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Formulation and evaluation of a herbal shampoo using flavonoid glycosides from *Dicerocaryum senecioides*

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Abstract

Trichophyton tonsurans affecting the scalp skin and hair shaft, is the most common public health problem in children in most countries. Multidrug *T. tonsurans* resistance has been observed leading to clinical treatment failure and relapse. In the present study, a herbal shampoo was formulated using flavonoid glycosides from *Dicerocaryum senecioides* as active principal components. Flavonoids glycosides were isolated using a bioassay directed protocol, Thin Layer Chromatography (TLC) p-iodonitrotetrazolium violet bio-autography. The formulated herbal shampoo efficacy was tested using the poisoned food assay with clinical strains isolated from patients showing resistance to conventional medicines that are marketed locally. Ten volunteers, 6 girls and 4 boys also participated in assessing the efficacy of the herbal shampoo. From the 10 participants, 3 were blindly given shampoos that did not consist of the flavonoids glycosides. Quality characteristics of the shampoo were determined by monitoring pH changes, microorganisms, colour and viscosity changes, and presence of rancid ordors. The herbal shampoo showed significant mycelial growth inhibitory activity of 93.2 ± 0.6% on the poisoned food assay. All the 7 participants administered with the experimental shampoo were healed by washing their scalp once per day and the condition did not appear again while for the 3 administered with the placebo the condition remained. The condition disappeared when they were administered the experimental shampoo later on. Sensory quality analysis shows that pH of 6.85, green leaf colour and smell were maintained throughout the period of study. The viscosity was also consistent. No microorganism or molds were found in the shampoo. The results obtained from this study showed that the herbal shampoo is an effective alternative option against *T. tonsurans* scalp skin and hair shafts infections. The flavonoid glycosides maybe utilized to make herbal shampoo for scalp infections.

Keywords: *Trichophyton tonsurans; Dicerocaryum senecioides; herbal shampoo; Flavonoids glycosides; Thin Layer Chromatography*

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Introduction

The most common form of hair treatment is shampooing, (Sharma *et al.,* 2011). Shampooing products are mainly aimed at cleansing the hair and the scalp. Basically a shampoo comprises of a detergent with other additives that have hair conditioning, lubricating and medicinal properties, (Vijayalakshmi *et al.,* 2018). The market is awash with synthetic, herbal, medicated and non-medicated shampoos, (Gokhale *et al.,* 2020). However, popularity of herbal shampoo among consumers is on the rise due to the belief that such products being of natural origin are safe and free from side effects, (Al Badi and Khan, 2014). Although synthetic surfactants are added to shampoo for their cleansing and foaming action, their regular use results in dryness, hair loss and irritation to the scalp and eyes, (Sunil, 2020). It is difficult to formulate cosmetics from purely natural raw materials even though herbal formulations are considered as alternative to synthetics. A large number of plants have been reported to have beneficial effects on hair and have been used in hair care formulations (Malpani *et al.,* 2020). The plants may be used as powders, in crude form, purified extracts or as derivative forms. A herbal shampoo consisting of a single natural material that would be milder and safer, with better foaming and detergency, than synthetic ones would be difficult if not impossible to make (Punyoyai *et al.,* 2018).

The leaves of *Dicerocaryum senecioides,* commonly known as Feso or Ruredzo in Shona, Inkunzane in Ndebele have traditionally been used in the Zimbabwean folklore systems since time immemorial for hair washing, (Rambwawasvika *et al.,* 2018). Feso produces a rich lather when shaken with water due to its high content of saponins, (Rambwawasvika and Parekh, 2017). Saponins exhibit antibacterial and antifungal activities. They are known to produce beneficial effects on hair and the skin, (Muyambo *et al.,* 2019). They are employed in hair care preparations as an anti-dandruff agent, hair growth promoter and also to strengthen hair.

This study was designed to formulate a herbal shampoo fortified with flavonoid glycosides from *D. seneioides* and evaluate its activity against *Trichophyton tonsurans,* a fungus affecting the scalp and the skin and hair shafts. The physicochemical and organoleptic properties of the shampoo were also evaluated.

Materials and Methods

Chemicals and reagents

All solvents, chemicals and reagents used were of A. R. grade. TLC plates ($60F_{254}$ 20 x 20 cm) for analytical TLC were supplied by Merck and ($60F_{254}$ 20 x 20 cm) for preparative TLC were supplied by Sigma-Aldrich.

Sample collection and plant identification

Fresh plant materials were collected in Mberengwa, Zimbabwe on 4 February 2021. The leaves of *Dicerocaryum senecioides* were cleaned and dried in air for two weeks under a shade. Identification and authentication was done by the National Herbarium and Botanical Gardens in Harare. Voucher specimens were deposited at the Bindura University of Science Education natural products section.

