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Original Article

A New Formulation of Cow Urine based Polyherbal Hair Conditioner and its Antifungal Activity Against *Candida albicans*

Chhangani J.A.*, Tiwari S.G.*, Rai M.M.**

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Abstract

Dandruff is a common embarrassing scalp disorder, affecting a large population.For the treatment of dandruff, shampoos containing imidazole derivatives and other chemicals are available but due to certain limitations such as poor clinical efficacy, compliance issues, side effects andinability to prevent recurrence, the people are attracted towards herbal products.Worldwide attraction and attention towardsherbal products for health care, health foods and natural cosmetic products including hair care formulations has increased. In the present work cow urine based polyherbal hair conditioner was developed as the medicinal properties of cow urine and herbs have been well described in ancient Indian medical science, Ayurveda and modern researchers have also reported their medicinal importance. Hair conditioner was formulated using coarse powder of *Sapindustrifoliatus, Acacia concinna,* mixture of *Emblicaofficinalis*, *Terminaliachebula* (retz.) and *Terminaliabelerica* in equal proportion, camphor, thymol and urine from indigenous cow. The developed formulation and available marketed products were compared for organoleptic characters such as pH, percent solids contents, dirt dispersion, detergency ability, ease of rinsing, luster of hair, foaming ability and stability. Evaluation of developed formulation for in-vitro anti-dandruff activity along with accelerated stability study was also carried out and presented.

Keywords: Cow Urine; Hair Conditioner; Candidaalbicans.

Introduction

History of mankind has witnessed the relationship between the beauty and cosmetics. Since ancient time, herbs are being used for maintenance and augmentation of human beauty. Hair care products are available in the marketin the form of shampoos, forcleansing the hair of accumulated sebum, scalp debris and residues of hair-grooming preparations [1]. Synthetic detergents have been claimedto be the most important factor in the growth of shampooproducts available in the market [2], in different forms such as liquid, lotion, cream, paste, gel, dry shampoo etc [3,4,5]. Sodium lauryl sulfate based detergents are most common but the concentration vary considerably from brand to brand and even within a manufacturer's product range [6,7]. For the treatment of dandruff, shampoos containing imidazole derivatives and other chemicals are also available in the market but due to their certain limitations, poor clinical efficacy or compliance issues or side effects andinability to prevent recurrence, people attracted towards other alternatives [8]. The hair conditioners or shampoos are toxic and found to have side effects like irritation of scalp, skin and mucous membrane of the eyes, dryness of scalp and hairs, discoloration, loss of hairs and variation in the individual response due to natural differences and chemicals used in them [9].

Medicinal plants due to their health benefit properties, areused as primary health care aid in the form of plant extracts or their active components [10].

Author's Affiliation: *Mahatma Gandhi Institute For Rural Industrialization, Wardha, **Center For Sericulture and Biological Pest Management, RTM Nagpur University, Nagpur,

Reprint's Request: J.Y.Changani, Mahatma Gandhi Institute For Rural Industrialization, Wardha, Maharashtra – 442 001, India
E-mail: jychhangani@gmail.com

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A remarkable increase in the use of medicinal plant products have been observed in the recent past decade. Today, herbs still found in 40% ofprescriptions, and the interest for use of herbal remediesinstead of chemical drugs is increasing because of lessside effects [11]. The importance of drugs of plant, animal and mineral origin for the treatment of various types of diseases has been well described in Ayurveda, the ancient Indian medical science.

The revered Indian cow, known as "Kamdhenu", in Indian Scripts, is believed to be a "Mobile Hospital", for most of the diseases. "Gou-Mutra", hereinafter referred as cow urine, has been used as a medicine in India, since ancient time. In Rigveda, cow urine has been compared with the nectar. Cow urine has clinical effects like appetizer, tranquilizer, anti-spasmodic and useful in swelling, inflammation, jaundice, anemia, leucoderma, dermatitis, itching and leprosy etc [11,12]. The U.S. patents granted for the "Gou-Mutra Ark"(cow urine distillate) to Council of Scientific and Industrial Research (CSIR) on "pharmaceutical preparation cow urine distillate" and "use of bio-active fraction from cow urine distillate as a bioenhancer of anti-infective, anti-cancer agents and nutrients" were the major breakthrough for the research on various medicinal properties of cow urine [13,14,15].

In this investigation an attempt has been made to develop an effective, economic and stable anti-dandruff hair conditioner by value addition of natural resources like herbs and cow urine, without using all traditionally incorporated synthetic active ingredients or preservative. The formulation was evaluated for its physicochemical properties and compared with marketed products andits efficacy against pathogen *Candida albicans*have been evaluated and discussed.

