Development of Cow Urine based Polyherbal Hair Conditioner and Evaluation of Antidandruff Activity

Chhangani, J.A.
Mahatma Gandhi Institute for Rural Industrialization, Wardha

Tiwari, S.G.
Mahatma Gandhi Institute for Rural Industrialization, Wardha

Rai, M.M.
Center For Sericulture and Biological Pest Management, R.T.M.Nagpur University, Nagpur

Bafana A.
Environmental Health Division, CSIR- National Environmental Engineering Research Institute, CSIR-NEERI, Nagpur.

Kannan Krishnamurthi
Environmental Health Division, CSIR- National Environmental Engineering Research Institute, CSIR-NEERI, Nagpur.

Abstract: Medicinal properties of cow urine (Gomutra) have been well described in ancient Indian medical science, Ayurveda and modern researchers have also reported its antimicrobial, antifungal, immunomodulatory and bio-enhancer properties. Considering these facts and with a view of value addition to this abundantly available natural rural resource, cow urine based herbal hair conditioner was formulated and prepared in laboratory and observed for its antidandruff activity, as dandruff is a common embarrassing scalp disorder, affecting a large chunk of population. For this, dandruff samples (test isolates) were isolated from scalp scrapping of sixteen women respondents belonging to the age group of twenty to twenty five years. The most commonly isolated test organism was identified as Malassezia furfur. Identification was carried out based on microscopic examination, cultural characteristics, biochemical and physiological tests. It is an opportunistic pathogen that causes diseases such as dandruff, pityriasis versicolor, seborrheic dermatitis etc. Antidandruff activity was determined by cup (well)-diffusion method and the zone of inhibition was recorded in millimeter. All the test isolates showed zone of inhibition ranging from 19mm-30mm, with one showing the least zone of 13 mm in diameter. Thus, innovative formulation prepared in laboratory using cow urine and medicinal herbs was found to be effective against test micro organism. The formulation also showed anti-lice activity.

Key words: Gomutra, dandruff, Malassezia furfur, lice, hair-conditioner

I. INTRODUCTION

Though considerable advances are made in the pharmaceutical sciences, especially in synthetic chemistry, plants and their derivatives continue to maintain their significance in medicines. Increased interest in natural drugs over synthetic drugs is because of a high degree of adverse side effects caused by the latter.[1]The world market is moving towards formulations based on value addition of natural resources for health care, healthy foods and for cosmetic purposes including hair conditioning. Hair conditioners may be described as a cosmetic preparation meant for the cleansing of hair and scalp of accumulated sebum, scalp debris and residues of hair-grooming preparations [2]. Dandruff is one of the most commercially exploited skin disease by the personal care industry all over the world, which affects 5% of the global population. [3] Dandruff is a common scalp disorder affecting half of the pubertal population of any ethnicity in both the genders (most prevalent in male population) between the age group of 20 and

60 years [4] .The most common symptoms of dandruff are hair falling, light brown or white patches on the skin, redness, itching, seborrhea, a chronic skin inflammation producing many scales and redness of the affected area with itching sensation. It is generally a major cosmetic problem that causes great public health concern both in developed and developing countries.[5,6]It is reported that approximately 30% of dermatophilic infections are due to the lipophilic yeasts.[7] Hereditary factors also play a major role in transmission of the disease.[8]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*.[9]*Malassezia furfur* is lipophilic yeast belonging to the class exobasidiomycetes and is generally characterized by globose, oblong-ellipsoidal to cylindrical yeast cells, but it can also grow in a mycelial phase. Currently available treatment options for the management of dandruff include therapeutic use of zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, and selenium sulphide, sulphur, piroctoneolamine, cipropiroxolamine and coal tar derivatives. However, these agents show certain limitations, either due to poor clinical efficacy or due to the compliance issues or side effects. Furthermore, these drugs are unable to prevent recurrence. [10]. The hair conditioners or shampoos are toxic and have side effects like irritation of scalp, skin and mucous membrane of the eyes, dryness of scalp and hairs, discolouration and loss of hairs and variation in the individual response due to natural differences and chemicals used in them.[11]

Nature has been a source of medicinal agents for thousands of years. The medicinal properties of plant, animal and mineral origin natural resources have been well described in ancient Indian medical science i.e. Ayurveda. More than 700 medicinal prescriptions have been included in Rig veda and Atharvanaveda[12].Plant products still remain the principal source of pharmaceutical agents used in traditional medicine.[13] Much work has been done on ethno medicinal plants in India.[14]