Preparation of plant extracts

The dried plant materials were pulverized separately using a blender to get smaller particles of about 0.5 mm in order to increase the surface area for extraction. The pulverized plant material (200 g) was exhaustively extracted using ethyl acetate by shaking for 12 hours on a laboratory shaker. Successive filtration using a mutton cloth followed by a Whatman No. 1 filter paper was done to remove the marc. The extraction process was done in triplicate using fresh ethyl acetate each time. The menstruum collected was bulked and concentrated on a rotary evaporator, (Bandiola, 2018) and dried in a fume-hood. Dried extracts were stored in amber bottles at 4°C until the time of use.

Thin Layer Chromatography and Phytochemical analysis.

Plant extracts were subjected to analytical Thin Layer Chromatography (TLC) for separation and qualitative detection of phytocompounds. Solutions of crude extracts,

with concentration of 15 mg/ mL were prepared by dissolving the extracts in the extracting solvent for chromatographic separation. The extracts were spotted on 5 × 10 cm TLC plates using a spotting capillary. The plates were subjected to different solvent systems for separation of phytocompounds. The crude extract was separated using the solvent system ethyl acetate; methanol; water (EMW, 10: 2: 1.5, v/v/v). A UV viewer cabin at 366 nm was used to view the developed plates to determine characteristic fluorescence and R_f values were determined Rambwawasvika *et al.,* (2018) and Rambwawasvika *et al.,* (2017). Flavonoids glycosides were detected by spraying the TLC plates with 1% $AlCl_3$ in ethanol according to the method specified by Dewanjee *et al.,* (2015) and Rambwawasvika *et al.,* (2019). Retention factor values were calculated using the following formula:

$$Rf = \frac{distance\ moved\ by\ extract}{distance\ moved\ by\ solvent} \qquad (1)$$

Thin Layer Chromatography-bioautography

Thin layer chromatography-direct bioautography was used. Ten microliters (0.1 mg mL^{-1}) of plant extract were loaded onto TLC plates in a narrow band and eluted using the solvent system ethyl acetate; methanol; water (EMW, 10: 2: 1.5, v/v/v). The developed plates were dried under a fan for three days to remove traces of solvent. The fungal strain was incubated separately in potato dextrose broth for 7 days. The culture was filtered through gauze cloth to remove mycelia. Spores were obtained by centrifuging. Dehydrated Potato Dextrose Agar was used to prepare spores to spray on the TLC plates. The PDA media inoculated with spores was rapidly sprayed on the plates. Inoculated TLC plates were incubated for 3 days at 25 ^{0}C. Iodonitrotetrazolium chloride was used as the revealing agent.

Isolation of antifungal compounds

Preparative TLC was done on the extracts for isolation of identified flavonoid glycosides. The same solvent system was used to develop the chromatograms on glass backed silica gel coated TLC plates ($60F_{254}$, 20 × 20 cm) followed by fan drying of the developed plates to evaporate all solvents. The different flavonoids glycosides bands were scratched individually into clean labelled beakers and re-dissolved in the extracting solvents. Thorough shaking ensued and the silica was removed by centrifuging for 15 minutes at 2500 rpm followed by vacuum filtration with Whatman No. 1 filter paper. The liquid extracts were put in pre-weighed Petri dishes and the solvent allowed to evaporate under a fume extractor until constant mass solids were obtained. Further analytical TLC to check purity was done and the dry extracts were kept in amber bottles in a refrigerator, Rambwawasvika *et al.,* (2018).

Formulation of the shampoo

Herbal anti-dandruff shampoo was formulated by adding the weighted ingredients as shown in the composition table (Table 1).

Poisoned food methodology for antifungal activity of shampoo formulation.

Anti-dermatophyte efficacy of the formulated shampoo (FA and FB) was determined using the 'Poisoned Food' technique according to the modified Balamurugan, (2014) protocol. Potato Dextrose agar (PDA) was prepared as per the manufacturer's instructions with no modifications and sterilized at 121^{o}C for 15 min. Pour plate method was aseptically done to pour the sterilized PDA into sterile 80 mm petri plates at 15 ml/plate. 40 µl of each sample at different concentrations was added to the molten agar and set to solidify after gentle swirling of each plate. 5 mm diameter disc of fungal mycelium taken from a 7 day old pure culture of *Trychophyton tonsurans* was inoculated in the middle of each plate. Inoculated PDA with no sample was set as the negative control. The fungicide Myconazole at 2 µl/ml/plate was used for comparison as the positive control. Plates were sealed with parafilm to minimize contamination and incubated at 28^{o}C. Growth was monitored daily and radial mycelial growth was measured periodically after day 7 of growth. Percentage inhibition of mycelial growth was calculated as below:

$$\% \text{ Inhibition} = (dc - dt)/dc \times 100 \ldots\ldots (2)$$

where dc= average increase in mycelial growth in control

dt= average increase in mycelial growth in treated

Table 1: Composition of formulated shampoo

Ingredients	*Percent composition %*	
	Sample A	Sample B
Water	76	78.5
SLES	10	10
CDE	6	6
sulphonic acid	3	3
Flavonoid glycosides	1	0.5
TEA	1	0.5
Coconut oil	1	0.5
Sodium chloride	1	0.5
Vitamin E	1	0.5

Microbiological assessment of Shampoo

The two shampoo formulations, A and B were evaluated for microbial contamination using the total bacterial count technique. Replicates of each formulation were serially diluted up to 10^{-5} in a sterile diluent. Aseptically 100 µl of each sample was plated in Mueller Hinton Agar and spread plate method was done. Uninoculated plates saved as the negative control while plates inoculated with *Staphylococcus aureus* were treated as the positive control for bacterial growth. Those inoculated with *Candida albicans* were positive control for yeasts. Plates were incubated at 37^{o}C for 24 hrs. Total bacteria count was done to enumerate using a colony counter and the colony forming units were recorded as CFU/ml, (Neza and Centini, 2016). The test was repeated periodically for 5 weeks to test the formulations for stability.

Efficacy assessment

The formulated herbal shampoo efficacy was tested using the poisoned food assay with clinical strains isolated from patients showing resistance to conventional medicines that are marketed locally. Ten volunteers, 6 girls and 4 boys also participated in assessing the efficacy of the herbal shampoo. From the 10 participants, 3 were blindly given shampoos that did not consist of the flavonoids glycosides. Participation was voluntary and the participants were made to sign consent forms. Names of the participants are not mentioned in the study for ethical reasons.

Evaluation of physicochemical parameters of the herbal shampoo Determination of pH

The pH of shampoo formulations was measured by preparing 10% v/v shampoo solution in distilled water. The pH was determined using a pH meter, (Sawant *et al.,* 2020).

Physical appearance

Colour, odour, dirt dispersion, pH, viscosity, foam stability and foaming ability were the physico-chemical parameters that were evaluated. Below is a detailed description of the evaluation procedures that were followed.

Foaming ability

Cylinder shake method was used on 50 ml of 1% shampoo placed in a 250 ml graduated flask. Total volume of foam produced was recorded after 1min of shaking. Foam stability was determined by recording foam volume 1 min and 4 min of shaking, (Madhusudhan *et al.,* 2021a).

Dirt dispersion

Two drops of shampoo were added to 10 ml distilled water in a test tube. A drop of ink was added, the test tube stoppered and shaken. The

amount of ink in the form was recorded as NONE,LIGHT or HEAVY, (Singh, 2018).

Viscosity

Viscosity of the shampoo was determined using a Brookfield viscometer DV-I Plus, LV, USA set at spindle speeds ranging from 0.5 to 10 rpm using spindle T95 keeping temperature and size of sample container constant, (Sharma *et al.*, 2011).

Stability studies

Short term stability studies were done for 3 months at intervals of 30 days. Small portions of the antifungal shampoo were kept at room temperature and other portion was refrigerated at 4^{o}C. Changes in physical properties and activity was be evaluated, (Madhusudhan *et al.*, 2021b).

Statistical analysis

All experiments were done in triplicate and data were expressed as mean ± standard deviation. Data on pH, foam stability, viscosity and mycelium diameter were analyzed using SPSS one –way analysis of variance (ANOVA) and Tukey HSD post hoc test.