Materials and Methods

The herbal materials purchased from local market were identified and authenticated by Dr. S.K.Padoley, Ex. Head,Deptt. of Botany, Porwal College of Science, Kamptee. The fruits were dried under shade for 10–15 days. The seeds were removed from the fruits, powdered and passed through sieve (mesh no. 60) to get fine powder. Cow urine was collected from healthy indigenous cows (*Gir*), kept under veterinary supervision at "Sewadham", Go-Vigyan Anusandhan Kendra, Dewalapar, Nagpur. It was ensured that urine sample collected was the first urine micturated early morning once the cattle awakened. All the chemicals and reagents used from Hi-Media (India) Ltd. and Merck India Ltd.

Formulation of Hair Conditioner

Composition of the developedproduct (DP) is summarized in Table 1. The coarse powder of Sapindustrifoliatus (*Reetha*), Acacia concinna (*Shikekai*) and mixture of *Emblicaofficinalis*, *Terminaliachebula* (retz.) and *Terminaliabelerica* (roxb.) in equal proportion i.e. *Trifala* powder (mixture of *Amla, Harad and Baheda*)were soaked in Cow urine for twelve hours, then concentrated to half of its volume by gentle heating. The concentrated liquid was filtered with nano-bolt cloth and cooled. Powder of camphor and thymol mixed to get liquid in a beaker and added to concentrated liquid to obtain the product as hair conditioner.

Evaluation of Formulation

The developed product (DP) and three samples of the products available in the market (MS1, MS2, MS3) were compared for following physico-chemical tests.

Organoleptic Evaluation

Various parameters such as physical appearance, color, odour and transparency by sensory organs of the developed product have been evaluated and discussed [16,17].

Determination of pH

A sample with 10% concentration was prepared by diluting the developed productusing distilled water. pHwas recorded using digital pH meter at room temperature [17].

Solid Contents

A clean dry China dish was weighedand 4 grams of developed product (DP) taken in the dish, placed on the water-bath untilthe liquid portion evaporated,then placed in oven at 110^0 C and weight of the dry content was calculated [17].

Dirt Dispersion

Two drops of developed product added in a large test tubecontaining 10 ml of distilled water. A drop of India ink wasadded; the test tube was stoppered and shacked ten times. The amount of ink in the foam was estimated as None, Light, Moderate, or Heavy.

Detergency Ability

Thompson method[18] was used to evaluate the

detergency ability of the samples. Briefly, a crumple of hair were prewashed with a 5% sodium lauryl sulphate solution**,** then dried divided into 3g weight groups. The samples (3g) were suspended in 20mln-hexane solution containing 10% artificial sebum and mixture was placed on shaker for 15 minutes at room temperature. Thethe solvent was evaporated at room temperature and weighed for determination of their sebum content. In the next step, each sample divided into 2 equal parts of 1.5 g each, one washed with 0.1 ml of the 10% test sample (DP) [18], the other sample was considered as the internal control and left untreated. After drying at 60^{0}C for 4 hours, the sebum remained in treated and untreated samples, were then extracted using 20ml hexane for 30 minutes on a rotary shaker. The sebum extracted from both the samples was weighed after complete evaporation of hexane solution. Detergency was evaluated as a percentage of sebum removed after treating with formulations using equation; Detergency = 100-(T x 100 / C) where T is the weight of sebum in treated sample and C is the weight of sebum in control sample.

Rinsing

The time taken to remove the detergent was performed by applying 5 ml of the shampoo and time taken for complete removal of frothing from wash water was determined.

Combing (Wet)

Ease of combing was performed by passing a comb through the wet hair and checking whether the comb glides smoothly.

Combing (Dry)

Ease of combing was performed by passing a comb through the dry hair and checking whether the comb glides smoothly.

Luster of Hair

The luster of hair was tested by checking the shine and smoothness of hair after drying.

Foaming Ability and Stability

Cylinder shake method with slight modification used for determining foaming ability. 100ml of the 10% shampoo solution taken into a 250 ml graduated measuring cylinder shaken for 10 times. The total volume of the foam contents after each 1 minute was recorded up to 5 minutes and foam volume generated was recorded after 10 minutes.

