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A COMPARATIVE STUDY ON ANTIFUNGAL ACTION OF DANDRUFF SHAMPOO'S AND DIFFERENT PLANT EXTRACTS OF *PUNICA GRANARUM* AND *RUBIA CORDIFOLIA LINN*

Khushboo Minj[1,2] and Wasim Raja[2*]

[1]School of Biology and Chemical Science, MATS University Raipur, Chhattisgarh 492010, India.
[2]Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh, India.

***Corresponding Author: Dr. Wasim Raja**
Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh, India.

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ABSTRACT

Dandruff a very common scalp disorder with high prevalence in population is caused by numerous host factors in conjunction with Malassezia furfur. Most of the commercially available anti-dandruff hair shampoos contain some form of antifungal agent(s) that appear to reduce the incidence of the disease. There are no good scientific studies done to prove the antifungal activity of commercially available hair shampoos. Dandruff, a prevalent scalp condition, results from the interplay between various host factor and *Malassezia furfur*. the majority of anti-dandruff hair shampoo on the market contain antifungal ingredients that effectively reduce disease occurrence. This investigation evaluated the antifungal efficacy of commercially available shampoos against a human dandruff isolate of *M. furfur.* The shampoos were head and shoulder, Dove, ketomac. Studies indicate that bioactive compound form plant extracts exhibit antifungal activity warranting further investigation for dandruff treatment. In this study commercially available shampoos were assessed for antifungal activity against a human dandruff isolate of M. furfur. The results demonstrated that all six of the assayed hair shampoos have some antifungal effect on growth of M. furfur. These products have poor efficacies, more side effects and give scope for recurrence of symptoms. The plant extracts were tested in different concentrations. This study held significance as it facilitated a comparative analysis of established plant products with antidandruff properties alongside commercial shampoos, highlighting their enhance efficacy at minimal concentration. The identified plant extracts can be combined to develop an efficacious polyherbal hair care product for dandruff management. These results confirm the antifungal activity of Punica granarum and Rubia cordifolia extract and support the traditional use of the plant in therapy of bacterial infection. This finding suggested the presence of antifungal activity in the tested plant material, exhibited by its bioactive compounds, and serving them as an alternative antifungal agent. This can help make a poly herbal mixture that could be incorporated in hair oil or shampoos for better anti-dandruff activity.

KEYWORDS: Dandruff, anti- dandruff hair shampoo, plant extracts.

INTRODCTION

Malassezia species formerly known as Pityrosporumis a lipophilic, dimorphic opportunistic yeast causing skin and hair infections like Pityriasis versicolar, seborrheic dermatitis and dandruff, etc (Gupta, et al, 2004; Vijayakumar, 2006). Dandruffmedically described as Pityriasis capitis is caused by Malassezia species like M. furfur, M. globosa, M. restricta (Shuster, 1984). It is a common scalp disorder and alsoa major cosmetic problem as it causes hair fall. It has been investigated and reported that there was no complete cure for this disease. This disease is of global prevalence and needs effective therapeutic remedy. There are natural effective remedies to control dandruff in Ayurveda (Ravichandra, 2004) but presently people are depending on commercial shampoos containingsome antifungal compounds like miconazole, ketoconazole, selenium sulphide etc. Plant products contain various compounds like alkaloids, flavanoids, tannins, terpenoids etc which have efficient antifungal activity (Saneesh, 2013). Plant that have one or more parts containing compounds useful for treating illness are known as medicinal plants. herbal medicinal are typically derived from various botanical components, including whole plants, leaves, roots, bark, seeds and flowers (Maurice M. Iwu et al,. 1999) In developing countries, there has been a growing trend in research focused on using medicinal plants to treat microbial infections (Santhi et al,. 2022) dandruff is a skin condition that mainly affects the scalp. The primary signs of dandruff are occasional itching and flaky skin. It is a widespread scalp condition and a significant cosmetic concern, as it can contribute to hair loss issues.

Dandruff is a common problem for people of every age (Kutcharlapati, 2019) dandruff predominantly affects individuals during the pubertal to middle-age period, coinciding with peak sebaceous gland activity dandruff is a condition that cannot be completely cured (Narshana et. al, 2017) this study undertook a comparative analysis of commercial anti-dandruff shampoo and natural plant products to assess their antifungal efficacy.