Results

Thin Layer Chromatography and Phytochemical analysis

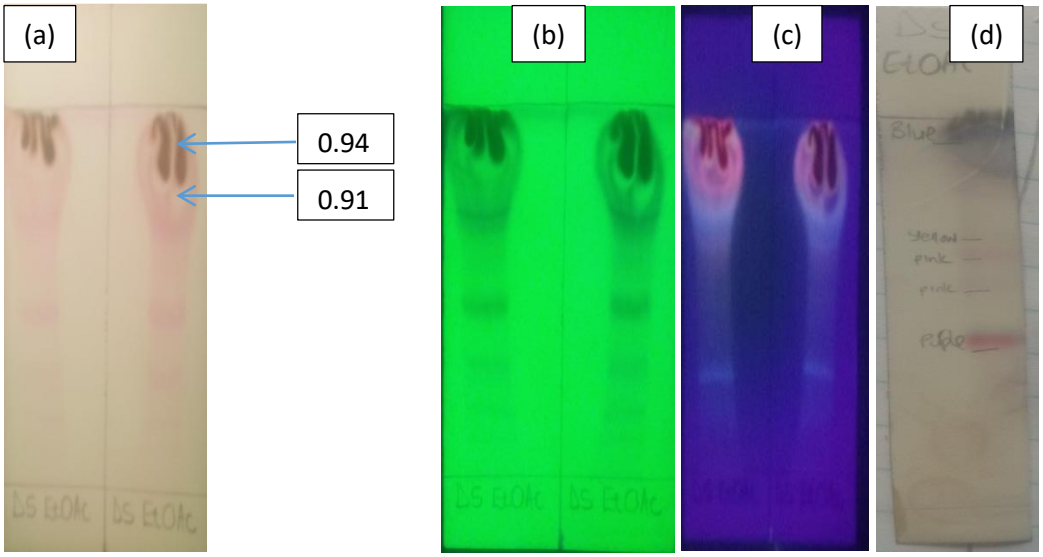

Figure 1 (a-d): TLC profile of ethyl acetate extracts of D. senecioides viewed under (a) UV at normal light, (b) 254 nm (c)366 nm and (d) ferric chloride test

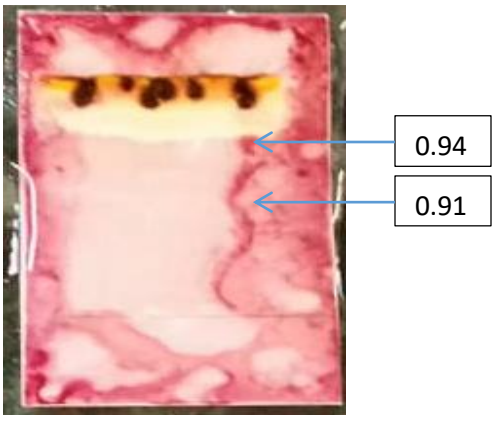

Fig 2: TLC-bioautography profile of D. senecioides acetate extracts against T. tonsurans

TLC separation of acetate extract showed 6 spots shown in Fig 1. The plates were subjected to phytochemical analysis. Bands with R_F values of 0.91 and 0.93 gave positive results with p-anisilaldehyde, ferric chloride and Shinoda's test. The results confirm the presence of flavonoid glycosides. The results correspond with Rambwawasvika (2017).

Thin Layer Chromatography-direct bioautography

Inhibitory activity following TLC separation of acetate extracts was found to be due to the presence of 2 major spots with R_F values of 0.91and 0.93 corresponding to identified flavonoids glycosides. These gave clear zones of inhibition of fungal growth for *T. tonsurans*. Figure. 2 illustrates the results.

Poisoned Food Methodology for Antifungal Activity of Shampoo Formulation.

Table 2 shows the results for poisoned agar assay for formulated shampoos at various concentration against *T. tonsurans.* Generally, formulation A exhibited greater inhibitory effect towards the test organism compared to formulation B. However, both formulations proved to have greater efficacy compared to Miconazole.

Table 2: Poisoned agar assay results

Treatment	***Treatment ID***	****Average Mycelium Diameter (mm)***		
		Control	Treated	%Inhibition
FAC1	Formulation A (conc. 1)	60	1.8	93.2
FAC2	Formulation A (conc. 2)	57	14.9	73.8
FAC3	Formulation A (conc. 3)	60	28.2	53.0
FAC4	Formulation A (conc. 4)	80	39.6	50.5
FA C5	Formulation A (conc. 5)	61	32.5	46.8
FBC1	Formulation B (conc. 1)	65	3.64	90.3
FBC2	Formulation B (conc. 2)	51	5.46	89.3
FBC3	Formulation B (conc. 3)	65	24	62.9
FBC4	Formulation B (conc. 4)	55	24.4	55.7
FBC5	Formulation B (conc. 5)	64	39	39.3
Myconazole		58	14.9	74.3
Untreated		66	68	-

**Values are mean for four replicates*

Microbiological assessment of Shampoo

Evaluation of the quality of the shampoo at microbiological level was done to confirm the adequacy of the non-addition of preservative to the shampoo in order to determine how long the shampoo samples would be good for use from a microbiological point of view, Francis and Boniface, (2017). At the end of each weekly interval of assessment done for 3 months, there was no evidence of bacteria or fungi growth on the culture kits from both samples. It can be concluded that the shampoo is not propitious to the growth of fungi and bacteria. Table 3 shows the microbiological assay results.

