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FORMULATION AND EVALUATION OF PIPER BETEL LEAF AND *TRACHYSPERMUM AMMI* LEAF FOOT SPRAY

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ABSTRACT

The most common problem nowadays is related to the Foot odour, which is one of the things that is quite disruptive to appearance. One of the causes of foot odour is the presence of bacteria, especially the bacteria *Bacillus subtilis*. Present work was focused on developing herbal antibacterial foot spray. Presently we have used ethanol extract of Piper betel leaf and *Trachyspermum ammi*, commonly known as khaupan and Ajwain Leaf, as the traditional Indian ayurvedic document describes several of its medicinal properties including as an effective antibacterial agent. The spray form was chosen because of its easy and pleasant use. This study aims to make a foot odor control product in the form of a spray with 3 concentrations of Betel leaf and Ajwain ethanol extract, 0.5%, 1.0% and 1.5% to study the antibacterial activity. The results showed that this ethanolic extract can be formulated into foot spray preparation with the results of the organoleptic examination of the three formulas are clear liquid and yellow slightly green color. This formula had a good appearance, fast-drying, non-greasy and thin coating on the skin surface after application. The pH of the preparation is in the range 5.10-5.92. In formula 3 (F3) with a concentration of 1.5% Betel and Ajwain ethanol extract, it has a strong bacterial inhibition zone with an average inhibition zone diameter of 12.5 mm.

Keywords: - Foot Spray, Ajwain, Odour Control, Betel Leaf

INTRODUCTION

One part of the body that is very important for humans is the feet, both for women and men, therefore feet need to be kept clean but sometimes they are often ignored, resulting in the appearance of odor problems on the feet. Foot odor was found to be derived from isovaleric acid, which is produced when *Staphylococcus epidermidis*, a resident species of the normal cutaneous microbial flora, degrades leucine present in sweat. In addition, *Bacillus subtilis* was detected in the plantar skin of subjects with strong foot odor, and this species was shown to be closely associated with increased foot odor. *Bacillus subtilis* is one of the normal flora species which is mostly found on the skin. The *Bacillus* SP bacteria group is known to have an important role in causing foot odor. *Bacillus Subtilis* was 11.5% in causing foot odor, while *Staphylococcus epidermis* was 86.5%.

Most commercial spray available today in the market is incorporated with chemical agents having antimicrobial activity with potential depilatory properties on skin pathogens. The problem now is most people do not know the long term consequence of using the commercial sprays. The drawbacks of commercial spray, led people now to be more inclined toward the use of herbal formulations. These problems of commercial spray have been reported to be successfully handled daily by using only what 'mother nature' has to offer to help you nourish your skin. Herbs are the natural products mostly found in the treatment of almost all diseases and skin problems owing to their high medicinal value, cost-effectiveness, availability and compatibility. The spray form is chosen based on the nature of the spray which can provide a concentrated content, but at the same time it has a fast drying profile so that it is easy to use and pleasant to the user.

Betel leaf possess strong aromatic flavor and have been long in use for the preparation of traditional Indian ayurvedic herbal in review. A couple of research articles have demonstrated the potential of the leaf extract on dermatophytes. On the other hand, Ajwain with its characteristic aromatic smell & has anti-aggregatory, anthelmintic, antihyperlipidaemic, antifilarial, insecticidal, kidney stone inhibitory, molluscicidal, mosquito repellent and nematicidal activities.

MATERIALS AND METHODS:

Fresh Leaves of Betel Plant and Ajwain were collected from the region of Kolhapur local market. Glycerin, Alcohol (96%), Menthol, Perfume, Polysorbate 20 were

purchased from a Chemical Store.

Method of Extraction:

Betel leaf (*Piper betle* L.) 100 gm of coarsely powdered leaf material of betel leaf (*Piper betle* L.) was subjected to maceration for 72 hours.

Ajwain Leaf (*Trachyspermum ammi*) 100 gm of coarsely powdered leaf material of **Ajwain Leaf (*Trachyspermum ammi*)** was subjected to maceration for 72 hours.

Procedure:

1. The ethanolic extracts of Betel Leaf and Ajwain was mixed together by stirring.
2. Glycerine, Alcohol, Methanol & Perfume was mixed together to form a solution.
3. The second solution was added to the extract solution with occasional stirring to form uniform solution.
4. Finally, water was added to adjust the volume up to 100 ml.

