



RESEARCH ARTICLE

HERBAL SALVE ELIXIR AGAINST FUNGAL PATHOGEN

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ABSTRACT

In this modern era, there is a remarkable growth in the field of science. In the same way microorganisms are becoming more resistant to the commercial antibiotics. Fungal human infections are more prevalent in these days. Treatment and control of fungal pathogens are difficult as they are eukaryotes, hence the drug designing is more complex. Synthetic antibiotics not only control the pathogen but may also cause many side effects. Hence herbal composition is required for their control. In this research we have made a herbal salve elixir against fungal pathogen especially dermatophytes causing dandruff in humans. They gave a promising growth control pattern at 1:2 ratio of oil:dry powder preparation, which was very similar to the commercially available ketoconazole lotion. Two organisms were selected namely – *Trichophyton sp* and *Malassezia sp*. The plant selected was *Phyla nodiflora*. The antifungal activity was tested and compared with standard ketoconazole lotion.

Key words: Dermatophytes, Dimorphic-fungi, *Phyla nodiflora*, dandruff, *Trichophyton sp*, *Malassezia sp*.

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INTRODUCTION

Phyla nodiflora is a member of family Verbenaceae. The family includes 75 genera and about 2500 species and the genus *Phyla* include 10 species. They are most commonly seen in wet lands at warmer area (Sharma RA et al., 2013). The aerial parts of this plant is used in the treatment of indigestion in children. Its decoction is considered as cooling agent and used as demulcent in cases of venereal diseases (Ali et al., 1974). Synonyms of *Phyla nodiflora* are *Lippia nodiflora*, *Lippia incisa* and *Phyla incisa*. *Phyla nodiflora* is fast growing perennial prostate herb. Leaves; obovate, obtuse, somewhat fleshy, and rarely subacute. Their surface is covered with fine hairs and color is grayish green. (Ranghunatha, 2003). Evelyn Priya et al., (2013) stated that bacteria develop resistant to many antibiotics leading to multi-drug resistant (MDR) strains and hence the discovery of efficient and novel antibacterial agents is essential. Crude extracts from aerial parts of *Phyla nodiflora* were screened for phytochemical and antimicrobial properties. The antidandruff activity of Indigenous medicinal herbs and its synergistic effect were tested. The leaves of *Lippia nodiflora* L, *Ziziphus jujube* L and *Wrightia tinctoria* L were procured from a field in and around Palakkad and Coimbatore, India. (Anitha et al., 2013) Dandruff is a common scalp disorder affecting half of the human population. Dead skin cells are shed in large oily clumps, which appear as

white or greyish patches on the scalp, skin and clothes is called dandruff. It is formed due to some factors. (Turner et al., 2012). The most important factor is oily skin due to sebum secretion, pollution, and unclean hair. It is most common caused by 2 fungi they are – *Trichophyton rubrum* (dermatophyte) and *Malassezia furfur* (dimorphic fungi). *M.furfur* colonies are creamy yellow brown and red. The lipase gene which have an important role in dandruff formation. Which is an extracellular enzyme degraded the long fatty acid chains and cause dandruff and other skin diseases. It is also a facultative pathogen, associated with a wide range of skin diseases. (Rajene Pereira nekes et al., 2005). The lipophilic yeast *Malassezia* utilizes sebum lipids as a nutrient source, and sebum production is hypothesized to be required to support growth of *Malassezia*. The role of the yeast *Malassezia* in dandruff and SD has been proposed since it was first shown in 1874 that levels of *Malassezia* species are elevated in dandruff. *Trichophyton rubrum* (*T. rubrum*) is a dermatophyte responsible for causing the majority of superficial fungal infections worldwide. Dermatophytes are a subset of fungi that have the ability to invade keratinized tissues, such as skin, hair, and nails. This group of fungi can cause infection anywhere on the skin, however, they most commonly affect the feet, inguinal region, scalp, and nails. About 80% of patients presenting with acute dermatophytosis respond well to topical antifungal treatment. However, the remaining 20% progress into a chronic state of dermatophytosis, which is resistant to antifungal treatment. The patients who present with a chronic *T. rubrum* infection, possibly contain a defective