Anti-dandruff Activity (In-vitro)

Candida albicans were employed for testing antifungal activity using well diffusion method. Sabouraud's agar medium prepared, autoclaved and 20ml of it was dispensed into sterilized petriplates. After solidification of the agar, 0.2 mL of 72 hour old suspension of *Candida albicans*poured in the centre of the sterilized petriplates using a micropipette and spread evenly on the agar using a sterilized glass spreader. Four wells (10 mm diameter) were made in to the agar at four corners of the petriplates taking care that the wells did not lie in close proximity to the edges of the petriplates or to each other. Well no. 1,2,3, and 4 werefilled with 0.05ml (100µL) of a solution of the 1g, 2g, 3g and 4g sample dissolved in 10 mL DMSO respectively. In other plate, four similar wells (10 mm diameter) were madeand filled with 0.05ml (100µL) of a solution of the 0.1g, 0.2 g, sample dissolved in 10 mL DMSO, pure sample and DMSO as controlrespectively.The plates were kept for diffusion at 4^{0}C for one hour and incubated at 30^{0}C for 48 hours. After incubation, the zone of inhibition measured in mm and compared [19].

Accelerated Stability

Accelerated stability of the developed product was evaluated at the 40±2^{0}C and 75±5% relative humidity for 90 days [20,21].

Results and Discussion

The knowledge about medicinal plants, their use seems to be well known to people of India. The recent interest of consumers in the herbal cosmetics has been stimulated due to decline in faith of modern synthetic cosmetics and belief that plant remedies are natural and thereby superior to synthetic cosmetics. These reasons have contributed to increased acceptance as well as manufacture of herbal cosmetics [22]. The ingredients used in this formulation were found rich source of novel drugs. The various quality control parameters were checked along with antifungal activity. The results obtained were encouraging and much better than the marketed products.

Organoleptic Evaluation

The developed formulation and marketed samples

were found to be semi liquid in nature with characteristic odour and various colours. The DP was not transparent whereas all the marketed samples were transparent (Table 2).

pH: The pH of the DP was 5.25 whereas that of marketed samples was in the range of 6-7.5. Though the pH of DP was lower than marketed samples, no harmful effect on scalp and hair was observed (Table 3).

Percent Solid Content

The percent solid content of marketed samples wasin the range of 15 to 17.5 but that of developed productwas 56.84 percent (Table 3).

Dirt Dispersion

The dirt should stay in water and should not concentrate in the foam, otherwise it is considered as of poor quality. The dirt stayed in the foam redeposits on the hair and is difficult to rinse. The developed formulation and all the marketed samples showed similar results [23,24] (Table 3).

Detergency

All the samples showed ability between 70 to 72.6 with highest detergency abilityrecorded for developed product andhence may be referred as active cleanser (Table 3).

Rinsing

In the case of ease of rinsing DP was rinsed out quickly, when compare to other marketed formulations(Table 3).

Combing (Wet and Dry)

In case of combing, all samples showed combing with friction in wet condition however at dry condition DP showed better results(Table 3).

Luster of Hair

In maintaining luster of hair, all the formulations showed fairly good luster but DF showed better results(Table 3).

Foaming Ability and Foam Stability

Normally it is considered that forming ability of natural shampoos or hair conditioners is not comparable with its synthetic counter parts. However the present investigation proves that natural formulation having combination of natural surfactants such as *Reetha* and *Shikakai* in optimized concentration can generate sufficient foam ability of shampoo. However, foam does not have much to do with the cleaning process. But it is of paramount importance to the consumer and is therefore an important criterion in evaluation of shampoos.[17]The DF showed better foaming ability as compared to marketed samples and unlike those of marketed samples, its foam remains as it is even after 10 minutes, which proves natural surfactants Reetha and Shikakai may be the best replacement for the harsh synthetic detergents which are commonly used in majority of shampoos (Table 4).

Table 1: Composition of Product

S. No.	Common Name of Ingredients	Botanical name/English name	Quantity
1.	Cow urine	----	5 L.
2.	Reetha	*Sapindustrifoliatus*	1.2 Kg.
3.	Shikekai	*Acacia concinna*	400g
4.	TrifalaChurna (Mixture of Amla, Harad and Baheda)	Mixture of *Embilicaofficinalis, Terminaliachebula (retz.)* and *Terminaliabelerica* (roxb.)	125 g (41.66g dried powder of each three)
5.	Kapoor	Camphor	40g
6.	Ajawain Sat	Thymol	25g