MATERIALS AND METHODS

SAMPLE COLLECTION

The mature and fresh, diseases free *Rubia cordifolia*, *pomegranate leaf* sample was collected from local market of Raipur (C. G) the collected sample were carefully stored in sterile Polyethene bags without tightening and authenticated at central laboratory facility, Chhattisgarh council of science and technology, Raipur Chhattisgarh.

CHEMICALS

All solvent used were of HPLC grade, methanol was acquired from limited (Mumbai India)

STERILIZATION OF GLASS WARES

All glass were used in the research work were washed with Millipore water rinse with distilled water and air dried, they were also sterilized on hot air oven and each material was wrapped with aluminum before sterilization.

REQUIREMENTS FOR EXTRACTION

FOR SOLVENT PREPARATION: - methanol and distilled water.

GLASSWERE: - Beaker petri plates, measuring cylinder, glass rod, conical flask etc.

INSTRUMENTS: - weighting machine, Extraction funnel, hot air oven, and incubator.

MISCELLANEOUS: - forceps, spatula, maker, filter paper.

EXTRACTION PROCEDURE: Punica granarum and Rubia cordifolia **(**100 g**)** was defatted with petroleum ether (1000 ml) and the residue was extracted in 50% methanol with the help of soxhlet extraction unit. The sample was collected and concentrated in water bath at 40-50^{o}C and dried in hot air oven at 40^{o}C. Final powder was kept in air tied box for further studies.

ANTIDANDRUFF ACTIVITY

ISOLATION OF CULTURE:- In the clinic study the organisms was isolated from scalp of person suffering from dandruff and maintained on PDA media (significant for fungi) plate enriched with 2% lipid source like coconut oil and incubated at temperature 30 ^{0}C for 48 hrs.

GROWTH AND IDENTIFICATION

Growth takes place within 48 hours then the organism was identified based on culture microscopic and biochemical methods.

CULTURE

The organisms growth pattern and colony morphology were investigated on PDA media enriched with coconut oil as a lipid source.

MICROSCOPIC

Gram stained smear of the culture was observed under microscope for cell morphology.

BIOCHEMICAL TEST

The organisms was biochemically tested by using litmus milk reaction, gelatin hydrolysis test, fermentation of carbohydrates like dextrose, xylose, rhamnose, raffinose and mannitol and the result were recorded.

DILUTION OF SAMPOOS

Commercial shampoo sample were diluted 10 fold and 20 fold with sterile distilled water incorporating active ingredients for antifungal assays.

Table 1: Active Ingredients In Different Commercially Available Shampoos.

Active Antidandruff ingredients	Shampoos
Pyrithione Zinc	Dove, head and shoulders
Ketoconazole	Ketomac

Table 2:- Different Plant Extracts Used And Their Common Name.

Scientific name	Plant used for extraction of active compound	Part used	Extracted i.e. Alcohol \ aqua	Dose used for antifungal activity %			
Rubia cordifolia	Manjistha	Strem	Methanol	100	75	50	25
Punica granatum	Anar	Leaf	Ethanol	-	-	-	-

ANTIFUNGAL ASSAYS

The antifungal of activity of antifungal shampoos and plant extracts was tested by disc diffusion method and agar well assays. Antifungal activity of *selected plant* extract was determined by agar disk diffusion method at four different concentrations i.e., 100, 75, 50 and 25 mg/ml agar was prepared according to the manufactur's instruction and the plates were seeded with appropriate antifungal spaces. Discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and sterilized. The discs were than impregnated with the extracts and solvent DMSO. The plates were incubated at 37^{o}C for 24 hrs and the zone of inhibition was measured with measuring scale (Nair, et, al., 2005). The above

experiment was carried out in triplicate for their confirmation.

RESULT

MORPHOLOGY

Malassezia furfur grew as white to tan cream colored colony with smooth pasty consistency on Sabouraud media and the cells appeared bottling shaped when observed microscopically.

BIOCHEMICAL TEST

The biochemical studies indicated that fermentation of dextrose and xylose produced acid but no gas maltose, lactose, raffinose and mannitol were not fermented.

ANTIFUNGAL ASSAY

PLANT EXTRACT

Patient 1- our present study shows that antidandruff activity of ethyl acetate extract of Rubia *cordifolia l.* Malassezia furfur is best in 100% concentration after 24 hours (2.1 cm zone of inhibition) Although 75% concentration having mild effect (1.6 cm zone of inhibition).

