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FORMULATION AND EVALUATION OF HERBAL ANTI-DANDRUFF SHAMPOO WITH *CARICA PAPAYA* LEAVES EXTRACT

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ABSTRACT:

Dandruff is a major dermatological scalp condition, characterized by excessive clusters and massive desquamation of stratum corneum which affects one's aesthetic value. The colonization of Malassezia furfur, a lipophilic yeast played an imperative role in pathogenesis of dandruff. Natural bioactive phytoconstituents, considered to be safer alternative over synthetic antidandruff shampoos which are associated with side effects upon long-term application. Our previous findings established anti-dandruff potential of *Carica papaya* leaves extract. Hence, the present study was undertaken to formulate and evaluate herbal anti-dandruff shampoo containing ethanolic extract of *Carica papaya* leaves. The shampoo was formulated by incorporating 1.8 % and 2% concentrations of *Carica papaya* leaves extract and subjected to various quality tests including, physical appearance, pH, solid content percentage, surface tension, dirt dispersion, foam height, skin irritancy and *in-vitro* antidandruff activity. The anti-dandruff shampoo with *Carica papaya leaves* extract at 1.8 % and 2 % concentrations exhibited anti-*Malassezia* activity and passed the quality tests. We suggest that *Carica papaya* leaves anti-dandruff shampoo serve as potential herbal anti-dandruff candidate for dandruff management.

KEYWORDS: *Carica papaya* leaves, anti-dandruff shampoo, herbal formulation, anti-fungal activity, *Malassezia furfur*, evaluation parameters

1. INTRODUCTION:

Dandruff is a common dermatological scalp condition, characterized by excessive clusters and massive desquamation of corneocytes in the form of white to yellowish flaky scales, accompanied by itching and inflammation[1][2][3]. Dandruff, an unpleasant common scalp condition affects worldwide population at the pre-pubertal age of any gender and affects one's aesthetic value[4]. The pathogenesis of dandruff mainly embraces,

hyperkeratinization of scalp epidermal cells, overproduction of sebum, individual susceptibility and microfloralmetabolism. The colonization of *Malassezia furfur*, a lipophilic yeast played a pivotal role in pathogenesis of dandruff by accelerating hyperproliferation of keratinocytes[5]. Commercially, diverse class of synthetic anti-dandruff shampoos are available in market for dandruff management with key ingredients like, salicylic acid, zinc pyrithione, selenium sulphide, imidazole derivatives (ketaconazole), tar derivatives, etc[6]. However, excessive application of synthetic antidandruff shampoos leads to various side effects like, erythema, dry skin, pruritus, edema, headache, difficulty in breathing and also developed fungal resistance following its chronic use [7,8,9]. Hence, the paramount approach to treat dandruff is to use natural herbal formulations containing active phytoconstituents or in combination with synthetic anti-fungal agents that might reduce its dose and lower the risk of side effects.

Carica papaya Linn, belonging to family *Caricaceae* is evergreen shrub medicinal plant commonly known as Papaya in English and Papita in Hindi. The plant was introduced to India in 16th century and is native to tropical America. The whole parts of the plant possess several medicinal properties. Interestingly, papaya leaves are rich in phytoconstituents such as, flavonoids (kaempferol and myricetin), alkaloids (carpaine, psuedocarpaine, dehydrocarpaine I and II), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compound (benzylglucosinolate)[10,11,12]. Papaya leaves also contains, amino acids, vitamin C and E, carotenoides namely, β- carotene, lycopene, anthraquinones glycoside. Owing to its diverse phytoconstituents, *Carica papaya* leaves exhibit pharmacological activities like anti-inflammatory, anti-malarial, wound healing property, hepatoprotective, cardioprotective, anti-cancer, antioxidant, antispaspmodic, anti-aging, antiviral, antibacterial and antifungal[12,13,14,15,16]. An array of evidence revealed antifungal effects of *Carica papaya* leaves against, *Candida albicans, Rhizopus stolonifer, Fusarium spp. and Colletotrichum gloeosporioides and Colletotrichum gloeosporioides*. Our recent data revealed *in-vitro* anti-dandruff potential of ethanolic extract of *Carica papaya* leaves against *Malassezia furfur*[6]. Therefore, the present investigation was undertaken to formulate and evaluate herbal anti-dandruff shampoo containing ethanolic extract of *Carica papaya* leaves. The shampoos were formulated by incorporating 1.8 % and2% concentration of *Carica Papaya* leaves extract and characterized for various quality attributes like, physical appearance, pH, solid content percentage, surface tension, dirt dispersion, foam height and skin irritancy. Moreover, *in-vitro* anti-dandruff activity of *Carica papaya* leaves extract shampoo against *Malassezia furfur* was evaluated by agar cup plate method using standard ketoconazole control.