Table 3: Microbiology assessment results

Sample	*Total Bacterial Count (CFU/ml)*					
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
FA	0	0	0	0	0	0
FA2	0	0	0	0	0	0
FA3	0	0	0	0	0	0
FA4	0	0	0	0	0	0
FA5	0	0	0	0	0	0
FB	0	0	0	0	0	0
FB2	0	0	0	0	0	0
FB3	0	0	0	0	0	0
FB4	0	0	0	0	0	0
FB5	0	0	0	0	0	0

FA- Formulation A; FA2- Formulation A after 1 week

Efficacy assessment

All the 7 participants administered with the experimental shampoo were healed by washing their scalp once per day and the condition did not appear again while for the 3 administered with the placebo the condition remained. The condition disappeared when they were administered the experimental shampoo later on.

Visual inspection and pH

Table 4 shows the results of visual inspection of the formulations. The results show that both formulations had desirable characteristics with respect to visual inspection and pH.

Table 4: Evaluation of formulation for physical characteristics and pH

Shampoo sample	*colour*	*pH*	*Foaming ability*	*Dirt dispersion*
A	green	6.85± 0.04	good	Heavy
B	green	6.84±0.03	good	Light

Foaming ability and foam stability

Ability to foam is not always proportional to cleansing effect. However, the former is an important parameter and criterion in evaluation of shampoos. The foam retention data is given in Table 5.

Table 5: Foam stability of shampoo

Time (min)	Foam volume (ml)	
	A	B
1	172	169
2	170	166
3	168	164
4	167	161

Figure 3 is a plot of foam volume against time. The graph shows that the foam volume and foam stability for formulation A was greater than that of B. Gradient of the plot for formulation B was steeper than that of A implying that formulation A foam was more stable than B.

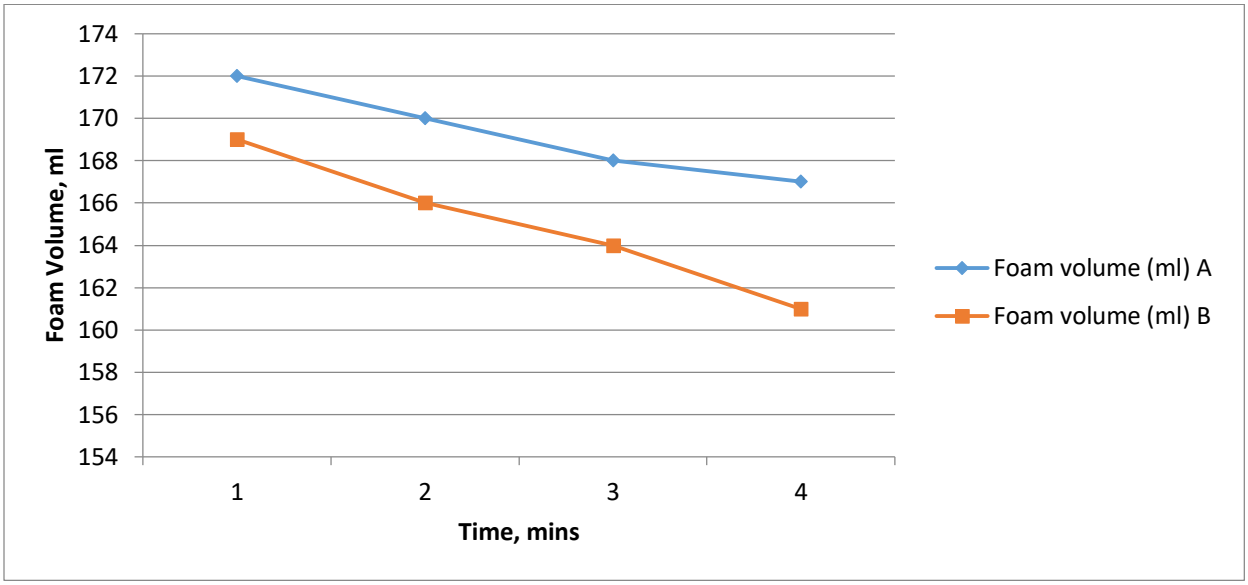

Fig 3. Variation of foam volume with time

Dirt dispersion

If ink is concentrated in the foam, the shampoo is regarded as poor quality. Dirt that stays in foam is difficult to rinse away and is redeposited in the hair, (Sawant *et al.,* 2020). Both shampoos indicated that dirt would not stay in the foam. Therefore, they are satisfactory formulations.

Viscosity

Viscosity of the formulation decreased with increase in speed of rotation. Table 6 shows viscosity data.

