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Screening of antimicrobial efficacy of traditionally used Indian plants against microorganisms associated with dandruff

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Dandruff is a clinical condition affecting the scalp, causes itching and relapsing inflammation. In the present study, a total of 32 plant extracts of 16 plants were prepared using methanol and ethyl acetate and screened for their anti-dandruff activity by agar well diffusion method. Dandruff causing microorganisms including *Staphylococcus epidermidis, Propionibacterium acne* and *Malassezia furfur* were selected for the study. Ethyl acetate extract of *Cinnamomum zeylanicum* and *Glycyrrhiza glabra* exhibited maximum activity with zone of inhibition of 18-20 mm while methanol extract of *Punica granatum* and *Syzygium aromaticum* demonstrated moderate activity against the studied microbes. On the basis of ZOI, PCA and MIC the results revealed that the ethyl acetate extract of *C. zeylinicum* bark and *G. glabra* root are most influential extracts followed by methanol extract of *P. granatum, G. glabra, S. aromaticum,* and ethyl acetate extract of *A. nilotica* in term of antimicrobial activity against the studied dandruff causing microbes. Therefore the active plant extracts can act as a potential source for the formulation of natural anti-dandruff agents.

Keywords: Anti-dandruff activity, *Cinnamomum zeylanicum*, *Glycyrrhiza glabra*, *Punica granatum*, PCA
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Dandruff is the most common scalp problem. It bothers about half of the population and causes significant discomfort. Dandruff may be heredity, known as seborrheic dermatitis. Sometimes this infection may become severe form with heavily affected 'continuously peeling off' scalp and this may affect other areas like nose corner, ears, eyelids. Dandruff sufferers frequently experience hair loss. Reasons of dandruff include a decrease in the level of hydration and of natural moisturizing factors (NMF); a disrupted barrier function; an altered proliferation and differentiation status on the scalp[1]. Sedentary life style, oily skin, stress, tiredness, temperature extremes, salty water, infrequent hair washing or obesity might increase the risk of dandruff[2].

Microorganisms such as *Malassezia furfur*, *Malassezia restricta,* and *Malassezia globosa* are generally considered to be the causative fungal species for dandruff[3]. The species involved differ depending on the host's geographical location. The bacteria *Propionibacterium acne* and *Staphylococcus epidermidis* inhabits on human scalp[4]. Basically, these microbes release many enzymes such as proteinases, lipases, hyaluronidases which may catabolised sebum into pro-inflammatory free fatty acid. These fatty acids damage top scalp layer of human, causing inflammation, skin cell flaking[5-7]. These days many treatments are available for the management of dandruff. Various antifungal agents such as ketoconazole, zinc pyrithione, selenium sulphide, cetyl alcohol are used in anti-dandruff preparations. But these compounds have many drawbacks; such as scalp dryness, deposition of salts on hair shafts, discoloration of hairs, toxicity to eyes, irritation, headache etc.[8]. Treatment of dandruff with available drugs is restricted due to microbial resistance, compliance and cost. Despite of the availability of a variety of antifungal agents the complete cure of dandruff is far from reach. Plant based products are eco-friendly and considered safe due to negligible side effects[9-11].

India has a rich heritage of traditional medicine which is flourishing us from many centuries. Various ethnopharmacological studies showed that botanicals such as amla, reetha, shikakai, lemon, methi, neem, heena are used in hair care[12,13]. Massage of *Aloe vera* on the hair scalp helps in hair growth and makes them silky[14]. *Lawsonia inermis* (Mehandi) leaves are finely ground and mixed with water to make a juice that is

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applied to the head for hair care. Decoction of *Murraya koenigi* (Curry), *Eclipta alba* (Bhringraj) leaves mixed in coconut oil and used as an excellent hair tonic to treat dandruff[15,16]. Aqueous extract of *Glycyrrhiza glabra* root is a good wash for falling and graying hair[17]. Medicinal plants containing secondary metabolites such as phenols, flavonoids, alkaloids, tannins, terpenoids etc., makes them effective against microbial infections but scientific validation is needed to justify their role[18-21].

Materials and Methods

Plant materials

Plant materials such as leaf of *Lawsonia inermis* (hena), *Azadirachta indica* (neem), *Aloe vera* (ghritkumari), *Acacia nilotica* (babul), *Emblica officinalis* (amla), *Tecomella undulata* (Ruhera), *Murraya koenigi* (curry patta); Rhizome of *Glycyrrhiza glabra* (mulethi), *Curcuma longa* (haldi); bark of *Cinnamomum zeylanicum* (dalchini); flower bud of *Syzygium aromaticum* (laung); fruit of amla, *Terminalia chebula* (harad), *Terminalia bellerica* (baheda); seed pod of *Acacia concinna* (shikakai) and fruit peel of *Punica granatum* (anar) were collected from Rohtak district of Haryana, India. The leaves of *T. undulata* leaves were procured from Mohindergarh district of Haryana. The plant samples were further identified using the Haryana Flora and by comparing herbarium specimens at Maharshi Dayanand University in Rohtak, India.