2. MATERIAL AND METHODS

2.1 Materials

The active *Carica papaya* leaves were collected from local suppliers and authenticated at Department of Botany, R.T.M., Nagpur University. Moreover, Sodium Lauryl Ether Sulphate, Glycerin, Cocobetain, Sodium Chloride, Methyl Paraben were procured from assured suppliers of pharmaceutical grade chemicals.

Figure 1.**The authenticated *Carica papaya* leaves**

2.2 Preparation of the plant extract

The papaya leaves were sorted, washed and shade dried at room temperature for 15 days. It was then crushed well into fine powder in an electronic grinder and kept into airtight polythene bags for further use and stored at room temperature [17]. Approximately 40 gm of papaya leaves powder were soaked and macerated in 400 ml of 96% ethanol for 3 days with occasional stirring. After extraction, the extracts were decanted and then filtered through Whatman filter paper. Ethanolic crude extract was obtained by evaporating the solvent using rotary evaporator and water bath at 60°C [18]. The ethanolic extract was weighed (3 gm) and then stored in the refrigerator at 4°C until use.

2.3 Formulation of anti-Dandruff Shampoo

Initially, three base formulations (F1, F2 and F3) were formulated to find the optimum shampoo formulation. In all trials, we fixed the quantities of Sodium Lauryl Ether Sulfate and glycerin, methyl paraben and varied the concentrations of Cocobetaine and Sodium Chloride as shown in Table 1. Firstly, Sodium Lauryl Ether Sulphate was added to the beaker, in that Glycerin and Methyl paraben was added. Then different variations of cocobetaine were added in three different beakers. The shampoo formulations were prepared by mixing the ingredients in distilled water using a magnetic stirrer operating at 500 RPM. The final pH of the shampoo was adjusted between 5-6 by 2% triethanolamine solution. The base shampoo trials were evaluated for physical appearance, and the most stable formulation F3 was selected for development of herbal anti-dandruff shampoo by incorporating ethanolic extract of papaya leaves at 1.8 and 2% concentrations, designated by S1, S2 respectively (Table 2).The formulated shampoos were stored in a suitable container and used for further evaluation[19].

2.4 Characterization of anti-Dandruff Shampoos

2.4.1*Organoleptic Properties*

The formulated shampoo was observed for their visual appearance, transparency, colour and consistency[20].

2.4.2. Determination of pH

The pH values of herbal anti-dandruff shampoo were determined using digital pH meter, by taking 10% shampoo solution in distilled water at room temperature 25º ± 0.2°C. The electrode was dipped into the sample and pH was recorded[21].

2.4.3. Determination of Solid Contents

Briefly, 4 gm of each shampoo formulation was poured into the dry, clean and pre-weighed petri dishes, and the final weight was noted. This was then placed in a convection oven set at 50°C for 1 hr or until shampoos were completely dried. The dried petri dishes were weighed again, and the solid content post drying was evaluated using the following formula [22].

Solid contents (%) = $W° - W_1/W°$

Where, W^o is the initial weight of the sample while W_1 is the weight of solid contents.

2.4.4. Determination of Surface Tension

Firstly, the shampoos were diluted using distilled water to make 10 % concentration and surface tension was measured using a stalagmometer. The flattened end of stalagmometer was dipped into the beaker and was standardised using distilled water . The distilled water was sucked until the level reaches the mark.The stalagmometer was fixed in the stand and allowed the water to run slowly from the mark. The no. of drops formed when level of liquid reached from A to B was counted. The procedure was repeated with the shampoo solution. The data was calculated using the equation below:

$$R_2 = \frac{(W_3 - W_1)\, N_1 X R_1}{(W_2 - W_1) N_2}$$

W_1 is weighed of empty beaker.

W_2 is weighed of beaker with distilled water

W_3 is weight of beaker with shampoo solution.

N_1 is no of drops of distilled water.

N_2 is no. of drops of shampoo solution.

R_1 is surface tension of distilled water at room temperature.