Table 6: Evaluation of viscosity of shampoo formulations

Speed rpm	*A*		*B*	
	% Tor	Viscosity	% Tor	Viscosity
0.5	20.23	80309	19.96	80132
1.5	40.18	49993	40.01	49870
2.5	50.67	37298	49.86	36667
5	66.78	24356	65.93	24112
10	83.49	14678	83.11	14043

Figure 4 shows the variation of viscosity with speed. The data suggests that the variation of viscosities for both formulations was the same. In both formulations' viscosity decreased with increase in speed.

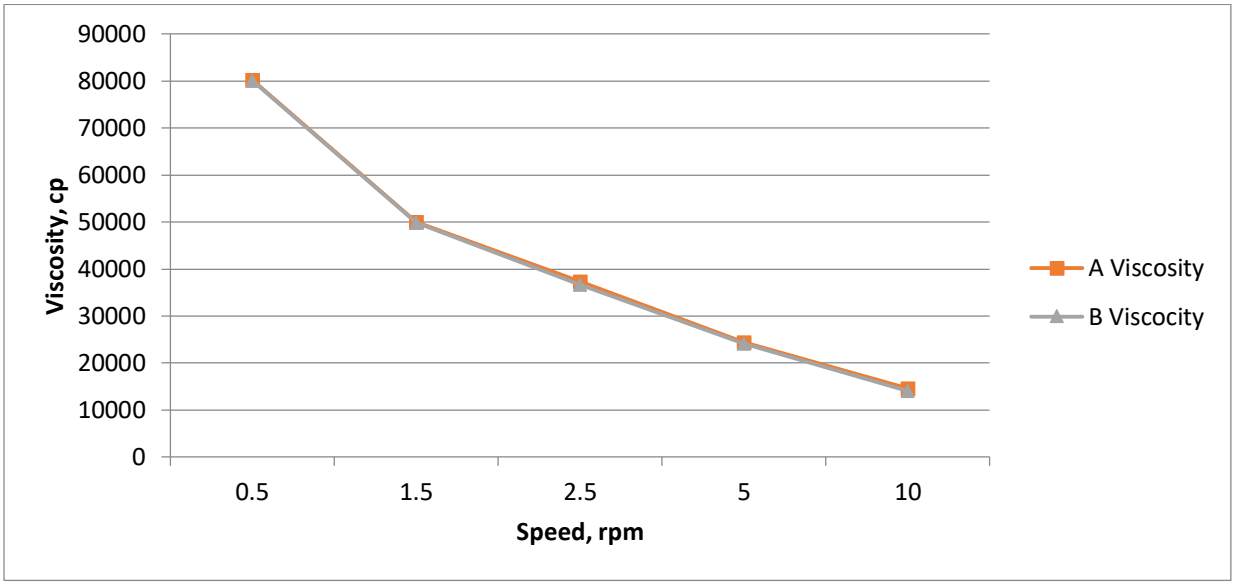

Figure 4. Variation of viscosity with speed

Stability studies

The stability results are listed in Table 7. Organoleptic characteristics evaluated in stability studies indicated that the formulations are chemically and physically stable.

Table 7: Stability studies for formulations

Parameters	1 month		2 months		3 months	
	A	B	A	B	A	B
Colour	green	green	green	green	green	green
Odour	none	none	none	none	none	none
Ph	6.85	6.84	6.85	6.84	6.85	6.84
Foaming ability + foam stability (ml)	168	164	168	163	168	163

Discussion

Extractable phytochemicals inarguably proved to be an excellent remedy for various ailments. Remedies derived from plant extracts are associated with fewer side effects in the patients and cost effective production processes. (Rambwawasvika *et al.,* 2019). The inhibitory effect of formulation A was 93.2%. *Trichophyton tonsurans,* a fungus affecting the scalp and the skin and hair shafts poses a public health threat in many countries (Gits-muselli *et al.,* 2017). *Trichophyton tonsurans* has been one of the causative agents of dermatophytosis, (Lee *et al.,* 2016). *Tinea capitis*, or scalp ringworm, remains a

major public health concern and is the most common superficial mycosis in children of school-going age.

In the present study, flavonoid glycosides extracted from *Dicerocaryum senecioides* have proven to have antifungal properties. Results obtained in vitro show significant activity against *T. tonsurans*. If the diverse forms of fungal infections are to be put into consideration, flavonoid glycosides have potential therapeutic effect on infections due to *Epidermophyton flocossum, C. albicans* and *Trichophyton rubrum*. Herbal formulations therefore are offering complementary and alternative remedy for managing topical fungal infections. The extract is yet to be tested for the treatment of fungal infections in vivo because this study did not go that far. Testing for the extract's effect in vivo will require laboratory mice.