Table 2: Organoleptic Evaluation

S. No.	Specifications	DF	MS 1	MS 2	MS 3
1.	Physical appearance	Semi liquid	Semi liquid	Semi liquid	Semi liquid
2.	Colour	Brownish	Green	Brown	Whitish
3.	Odour	Characteristic	Characteristic	Characteristic	Characteristic
4.	Transparency	Not transparent	Transparent	Transparent	Transparent

Table 3: Physico-chemical and Generalobservations of DP and other marketed products

S. No.	Test	DF	MS 1	MS 2	MS 3
1	pH	5.44	6.1	6.02	7.28
2	Solid content (%)	56.84	16.04	15.72	17.24
	Dirt dispersion	None	None	None	None
3	Detergency ability	72.6	72.2	70.5	70.2
4	Cleaning action	96.94	89.37	91.58	90.15
5	Ease of rinsing	***	**	**	**
6	Ease of combing (wet)	**	**	**	**
7.	Ease of combing (dry)	**	*	*	*
8	Luster of Hair	***	**	**	**

* Good, ** Better, *** Best

Table 4: Foam formation and foaming stability

Time (minutes)	Foam volume (ml)			
	DF	MS 1	MS 2	MS 3
1	248	247	245	247
2	246	245	243	245
3	244	242	241	242
4	242	240	238	240
5	242	240	236	240
10	150	120	125	128

Table 5: Antimicrobial activity of DP against Candida albicans

	Concentration wise zone of inhibition (in mm)						
Conc.	1%	2%	10%	20%	30%	40%	Pure sample
Zone	Nil	13	18	20	23	25	31

Table 6: Accelerated Stability Testing

Test	Initial month	After 1 month	After 2 months	After 3 months
Physical appearance	Semi liquid	Semi liquid	Semi liquid	Semi liquid
Colour	Brownish	Brownish	Brownish	Brownish
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Transparency	Not transparent	Not transparent	Not transparent	Not transparent
pH	5.44	5.45	5.46	5.46
Percent solid content	56.84	56.84	56.84	56.84
Dirt dispersion	None	None	None	None
Detergency ability	72.2	72.00	71.21	71.20
Foam volume	242	240	240	240
Thermal Stability	OK	OK	OK	OK
Degradation of product	Nil	Nil	Nil	Nil
Microbial count (cfu/g)	$0x10^20x10^2$	$0x10^20x10^2$	$0x10^20x10^2$	$0x10^20x10^2$

1. 10%, 2.20%, 3. 30% and 4.40% concentration

1.1%, 2.2% concentration, 3. Pure sample and 4. DMSO solvent

Fig. 1: Zone of inhibition (mm) of DP against one of the test isolates.

In-Vitro Anti-Dandruff Activity

Dandruff has worldwide occurrence. The dermatophilic fungi on the scalp utilizes the secretions of the sebaceous glands for its growth and causes infections like dandruff and associated secondary infections. Anti-dandruff shampoos based on chemicals are being used for its treatment but due to their side effects or recurrence problem, the people are attracted towards alternatives based on natural resources. Medicinal plants are increasingly of interest as antimicrobial agents and have been widely used in traditional medicines [25].

The antifungal activity of developed formulation showed positive resultsagainst the tested fungal pathogen *Candida albicans*, in all concentration except at 1% which may be considered as MIC, as 2% concentration showed zone of inhibition 13mm. Increase in concentration showed increase in zone of inhibition and the pure sample of DP showed maximum zone of inhibition 31 mm (Table 5). The higher antifungal activity of DP may be because of the synergistic activity of all herbal ingredients along with cow urine's enhancing properties.

Accelerated Stability Study

Accelerated stability testing of the developed formulation conducted at 40±2^{0}C temperature and 75±5% relative humidity for 90 days (Table 6).

Conclusion

In the present study, a new formulation of cow urine based polyherbal hair conditioner was developed and compared with three marketed products for various physico-chemical properties. The results obtained were encouraging and much better than the marketed products.The antifungal activity of developed formulation showed positive results against the tested fungal pathogen *Candida albicans*, in all concentration except at 1%. The main objective of this study was development of effective, economic and stable antidandruff formulation withoutusing any kind of synthetic additives, which are normally incorporated in such formulations. The technology of this value added product is ready for dissemination for entrepreneurship development and employment generation as well as societal benefit in the form of good health.

