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Research article

COMPARATIVE EVALUATION OF MARKETED HERBAL AND NON HERBAL SHAMPOO PRODUCTS AGAINST PATHOGENIC BACTERIAL AND FUNGAL CULTURES

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ABSTRACT: Scalp flora serves as repository of pathogenic microorganisms and may prove fatal in scenarios with depleted immune system. Dandruff is among the unbiased and most prevailing scalp infection that requires cosmetic managements. The present study was undertaken to evaluate the antibacterial and antifungal potential of the commercially available herbal and non herbal shampoo products. Agar well diffusion method and Kirby Baur (disc diffusion) method was used for the comparative assessment of antimicrobial activity. A spectrum of pathogenic bacterial and fungal strains known to cause scalp infection including *Staphylococcus aureus, Pseudomonas aeruginosa, Streptobacilli, Penicillium, Alternaria, Cladosporum, and S.cerevisiae* were employed. It was observed that non herbal products showed comparatively higher antimicrobial and antifungal activity against the test isolates. The potent antimicrobial activity of non herbal products could be attributed to its active ingredient zinc pyrithione. Present study provides an insight to the putative commercially available anti-dandruff shampoo products and highlights the need for understanding the bioactive ingredients of the herbal products.

Key words: Antimicrobial, Dandruff, *Staphylococcus aureus*, Scalp infection

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INTRODUCTION

The normal flora of the head and neck exists in a fragile balance within tightly regulated ecologic niche, counter balanced by a highly efficient innate immune system of the host (Cogen AL, *et al.*, 2008). Invasion by the pathogenic flora is rare when mucosal defenses remain intact. Under condition of external stress that induces depletion in innate immune barrier, scalp flora serves as repository for infection by pathogenic microbes. In head and neck cancer radiotherapy, irradiated patients are at higher risk of infections by scalp flora due to weekend immune barrier. There is a contrasting belief to categorize dandruff as a disease or a disorder; however, it is eminent to deteriorate the human health by accumulation pathogenic flora. The skin flora, commonly referred as the skin micro biota, contains around 1000 species from 19 phyla (Grice EA, *et al.*, 2009, Pappas S., 2009) found in the superficial layers of the epidermis and the upper parts of hair follicles. Skin flora is usually non-pathogenic, and either commensals (not harmful to their host) or mutualistic (offers a benefit). In mutualism presence of bacteria may confer prevention form transient pathogenic organisms to colonize the skin surface.

The word dandruff (dandriffe) is of Anglo-Saxon origin, a combination of 'tan' meaning 'tetter' and 'drof' meaning 'dirty'. It is a common scalp disorder prevailing in half of the population at the pre-pubertal age without gender, geographical and ethnic biasing (Cogen AL, *et al.*, 2008, Grice EA, *et al.*, 2009, Badi KA and Khan SA, 2014).

Dandruff is characterised by shedding of about 800,000 cells/sq cm from the scalp post detergent treatment compared to physiological threshold of 487,000cells/sq cm get under normal conditions (San Philippo A and English JC, 2006). Role of keratinocytes has been well documented in the expression and generation of immunological reactions during dandruff formation (Pappas S., 2009). The severity of dandruff may fluctuate with season as it often worsens in winter Ranjith MS, et al., 2002). Though multiple microbes have been isolated and characterised from the scalp infections, the mechanism of dandruff formation from the normal physiological spectrum of scaling is yet to be understood. The presence of flakes on scalp followed by itching marks the onset of scalp infection. The lipophilic yeast, *Malassezia*, is known to cause the infection in large amplitude. Other causative microorganism may contribute in the infection during the cuts, other head injuries or transient pathogenic flora from other infected places of the body.

An array of compounds exhibiting antibacterial and antifungal properties has been explored against scalp infection. By using different herbal and non herbal active compounds especially antifungal compounds it is possible to inhibit the prevalence of dandruff. Several herbal substances such as tea tree oil (San Philippo A and English JC, 2006), neem extract, clove oil, pepper extract have been shown to play a pivotal role in eradicating infection from the scalp because of their antimicrobial activity. The treatment which is very common to control the scalp infections is using an emulsion commonly known as shampoo (Urbano CC, 1995). Various active ingredients such as zinc pyrithione (Schwartz J, et al., 2005), ketoconazole, selenium sulphide with potent bioactivity are added in the shampoos for controlling the dandruff as well as other infection causing microorganisms (Pierard-Franchimont C, 2006). Multiple products are available in the market under different brand names which intend to possess antimicrobial activity (Badi KA and Khan SA, 2014). Present study highlights the comparison of the antimicrobial activity of the commercially available herbal and non herbal shampoo products. Interestingly, non herbal shampoo products showed potent antimicrobial activity compared to herbal products. Present observations further provide an insight to the efficacy of the commercially available products and need for judicial use.