R_2 is surface tension of shampoo solution[23].

2.4.5. Determination of foam volume and stability

The foam volume and foaming stability of the prepared shampoos was determined by the shake cylinder method[24]. Briefly, 50 ml of the 1% shampoo solution was placed into 250 ml graduated cylinder and shook 10 times upside down. The total volume of foam contents after 1 minute of shaking was recorded. Foam stability was evaluated by recording the foam volume after 5 min.

2.4.6. Determination of dirt dispersion

Two drops of shampoo were added to 10 ml of distilled water in a large test tube. In that 1 drop of India ink was added. The test tube was stoppered and was shaken 10 times. The amount of ink was estimated in the foam as none, light, moderate and heavy[25]. Shampoos that caused the colour to stay in the foam were considered low quality.

2.4.7. *Determination of* consistency

The consistency of formulated shampoo was determined by hand. A pinch of shampoo was taken and rubbed against finger[20].

2.4.8. Skin Irritancy

Skin irritancy of shampoo was evaluated by taking small amount of shampoo on skin, after few min, if any, local irritation or inflammation reaction produced was evaluated[26].

2.4.9. *In vitro* antifungal assays of anti-dandruff shampoo

Agar cup plate method

The antifungal activity against *M. furfur* was investigated by agar cup plate method according to the procedure described by [27, 28] with slight modifications. Sabouraud dextrose agar (SDA) medium was prepared and poured into the sterilized petri plates. After the medium got solidified, the 0.5 ml of diluted fungal suspension was swabbed on respective nutrient agar plates. Then, a hole with a 1 cm diameter was punched aseptically with a sterilized cork borer. The 100 µL (0.1 ml) of herbal antidandruff shampoos containing ethanolic papaya leaves extracts of 1.8 % and 2 % concentrations were added into each wells. Ketoconazole (1%) solution was used as a reference standard control. The plates were incubated at 28 °C for 48 hrs. The antifungal activity was determined by measuring the diameter of zone of inhibition (mm) around the well by vernier caliper. The data were recorded in terms of mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Formulation of herbal anti-dandruff shampoo

Recently, our previous research demonstrated *in-vitro* anti-dandruff potential of ethanolic extract of *Carica papaya* leaves against *Malassezia furfur* [6].Therefore,in present study, an attempt was made to develop

and characterize the herbal anti-dandruff shampoo containing ethanolic extract of *Carica papaya* leaves with an aim to develop herbal, cost-effective anti-dandruff shampoo that could lower the risk of side effects over synthetic shampoos. Initially, three base formulation trials (F1, F2 and F3) were formulated and were evaluated for their physical appearance, pH and daylong stability. The trial F3, base formulation was found to be more stable than any other formulations after keeping it at 45°C for 24 hr, and had a pH value of 5.5, which was close to the pH of hair and scalp [19]. Moreover, the physical appearance of F3 base shampoo was more appealing in terms of colour, odour, texture, consistency and clarity. Hence, F_3 base shampoo was selected for incorporation of ethanolic extract of *Carica papaya* leaves at 1.8 % and 2%concentrations.

3.2. Organoleptic Properties

The formulated herbal anti-dandruff shampoos (S1 and S2) were evaluated for colour, odor, and texture. The results of organoleptic evaluations are summarized in Table 3. In the formulated shampoos, we did not add any colour or perfume; however, the incorporation of *Carica papaya* leaves extract resulted in green colored shampoo formulations. The formulations were viscous, non-transparent with smooth consistency and acceptable. All the formulations displayed pleasant organoleptic characteristics.

3.3. pH measurements

The pH balance of the shampoo seems to be imperative parameter for improving and enhancing the hair qualities, minimizing hair damage and irritation to the eyes and stabilizing the ecological balance of the scalp[29]. The maintenance of lower pH of the shampoos prevents cuticle damage and promotes shine[19]. The pH of the herbal *Carica papaya* leaves anti-dandruff shampoos containing 1.8% and 2% extract were found to be 5.6 and 5.8 respectively (pH range as per BIS: 4.0-9.0), which was comparable with pH of hair and scalp (Table 3).

3.4. Percentage solid contents

The percentage range of solid contents of ideally formulated shampoo should be between 20-30% that promotes easy application, rinsing and its removal in appropriate manner [30]. On the similar lines, our formulated shampoos S1 and S2 were found to have 23.24% and 22.43% solid contents respectively. The herbal *Carica papaya* leaves anti-dandruff shampoos had a lower range of solid contents, thus considered excellent.

