

***In vitro* Evaluation of Indigenous Medicinal Plants for Their Antidandruff Hair Oil Preparation**

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Abstract: 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. The ethanol and water extraction was carried out for 48 hrs in room temperature. Phytochemicals screening of leaf extracts of the above plants showed the presence of Saponin, Flavanoids, Alkaloids, Terpenoids, Aminoacids and Cardio glycosides in the leaf extracts. *In vitro* antidandruff activity showed that *Malassezia furfur* was very sensitive to all the concentrations of both ethanol and water extracts of medicinal plants used in this study. *In vitro* MIC showed that the water extract of *Wrightia tinctoria* L was rapidly inhibiting the dandruff causing microorganism at highest and also in least concentration. Poly 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 nill” in all the 6 volunteers was observed. The synergistic outcome of medicinal plants providing an attractive surveillance which can well be applied as a development strategy in hair care products, targeting the control of dandruff. Further studies should be carried out to unravel the identity of the active ingredients as well as its medicinal properties.

Key words: Dandruff • *Wrightia tinctoria* L • *Ziziphus Jujube* L • *Lippia Nodiflora* L • *Malassezia furfur* • Antidandruff activity • Polyherbal hair oil

INTRODUCTION

Dandruff is a condition of the scalp and other hairy areas of the body characterized by the presence of abundant flakes that break off and accumulate in the hair. It manifest as profuse white to silvery powdery scales in the scalp region often with moderate to severe itching [1]. Dandruff is also called as Pityriasis versicolor disease which is a chronic, superficial fungal infection of the skin caused by the lipophilic, yeast like fungus *Malassezia* [2]. It is a common scalp disorder affecting almost half of the pubertal population of any ethnicity in both genders but most prevalent in male population between the age group of 20 and 60 years [3]. The most

common symptoms of dandruff are hair falling, light brown or white patches on the skin, redness, itching, seborrhea (which is a chronic skin inflammation that produces many scales and redness of the affected area with itching sensation).

In the current scenario, many chemical substances (shampoos) are used for treating dandruff by controlling the abundance of fungi on the scalp. Shampoos are toxic and found to have side effects like dryness of the scalp and hair, oiliness of the scalp and hair, irritation of the scalp, skin and mucous membrane of the eyes, discolouration of hair, loss of hair and variation in the individual response due to the natural differences and due to chemicals used in different preparations [4].

To avoid these problems, Herbs are widely used as remedial agents because such drugs are easily available at low cost and comparatively safe and the people have good faith in such remedies. In India, Ayurvedic system evolved over 5,000 years ago and is still in practice. The Rig veda and Atharvanaveda have included more than 700 medicinal prescriptions [5]. In Ayurvedic medicine, herbs are used as an integral part of health care systems. Besides health care it is also used for beautification of the body and for preparation of various cosmetics and colours. With this the present study focuses on the antidandruff activity of Indigenous medicinal herbs and its synergistic effect against dandruff causing microorganisms specifically *Malassezia furfur*.

MATERIALS AND METHODS

In light of the recent development in scientific and technological world, even today herbs are widely used as remedial agents. WHO currently encourages, recommends and promotes traditional (or) herbal remedies in National health care programs because such drugs are easily available at low cost and comparatively safe, the people have good faith in such remedies [4]. With this concern an attempt has made in some indigenous medicinal plant which is suspected to have the antidandruff activity.

Collection of Plant Material: Fresh leaves of *Wrightia tinctoria* Linn (Family: *Apocynaceae*; Common Name: Ivory wood (English)); *Ziziphus Jujube* Linn (Family: *Rhamnaceae*; Common Name: Red date (English) and *Lippia Nodiflora* Linn (Family: *Verbenaceae*; Common Name: Frog fruit (English) were collected from Palakkad District and Botanical garden, Agricultural University, Coimbatore District and authenticated by Botanical Survey of India, Coimbatore.

Preparation of Solvent Extraction: The leaf samples were dried in shade, powdered and extracted with aqueous and ethanol as solvent system for overnight according to the method of Mbakwem *et al.* [6]. The mixture was heated in a boiling water bath at 80°C for 15 mins. The extracts were completely dried under vacuum to obtain pure powders. The percentage of yield was calculated and the dried plant extract was used for phytochemical screening [7].