MATERIAL AND METHODS

Preparation of samples

A plethora of herbal and non herbal shampoo products were procured from the local merchandisers of Dehradun (Uttarakhand). The shampoo products employed in the present study were from pioneer and popular brands in Uttarakhand (India) market (names not disclosed to avoid any commercial implication). List of shampoos are shown below in table no. 1:

Table-1: List of Herbal and Non herbal shampoos

S.No	Herbal shampoos	Non herbal shampoos
1	Hi	H
2	Nu	C+
3	S	D
4	V	Me
5	Kr	G
6	Ar	Sp
7	A	Cl
8	T	F
9		Re
10		Ch
11		Sn
12		L
13		P

Isolation of test microorganism

Isolation of the microorganisms (bacteria, fungi and yeast) is done from the flora present on scalp and hairs. Flakes present on the scalp were place on media and incubated for 24h at 37^0C. Microflora appeared was biochemically and morphologically characterised by using following standard tests: Gram's staining (Todar K, *et al.*, 2005), Indole test, Catalase test, Carbohydrate degradation test (Bhattacharya.C, *et al.*, 2014) Starch hydrolyses (De Oliveria, N, 2007). Isolated microorganisms were maintained as pure culture using Potato dextrose agar (Jianfeng Wang, *et al.*, 2000), Nutrient agar (Satti, L, *et al.*, 2012) and Rose Bengal agar.

Preparation of 0.5 Mcfarland standard

0.5 ml of 0.048M $BaCl_2$ (1.17% w/v $BaCl_2$) was added to 99.5 ml of 0.18M H_2SO_4 (1% w/v) with constant stirring. The O.D. of the solution was recorded; it should be in the range of 0.08-0.1 at 625 nm ($1.5x10^8$ cells/ml for yeast). Standard was stored in amber colour bottle to prevent it from light at room temperature. Standard was briskly vortexed on a vortex mixer prior to use. (NCCLS, 1997).

In vitro antimicrobial activity (Kumar GS, 2007)

Agar well diffusion assay,

Wells of 5mm diameter was excavated by using pre sterilized cork borer in (Jianfeng Wang, *et al.*, 2000), Nutrient agar (Satti, L, *et al.*, 2012) and Rose bengal agar. 20 µl of the prepared samples from different herbal and non herbal products were dispensed in the well. Solvent used for making dilutions of the concentrated products was used as control. Dispensed samples were allowed to diffuse for 15 min and were sealed with molten agar. After 15 min, the plate was swabbed with 16-18h old 0.5 McFarland adjusted culture of respective test isolated micro-organism. Plates were incubated at 37^0C for bacteria and 25^0C for fungi for 24h. Antibacterial and antifungal activity was determined by measuring the diameter of zone of clearance also known as halo formed. Three independent experiments were performed in triplicates.

KB disc diffusion assay

5mm size discs were used with loading capacity of 30µl. Prepared samples were loaded in sterile discs and dried under aseptic conditions. Plates containing respective media were swabbed with 0.5 McFarland adjusted 16-18 hour old culture of the isolated test organisms. Sample loaded discs were then placed on the swabbed media plates and incubated at 37^0C overnight for 24h. Disc loaded with solvent used for dilution served as control. Antibacterial and antifungal activity was determined by measuring diameter of zone of inhibition.

RESULTS

Antimicrobial activity of herbal and non-herbal shampoo products against the isolated pathogenic bacteria

Plethora of pathogenic microorganisms was isolated from the flakes of scalp. These isolated microorganisms were characterised and employed to evaluate the antimicrobial potential of the test samples. Among the potential pathogenic microflora *Staphylococcus aureus, Pseudomonas aeruginosa, Streptobacilli, Bacillus cereus, Cocci* and *Serratia* were chosen to challenge the test samples. Intriguingly, few of the test samples exhibited antibacterial activity with the maximum zone of inhibition obtained in shampoos Cl (28 mm, *Staphylococcus aureus*) , Cl (30 mm, *Pseudomonas aeruginosa*), Kr (48 mm, *Streptobacilli*), Kr (33 mm, *Bacillus cereus*), H (33 mm, *Cocci*), Cl (31 mm, *Serratia*) (Fig. 1, table no. 2).