3.5. Surface tension

The cleansing ability of shampoos is considered to be directly related with surface tension.The lesser the surface tension, higher the cleaning efficacy due to its ability to reduce the surface tension. Ideal shampoos should reduce the surface tension of pure water, from 72.28 dyne/cm to about 40 dyne/cm[31]. The measured surface tension of *Carica papaya* leaves shampoos (S1 and S2) was 32.057±0.66 dyne/cm and 31.432 ±0.89 respectively (Table 3). Thus, herbal *Carica papaya* leaves shampoos significantly reduced the surface tension of water and exhibited good cleaning efficacy.

3.6. Foam volume and Foam stability

Foaming or lathering seems to be important quality parameter in evaluation of shampoo as it directly correlates with its cleansing power of hair care formulations [32].The foam volume of herbal *Carica papaya* leaves anti-dandruff shampoos S1 and S2was found to be 155ml and 160 ml respectively (Table 3). The foam produced by formulated herbal anti-dandruff shampoo remained unchanged within the 5-minute time period, thus suggesting good foam stability. The presence of surfactant could have reduced surface tension resulted in bubble formation as revealed by foaming of shampoos.

3.7. Dirt Dispersion

Dirt dispersion is an essential quality characteristic of shampoos, and it is associated with the cleansing action of shampoos. The shampoos that cause the ink to stay in the foam are considered of poor quality because of difficulty in washing away the ink, subsequently, the dirt would remain trapped and difficult to remove from the hair[25]. The formulated herbal *Carica papaya* leaves shampoos S1 had light ink in their foam section while no ink was detected in the S2 formulation (Table 3).

3.8. *in-vitro* anti-dandruff activity of *Carica papaya* leaves shampoo

In the present study, the *Carica papaya* leaves antidandruff shampoo exhibited significant anti-*Malassezia* effect in agar cup plate method. The papaya leaves anti-dandruff shampoos S1 and S2(1.8% and 2%)showed anti-fungal activity with zone of inhibition of 10±0.35 mm and 11 ± 0.22 mm respectively (Figure 2) relative to ketoconazole control (12 ± 0.43 mm). Table 4. summarizes the dose-dependent zone of inhibition of anti-dandruff shampoo at 1.8% and 2% concentration against *M. furfur*. The present findings confirmed the antidandruff potential of *Carica papaya* leaves antidandruff shampoo as revealed by inhibitory effect against the dandruff causing *M. furfur* agent.

The results of present study corroborate previous findings. The ethanolic extract of *Carica papaya* leaves demonstrated significant antifungal activity against *Candida albicans, Rhizopus stolonifer, Fusarium Spp. and Colletotrichum gloeosporioides*[9,16,33,34,35]. Moreover, papaya leaves found to be enriched with several phytoconstituents like flavonoids , alkaloids, tannins which attributes to its antifungal and antimicrobial effects[16]. Carpaine, a major alkaloid found in *Carica papaya* leaves that contributes to its antimicrobial properties[36].Tannins found to inhibit the cell wall synthesis by irreversible complexes with proteins[37]. Altogether, herbal anti-dandruff shampoo containing *Carica papaya* leaves extract demonstrated substantial *anti-Malesezzia* activity, hence could serve as safe, novel and excellent alternative over marketed anti-dandruff shampoos.

3.9. Skin irritancy

Skin irritancy test is performed to evaluate whether the product or formulation is allergic to skin or not. If the skin exhibits redness (erythema) or have any irritation like itching, inflammation it is found to be irritant. If it does not show any effect even after applying for around 5-10 minutes, it is considered safe and non-irritant/non-allergic[26]. The formulated *Carica papaya* anti-dandruff shampoo was found to be non-irritant after applying for around 10 minutes. In the present study, application of S1 and S2 formulations containing *Carica*

papaya leaves extract (1.8% and 2%) failed to produce any redness, irritation or inflammation on the skin and hence considered safe and non-irritant.