Foot spray was made with four formulas using extracts with different concentrations. *Bacillus subtilis* was obtained from the Laboratory of Microbiology, Anandi Pharmacy College, Kalambe Tarf Kale.

Table 01: Formulation Table

Material	Formula %			
	F0 (Blank)	F1	F2	F3
Ethanolic Extract of Betel Leaf	-	0.5	1	1.5
Ethanolic Extract of Ajwain Leaf	-	0.5	1	1.5
Glycerin	0.2	0.2	0.2	0.2
Alcohol (96%)	40	40	40	40
Methanol	0.5	0.5	0.5	0.5
Perfume	0.5	0.5	0.5	0.5
Polysorbate 20	4.3	4.3	4.3	4.3
Distilled Water (qs)	100 ml	100 ml	100 ml	100 ml

Evaluation of foot spray preparations is by organoleptic testing including color, odor, pH test using a pH meter, antibacterial activity test and stability test observed for 12 weeks at room temperature conditions.

The antibacterial activity test of foot spray preparation containing Betel leaf and Ajwain Leaf extract was carried out against *Bacillus subtilis* by using the well diffusion method for formulas one (F1), two (F2), three (F3) and negative control or without extract (F0).

The test was carried out three times for all formulas. The materials used in the antibacterial power test were MHA (Mueller Hinton Agar), Mac Farland standard solution to make test bacterial suspensions, sterile distilled water as a solvent for testing bacteria and *Bacillus subtilis* bacteria.

RESULTS AND DISCUSSION:

Phytochemical analysis was conducted to know alkaloids, flavonoids, saponin, tannins, steroid and triterpenoid. Based on the results of the phytochemical screening test, it was found that the ethanol extract of betel leaf & ajwain leaf was positive for alkaloids, flavonoids and tannins. Result of phytochemicals this extract is in table 02 below.

Table 02: Presence of Phytochemicals

Secondary Metabolite	Betel Leaf	Ajwain Leaf
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	+
Tannin	+	-
Steroid	-	-
Triterpenoid	+	-

There are four formulas of foot spray of betel leaf and ajwain leaf extract consisting of 0.0% (F0), 0.5% (F1), 1.0% (F2), and 1.5% (F3). The results of the formulas show that there is relationship between concentrations of the extract with color, the higher the concentration of extract (active ingredient), the color is getting darker.

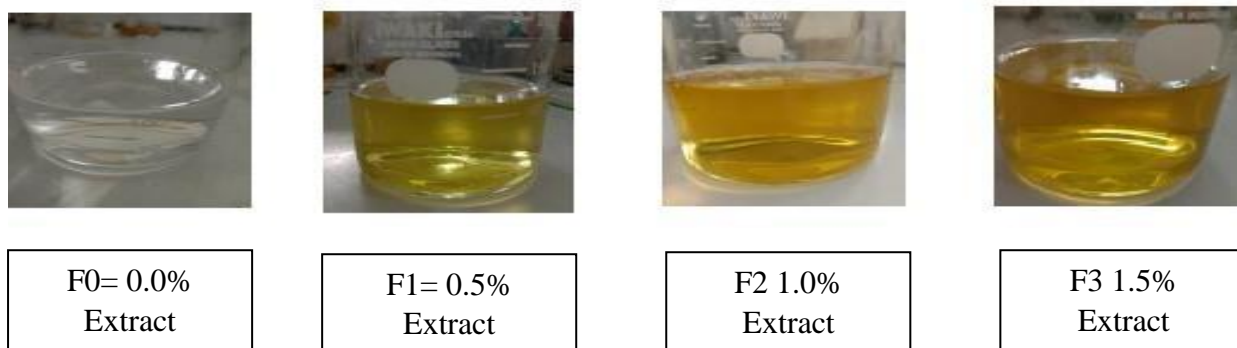


Figure 01. Foot Spray of Betel Leaf and Ajwain Leaf Extract

The observation shows that foot spray containing extracts of Betel Leaf and Ajwain leaf 0.5 % (F1), 1.0% (F2) and 1.5% (F3) have the same characteristics as F0, but have a

yellow slightly green color (Table 3). The colors of F2, F3, and F4 were derived from the ethanol extract of Betel Leaf and Ajwain leaf.