In vitro Antidandruff Activity of the Extracts

Organism Used (Dermatophyte): Test organisms used in this study are *Malassezia furfur* (MTCC 1372), *Trichophyton mentagrophytes* (MTCC 8276) and

Microsporum gypseum (MTCC 2819) were obtained from Microbial Type Culture Collection, Chandigarh, India. The freeze dried cultures were inoculated into broth and maintained in Sabouraud's Dextrose Agar (SDA) slant and stored at 4°C for further studies.

Antifungal Susceptibility Testing-Disk Diffusion

Method: *In-vitro* antidandruff activity was performed using the disk diffusion assay described by Richa *et al.* [8]. Suspension containing 5×10^6 CFU/ml of dandruff causing organisms was swabbed on the surface of the sterile SDA plates using a sterile cotton swab. Sterile filter paper discs impregnated with the extracts ranging from 25 µg/ml, 50 µg/ml and 75 µg/ml per disc was aseptically placed over the seeded SDA plates. Similarly, standard antibiotic disc of fluconazole was used as standard drug for comparison of antifungal activity. The plates were incubated at 27°C for 28 hrs and at the end of incubation the diameter of the inhibition zones was measured and tabulated.

Determination of Minimum Inhibitory Concentration

(MIC): Different concentrations of plant extracts (0.5 to 2.0 mL) was prepared and dispensed into test tubes and made up to 5 mL with sterile SD broth. One drop of an overnight broth culture of dandruff causing microorganisms was inoculated and incubated for 22-28 hrs at 25°C to determine the MIC. The culture inoculated into the sterile SD broth and placed at 2°C in a refrigerator overnight was used as standard. The MIC value was taken as the lowest concentration of compound at which there is no visible growth of the test organisms after 22-28 hrs of inoculation at 25°C.

Preparation of Polyherbal Hair Oil (PHO): The formula of base contains coconut oil. Mixed equal volume of *Lippia Nodiflora* L, *Ziziphus Jujube* L and *Wrightia tinctoria* L powders properly and then added Juice of Aloe Vera, curry leaves and cumin seed (Hair growth promotion) for getting uniform paste. Into a heated 500 mL pure coconut oil added the above paste and boiled for 1hr on medium flame with intermittent shaking. Thereafter, the oil was cooled, filtered and tested for its potency as a novel antidandruff agent [9].

Preclinical Studies of Polyherbal Oil for Antidandruff

Activity: Six volunteers between the age group of 18-25 years studying in our college, having different scaling of dandruff were included in this study. PHO (10-15 ml/day) was used every day instead of commercial hair oil. During PHO usage, all the volunteers are requested to abstain

from the use of other antidandruff shampoos/hair cream or any other antifungal medicament. They were examined three days once up to 10 days. Scalp scrapings were collected from the volunteers, prior and after oil application. The scraped materials were inoculated in SDA and incubated at 37° C for 3 days. The total spore burden on the scalp before and after the use of PHO was assessed to determine the severity of the scalpel scaling and to determine the efficacy of Polyherbal hair oil against dandruff [1].

Statistical Analysis: Samples were analyzed in triplicates and the results were given as Mean \pm SD.

RESULTS AND DISCUSSION

The collected aqueous (water) and solvent (ethanol) extracts of *W.tinctoria* L, *L. nodiflora* L and *Z. jujube* L were analyzed for the presence of phytochemicals. It showed the presence of saponin, flavanoids, alkaloids, terpenoids, amino acids and cardio glycosides in the leaf extracts. Similarly polyherbal mixture of Pomegranate fruit rind, *Azadirachta indica* and *Datura metal* which was rich in Alkaloids, Flavanoids, Phenolic compounds, Terpenoids, Tannins has been reported by Prabha *et al.* [10]. Sibi *et al.* [11] revealed the presence of Flavonoids, Saponins, Tannins and Terpenoids in various solvent extracts of *R.communis* leaves. Likewise phytochemical screening of *Albizia amara*, *Achyranthes asperan*, *Cassia fistula*, *Cassia auriculata*, *Datura stramonium* and *Azadirachta indica* showed the presence of Alkaloids, Terpenoids, Coumarin and Saponins was illustrated by Suresh *et al.* [1].