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cellular immune responses. There are natural effective remedies to control dandruff in Ayurveda but presently people are depending on commercial shampoos containing some antifungal compounds like miconazole, ketoconazole, selenium sulphide etc. Plant products contain various compounds like alkaloids, flavanoids, tannins, terpenoids etc which have efficient antifungal activity. These compounds can be used in combination as polyherbal mixtures for controlling dandruff. The present work was a comparative study of the effect of commercial anti dandruff shampoos and natural plant products to evaluate their anti fungal efficacy. There are no reports of such comparative study and this study gives significant information about the higher antifungal efficiency of natural products at low concentration which can be exploited for commercial poly herbal preparations.

MATERIALS AND METHODS

Collection of plant materials

Phyllanthus nodiflorus plant leaves were collected at Thalavasal (Manivillunthan village), Salem district, Attur taluka, Tamil Nadu, India during the months of January 2018.

Specimen collection

The dandruff sample was collected from the scalp of infected person. A portion of hair was removed and with the help of a sterile scalpel the dandruff sample was scratched from the scalp and taken for further studies.

Plant bioactivity testing (Evelyn Priya S et al., 2015)

Fresh and healthy plant part, namely leaf were collected and washed to water remove any dust particles. They were then cut into small pieces and fixed in 5 mL of formalin, 5 mL of acetic acid, and 90 mL of 70% ethyl alcohol for 24 hrs. The materials were dehydrated with graded series of tertiary butyl alcohol (TBA). Infiltration of the specimens was carried out by gradual addition of paraffin wax until TBA solution attained supersaturation. The specimens were embedded into paraffin blocks for sectioning. The paraffin-embedded blocks were mounted on wooden stubs and microtome sections 8-14 μ m were cut using a Spencer rotary microtome. The resulting paraffin ribbons were stained with alcoholic safranin (0.5% w/v) and counterstained with fast green (0.25%, w/v) solution. After staining with safranin, the slides were dehydrated by employing a graded series of ethyl alcohol (30%, 50%, 70%, 90%, and absolute alcohol). The slides were stained with fast green in clove oil and xylol:alcohol (50:50). They were then passed through 100% xylol and finally mounted in distyrene plasticizer and xylene mountant. Then observed microscopic.

Preparation of plant extracts (T. Regupathi et al., 2014)

The collected plant leaves were washed with water and dried under shade and it was made into a fine powder using a blender. 20g of powder was taken in different solvents namely chloroform, toluene, dimethyl formamide and water of 100ml each. It was kept on a shaker for 3 days and filtered using a filter paper. The extracts were dried in a hot air oven and the dried powder was weighed and dissolved in the respective solvent to make 1:1 dilution and used for further studies.

Herbal salve elixir

10 ml of coconut oil was taken and heated to which plant leaf powder was added at different concentrations – 10g, 20g, 30g and prepared separately. It is allowed to cool and filtered with Whatman No. 1 filter paper. The filtrate was used for further procedures.

Phytochemical analysis of the plant (Evelyn Priya S et al., 2015)

The following tests were made based on the standard procedures with plant extract to find the phytochemical analysis of the plant. They are: 1. Proteins (Biuret), 2. Amino acids (Ninhydrin), 3. Starch (KOH), 4. Alkaloids (Dragendorff), 5. Flavanoids (Ferric chloride, $\text{NH}_3\text{-HCl}$, 1% AlCl_3), 6. Tannins (5% ferric chloride, iodine, $\text{H}_2\text{SO}_4\text{-HCl}$, $\text{K}_2\text{Cr}_2\text{O}_7$, 10% lead acetate), 7. Phlobatannins (1% HCl), 8. Steroids (Liebermann Buchard), 9. Terpenoids (Salkowski), 10. Saponins (foam), 11. Cardiac glycosides (Keller Killiani), 12. Antraquinones (Borntragers).

Isolation and identification of fungal pathogen

The collected dandruff samples were plated on PDA and SDA medium. The individual colonies are separated based on the colony morphology and microscopic observation on LCB mount. The isolated colonies were further stored in PDA slant (*Trichophyton sp*) and PDA with 2% coconut oil slant (*Malassezia sp*).