Different products obtained from cow, like urine, dung, milk, ghee and curd are used widely in number of Ayurvedic formulations.[15]The pharmacological importance of Cow urine is stupendous and its medicinal applications for prevention and cure of diseases are mentioned in Ayurveda. It is widely used in the Ayurvedic formulations for enhancing the properties of many drugs by repeated trituration (bhavana), for detoxification of toxic herbs and metals (shodhana) used in therapeutics. An exhaustive reference of cow's urine having curative properties in skin diseases is referred to in Carakasamhita. Cow urine is found to be effective against reversal of certain cardiac and kidney diseases, indigestion, stomach ache, edema, skin disease etc. [16].Cow urine exhibits both antioxidant and antimicrobial activities which was confirmed by Edwin [17]. The cow urine distillate has been patented as having antioxidative properties as well as an activity enhancer and availability facilitator for bio molecules including anti- infective and anti-cancer agents [18], [19], [20], [21]

The present study is undertaken to develop and evaluate antidandruff activity of cow urine based polyherbal hair conditioner with a vision of value addition of natural resources to promote rural industrialization for entrepreneurship development based on eco-friendly, effective and cheaper products.

II. MATERIAL AND METHODS

The herbal materials purchased from local market were identified and authenticated by Dr. S.K. Padoley, Ex. H.O.D., Deptt. Of Botany, Porwal College of Science, Kamptee. The fruits were dried under shade for 10–15 days. Then the seeds were removed from the fruits, powdered and passed through # 60 sieve 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 were awakened. All the chemicals and reagents were purchased from Hi-Media (India) Ltd. and Merck India Ltd.

Formulation of Hair Conditioner:

Composition of the developed formulation is summarized in the table (Table No.1). All the materials had been used as per Ayurvedic pharmacopoeial quality. The standards of cow urine used had been summarized in Table no. 2. The coarse powder of *Sapindus trifoliatus*, *Acacia concinna* and *Trifala* powder were soaked in Cow urine for twelve hours. Then it was concentrated to half of its volume by gentle heating. The concentrated liquid was filtered with nanobolt cloth and cooled. Powder of camphor and thymol was then mixed to get liquid, which was then added to concentrated liquid to obtain hair conditioner.

Table 1. Composition of formulation:

S.no.	Common Name of Ingredient	Botanical name/English Name	Quantity
1.	Cow urine	--	5 L.
2.	Reetha	*Sapindus trifoliatus*	1.2 Kg
3.	Shikekai	*Acacia concinna*	400 g
4.	Kapoor	Camphor (From *Cinnamomum camphora*)	40 g
5.	Ajwain Sat	Thymol (From *Corum copticum*)	25 g
6.	TrifalaChurna (Mixture of Amla, Harad and Baheda)	Mixture of *Emblica officinalis*, *Terminalia chebula* (retz.) and *Terminalia belerica* (roxb.)	125 g (41.66 g dried powder of each)

Table 2 Standards of Cow urine used for formulation:

S.no.	Parameters	Observations
1	General observations	Shining yellow, clear, typical odour of gomutra, gets brown colour on exposure
2	Weight / ml	1.034 ---1.041
3	Total solid (% w / v)	5.79 – 7.61
4	Conductivity	645--803
5	pH	8.08—8.69
6	Alkalinity	0.245—0.382
7	Total phenol – Assay (mg / 100 ml)	87—126
8	Nitrogen estimation (mg / 100 ml)	514--755
9	Test for heavy metals	Within permissible limits
10	Microbial Count	Not more than permissible limits.

GC-MS study of Cow urine extract:

The identification of compounds in methanolic extract of cow urine was carried out by gas chromatography-mass spectrometer (GC-MS, Saturn Model Varian) with capillary column 50 Q c2 / FFAP, MS column (M/s. J &W Scientific, USA 50 m×0.22 mm id×0.25 μ) and helium gas as carrier. The injector temperature was 150^0C. The oven was programmed at 40^0C for 2 min, raised to 200^0Cat 10^0C/min. The compounds were identified by computer search of the National Institute of Science & Technology (NIST-1998) Library of Mass Spectra on the basis of retention time and mass fragmentation pattern.