Table 03: Organoleptic Properties

Formula	Organoleptic Properties		
	Appearance	Colour	Smell
F0	Clear, Liquid	Colourless	Floral Fruity Smell
F1	Clear, Liquid	Yellowish, Slight Green	Floral Fruity Smell
F2	Clear, Liquid	Yellowish, Slight Green	Floral Fruity Smell
F3	Clear, Liquid	Yellowish, Slight Green	Floral Fruity Smell

The results of the pH examination, the pH range of the formula F0, F1, F2 and F3 was obtained between 4.58 - 5.92. The results of checking the pH of the four preparations are shown below.

Table 04: pH Test

Formula	pH
F0	4.58
F1	5.10
F2	5.92
F3	5.92

The degrees of acidity (pH) of the formula without extract (F0), F1, F2, F3 is still included in the skin pH range of around 4.5 - 6.5. This indicates that the pH of 5.10-5.92 in the foot spray with Betel Leaf & Ajwain Leaf extract is still in the pH range of the foot skin so it can be used safely for feet.

Antibacterial Test

The results of the antibacterial test using the well diffusion method, it is found that all formulas provide the inhibition zone diameter and the results of the examination can be seen in Figure below and the inhibition zone can be seen in Table 5.

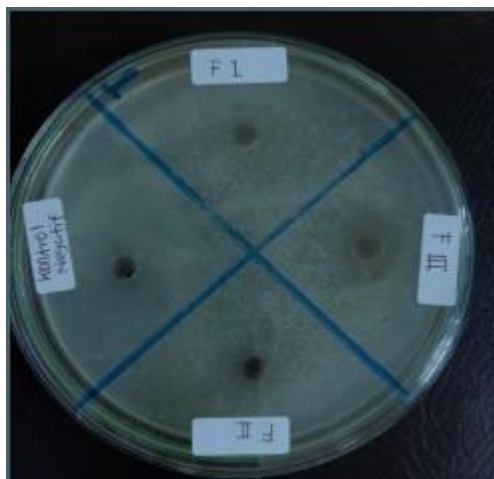


Fig 2: Antibacterial Activity Test

Table 05 Antibacterial Activity Test

Formula	Inhibition Zone Diameter
F0	4.5 mm
F1	6.7 mm
F2	9.3 mm
F3	12.5 mm

From the above observations it can be seen that the increasing concentration of the extract also increases the inhibitory zone of the bacteria *Bacillus subtilis*. Formula 0 without extract provides 4.5 mm inhibition and shows a weak inhibition zone, and with the addition of the extract strengthens the inhibition zone of bacteria to a diameter of 12.5 mm for formula 3 with the addition of extract was 1.5%.

Formula 0 without extract provides a weak inhibition zone of 4.5 mm which can be caused by the ethanol content of 40% w / w in the formula. Alcohol is used in this formula as a solvent, provides a cool sensation to the preparation and makes the sprayed preparation dry on the feet quickly after spraying.

From the above results, formula three with a concentration of 1.5% betel leaf and ajwain leaf ethanol extract is effective in blocking the development of the *Bacillus subtilis* bacteria which is a group of gram-positive bacteria. The strength of the extract in blocking bacterial development is due to the cell wall properties of the tested bacteria. Gram positive bacteria have a simpler cell wall structure that makes it easier for antibacterial compounds to enter the cells and reach their workplaces, while gram-negative bacteria have better resistance than gram-positive bacteria to antibacterial

compounds. Bacteria will die immediately if there is no cell wall, because they are unable to survive the influence of the surrounding environment.

Formulas 1, 2 and 3 that are produced are tested by putting them in a spray bottle, from the results of testing formulas 1, 2 and 3 can be sprayed, because formulas 1, 2 and 3 are clear liquids.



Fig. 03 Polyherbal Foot Spray Formulation

CONCLUSION

Betel Leaf and Ajwain leaf ethanol extract can be formulated into foot spray preparation and have antibacterial activity against *Bacillus subtilis in vitro*. Formula 3 (F3) of foot spray on with the addition of 1.5 %. This formula most excellent and effective against *Bacillus subtilis* and stable in storage for 12 weeks at room temperature (28-30 °C).

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