatological diseases like anhydrotic dysplasia and other stimuli [5]. The smelly odour arises when the bacteria consume sweat for their metabolism. A bacterium also ingests various salts and minerals for their proliferation and excretes waste products. The waste product isovaleric acid is a fatty acid that causes the odour to sweaty feet. The unpleasant smell of gases produce by the bacteria were similar to those bacteria that been used in producing cheese [6].

Past research findings revealed that bacteria’s like Bacillus subtilis, Staphylococcus epidermidis, Corn bacterium and Brevibacteria were responsible to cause unpleasant smell in foot hyperhidrosis. In this study, Staphylococcus epidermidis was selected for its invtro evaluation against formulated herbal foot antiperspirant powder from mulberry leaves. The study was designed as an alternative to the available synthetic antiperspirants; the major side effects of synthetic made formulations are reported for its localized burning, stinging, allergy and irritation to the skin.

The adverse effects caused by chemical antiperspirants that contain aluminum salts could be avoided by using natural herbal extracts that have similar properties for reduce sweating. In the present study, Mulberry (Morus) leaves was chosen to form a simple combined formula as foot powder and further evaluated to find its effectiveness against foot hyperhidrosis. The selected herbal was supported through reported studies for its antiperspirant activity and also used to deodorize the unpleasant smell caused by sweating. Mulberry leaves are traditionally used as antiperspirant and were recorded in China ancient record. These herbs were reported for its significant astringent and antimicrobial properties.

Mulberry is a flowering plant belongs to the family Moraceae. It includes many species and commonly known are morus alba L, morus nigra L and morus rubra L. Mulberry is used as the primary food source for silkworms and it is cultivated for silk production in many regions of Asia. Mulberry grows in both temperate and topical areas and is a medium-sized, monecious plant, deciduous tree growing up to 30 m tall and 1.8 m wide. The characteristic of mulberry leaves is alternate, oval in shape; 3 to 5 inches long with toothed margins and may be lobed or unlobed. The fruit is usually red or black and also white to pinkish in colour. The antiperspirant effects of Mulberry has been
clearly recorded in the “Shennong’s Classic of Materia Medica”. The effectiveness of Mori follium (mulberry leaves) as antiperspirant also have been reported in Ming Dynasty - China. The observed results proved that the extracts from Mori follium had the similar effects as the aluminum polychloride that been used in chemical antiperspirant formulations. Sweating is initiated through the release of acetylcholine in sympathetic system.

Acetylcholine esterase shows inhibitory action on acetylcholine, the cellular activity of sweat gland can be revealed through assessment of specific enzyme activities under the influence of herbal agents. Thus, mulberry extracts enhanced acetylcholine esterase activities in inhibiting formation of acetylcholine [7]. Mulberry leaves is rich in phenolic compounds such as kuwanon E, kuwanon U, morusin and moracin these constituents were believed for its antibacterial activity [8].

Materials and Methods

Collection and Identification

About 2.5 kg of fresh mulberry leaves was collected from a farm at Kg. Tanah Liat, Bukit Mertajam Pulau Pinang, and Malaysia. The plant was authenticated by the botanist Mr. Suhaimi Bin Hj. Din, at plant bio security division Pulau Pinang, Malaysia and the herbarium was recorded and stored for future reference.

Preparation of Extract

Fresh leaves were selected and washed thoroughly using tap water to remove dust and foreign materials. The washed leaves were subjected for drying under partial sunlight to remove excess moisture. Furthermore, the leaves were kept in hot air oven for drying at 35°C for 72 h upon desired drying; the leaves were grounded to coarse powder using the household blender.

The powdered sample were collected, weighed and stored accordingly in an air tight container until further study. The coarse powder was extracted by Continuous hot extraction method using ethanol as solvent, however defatting of the plant material was initially performed with petroleum ether before the main extraction process. About 200 g of grounded mulberry leaves were weighed and packed in the extractor and was defatted using petroleum ether for 4 days at 45°C by continuous hot extraction method. Upon defatting, the marc was dried and repacked into the extractor and was extracted with ethanol at 50°C for 5 days. Extraction process was continued until maximum extraction was observed, the extracted solvent was collected, filtered and then concentrated using rotary evaporator under control temperature and pressure. The collected crude extract was weighed, packed in air tight containers and stored in desiccator for its further usage.

Qualitative Phytochemical Analysis

The stock solution was prepared from the crude extracts by dissolving with suitable amount of mother solvent. The obtained stock solutions were subjected to preliminary phytochemical screening [9].

Test for Alkaloids

Dragendorff’s test Added 1ml of Dragendorff’s reagent into the extract. An orange red precipitate indicates the presence of alkaloids. Wagner’s test To few drops of Wagner’s reagent into the extract. A reddish-brown precipitate indicates the presence of alkaloids. Mayer’s test Add 1ml of Mayer’s reagent into the extract. A dull white precipitate indicates the presence of alkaloids.