Antifungal activity testing (Mounyr Balouiri et al., 2016)

Poisoned food method

Poisoned food method is mostly used to evaluate the antifungal effect against molds. The antifungal agents or the extracts are incorporated into the molten agar at a desired final concentration and mixed well. Then, the medium is poured into petridishes. After the overnight pre-incubation, the inoculation can be done by a mycelia disc ranging from 2 to 5mm, which is deposited in the centre of the plate. After further incubation under suitable conditions for the fungal strain, the diameter of the fungal growth in control and sample plates are measured.

Antifungal effect is estimated by the following formula:

$$\text{Antifungal activity (\%)} = ((D_c - D_s) / D_c) \times 100$$

Where D_c is the diameter of growth in control plate and D_s is the diameter of growth in the plate containing tested antifungal agent. Sporulation can be also compared to the control.

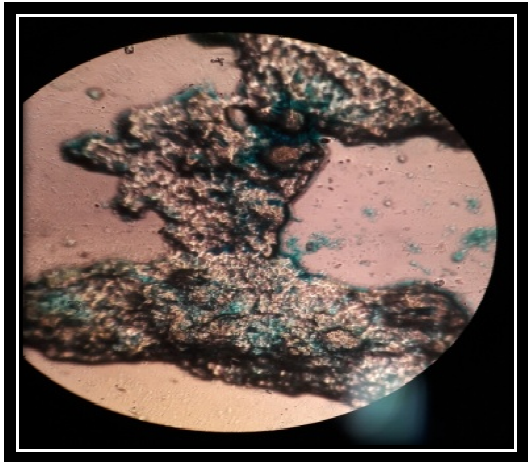
RESULTS AND DISCUSSION

Plant bioactivity testing

The plant was identified based on the morphology of the leaf and flower. Sharma RA *et al.*, 2013 stated that the leaves arise in pairs at stem nodes and are rounded 10 to 10mm long and 3 to 7 mm width. It contains white to purple color produced in heads 10mm in diameter on long peduncles. On microscopic observation of the transverse section of the leaf the epidermis was observed. It was made up of small vertically elongated parenchyma cells. Granular trichomes are seen in small depressions. It also showed chlorenchymatous cells.



Phyla nodiflora



Transverse section of leaf

On the phytochemical analysis the chloroform extract showed the positive results for most of the substances. In chloroform extract the plant showed the presence of proteins, amino acids, alkaloids, flavanoids, steroids, terpanoids, cardiac glycosides, antroquinones.



Phytochemical analysis with chloroform extract



Phytochemical analysis with Toluene extract



Phytochemical analysis with Water extract



Phytochemical analysis with Dimethyl formamide extract

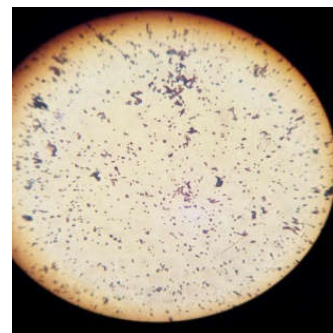
In LCB mout the hyphae with vacuoles. The shape of microconidia vary from slender clavate to pyriform. Macroconidia were very few. In PDA plates slow growth was seen. Surface pigmentation may vary from colorless to yellowish to yellow brown to wine red.



Microscopic observation



Colony morphology in PDA



Microscopic observation



Colony morphology in PDA

In LCB mount yeast like conidia were seen. The cells are ellipsoidal shape which are round at one end. Bottle shaped budding cells were seen. In PDA with coconut oil *Malassezia* colonies are raised and smooth initially and get dry and wrinkled in time. The color is creamy yellow to brown. On antifungal activity testing by poisoned food method with herbal salve the growth of both the yeast and dermatophytes were seen in 10g concentration. But in 20g and 30g concentration the growth was not seen. Which shows that the plant *Phyla nodiflora* is a fungistatic against both the *Malassezia sp* and *Trichophyton sp*. Anitha *et al.*, 2013 experimented the antidandruff activity of Indigenous medicinal herbs and its synergistic effect were tested. *In vitro* MIC showed that the water extract of *Wrightia tinctoria* was rapidly inhibiting the dandruff causing microorganism at highest and also in least concentration.