Activity of Fractionized Extracts of Medicinal Plants Against Dematophytes: The ethanol and water extracts of the leaf parts of *W. tinctoria* L, *Z. jujube* L and *L. nodiflora* L were tested for their efficacy against dandruff causing microorganisms. The concentrations of the extract used were 25, 50, 75 μ g/mL and that of the antibiotic of 25 mg/disc.

M. furfur was sensitive to all the extracts of plants tested. Ethanol extract of *Z. jujube* L showed the inhibition of 0.77 ± 0.25 , 1.47 ± 0.25 and 2.17 ± 0.12 when used in 25, 50 and 75 μ g/mL concentrations where as flucanazole showed zone of 2.03 ± 0.15 . For the water extract, the inhibition of 1.07 ± 0.21 , 1.47 ± 0.23 , 2.57 ± 0.60 at the concentration of 25, 50 and 75 μ g/mL and for the antibiotic 2.3 ± 0.62 was observed. The lower concentrations (25 and 50 μ g/mL) of ethanol and water extracts also had significant inhibition percentage.

The water extract of *L. nodiflora* L showed greater inhibition than the ethanol extract when 25, 50 and 75 μ g/mL concentrations was used. The inhibition produced by water extract at these concentrations was 0.97 ± 0.42 , 1.40 ± 0.53 and 2.40 ± 0.30 cm. For ethanol extract the inhibition was found to be 0.6 ± 0.53 , 1.07 ± 0.55 and 1.97 ± 1.00 cm at the same concentration. The inhibitory zone of antibiotic was found to be 1.30 ± 1.04 and 1.63 ± 0.85 respectively.

Similar zones were found for ethanol (1.9 ± 0.30 and 2.3 ± 0.20) and water extracts (1.9 ± 0.30 and 2.3 ± 0.20) of *W. tinctoria* L at 50 and 75 μ g/mL concentrations. The inhibition produced by ethanol and water extract was 1.40 ± 0.26 and 1.53 ± 0.57 respectively at 25 μ g/mL concentration. In control the activity was found to be Nil.

The ethanol extracts of *L. nodiflora* L and *Z. jujube* L showed greater inhibition (2.26 ± 0.00) compared to *W. tinctoria* L (1.93 ± 0.21) at 75 μ g/mL concentration. At 25 and 50 μ g/mL concentration of *L. nodiflora* L showed the inhibition of 1.03 ± 0.00 and 1.43 ± 0.00 . The inhibition produced by ethanol extract of *W. tinctoria* at 25 and 50 μ g/mL was 0.67 ± 0.45 and 1.13 ± 0.21 respectively.

The inhibition produced by 25, 50 and 75 μ g/mL of water extract of *L. nodiflora* L was found to be similar as of ethanol extract whereas *W. tinctoria* L showed slightly lower inhibition than the ethanol extract. The antibiotic showed the inhibition of 1.27 ± 0.75 , 2.0 ± 0.00 and 2.1 ± 0.00 against *T. mentagrophytes*.

The ethanol and water extract extracts of *Z. jujube* L showed significant inhibition with 1.43 ± 0.00 and 0.53 ± 0.42 at 25 μ g/mL and 1.9 ± 0.00 and 1.2 ± 0.26 at 50 μ g/mL concentrations respectively. The 75 μ g/mL of concentration of ethanol and water extracts had controlled the growth at a zone of inhibition 2.26 ± 0.00 and 1.43 ± 0.21 respectively.

Like *M. furfur*, *T. mentagrophytes*, the dandruff causing *M. gypseum* was also sensitive to 75 μ g/mL of ethanol extract of the selected medicinal plants. The inhibition produced by ethanol extract of *L. nodiflora* L was 2.7 ± 0.56 at 75 μ g/mL where as the antibiotic showed zone of 2.23 ± 0.31 . The zone of inhibition at 25 and 50 μ g/mL concentrations are of 1.56 ± 0.40 and 2.07 ± 0.25 . The water extract did not showed greater inhibition like ethanol extract at 25 and 50 μ g/mL concentrations.