Collection of test isolates:

Scalp scraping i.e. dandruff samples were taken from human female respondents belonging to the age group of 20-25 years from local area using sterile cotton buds, forceps and sterile combs. The scales were collected in sterile test tubes and immediately transferred to sterile peptone water broth (PWB). The inoculated PWB tubes were labeled according to the sample history and kept for incubation at 32^0 C for 48 hours.[22]

Isolation of organism:

The growth observed in incubated PWB tubes after 48 hours was again inoculated in to petriplates containing Sabouraud's dextrose agar (SDA), MacConkey agar and *Candida* medium. Then the plates of SDA and *Candida* medium were incubated at 32^0 C for 48 hours and the plates of MacConkey agar medium were incubated at 37^0 C for 24 hours.[22]

Identification of test isolates:

A] Morphological characteristics:
Scalps scraping were digested in PWB and stained with lacto phenol cotton blue and observed under high power (40x) objective of compound microscope.
Smears were also prepared from colonies formed on SDA and stained with lacto phenol cotton blue and observed under a compound microscope.[22]

B] Cultural characteristics:
Colonies on MacConkey agar medium were inoculated into selective medium i.e. *Pseudomonas* isolation agar (PSI), Xylose-lysine deoxycholate agar (XLD), Christensen's urea agar (CUA), Bismuth sulphite agar (BSA) and then all the PSI, XLD, CUA and BSA plates were incubated at 37^0 C for 48 hours.

C] Biochemical characteristics:
The colonies on MacConkey agar medium were inoculated in to nutrient broth and incubated at 37^0 C for 24 hours. The colonies on SDA medium were inoculated in SDB and incubated at 32^0 C for 48 hours. The growth obtained on nutrient broth was then subjected to biochemical tests namely IMVic, sugar fermentation, urease, triple sugar iron (TSI) and all the tests were incubated at 37^0 C for 24 hours. The growth obtained on SDB was also subjected to biochemical tests namely Carbohydrates (sugar) fermentation test i.e. glucose, lactose, mannitol, sucrose, gelatin hydrolysis test and litmus milk reaction test. All the tests were incubated at 32^0 C for 48 hours.[23]

D] Physiological characteristics:
Catalase reaction: The reaction was carried out by applying a drop of H_2O_2 (Hydrogen peroxide) (10volume) on a portion of colony of SDA medium on glass slide or directly on colonies of SDA medium culture plates and results were observed.[24]

Evaluation of antidandruff activity:
Broth culture of test isolates were prepared in SDB medium and incubated for 6-8 hours at 30 ± 2^0C. SDA plates were prepared for each test isolates of which one is considered as control plate and other as an experimental. SDB culture of test isolates was swabbed as lawn over SDA plates by using sterile glass spreader under aseptic conditions. Wells were made in the center of each plate with the help of sterilized cork borer as per well diffusion method. With the help of micropipette, 300µl (0.3mL) of hair conditioner was transferred in each well of each SDA plate except control plate. The experimental and control plates were incubated at 30 ± 2^0C for 7 days. Then the zone of inhibition was observed and the diameter was measured with the help of zone interpretation scale.[25]

Anti-lice activity
Anti-lice activity of the prepared formulations was performed by placing ten live lice in petri dishes containing 1 ml of 10 % of the formulation. The time taken by the last lice to the mortality [mortality was defined as lack of limbs and gut and failure to respond when the legs were stroked with forceps12] faint was checked and noted.[26]

III. RESULTS AND DISCUSSION

Microbial development of resistance, as well as economic incentives, have resulted in research and development in the search for new antimicrobial formulations. Natural plant products are important sources to control bacterial pathogens. [27] In ancient traditional medicine system of India, there is a practice of using cow urine for such purposes. Now-a-day the usefulness of herbs in the production of hair care products has extensively increased and there is a great demand for these herbal products because various synthetic compounds, like Sodium lauryl sulfate etc. being used in cosmetic preparations have been proved to cause various skin diseases with numerous side effects. Considering these facts cow urine based polyherbal hair conditioner was developed and evaluated.

The developed formulation was found to be brown coloured, semi liquid, non transparent in nature with characteristic odour. The GC-MS results for compounds identification in methanolic extract of cow urine are summarized in Table no. 3. On the basis of morphological, cultural, biochemical and physiological characteristics, the test isolates (dandruff samples) were identified as *Malassezia furfur* (Table no. 4 and 5) which is a lipophilic,

dimorphic and yeast-like fungus responsible for skin diseases including dandruff. An excellent antidandruff activity was shown by cow urine based Polyherbal hair conditioner which is shown in Table no. 6 and Fig. 1. Anti-lice activity was performed by using live lice and measuring the fainting time. The fainting time was found to be 5 minute which was comparable one. The results are shown in Table 7.