Antimicrobial activity of herbal and non-herbal shampoo products against the isolated Fungi and Yeast

From the spectrum of microflora obtained *Penicillium, Aspergillus, Alternaria, Cladosporum, and S.cerevisiae* were employed to investigate the microbicidal potential of the test samples. Interestingly, multiple test samples exhibited microbicidal activity against both fungi and yeast with the maximum zone of inhibition observed in shampoos Cl (42 mm, *Penicillium*), Cl (33 mm, *Aspergillus*), Cl (45 mm, *Alternaria*), H (42 mm, *Cladosporum*) and Cl (60 mm, *S. cerevisiae*) (Fig. 2 and table no. 3).

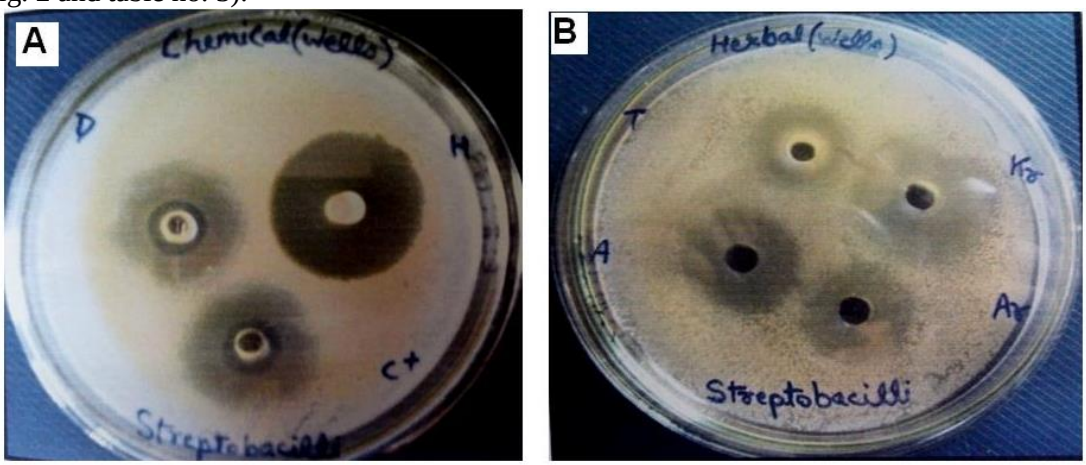

Figure 1. Antibacterial activity of herbal and non herbal products. Zone of inhibition against S*treptobacilli* induced by (A) non-herbal shampoo and (B) herbal shampoo.

Table 2. Anti-bacterial activity of herbal and non herbal shampoos. Potential of herbal and non herbal shampoos against pathogenic bacteria involved in scalp infection was evaluated by measuring the zone of inhibition using KB disc diffusion assay and Agar well diffusion assay.

S.No	Test Organisms	Test shampoos (herbal/non herbal)	ZOI (diameter in mm)	Putative shampoo
1	*Staphylococcus aureus*	Cl	**28**	Cl
		Re	20	
		F	22	
2	*Pseudomonas aeruginosa*	Cl	**30**	Cl
		Re	18	
		F	21	
3	*Streptobacilli*	D	22	H,Kr
		H	**48**	
		C+	25	
		T	20	
		A	25	
		Kr	**43**	
		Ar	23	
4	*Bacillus cereus*	A	21	Kr
		T	20	
		Kr	**33**	
		Ar	26	
5	*Cocci*	D	28	H
		C+	30	
		H	**33**	
6	*Serratia*	Cl	**31**	Cl
		F	26	
		re	24	