Flavonoids were positively identified on TLC plates by spraying with ferric chloride reagent. A dark colour characterized flavonoids (Figure 1). The observed chromatograms were correlating with the findings of Rambwawasvika *et al.,* (2018). Other phytocompounds, steroidal glycosides and triterpenoids in the ethyl acetate extract also exhibited significant antifungal activity compared to the negative control. Their performance was however significantly lower than that of flavonoid glycosides and the standard drug miconazole. Future studies are required to determine their structure as well as the active sites. There was no major difference between the blank control and the ethylacetate only indicating that the extracting solvent has no influence in the inhibition of fungal growth.

Basing on efficacy tests, flavonoid glycosides as active antifungals is a very important discovery because they also have antioxidant properties. Cosmetic formulations contain antioxidants to reduce the undesirable effects of ultra violet (UV) radiation on hair, (Rambwawasvika *et al.,* 2019). Flavonoids also have great therapeutic potential due to their wide biological and pharmacological actions, Astuya, (2017). Therefore use of the formulation provides a conducive environment for hair regrowth by providing decent food nutrients without fungal toxins. The activity of these flavonoid glycosides can also be attributed to their saponification value where the cleansing action is responsible for sloughing off dead skin thus the opening of scalp pores. Astuya, (2017) suggests that flavonoids can also act by inhibiting the activity of enzymes such as aldose reductase, cyclooxygenase, lipoxygenase among others.

Quality control parameters were checked carefully. The necessary evaluation parameters gave positive and acceptable results. The results obtained in the current study show that when these active plant extracts are incorporated in shampoo, they render it a more stable and effective product improved patient compliance. The pH of shampoo formulations is very important in enhancing the quality of hair, reducing eye irritation as well as maintaining the scalp micro-biome, (Sharma *et al.,* 2011). Shampoos of low pH are currently trending as mitigation against hair damage. Mild acidity prevents swelling, tightens scales thus inducing shine. Both shampoos were acid balanced. The pH values exhibited by the formulations are closer to that of the skin (table 4). The pH of the shampoo is good which helps in improving and enhancing the quality of hair, minimizing the irritation to the eyes and stabilizing the ecological balance of the scalp, (Lodha, 2019).

Lathering and stability are imperative parameters in the evaluation of a shampoo, (Sekar and Noordin, 2016). Formulated shampoos exhibited compact, uniform and dense foam. Foam volume decreased negligibly over 4 minutes in formulation A suggesting that the foam produced was stable. Generally, the two shampoo formulations showed almost similar foaming characteristics with A exhibiting better foam stability (table 5 and 6). Rheological evaluation of the formulations suggested that their viscosities gradually decreased with increase in the speed of rotation. At low rpm values, the formulations showed high viscosity. This implies that the formulations were shear thinning or pseudo plastic in nature. Pseudo plastic behaviour is desirable in shampoos, (Sbhatu *et al.,* 2020). This shows the ease with which the shampoo spreads on hair, (Sawant *et al.,* 2020).

Conclusions

Conclusively, shampoo fortified with flavonoid glycosides from *D. senecioides* showed potential

antifungal activity against *T. tonsurans*. The shampoo was found to be cost effective, simple to prepare, skin friendly and is a remedy to drug resistance strains. The antifungal activity of the fortified shampoo opens a new door for the new range of cosmeceuticals.

References

Al Badi, K., & Khan, S. A. (2014). Formulation, Evaluation and Comparison of the Herbal Shampoo with the Commercial Shampoos. *Beni-Suef Univ. J. Basic Appl. Sci. 3* (4), 301–305.

Balamurugan, S. (2014). *In Vitro* Antifungal Activity Of *Citrus Aurantifolia* Linn Plant Extracts Against Phytopathogenic Fungi *Macrophomina Phaseolina. International Letters of Natural Sciences Online: 2014-04-12 ISSN: 2300-9675, Vol. 13, pp 70-74 doi:10.18052/www.scipress.com/ILNS.13.70 2014 SciPress Ltd, Switzerland.*

Bandiola, T. M. (2018). Screening of Medicinal Plants For. *Int. J. Pharm.*, *8* (1), 137–143.

Dewanjee, S., Gangopadhyay, M., & Bhattacharya, N. (2015). Bioautography and Its Scope in the Fi Eld of Natural Product Chemistry. *J. Pharm. Anal*, *5* (2), 75–84.

Francis, O. O., & Boniface, O (2017). Formulation and Antimicrobial Activity of Triclosan-Based Conditioning Medicated Shampoo, *13* (4), 787–798.

Gits-muselli, M., Benderdouche, M., Hamane, S., Mingui, A., Chauvin, M. F. De., Guigue, N., Picat, M., Bourrat, E., Petit, A., & Bagot, M., *et al*. (2016). Continuous Increase of *Trichophyton tonsurans* as a Cause of *Tinea capitis* in the Urban Area of Paris , France : A 5-Year-Long Study, No. October 2016, 476–484.