Patient 2 – Our present study shows that antidandruff activity of Ethly acetate extract of *Punica granatum.* Against Malassezia furfur is best in 50%concentration after 24 hours (2.1 cm zone of inhibition).

SHAMPOO- Among the antidandruff shampoos tested every shampoo showed a very good zone of inhibition the Highest was for ketomac followed by dove and head and shoulders.

PATIENT 1 – In ketomac 100%and 50% concentration having 2.7 cm zone of inhibition 75% concentration of shampoo is having good antidandruff active at maximum 2.4 cm zone of inhibition head and shoulder neem in 100 % concentration have 1.9 cm zone of inhibition.

PATIENT 2 – In dove 100% concentration having 2.9 cm zone of inhibition 75% concentration have 1.9 cm zone of inhibition.

The study of anti- dandruff activity of different plant extracts using diffusion method.

Extract	Malassezia furfur (patient 1) zone of inhibition (in cm)				Malassezia furfur (patient2) zone of inhibition (In cm)			
	100%	75%	50%	25%	100%	75%	50%	25%
R. cordifolia	0.8±0.06	0.8±0.38	1.0±0.21	0.8±0.8	0.0±0.20	0.9±0.23	0.6±0.19	0.4±0.23
P. granatum	1.6±0.3	1.3±0.20	1.4±0.30	1.1±0.05	2.0±0.26	1.8±0.15	1.7±0.12	0.9±0.05

The study of anti-dandruff activity of different shampoo by using diffusion method

Shampoo	Malassezia furfur (patient 1) Zone of inhibition (in cm)				Malassezia furfur (patient 2) Zone of inhibition (in cm)			
	100%	75%	50%	25%	100%	75%	50%	25%
Dove	1.4±0.31	1.5±0.28	1±0.35	0.9±0.33	2.9±0.51	1.9±0.34	1.5±0.47	1.3±37
Head and shoulder (basic)	1.5±0.75	1.6±0.08	1.0±0.26	0.6±0.41	1.2±0.41	0.7±0.05	0.6±0.05	0.8±0.26
Head and shoulder (neem)	1.9±0.03	1.4±o.14	1.4±0.16	0.8±0.18	1.0±0.23	1.2±0.18	0.8±0.18	0.4±0.21
Ketomac	2.7±0.14	2.5±0.11	2.5±o.20	2.7±0.28	1.6±0.26	1.5±0.17	1.2±0.17	0.8±0.11

DISCUSSION

Dandruff is a common disease caused by Malassezia species especially Malassezia furfur. The lipolytic activity of these organisms induces hydrolysis of human sebum tri-glycerides in to free fatty acids that cause both hair loss and scalp (Yvonne, 2005). Medically significant fungi are known to grow on Sabouraud's agar medium. The present isolate being lipolytic grewwell on olive oil and Butter enriched medium this is in accordance with other reports on growth of Malassezia (Vijayakumar, 2006). All the antidandruff shampoos had good antifungal activity but there is considerable variation in the potency of their antifungal activity depending on the active compound and its concentration. In the present study the best antidandruff shampoo was Vivel Ultra Pro as it contains Ketokanozole which is reported tobe anti-malassezial agent (Nikam, 2013). This was followed by Dove and Head and Shoulders as they contain antifungal compounds like Zinc Pyrithione. Most of the plant extracts were showing good antifungal activity almost equivalent to that of commerciallyavailable shampoos. Lemon, Henna, Soap nut, Amlahad more antifungal activity and this could bebecause of their active compounds like Citric acid in Lemon and Amla and Saponins in Soap nut(Prabha, 2012). Asthere are no reports of such comparative aspect thepresent study gives significant information about thehigher antifungal activity of natural products at lowconcentration which can be exploited for commercialpoly herbal preparations. Use of natural plant products is not only cost effective but also negligibleside effects (Krishnamoorthy, 2006; 2020).

CONCLUSION

The present study was significant as not only efficient known plant products with anti-dandruff activity could be compared with commercially available shampoos but also their better efficacies at minimum concentrations could be identified. Further this research work can help make a polyhedral mixture that could be incorporated in hair oil or shampoos for better anti-dandruff activity.

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