Plant extracts preparation

Plant extracts were prepared by cold percolation method[22]. Collected plant samples were washed, shade dried and powdered. 10 g of the prepared sample was dissolved in 100 mL of solvent (methanol and ethyl acetate) and rotated in an incubator shaker for two days. After that, it was filtered, lyophilized and stored for later use.

Antimicrobial activity

The antimicrobial activity of plant extracts against microorganism strains; *S. epidermidis* (MTCC 435), *P. acne* (MTCC 1951) and *M. furfur* (MTCC 1374) was determined using the agar well diffusion method[23].

Minimum inhibitory concentration (MIC)

MIC of effective plant extracts were determined by using 96 well plate methods[23,24].

Principal component analysis (PCA)

Principal component analysis was done by latest version of XLSTAT statistical software for evaluate correlation between plant extract and antimicrobial activity.

Results

In the present studies, plant extracts of selected medicinal plants were prepared for checking antimicrobial activity against dandruff causing microorganisms; *Staphylococcus epidermididis, Propionibacterium acne and Malassezia furfur*. The antimicrobial microbial activities were varied in the range of 8-20 mm (Table 1) against the studied microbes. Among all the extracts the ethyl acetate extract of *C. Zeylanicum*, *G. glabra* and *A. nilotica* showed the significant antimicrobial against all the tested microbes. Similarly, methanol extract of *P. granatum*, *S. aromaticum* and *T. bellirica* showed the antimicrobial activity against the tested pathogens (Table 1). The results were compared with standard antibiotic Piperacillin.

The horizontal axis explained 90.02% of the total variance in the Principal component analysis (PCA), while the vertical axis explained 13.23%. F1 and F2 are horizontal and vertical axis of PCA graph respectively which help in calculation of correlation as shown in Figure 1. Plant extract activity against *M. furfur* positively lies on F1 axis followed by *S. epidermidis*. Plant activity against *P. acne* positively linked with F2 axis. Ethyte acetate extracts of *C. zeylinicum* bark (S8) and *G. glabra* root (S2) have the highest F1 score followed by methanol extract of *P. granatum* (S15), *G. glabra* (S1), *S. aromaticum* (S17) and ethyl acetate extract of *A. nilotica* (S20), other extracts showed lower F1 score as shown in Table 2.

Minimum inhibitory concentration (MIC)

The MIC of selected plants extracts has been illustrated in Figure 2. *G. glabra*, *C. zeylanicum* extract showed the lowest MIC (0.19 mg/mL) against *P. acne* and *M. furfur* while *P. granatum* against *S. epidermidis* at same concentration.

Discussion

Nature has bestowed medicinal plants to mankind for various ailments and there is a cure for every diseases, man has to find it out. The extract preparation as methanol dissolves most of the polar compounds while ethyl acetate dissolves non- polar compounds. The antimicrobial activity of *Glycyrrhiza glabra* is well known[25]. *G. glabra* secondary metabolites such as glycyrrhizin, 18 β-glycyrrhetinic

Table 1 — ZOI of plant extracts against *P. acne*, *S. epidermidis* and *M. furfur*