Gokhale, S., H. Pawshe, A., P. Patil, S., M. Pitambare, R., S. & Pawar, P. (2020). Extraction, Formulation And Evaluation Of Moringa Herbal Shampoo. *Int. J. Adv. Res.*, *8* (6), 1392–1396.

Recommendations

Based on the results of this study, it is recommended that herbal formulations of shampoo can be considered for use in managing fungal infections of the scalp.

Lee, W. J., Sim, H. B., Jang, Y. H., Lee, S., Kim, D. W., & Jun, J. B. (2016). Skin Infection Due to Trichophyton Tonsurans Still Occurs in People in Korea but Not as Outbreaks, No. 2015, 296–300.

Madhusudhan, M., Rao, M. K., Radha, G. V., & Ganapathy, S. (2021a). Formulation, Evaluation and Comparison of the Polyherbal Shampoo with the Commercial Shampoos. *Asian J. Pharm. Res. Heal. Care* , *13* (3), 254–265.

Madhusudhan, M., Rao, M. K., Radha, G.V., & Ganapathy, S. (2021b) Use Of Traditional Herbs For The Formulation Of Herbal Powdered Shampoos And Their Evaluation. *PLANT Arch.*, *21* (No 1).

Malpani, T., Jeithliya, M., Puri, P., & Pal, N. (2020). Formulation and Evaluation of Pomegranate Based Herbal Shampoo. ~ *1439 ~ J. Pharmacogn. Phytochem*, *9* (4), 1439–1444.

Muyambo, S., Chikurunhe, I., & Moyo, D. N. (2019). Intrinsic Viscosity of Stud Plant Mucilage (*Dicerocaryum zanguebarium*): Polymeric Studies at Infinite Dilution , *10* (1), 28–39.

Neza, E., & Centini, M. (2016). Microbiologically Contaminated and Over-Preserved Cosmetic Products According Rapex 2008–2014.

Punyoyai, C., Sirilun, S., Chantawannakul, P., & Chaiyana, W. (2018). Development of Antidandruff Shampoo from the Fermented Product of *Ocimum anctum Linn*. *Cosmetics*, *5* (3).

Rambwawasvika, H.; Parekh, C. T. (2017). Extraction and Characterisation of Mucilage from the Herb *Dicerocaryum senecioides* and Its Use a Potential Hair Permanent., *13* (3), 691–705.

Rambwawasvika, H., Dzomba, P., & Gwatidzo, L (2018). In Vitro Study of Phytochemical Composition and Antifungal Activity of *Dicerocaryum senecioides* Leaf Extract, *Pharmacologia, 9: 137*, *143*. .

Rambwawasvika, H., Dzomba, P., & Gwatidzo, L. (2019). Hair Growth Promoting Effect of *Dicerocaryum senecioides* Phytochemicals. *Journal of Medicinal Chemistry,* (1),1-10.

Sawant, P. S., Sankpal, P. B., Jagtap, A. M., Gavade, A. S., & Vambhurkar, G. B. (2020) Formulation and Evaluation of Herbal Shampoo. *Res. J. Top. Cosmet. Sci.*, *11* (1), 01.

Sbhatu, D. B., Berhe, G. G., Hndeya, A. G., Abraha, H. B., Abdu, A., Gebru, H. A., Taye, M. G., Mulugeta, A., Weldemichael, M. Y., & Tekle, H. T., et al. (2020). Formulation and Physicochemical Evaluation of Lab-Based *Aloe Adigratana* Reynolds Shampoos. *Int. J. Anal. Chem.*, *2020*.

Sekar, M., H. A. M. N. N. M. (2016). Formulation and Evaluation of Herbal Shampoo Containing Rambutan Leaves Extract. *Int. J. Pharma Bio Sci.*, *7* (4), P146–P151.

Sharma, R. M., Shah, K., & Patel, J. (2011). Evaluation of Prepared Herbal Shampoo Formulations and to Compare Formulated Shampoo with Marketed Shampoos. *Int. J. Pharm. Pharm. Sci.*, *3* (4), 402–405.

Singh, A. (2018). *Formulation and Evaluation of Herbal Anti-Dandruff Shampoo from Bhringraj Leaves*; Vol. 4.

Sunil, S. (2020). Formulation And Evaluation Of Herbal Shampoo, Vol. 8.

Vijayalakshmi, A., Sangeetha, S., & Ranjith, N. (2020). Formulation and Evaluation of Herbal Shampoo. *Asian J. Pharm. Clin. Res.*, *11* (Special Issue 4), 121–124.