The water extract of *Z. jujube* L showed little inhibitory activity with 0.3 ± 0.17 , 0.67 ± 0.21 and 1.13 ± 0.15 at 25, 50 and 75 μ g/mL concentrations but the ethanol extract showed better inhibitory activity with, 1.2 ± 0.26 , 1.53 ± 0.25 and 2.2 ± 0.20 at same concentrations. Similarly lesser inhibitory activity was showed by the ethanol and

Table 1: Effect of fractioned extracts of medicinal plants against dermatophytes

Test organism	Plant Name	Extract	Growth index (in cm)				
			Control	Antibiotic	Concentration of the extract in µg/mL		
					25	50	75
<i>M. furfur</i>	<i>Wrightia tinctoria</i> L	Water	0.0	1.37 ± 0.15	1.53 ± 0.57	1.87 ± 0.35	2.03 ± 0.25
		Ethanol	0.0	2.03 ± 0.25	1.40 ± 0.26	1.9 ± 0.30	2.3 ± 0.20
	<i>Ziziphus jujube</i> L	Water	0.0	2.3 ± 0.62	1.07 ± 0.21	1.47 ± 0.23	2.57 ± 0.60
		Ethanol	0.0	2.03 ± 0.15	0.77 ± 0.25	1.47 ± 0.25	2.17 ± 0.12
	<i>Lippia nodiflora</i> L	Water	0.0	1.30 ± 1.04	0.97 ± 0.42	1.40 ± 0.53	2.40 ± 0.30
		Ethanol	0.0	1.63 ± 0.85	0.6 ± 0.53	1.07 ± 0.55	1.97 ± 1.00
<i>T. metagrophyte</i>	<i>Wrightia tinctoria</i> L	Water	0.0	1.2 ± 0.66	0.4 ± 0.20	0.93 ± 0.38	1.47 ± 0.61
		Ethanol	0.0	1.27 ± 0.75	0.67 ± 0.45	1.13 ± 0.21	1.93 ± 0.21
	<i>Ziziphus jujube</i> L	Water	0.0	1.07 ± 0.45	0.53 ± 0.42	1.2 ± 0.26	1.43 ± 0.21
		Ethanol	0.0	2.0 ± 0.00	1.43 ± 0.00	1.9 ± 0.00	2.26 ± 0.00
	<i>Lippia nodiflora</i> L	Water	0.0	2.1 ± 0.00	1.03 ± 0.00	1.43 ± 0.00	2.26 ± 0.00
		Ethanol	0.0	2.1 ± 0.00	1.03 ± 0.00	1.43 ± 0.00	2.26 ± 0.00
<i>M. gypseum</i>	<i>Wrightia tinctoria</i> L	Water	0.0	1.2 ± 0.00	0.60 ± 0.00	1.07 ± 0.00	1.4 ± 0.00
		Ethanol	0.0	1.8 ± 0.26	1.2 ± 0.10	1.43 ± 0.15	1.97 ± 0.15
	<i>Ziziphus jujube</i> L	Water	0.0	1.00 ± 0.20	0.3 ± 0.17	0.67 ± 0.21	1.13 ± 0.15
		Ethanol	0.0	1.97 ± 0.31	1.2 ± 0.26	1.53 ± 0.25	2.2 ± 0.20
	<i>Lippia nodiflora</i> L	Water	0.0	0.97 ± 0.45	0.43 ± 0.21	0.63 ± 0.21	1.17 ± 0.15
		Ethanol	0.0	2.23 ± 0.31	1.56 ± 0.40	2.07 ± 0.25	2.7 ± 0.56