CONCLUSION

The current study revealed the effective formulation of anti-dandruff shampoo containing papaya leaves extract at different concentration of 1.8 % and 2 %. The formulated shampoo exhibited excellent properties in terms of pH, consistency, foam volume, solid content, dirt dispersion and *in vitro* anti-*Malassezia* activity. The efficacy of formulated shampoo can be further warranted through subjective evaluation. We suggest that anti-dandruff shampoo with *Carica papaya* leaves extract could serve as novel, potent, cost-effective herbal formulation to ameliorate dandruff conditions and might alleviate side effects associated with current synthetic anti-dandruff shampoos.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Table 1. Formulation of Anti-Dandruff shampoo base

Sr.no	Ingredients	Quantity for 100ml			Uses
		F1	F2	F3	
1.	Sodium Lauryl Ether Sulfate	50 ml	50 ml	50 ml	Surfactant
2.	Glycerin	2 ml	2 ml	2 ml	Humectant
3.	Cocobetaine	4 ml	5 ml	6 ml	Surfactant
4.	Sodium Chloride (NaCl)	0.2 gm	1 gm	1 gm	Thickener
5.	Triethanolamine	0.4ml	0.5ml	0.5ml	pH adjuster
6.	Methyl paraben	0.2 gm	0.2 gm	0.2 gm	Preservative
7.	Water	43.2 ml	41.3 ml	40.3 ml	Solvent

Table 2. Formulation of Anti-Dandruff shampoo with papaya leaves ethanolic extract

Sr.no	Ingredients	Quantity in 100% (S1)	Quantity in 100%(S2)
1.	Sodium Lauryl Ether Sulfate	50 ml	50 ml
2.	Glycerin	2 ml	2 ml
3.	Cocobetaine	6 ml	6 ml
4.	Sodium Chloride (NaCl)	1gm	1gm
5.	Triethanolamine	0.5 ml	0.5 ml
6.	Methyl paraben	0.2 gm	0.2 gm
7.	Water	Upto 100ml (38.5)	Upto 100ml (38.3)
8.	Papaya leaves extract	1.8%	2%

Table 3. Evaluation parameters of Anti-dandruff Shampoo

Evaluation parameters	Anti-dandruff shampoo (S1)	Anti-dandruff shampoo (S2)
Colour	Green	Green
Transparency	Non-transparent	Non-transparent
Texture	Smooth	Smooth
pH	5.6	5.8
% Solid content	23.24%	22.43%
Foam volume and stability	155 ml	160 ml
Surface tension	32.057±0.66 dyne/cm	31.432±0.89 dyne/cm
Dirt Dispersion	Light	None

Table 4. Anti-dandruff efficacy of papaya leaves shampoo against *M.furfur*

Sr. No	Name of the Sample	Concentrations of shampoo	Zone of inhibitions
1	Antidandruff shampoo with ethanolic papaya leaves extract	1.8 %	10±0.35 mm
2	Antidandruff shampoo with ethanolic papaya leaves extract	2 %	11±0.22 mm
3	Ketoconazole (Control)	1%	12±0.43 mm

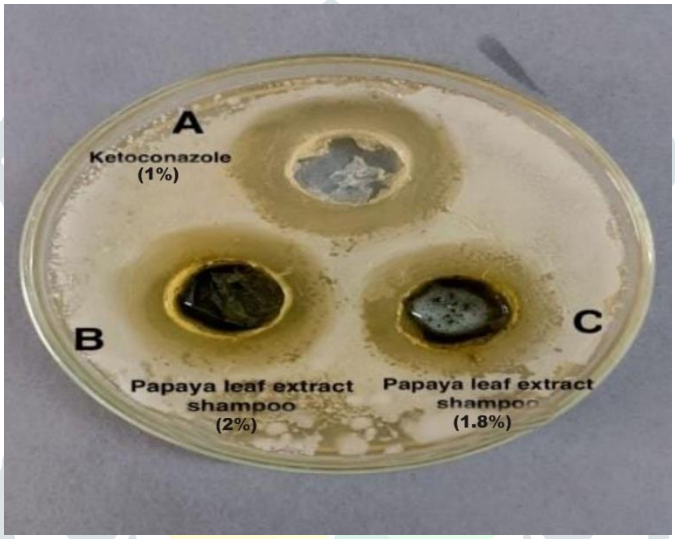

Figure 2. Zone of Inhibitions of herbal *Carica papaya* leaves shampoo and Ketoconazole (control) against *M. furfur* at different concentrations using agar plate method.

A – Ketoconazole (1%)

B - Papaya leaves extract shampoo (2%)

C - Papaya leaves extract shampoo (1.8%)

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