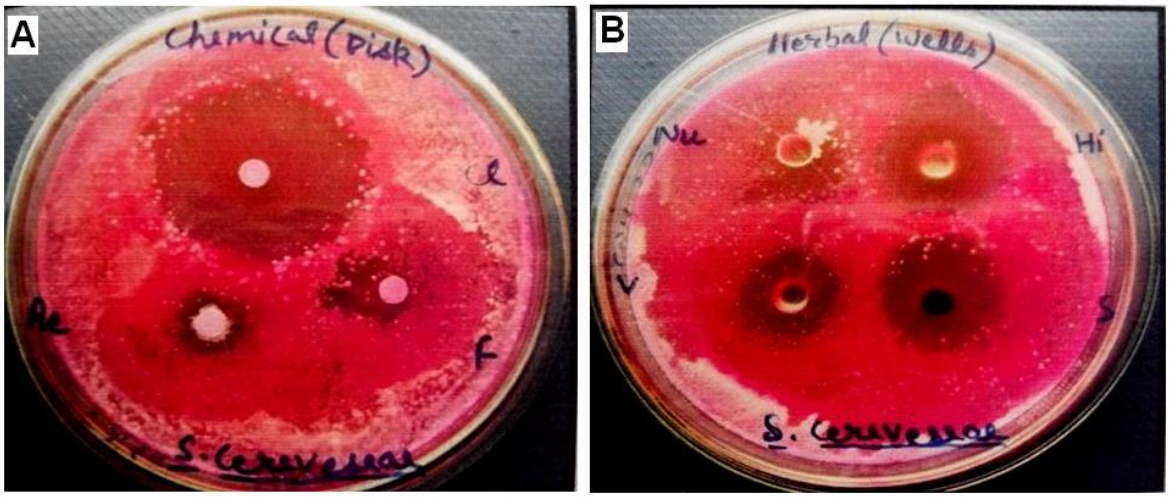

Figure 2. Yeast inhibitory activity of herbal and non herbal products. Zone of inhibition against *S. cerevisiae* induced by (A) non-herbal shampoo and (B) herbal shampoo

Table 3. Anti-fungal and yeast inhibitory activity of herbal and non herbal shampoos. Potential of herbal and non herbal shampoos against pathogenic fungus and yeast involved in scalp infection was evaluated by measuring the zone of inhibition using KB disc diffusion assay and Agar well diffusion assay.

S.No	Test Organisms	Test shampoos (herbal/non herbal)	ZOI (diameter in mm)	Result
1	*Penicillium*	Cl	**42**	Cl,H
		Re	18	
		F	17	
		D	25	
		C+	24	
		H	**35**	
2	*Aspergillus*	Cl	**33**	Cl,H
		Re	20	
		F	15	
		D	24	
		C+	24	
		H	**31**	
3	*Alternaria*	Nu	20	Hi,Kr,Cl
		Hi	**40**	
		S	20	
		V	21	
		T	22	
		Kr	**42**	
		Ar	38	
		A	39	
		Cl	**45**	
		F	24	
		Re	28	
4	*Cladosporum*	Re	25	H,Cl
		Cl	**35**	
		D	22	
		H	**42**	
		C+	20	
5	*S.cerevisiae*	Cl	**60**	Cl,Hi
		F	15	
		Re	12	
		Nu	16	
		Hi	**53**	
		S	25	
		V	18	

DISCUSSION

Shampoos are routinely being used as cosmetic products for cleansing hairs and scalp (Badi KA and Khan SA, 2014). A shampoo encompasses surfactant which is applied under specific conditions to confiscate surface grease, dirt and skin debris from the hair shaft without imparting any side effects to the user. Multiple synthetic, herbal, medicated and non medicated shampoos are commercially available for consumers but owing to the natural origin and no side effects, herbal shampoos are of prime choice for fighting against scalp infection (Nimisha, *et al.*, 2013).

Dandruff is common scalp infection characterised by the shedding of dead scalp skin. Scalp infection poses a threat by acting as reservoir of pathogenic microbes that may prove fatal during immune depleted scenarios (Preethi JP, 2013). Notionally, dandruff is a form of physiologic scaling that requires a cosmetic management against which the response is swift, but transient. Dandruff is a high degree cohesive huddle of corneocytes that tend to adhere strongly with one another and are detached from the scalp in a cluster from the stratum corneum (Kloos I, 2013).

The size and abundance of scales depends on the severity of the clinical manifestations, which may also be influenced by seborrhea (Saad and Khadim, 2011). Though dandruff is non-inflammatory in nature but other scalp infections like seborrhoeic dermatitis mount an inflammatory response. Thus a dire need exist to combat against microbial flora mediated scalp infections by employing agents with putative antimicrobial activity. Plethora of commercially available herbal and non herbal shampoo products are used as remedy against dandruff (Nimisha, *et al.*, 2013). Present study was undertaken to have a relative assessment of the available products in terms of their antibacterial and antifungal activity. Random and unbiased samples were collected from herbal and non herbal shampoos.