Test for Proteins

Biuret test 1ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution was added into the extract. A violet colour indicates the presence of proteins.

Test for Carbohydrates

Molisch test 1ml of a-naphthol solution was added into the extract and concentrated sulphuric acid was added along the side the test tube. Purple or reddish violet colours at the junction between the two liquids indicate the presence of carbohydrate.

Test for glycosides

Legal test the extract was dissolved on pyridine. Sodium nitroprusside solution was added and made it into alkaline. Pink or red colour indicates the presence of glycosides Baljet test Sodium picrate was added to the extract. Yellow to orange colour indicate the presence of glycosides.
Test for Fixed Oils
Spot test a small quantity of extract was pressed between two filter papers. Oil stains on filter paper indicates presence of fixed oil.

Test for Tannins
Ferric chloride was added to the extract. Dark blue or greenish black colour indicates the presence of tannins. Potassium dichromate solution was added. The precipitate indicates the presence of tannin the presence of tannin.

Test for Flavonoids
Magnesium turnings were added to the test extract, followed by addition of concentrated hydrochloric acid. A red colour indicates the presence of flavonoids.

Test for Phenols
1ml of extract were added with 2ml of water. Then, few drops of 10% ferric chloride were added. The positive result shows blue green colouration.

Test for Reducing Sugar
3ml of extract was added to 1ml of water and 20 drops of Fehling solution. The mixture was boiled in water bath for 5 minutes. The positive result is presence of red brick solution.

Test for Saponin
10ml of water was added with 2.5ml of extract and shaken vigorously. The mixture was allowed to stand for few minutes formation of foam indicates presence of saponin.

Determination of Antibacterial Activity
Staphylococcus epidermidis was used as test organism in the determination of antibacterial activity with Mulberry leaves extract.

Preparation of Agar Plate
The nutrient agar medium was prepared using the ingredients as shown in Table no. 1. Appropriate quantities of each ingredient was weighed and transferred to a clean 1000 ml conical flask and dissolved using distilled water. The content of the flask was heated to dissolve completely. The conical flask was plugged with sterile cotton and covered with aluminium foil, and kept in autoclave for 15 minutes at 121°C. Final pH was adjusted to 6.8. The warm nutrient agar solution was then poured into the sterile petri plates under the laminar flow and remained undisturbed to get completely solidified.

<table>
<thead>
<tr>
<th>Table 1: Ingredients of nutrient agar medium</th>
<th>Quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>1.2 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>500 ml</td>
</tr>
</tbody>
</table>

Antimicrobial Activity of the Extract
Bacterial strains of Staphylococcus epidermidis was inoculated followed by incubation at 37± 2°C for 24 hours. The colonies from the sample was further sub cultured in the nutrient broth and incubated at 37+ 2°C for 24 hours for the further studies. The current study was conducted using two concentrations of mulberry extract using 200mg and 400mg of extract in 1ml of DMSO respectively. 50µl of broth was then poured in each of the nutrient agar plates and spreaded using cotton swab.

Standard discs containing Ofloxacin was placed on each plate as control, mulberry leaves extract was impregnated to the empty discs and allowed to dried before placing to the agar plate, DMSO containing disc kept as blank. Once the disc have been placed in the nutrient agar plates, the plate was inverted and kept for incubation at 37± 2°C for 24 h. The zone of inhibition on each plate was measured and recorded in mm scale.

Formulation of Herbal Antiperspirant Foot Powder
The ingredient of the formulated herbal antiperspirant foot powder was shown in Table.no. 2. The powder was formulated using trituration method. To 5g of mulberry leaves extract added 10g of magnesium oxide powder and triturated to a homogenous mixture and kept as mixture A. Mixture B was prepared using 3g of citric acid added with 2g of magnesium oxide powder and mixed thoroughly. Finally both the mixtures were triturated together until homogenous powders are obtained. In order to have a desired flow property 20g of starch and 5g of
talc were added to the above mixture and trituted to a homogenous mixture was obtained. The formulated herbal foot powder was further subjected to sieving for the desired particle size of 45µm; the process was continued until desired quantity was obtained. The formulated powder was shown in Fig.2.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantities</th>
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<tbody>
<tr>
<td>Mulberry leaves extract</td>
<td>5g</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3g</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>17g</td>
</tr>
<tr>
<td>Talc</td>
<td>5g</td>
</tr>
<tr>
<td>Starch</td>
<td>20g</td>
</tr>
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</table>

**Fig. 2: Formulated herbal foot antiperspirant powder from the extract of mulberry leaves**

**In Vitro Evaluation of Formulated Herbal Antiperspirant Foot Powder**

**Physical Evaluation**

The formulated powder was tested for its colour and homogeneity by visual appearance. For testing the grittiness, small quantity of the sample was rubbed between the index finger and thumb to determine the presence of gritty particles.