Table 3. GC-MS results for compounds identification in methanolic extract of cow urine

P.no.	Name of Compound	M.W. Formula	Ret.time	Area
1.	ETHANETHIOL, 2-PHENOXY-	154 C8H10OS	7.52	4995165
2.	2(3H)-FURANONE, DIHYDRO-3-HYDROXY-4,4-DI	130 C6H10O3	8.4	1858205
3.	PHENOL, 3-METHYL-	108 C7H8O	9.13	23616954
4.	CYCLOHEXANECARBOXYLIC ACID	128 C7H12O2	9.76	1133074
5.	BENZOIC ACID	122 C7H6O2	11.47	456436288
6.	PROPANEDIOIC ACID, PHENYL-	180 C9H8O4	12.1	321667200
7.	2,4-IMIDAZOLIDINEDIONE, 5-METHYL-	114 C4H6O2N2	12.14	76127840
8.	O-TOLYLACETIC ACID	150 C9H10O2	13.06	14539599
9.	2(3H)-BENZOFURANONE, 3-METHYL-	148 C9H8O2	13.7	1125883
10.	2-BUTANOL, 2,3-DIMETHYL-	102 C6H14O	14.31	2554975
11.	8-HYDROXYISOTRICHODERMIN	308 C17H24O5	14.92	5528799
12.	13-METHYL-E,E-9,11-TETRADECADIEN-1-OL AC	266 C17H30O2	15.01	4289363
13.	1(2H)-NAPHTHALENONE, 6-(1,1-DIMETHYLETHY	236 C16H28O	16.92	4718378
14.	2-BUTYLOXYCARBONYLOXY-1,1,10-TRIMETHYL-6	326 C18H30O5	17	200661988
15.	4,8-DECADIENOIC ACID, 2-ACETYL-2,5,9-TRI	280 C17H28O3	17.14	8039207
16.	2(1H)-QUINOLINONE	145 C9H7ON	17.41	2239714
17.	CYCLOHEXANONE, 2-(1-METHYL-2-NITROETHYL)	185 C9H15O3N	17.77	2897018
18.	2-CYCLOHEXEN-1-ONE, 4-[3-(.BETA.-D-GLUCO	554 C27H38O12	18.48	737895
19.	L-PROLINE, 4-HYDROXY-2-(2-PROPENYL)-, TR	171 C8H13O3N	18.65	651857
20.	PICROTOXININ	292 C15H16O6	18.9	4779988
21	1-(4-HYDROXYPHENYL)-2-(3-HYDROXYPHENYL)E	214 C14H14O2	25.4	8027120
22.	OCTANE, 1-ETHOXY-	158 C10H22O	25.56	4434299
23.	CEDRAN-DIOL, 8S,14-	238 C15H26O2	35.66	135100

Table 4. Morphological, Biochemical and Cultural test results for test isolates from MacConkey (MCA) medium

Test Isolate	Mac-Conkey agar (MCA)	Gram's staining	Motility	IMVic test				Sugar Fermentation test			Urease slant	Triple Sugar Iron (TSI)	Pseudomon-as isolation agar	Xylose-lysine deoxycho-late agar(XLD)	Christensce's urea agar (CUA)	Bismuth sulphite agar
				Indole	Methyl red	Vogesprouskauer	Citrate	Glucose acid gas	Lactose acid gas	Manitol acid gas						
1	Growth observed	-	-	-	+	-	-	+	-	-	-	Alkaline slant, acidic butt	No growth	No growth	No growth	No growth
2	Growth observed	-	-	-	+	-	+	-	-	-	-	Alkaline slant acidic butt	No growth	No growth	No growth	No growth
3	Growth observed	-	-	-	+	+	-	(+)	-	(+)	-	Alkaline slant acidic butt	No growth	No growth	No growth	No growth
4	Growth observed	-	-	-	+	-	-	-	-	-	-	Alkaline slant acidic butt	No growth	No growth	No growth	No growth
5	Growth observed	-	-	-	+	+	-	(+)	(+)	+	+	Alkaline slant acidic butt, CO_2production	No growth	No growth	No growth	No growth
6	Growth observed	-	-	-	+	-	-	+	-	-	-	Alkaline slant acidic butt	No growth	No growth	No growth	No growth
7	Growth	-	-	-	+	+	+	-	-	-	-	Alkaline slant acidic	No growth	No growth	No growth	No growt