The efficacy of a shampoo is dependent not only on the concentration of the active ingredients but also on the shampoo formulation. Interestingly, we observed that though available products showed potent antimicrobial efficacy, non herbal products exhibited relatively higher potential against the test microbes especially against *Staphylococcus aureus, Pseudomonas aeruginosa, Streptobacilli, Bacillus cereus, Cocci, Serratia,* and *Penicillium, Aspergillus, Alternaria, Cladosporum, S.cerevisiae*. These results provide an insight to the variable degree of antimicrobial activity of the commercially available products. Further, these observations highlight the need of better understanding of the active ingredients in herbal preparations.

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REFERENCES

Cogen AL, Nizet V, Gallo RL (2008). "Skin microbiota: a source of disease or defence?". Br J Dermatol 158(3):442–55.

Grice EA, Kong HH, Conlan S (2009). "Topographical and Temporal Diversity of the Human Skin Microbiome". Science 324: 1190-1192.

Pappas S. (2009). Your Body Is a Wonderland ... of Bacteria. Science NOW Daily News.

Ranjith MS, Gokul Shankar S, Ranganathan S, Sivaramakrishnanan M,Natarajan V, Rasool SK (2002). Role of ABO blood group in the infection rate of dandruff caused by Pityrosporum ovale. Indian J of Dermatol. 47(1): 21-23.

San Philippo A, English JC (2006). An overview of Medicated shampoos used in Dandruff treatment. *P&T*. 31(7): 396-400.

Urbano CC (1995). 50 years of hair care development. Cosmet Toiletries. 110: 85–104.

Schwartz J, Cardin C, Dawson Jr. T. Dandruff and seborrheic dermatitis. In: Barran R, Maibach H (2005). Textbook of Cosmetic Dermatology, 3rd Edn: Taylor & Francis: New York. p. 259–272.

Pierard-Franchimont C, Xhauflaire-Uhoda E, Pierard GE (2006). Revisiting Dandruff. International Journal of cosmetic Science. 28: 311- 318.

Todar K., Ubukata M., and Hamada M. (2005).Microbiology of Human Perspective. McGraw Hill Publisher, London.

Bhattacharya C, et al., (2014); Isolation and Characteriazation of Bacterial Isolates From
Agricultural Soil at Durg District. Indian J. Sci. Res. 4 (1): 221-226

De Oliveria N, de Oliveria Andrade L A J S and Chagas J A F, (2007). Rhizobia amylase production using various starchy substances as C substrates. Braz. J. Microbiol. 38: 208-218.

Kumar GS, Jayaveera KN, Ashok kumar CK, Sanjay PU, Vrushabendra Swamy BM, Kishore Kumar DV (2007). Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Tropical Journal of Pharmaceutical research. 6 (2): 717-723.

Jianfeng Wang, Guiling Li, Huaying Lu, Zhonghui Zheng, Yaojian Huang, Wenjin Su, (2000). Taxol from *Tubercularia* sp. strain TF5, an endophytic fungus of *Taxus mairei.* FEMS Microbiology Letters 193: 249-253

Satti L, Abbasi S and Faiz U, (2012). Evaluation of nutrient agar for the culture of *Mycobacterium tuberculosis*using the microcolony detection method. Int J tuberc lung dis 16 (7):908–910

Kloos I, Straubinger RK, Werckenthin C, Mueller RS. (2013). Residual antibacterial activity of dog hairs after therapy with antimicrobial shampoos. Vet Dermatol. 24(2):250-e54.

Jaya Preethi P., Padmini K., Srikanth J., Lohita M., Swetha K., Vengal Rao P. (2013). A Review on Herbal Shampoo and Its Evaluation. Asian J. Pharm. Ana. 3(4):153-156

Saad and Khadim (2011). Formulation and evaluation of herbal shampoo form Ziziphus Spina leaves extract. IJRAP 2(6): 1802-1806

Namita and Nimisha (2013). Formulation and evaluation of herbal shampoo having antimicrobial potential. Int J Pharm Pharm Sci 5(3): 708-712

Khaloud Al Badi and Shah A. Khan (2014). Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoos. Journal of basic and applied sciences 3: 301-305

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