Plant Name	Methanol(M) / ethylacetate (EA) extracts	Sample code	Zone of Inhibition(mm) at 1.5 mg/well		
			P. acne	*S. epidermidis*	*M. furfur*
G. glabra	M	S1	16.11±1.0	12.83±0.76	16. 67±1.52
	EA	S2	20.33±1.15	18.16±0.29	20±1.0
L. innermis	M	S3	12.33±0.57	13.28±0.28	12.34±1.54
	EA	S4	11.67±0.58	11.5±0.5	12.33±0.57
A. indica	M	S5	13.66±0.58	-	11.44±1.15
	EA	S6	17.33±1.15	10.33±0.58	-
C. zeylanicum	M	S7	12±1.0	13.5±0.5	11.23±1.05
	EA	S8	19.21±1.52	19.67±1.53	20±1.2
C. longa	M	S9	9.67±0.58	10 .17±1.04	11.23±1.05
	EA	S10	10.67±0.57	10.33±0.57	20±1.2
T. chebula	M	S11	12.33±1.53	13.83±0.28	14.12±0.67
	EA	S12	13.66±1.52	15.16±1.04	14.5±0.45
A. vera	M	S13	11±1.73	-	-
	EA	S14	13.33±0.58	9±1.0	13.8±0.73
P. granatum	M	S15	15. 67±1.52	19.33±1.53	16.66±1.53
	EA	S16	11.33±1.15	15.5±0.5	10.34±0.56
S. aromaticum	M	S17	15.66±0.57	15.16±0.29	15.5±0.5
	EA	S18	12.5±0.5	13.34±1.12	13.33±0.58
A. nilotica	M	S19	11.67±1.15	13.33±0.38	13.34±0.33
	EA	S20	17±1.0	14.16±1.03	15.33±1.08
E. alba	M	S21	-	10 .16±1.03	9.12±0.57
	EA	S22	-	-	9.67±0.57
T. undulata	M	S23	10.66±0.58	10.5±0.5	13.33±0.58
	EA	S24	-	9.5±0.5	14.34±0.66
M. koenigi	M	S25	9±1.0	11±0.5	11±1.74
	EA	S26	-	9.4±0.66	-
E. officinalis	M	S27	-	-	-
	EA	S28	12.5±0.5	-	11.56±0.44
T. bellirica	M	S29	14.33±0.57	14.5±0.5	14.5±0.5
	EA	S30	12.83±0.76	13.2±0.23	11.33±67
A. concina	M	S31	-	-	-
	EA	S32	-	-	13.43±0.57
Piperacillin disc used as standard		S33	16±0.31	15±0.12	16±0.25

acid has antimicrobial activity against *S. epidermidis*[26], glabridin against *Staphylococcus aureus*, and *S. epidermidis*[27,23]. Previously, MIC of glabridin was reported in the range of 31.25-250 µg/mL against *Candida albicans*[28]; glabrene, glabrol against *S. epidermis*[23]. Glycyrrhizic acid (Glycyrrhizin) has anti-irritant and anti - inflammatory properties[29].

Ethyl acetate extract of *C. zeylinicum* bark showed the significant activity against dandruff associated microorganism. *C. zeylinicum* bark contains cinnamaldehyde, cubenol, coumarin, a-copaene, a-muurolene, beta-bisabolene, caryophyllene, 1,8-cineole, humulene, trans calamenene, t- muurolol are the main active constituents. Cinnamaldehyde has antibacterial and anti-biofilm activity against *S. epidermidis*[30,31] and *M. furfur*[32]. Cinnamaldehyde also showed the anti-inflammatory activity[33]. Eugenol has antibacterial activity against *S. epidemidis*[31], *P. acnes*[34] and *M. furfur*[35].

Table 2 — Correlations between plants extract activity against dandruff associated microorganisms

Plant Sample	Code	F1-axis	F2-axis
C. zeylinicum	S8	2.657	-0.082
G. glabra	S2	2.614	0.177
P. granatum	S15	1.961	-0.327
A. nilotica	S20	1.446	0.307
G. glabra	S1	1.370	0.196
S. aromaticum	S17	1.437	0.043

Methanolic extract of *P. granatum* showed high activity against *S. epidermidis* and moderate activity against *P. acne* and *M. furfur*. *P. granatum* peels contain bioactive compound such as ellagitannins, quercetin, kaemferol, proanthocyanidin gallagic acid, ellagic acid, anthocyanins[36]. Punicalagin is the main