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References

1. B.M.Mithal,"Text Book of Forensic Pharmacy." Vallabh Prakashan. Delhi, 1994; 10.
2. Naresh C. Ghose, DipankarSaha, Anjali Gupta. Synthetic Detergents (Surfactants) and Organochlorine Pesticide Signatures in Surface Water and Groundwater of Greater Kolkata, India. J. WaterResource and Protection 2009; 4: 290-98.
3. Anne Churchill, Michael Meyners, Louisa Griffiths,Pippa Bailey. The Cross-Modal effect of fragrance inshampoo: Modifying the perceived feel of bothproduct and hair during and after washing. FoodQuality and Preference 2009; 20: 320-28.
4. Mitsui T. editor. New cosmetic science. 1st ed.Amsterdam: Elsevier; 1998; p. 158.
5. Bennett JE. Antifungal Agents. In: Brunton, LL, Lazo JS,Parker KL. editors. Goodman and Gilman2 s. ThePhrmacological Basis of Therapeutics. 11th ed. NewYork: McGraw-Hill; 2006; p. 1240.
6. Marrakchi S, Maibach HI. Sodium lauryl sulfate-inducedirritation in the human face: regional and age-relateddifferences. Skin Pharmacol Physiol. 2006; 19(3): 177–80.
7. Piret J, Désormeaux A, Bergeron MG. Sodium laurylsulfate, a microbicide effective against enveloped andnonenveloped viruses. *Curr Drug Targets* 2002; 3(1):17–30.
8. Ravichandran G, Shivram BV, Kolhapure SA. Evaluation of the clinical efficacy and safety of anti-dandruff shampoo in the treatment of dandruff. Antiseptic 2005; 102: 1-5.
9. Mohamed Halith, S., A. Abirami., S. Jayaprakash, ChitraKarthikeyan, K. KulathuranPillai and P.U. Mohamed Firthouse, Effect of Ocimum sanctum and Azadiractaindica on antidandruff herbal shampoo powder Der. Pharmacia Letter, 2009; 1(2): 68-76
10. Word Health Organisation. (2008). Traditional

medicine. Retrieved 29- 07-2010, from http://www.who.int/mediacentre/factsheets/fs134/en/.

11. Bhavprakash, Mishra, Prakaran, Adhyaya 19, Shloka 1-8
12. Ashtanga-Hrudaya, Sutra-Sthana, Adhyaya 6, Shloka 80-81
13. US Patent No 6410 059/2002.
14. Patent no. 6896907
15. K. Krishnamurthi, Dipanwita Dutta, S. Saravana Devi and T. Chakrabarti. Ameliorative property of distillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals. Biomedical and Environmental Sciences. 2004; 17; 86-93. (IF=1.0)
16. Swati Deshmukh, Bindurani Kaushal and Shweta Ghode. Formulations and evaluation of herbal shampoo and comparative studies with herbal marketed shampoo, Int J Pharm Bio Sci; 2012; 3(3): 638 - 645.
17. Richa Madhu Sharma, Kinjal Shah andJanki Patel. Evaluation of prepared herbal shampoo formulations and to compare formulated shampoo with marketed shampoos, Int J Pharm Sci, 2011; 3: 402-405
18. Thompson D, Lemater C, Allen R and Whittam J. Evaluation of relative shampoo detergency. J. SocCosmetChem 1985; 36: 271-286
19. Ternikar, S. G,Alagawadi, K. R., Ismail Pasha, Dwivedi, S, Mahammed Rafi and Shar-ma.T. Evaluation of antimicrobialand acute antiinflammatory activity of Sidacordifolia Linn Seed Oil, J. Cell Tissue Research; 2010; 10(3): 2385-2388.
20. Pounikar Y, Jain P, Khurana N, Omray L. K. Patil S. Formulation And Characterization Of Aloe Vera Cosmetic Herbal Hydrogel. Int J Pharmacy Pharm Sci, 2012; 4(4): 85-86.
21. Adriana D, Marcos R, Eugenia A, Alicia S R. Topical Antiinflammatoryactivity Of TripodanthusAcutifolius Flowers Gel Formulation.J Pharmacy Pharm Sci, 2012; 4(1): 183-186.
22. Kole PL, Jadhav HR, Thakurdesai P and Nagappa AN, Cosmetic potential of herbal extracts, Pharmacy group, Birla institute of technology and science, Rajasthan, 2005; 14(4): 315-321.
23. Neu GE. Techniques of foam measurement. J. SocCosmetChem 1960; 11:390-414
24. Ross J and Miles G.D. An apparatus for comparision of foaming properties of soap and detergents. Oil Soup 1941; 18(5): 99-102
25. Chee HY and Lee MH, In vitro activity of erlery essential oil against *Malassezia furfur*. Mycobiology, 2009; 37(1): 67-68.