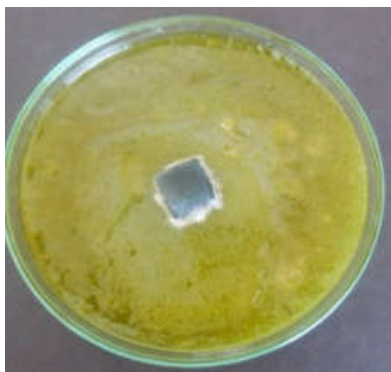


Test plate (20g)

Malassezia sp



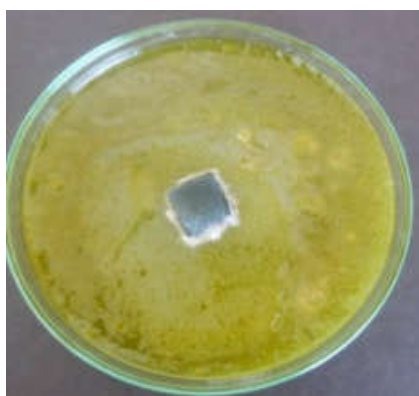
Control



Control



Test plate (10g)



Test plate (10g)



Test plate (20g)

S.No	Tests	Chloroform	Toluene	Water	Dimethyl formamide
1.	Protein - Biuret test	+	-	-	+
2.	Amino acids - Ninhydrin test	+	-	-	+
3.	Starch - KOH test	+	+	+	+
4.	Alkaloids - Dragondroff test	+	+	+	+
5.	Flavanoids - Ferric chloride	+	+	+	-
6.	Flavanoids - Ammonia HCl	-	+	+	+
7.	Flavanoids - Aluminium chloride	+	+	+	+
8.	Tannins - Ferric chloride	+	+	+	+
9.	Tannins - Iodine	+	+	-	-
10.	Tannins - H ₂ SO ₄ & HCl	+	+	+	+
11.	Tannins - Potassium dichromate	+	+	+	+
12.	Tannins - Lead acetate	-	+	+	+
13.	Phlobatannins - 1% HCl	-	-	+	-
14.	Steroids - Liebermann Buchard	+	+	-	+
15.	Terpanoids - Salkowski	+	-	-	+
16.	Saponins - Foam	-	-	-	-
17.	Cardiac glycosides - Keller Killiani	+	+	+	+
18.	Anthraquinones	-	-	-	-

oly herbal hair oil was prepared using the above mentioned medicinal plants and preclinical trials were performed with human volunteers. After 8 days of treatment with PHO, there was reduction in the dandruff scaling from "severe to mild" and traces to nil" in all the 6 volunteers was observed. Sharma RA *et al.*, reported The antifungal activity of crude extracts of *L. nodiflora* L. against the human pathogenic fungi was reported. The crude extract of various solvents of *Phyla nodiflora* had been screened for Antifungal activity against a *spergillus niger*, *A. Flavus*, *Paecilomyces varioti*, *Microsporum gypseum*, *Trichophyton rubrum*. All crude extract had significant inhibition activity against most of the fungi. Ethanol extract had maximum inhibition activity (100 %) against tested organism as compared to aqueous (82.6%), Methanol (61 %), Ethyl acetate (87 %) extracts.³⁴ There are some terpenoids in *L. nodiflora* composition, it shows antifungal activity.

Conclusion

Most of the human fungal pathogens are becoming resistance to commercial antifungal agents. Commercial antifungal agents like ketaconazole, meconazole, posaconazole etc though has a promising effect against fungal pathogens they cause side effects in most of the persons. Herbal remedies for such pathogens are in need to overcome this problem. In this current study the selected antifungal pathogens – *Trichophyton sp* and *Malassezia sp* were able to grow well in the presence of coconut oil. Hence herbal salve is prepared against these pathogens by mixing the dry powder of the plant with coconut oil in 3 different concentrations. Among which 20g per 10ml concentrations showed the similar results as the commercially available antifungal lotions. Hence dandruff control can be achieved by applying the herbal salve on the scalp as it acts as a very good fungistatic agent.

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