* Zone of inhibition in cm, Values are mean ± SD of triplicates

Table 2: MIC concentrations of medicinal plants against dermatophytes

			OD at 660 nm			
			Concentrations			
Test organism	Medicinal plants studied	Extracts	0.5ml	1ml	1.5ml	2ml
<i>M. furfur</i>	Fluconazole	-	0.65	0.58	0.46	0.38
	<i>Wrightia tinctoria</i> L	Water	0.21	0.17	0.14	0.10
		Ethanol	0.70	0.58	0.36	0.27
	<i>Ziziphus jujube</i> L	Water	0.29	0.24	0.21	0.17
		Ethanol	1.48	1.27	0.67	0.42
	<i>Lippia nodiflora</i> L	Water	0.47	0.46	0.42	0.40
Ethanol		0.50	0.48	0.38	0.35	
<i>T. metagrophyte</i>	Fluconazole	-	0.56	0.52	0.48	0.43
	<i>Wrightia tinctoria</i> L	Water	0.40	0.24	0.19	0.12
		Ethanol	1.74	1.24	0.53	0.24
	<i>Ziziphus jujube</i> L	Water	0.60	0.57	0.51	0.42
		Ethanol	0.52	0.47	0.43	0.39
	<i>Lippia nodiflora</i> L	Water	0.90	0.82	0.78	0.53
Ethanol		1.56	1.23	0.71	0.42	
<i>M. gypseum</i>	Fluconazole	-	0.49	0.45	0.41	0.39
	<i>Wrightia tinctoria</i> L	Water	0.15	0.12	0.10	0.07
		Ethanol	1.97	1.58	1.14	1.07
	<i>Ziziphus jujube</i> L	Water	0.42	0.37	0.31	0.22
		Ethanol	0.68	0.52	0.41	0.37
	<i>Lippia nodiflora</i> L	Water	1.95	1.23	0.59	0.42
Ethanol		0.37	0.23	0.17	0.10	

water extracts of *W. tinctoria* L at 25, 50 and 75 µg/mL concentrations (Table 1). From the above results it was found that the dermatophytes were more sensitive to all the concentrations of ethanol extract of all the three medicinal plants than the water extract.

There are several reports on the aqueous and ethanolic extract of *Terminalia bellerica* showed a dose-dependent antidandruff activity against *Malassezia furfur*. The synergistic antidandruff studies were performed by making the aqueous mixtures of *Terminalia*

bellerica and *Lantana camara* and ethanolic mixtures of *Terminalia bellerica* and *Lantana camara* in equal proportions. Also the results showed no synergy between *Terminalia bellerica* and *Lantana camara* in any of the solvents tested [12]. Similarly the ethanolic extract of *Ficus exasperatavahl* inhibited the growth of the fungi *Microsporum*, *Trichophyton* and *Malassezia furfur* to a high degree was reported by Mbakwem *et al.* [6]. Sibi *et al.* [11] revealed that the methanolic extracts of *R. commuis* exhibited significant activity (8.20 ± 0.3) against *Malassezia* species. Whereas aqueous extracts of the leaves recorded appreciable inhibitory activity (5.74 ± 0.8) when compared with chloroform (1.66 ± 1.2) and petroleum ether extracts.

Minimum Inhibitory Concentration (MIC): Table 2 showed the minimum inhibitory concentrations of the water and ethanol extracts of medicinal plants against dandruff causing microorganisms. The highest MIC value of 0.10 (OD) was obtained for water extract of *W. tinctoria* L and least MIC value of 0.42 (OD) was obtained for water extract of *Z. jujube* L at 2 ml concentration against *M. furfur*. The water extracts showed greater inhibitory activity than the ethanol extracts against *M. furfur*.

The highest MIC value of 0.12 (OD) was obtained for *W. tinctoria* L and least MIC value of 0.53 (OD) was obtained for *L. nodiflora* L of water extract at maximum (2 ml) concentration. At lower concentration (0.5 ml) highest MIC of 0.40 was obtained for water extract of *W. tinctoria* L and least MIC of 1.74 for ethanol extract of *W. tinctoria* L. The water extracts was proved to be best against *T. mentagrophyte*.

The highest MIC value of 0.07 (OD) at 2 ml and 0.15 (OD) at 0.5 ml was obtained for water extract of *W. tinctoria* L against *M. gypseum*. Likewise least MIC value of 1.07 (OD) at 2 ml, 1.97 (OD) at 0.5 ml for ethanol extract of *W. tinctoria* L was obtained. The ethanol extract failed to produce higher activity than water extract against

M. gypseum. From the above it has been observed that water extract of *W. tinctoria* L was rapidly inhibiting the multiplication of the dandruff causing microorganism in test tube at highest and also in least concentration than the antibiotic fluconazole. Likewise evaluation of Minimum Inhibitory Concentrations (MICs) of two standard antifungal drugs (Ketoconazole and Fluconazole) against *Malassezia furfur* and their comparison with botanicals was done by Amit *et al.* [13]. MICs of Fluconazole, Ketoconazole, Eugenol and *Micromeria biflora* essential oil against *Malassezia furfur* were found to be 10.538 mg/ml, 6.438 mg/ml, 6.956 mg/ml and 8.928 mg/ml respectively.