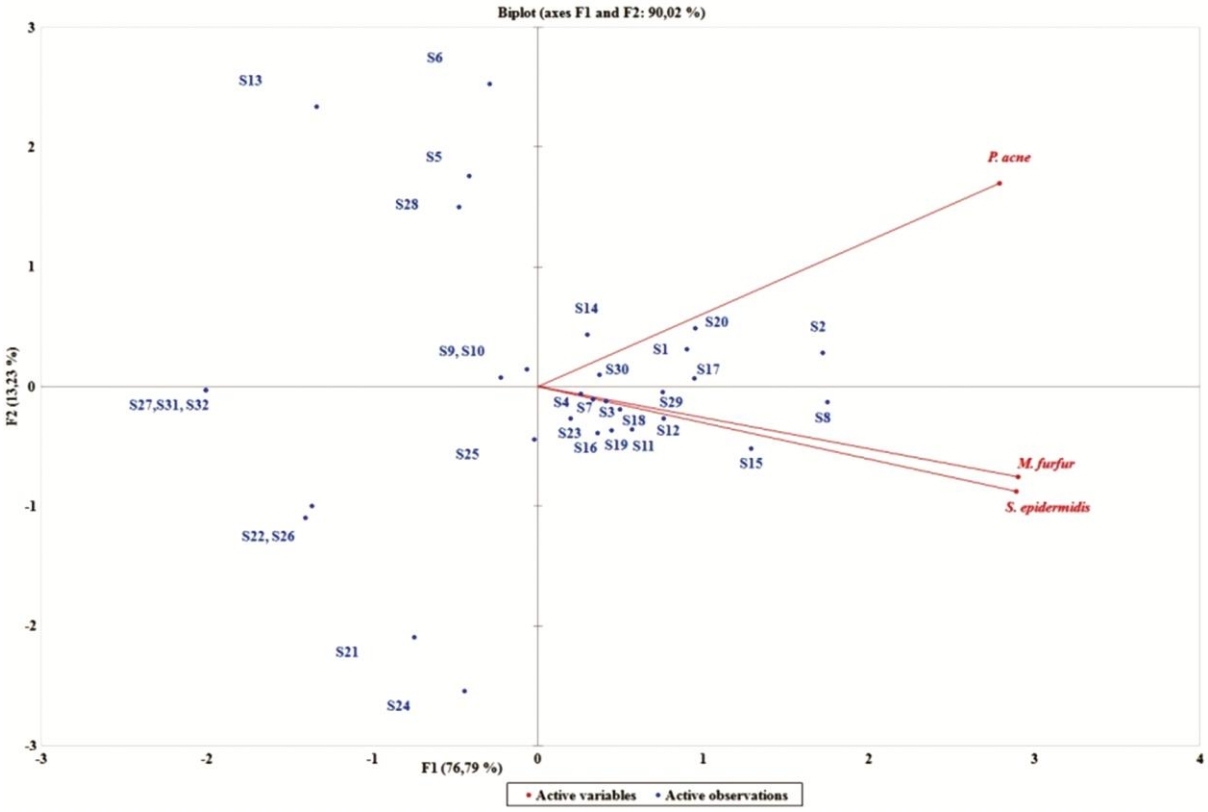

Fig. 1 — PCA analysis of plant extracts activity against dandruff associated microbes.

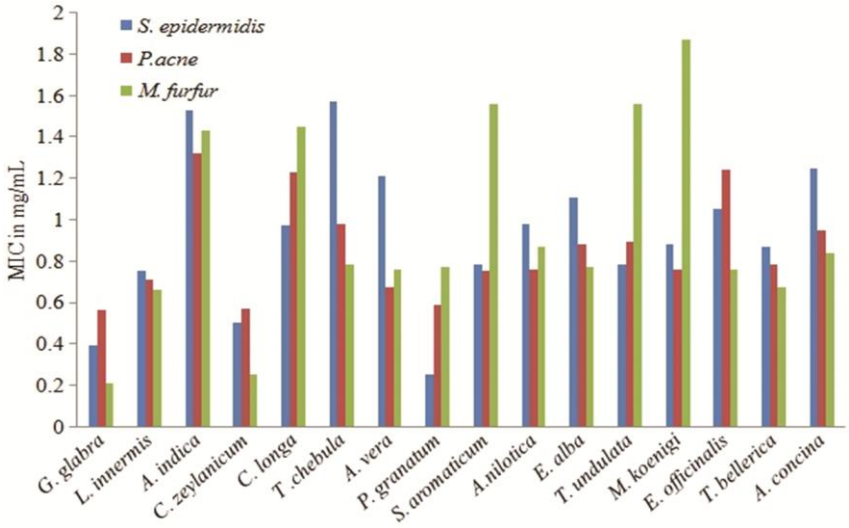

Fig. 2 — MIC of extracts against dandruff associated microbes

ellagitannin which is responsible of the antioxidant efficiency of pomegranate[36]. Pomegranate peel methanol extract showed the potential antimalarial, antioxidant and anti-inflammatory effects[37]. While *A. nilotica, S. aromaticum, A. indica, L. innermis, C. longa, T. chebula* and *T. bellerica* showed moderate activity against all three pathogens. *A. vera*, *E. alba*, *T. undulata*, *M. koenigi*, *E. officinalis* and *A. concina* showed very less activity among the extracts.

Conclusion

Herbal extracts formulations are a viable alternative to synthetic antidandruff drugs. Preliminary screening outcome of medicinal plants

provides an attractive way to develop strategies for hair care products, targeting the control of dandruff. Among all the extracts ethyl acetate extract of *C. zeylanicum* and *G. glabra* showed highest activity i.e., 18-20 mm while methanolic extract of *P. granatum* and *S. aromaticum* showed moderate activity against all the three microbes. Thus the active plant extracts can be a potential source for the formulation of natural anti-dandruff agents. Further studies should be carried out to unravel the identity of the active ingredients present in these plant extracts.

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Conflict of Interest

The authors confirm that this article content has no conflict of interest.

Authors' Contributions

RCM designed the study and performed all of the experiments; RK wrote the manuscript and analyzed all of the data and JPY supervised the whole study and prepared the final manuscript.

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