	observed	-	-	-	+	-	-	butt		No growth	No growth	No growth	No growth	h
8	Growth observed	-	-	-	+	-	-	Alkaline slant acidic butt, CO_2production	-	No growth	No growth	No growth	No growth	No growth
9	No Growth observed	-	-	-	-	-	-	No change	-	No growth	No growth	No growth	No growth	No growth
10	No Growth observed	-	-	-	-	-	-	No change	-	No growth	No growth	No growth	No growth	No growth
11	No Growth observed	-	-	-	-	-	-	No change	-	No growth	No growth	No growth	No growth	No growth
12	Growth observed	-	-	+	-	+	+	Alkaline slant acidic butt	-	No growth	No growth	No growth	No growth	No growth
13	No Growth observed	-	-	-	-	-	-	No change	-	No growth	No growth	No growth	No growth	No growth
14	Growth observed	-	-	+	+	+	+	Alkaline slant acidic butt	-	No growth	No growth	No growth	No growth	No growth
15	Growth observed	-	-	+	+	+	-	Alkaline slant acidic butt, CO_2production	-	No growth	No growth	No growth	No growth	No growth

16	Growth observed	-	-	-	+	-	+	+	-	-	-	Alkaline slant acidic butt, CO_2 production	No growth	No growth	No growth	No growth

Table 5. Morphological, Biochemical and Cultural test results for test isolates from Sabouraud's dextrose agar (SDA) medium

Test - Isolates	Sabouraud's dextrose agar (SDA)	Litmus milk reaction test	Candida medium	Lactophenol cotton blue stain (LCB) (Microscopic observation)	Sugar fermentation test				Gelatin hydrolysis test(+) or (-) rate of hydrolysis			Catal-ase test
					Gluco-se acid gas	Lacto-se acid gas	Mannit-ol acid gas	Sucro-se	2 days	7days	Slow/Ra-pid	
1	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	+	-	Rapid	+
2	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
3	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
4	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
5	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
6	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
7	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
8	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	+	-	Rapid	+
9	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
10	Growth	Acidification	No	Spherical, unicellular yeast	+	-	-	-	+	-	Rapid	+

	observed	with curd	Growth	observed							
11	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	Slow	+
12	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	+	-	Rapid	+
13	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
14	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
15	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+
16	Growth observed	Acidification with curd	No Growth	Spherical, unicellular yeast observed	+	-	-	-	-	+	Slow	+

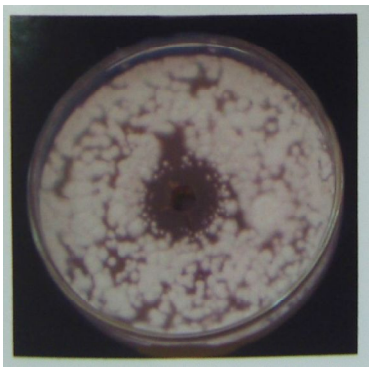
Table 6. Zone of inhibition (mm) of Cow urine based Polyherbal hair conditioner against test isolates

	Test isolates (Dandruff samples)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1
Antidandruff activity Zone of inhibition (mm)	26	25	22	19	26	28	30	27	30	20	20	25	24	13	20	2

Table 7. Anti-Lice Activity For Cow urine based Polyherbal hair conditioner

Fainting time of lice (in minutes)	No. of lice
1	-
2	-
3	-
4	1
5	7
6	8
7	10
8	11
9	-
10	12

Figure 1. Zone of inhibition (mm) of Cow urine based Polyherbal hair conditioner against one of the test isolates



IV. CONCLUSION

The zone of inhibition measured in mm for antidandruff activity showed variation in size ranging from 19mm to 30mm except one showing least zone of 13mm in diameter. Thus Cow urine based Polyherbal hair conditioner was found to be effective against test organism i.e. *Malassezia furfur* and the excellent activity might be due to synergistic antifungal action of its ingredients. Therefore it may be concluded that the formulation is effective, safe, easily affordable for common man and is best suitable for rural industries and employment generation as it is based on value addition of commonly and abundantly available natural resources.

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