One study reported from India, showed MIC of Ketoconazole 2.5 µg/ml, Fluconazole 2.5 µg/ml, Clove oil 1000 µg/ml, Coleus oil 25 mg/ml and Basil oil 10 mg/ml against *Malassezia furfur* by disc diffusion method [14]. Another study done by Miranda *et al.* [15] which MIC ranges were <0.03-4 µg/ml for Ketoconazole and <0.125 to >64 µg/ml for Fluconazole against *Malassezia*. Eugenol and *Micromeria biflora* oil recorded a very good activity among the herbal ingredients. MIC of Eugenol was very close to Ketoconazole which is a popular synthetic antifungal. The minimum inhibitory concentrations of the ethanolic extracts of *Ficus exasperatavahl* showed the highest MIC value of 44.67mg/ml was obtained for *Ficus exasperatavahl* against *Epidermophyton* and least MIC value of 25.12mg/ml was obtained against *Malassezia furfur* [6].

Preclinical Studies of Polyherbal Oil for Antidandruff Activity: In the clinical studies, after 8 days of treatment with PHO, there was a reduction in the scaling from “severe to mild” and traces to nil” in all the 6 volunteers. Thus PHO was found to be very effective against *M. furfur* *in vitro*. There was clear symptomatic relief from dandruff in all the volunteers after 10 days of use. Further, the isolation of *M. furfur*, the causative organism of the dandruff was not found after the use of PHO (Table 3).

Table 3: Effect of PHO on *Malassezia furfur* in the scalp

S.No	Scaling range of volunteers	No of Volunteers	Isolation of Pre use	After PHO use		
				Day 3	Day 6	Day 10
1.	Severe	V 1	TNTC	500	120	5
		V 2	TNTC	650	225	12
		V 3	TNTC	300	75	No colonies
2.	Moderate	V 4	1200	135	28	3
3.	Mild	V 5	400	40	10	No colonies
		V 6	520	32	8	No colonies

* TNTC-Too Numerous To Count

The PHO contains all good characters and it was found more efficient and profitable. Whereas isolation of *P. ovale* was not possible in all the volunteers after 8 days was documented by Suresh *et al.* [1]. In case of Dano, the isolation of dandruff causing microorganism was possible after 8 days also [16].

In contrast, Mohamed *et al.* [4] prepared and evaluated an antidandruff herbal shampoo powder using natural ingredients with *Ocimum sanctum* (Tulsi) and *Azadiracta indica* (Neem). They reported that the powder contain all good characters of an ideal shampoo and it was found to be harmless, more effective and economic against strains of Gram +ve, Gram-ve organisms and fungal organism such as *Candida albicans*. Likewise, herbal shampoo powder using bahera, amla, neem tulsi, shikakai, henna and brahmi were formulated by Sachin *et al.* [17] for hair care. They evaluated for organoleptic, powder characteristics, foam test and physical evaluation which was reported as useful for the standardization of herbal shampoo powder.

From this study it can be concluded that the very significant antidandruff activity of *Wrightia tinctoria* L, *Ziziphus Jujube* L and *Lippia Nodiflora* L present them as natural precious antidandruff agents against controlling the growth of *Malassezia furfur*, *Trichophyton mentagrophytes* and *Microsporeum gypseum* and also in the treatment of fungal related disorders. The formulation of herbal hair oil was found to be very effective against *M.furfur in vitro*. The PHO contains all good characters and it was found more efficient and profitable. The synergistic outcome of medicinal plants providing an attractive surveillance which can well be applied as a development strategy in hair care products, targeting the control of dandruff. Further studies should be carried out to unravel the identity of the active ingredients as well as its medicinal properties.

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