**Angle of Repose**

The experiment set up was followed as per standard procedure. The stem orifice of funnel was blocked by thumb and about 10g powder was transferred. Height of the funnel to the glass plate was adjusted and maintained to 3 cm between the bottom of funnel stem and top of powder pile. The angle of repose was measured when powder is emptied from funnel. The outer edges of pile on graph paper were made with pencil and the diameter was recorded. The height of pile was measured and the angle of repose was calculated using the following formula: \( \theta = \tan^{-1} \left( \frac{\text{height}}{\text{radius}} \right) \)

**Bulk Density**

The bulk and tapped densities were determined using the standard method [10]. Powder was passed through desired sieve mesh to break up agglomerates and 10g of powder was transferred into a pre-weighed 50ml graduated cylinder with 0.5ml markings. The volume was recorded and bulk density was calculated using the formula given below:

\[ \rho = \frac{\text{mass}}{\text{untapped volume}} \]

**Tapped density**

Weighed about 10g of formulated powder and was transferred into a 50ml of graduated cylinder. The cylinder was tapped 50 times per minute manually and the tapped volume was recorded. Tapped density was calculated using the formula given below:

\[ \bar{D} = \frac{\text{Mass}}{\text{Tapped volume}} \]

**Hausner Ratio and Carr’s Compressibility Index**

Hausner ratio [11] and Carr’s compressibility index [12] were used to measure the flow ability of the formulated powder. The results were calculated using values obtained in bulk and tapped densities.

\[ \text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \]

\[ \text{Carr's compressibility index} = \left( \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \right) \times 100 \]
Results and Discussion

The qualitative phytochemical analysis on the mulberry leaves ethanol extract was performed and the results were shown in Table no. 3. The antibacterial activity of mulberry leaves extract and the formulation was evaluated through disc diffusion method against the gram positive bacteria Staphylococcus epidermidis. Disc diffusion method was used as it was simple, rapid, reproducible, and inexpensive. The zone of inhibition was measured and recorded as shown in Table. No. 4, as the concentration of mulberry leaves extract and formulated powder increases the zone of inhibition was also found to increase, however the standard drug have shown significant zone of inhibition with 30.65mm. The maximum zone of inhibition for the mulberry extract was 12.15mm while the herbal foot powder was recorded as 13.55mm. The zone of inhibition of the extract and the formulation was shown in Fig. 3 and Fig.4.

<table>
<thead>
<tr>
<th>Compound group</th>
<th>Mulberry leaves extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>2. Proteins</td>
<td>+</td>
</tr>
<tr>
<td>3. Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>4. Glycoside</td>
<td>-</td>
</tr>
<tr>
<td>5. Fixed oils</td>
<td>-</td>
</tr>
<tr>
<td>6. Tannins</td>
<td>+</td>
</tr>
<tr>
<td>7. Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>8. Phenol</td>
<td>+</td>
</tr>
<tr>
<td>9. Reducing sugar</td>
<td>+</td>
</tr>
<tr>
<td>10. Saponin</td>
<td>+</td>
</tr>
</tbody>
</table>

+: Present, - : Absent

Table 4: Diameter of inhibition zones (mm) caused by extract and formulated powder against Staphylococcus epidermidis in the disc diffusion method.

<table>
<thead>
<tr>
<th>Sample Concentration</th>
<th>Mulberry leaves extract</th>
<th>Herbal foot powder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200mg/ml</td>
<td>400mg/ml</td>
</tr>
<tr>
<td>Zone of inhibition (mm)</td>
<td>10.00</td>
<td>12.15</td>
</tr>
</tbody>
</table>

Fig. 3: Antibacterial activity of Mulberry leaf extract

Fig. 4: Antibacterial activity of formulated herbal foot powder

The formulated herbal foot powder was tested by various micrometric studies including angle of repose, bulk density, tapped density, Haussler's ratio and Carr's
The results showed poor flow property on the formulated powder as the particle size was very fine in nature. The results were shown in Table no. 5.

Table 5: Micrometric study of formulated herbal foot powder from mulberry leaves extract.

<table>
<thead>
<tr>
<th>Test Parameters</th>
<th>Result observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angle of repose</td>
<td>13°</td>
</tr>
<tr>
<td>Bulk density</td>
<td>0.47g/ml</td>
</tr>
<tr>
<td>Tapped density</td>
<td>0.83g/ml</td>
</tr>
<tr>
<td>Hausner ratio</td>
<td>1.77</td>
</tr>
<tr>
<td>Carr's compressibility index</td>
<td>43.37</td>
</tr>
</tbody>
</table>

**Conclusion**

The present study was developed as prototype herbal foot powder formulation from the mulberry leaves extract for its antiperspirant and deodorant activity. Due to its commendable antibacterial activity against Staphylococcus epidermidis, formulation made from the extract can be recommended for the proposed activity. However, the formulated powder has to be investigated further for its better flow property and effectiveness in human volunteers in order for commercialization as an antiperspirant and deodorant foot powder in the market.

**References**