SUN PROTECTION FACTOR EVALUATION OF PREPARED HERBAL SUNSCREEN CREAMS FROM *Mansonia gagei* J.R. DRUMM (KALAMET) STEM

Thin Wut Soe[1], Cho Lwin Lwin Khine[2], May Zin Oo[3], Su Su Aung[4], Myat Myat Thaw[5]

Abstract

In this project, *Mansonia gagei* J. R. Drumm (Kalamet) stem in the tropics of Myanmar is investigated for sunscreen creams preparation. The sample was collected from Kaunghmudaw Pagoda Market, Sagaing Region, in June 2023. The preliminary phytochemical tests revealed the presence of α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, and saponins in the stem of *M. gagei*. In addition, sunscreen creams were prepared by maxing ingredients with water and ethanol extracts from selected sample for the protection of skin from UV radiation. Prepared herbal sunscreens were evaluated for physicochemical characteristics such as color, odor, appearance, homogeneity, consistency, washability, viscosity, skin irritancy, pH, and sun protection factor (SPF). Results showed that both herbal sunscreen creams had good consistency, easy washability, were within the permitted viscosity range, and contained no irritants. The pH values of the prepared sunscreen creams were observed not to be significantly changed during testing, and those were in the range of 6.4 to 6.6. SPF values of prepared sunscreen creams were observed to be 15.17 (cream-H_2O) and 17.91 (cream-EtOH). The antibacterial activity of the prepared sunscreen creams against *Staphylococcus aureus* in topic therapy was investigated using the agar-well diffusion method. The inhibition zone diameters were found to be 11 mm (cream-H_2O) and 14 mm (cream-EtOH). In comparison to prepared creams, cream-EtOH was shown to be more stable, effective, with a high SPF, and potent antibacterial activity. It can enhance and effectively contribute to the UV-absorbing properties of conventional sunscreen. It could also have the greatest advantage of avoiding the adverse and undesired effects of synthetic sunscreen compounds.

Keywords: *Mansonia gagei* J.R. Drumm, sunscreen creams, water and ethanol extracts, sun protection factor, antibacterial activity

Introduction

Sunscreens are formulations for skin application that contain substances that can absorb, reflect, or disperse solar radiation, reducing its biological effects on the skin. Most active sunscreen ingredients have been used globally for more than 15-30 years and are considered to be safe for humans (Hanrahan, 2012). Herbal cosmetics are formulated by using different cosmetic ingredients to form a base in which one or more herbal ingredients are used to treat various therapeutic skin elements. Cosmeceuticals have medicinal benefits that affect the biological functioning of the skin, depending on the type of functional ingredients they contain. These are modern trends in the fields of beauty and fashion (Pandey *et al.*, 2021).

Mansonia gagei is a medium-sized evergreen tree with a bole that can be up to 70 cm in diameter. Leaves are simple, faintly dentate, sometimes with domatia. Flowers are mostly

[1] Department of Chemistry, Sagaing University of Education
[2] Department of Chemistry, Sagaing University of Education
[3] Department of Chemistry, Sagaing University of Education
[4] Department of Chemistry, Sagaing University of Education
[5] Department of Chemistry, Sagaing University of Education

hermaphroditic, zygomorphic, fragrant, and have two stamens per staminal bundle. It is a common flowering shrub native to Myanmar, India, and tropical West Africa. (Kubitzki and Bayer, 2013). It has been used for many traditional uses, such as to treat cardiac stimulants, antiemetics, antidepressants, tonic reagents, urinary disorders, and anemia (Nishina *et al*., 2018). The paste is also applied topically to the body for a cooling effect and to alleviate itches (DeFilipps and Krupnick, 2018). Pharmacological studies suggest that the plant possesses antifungal, antibacterial, antioxidant, antiestrogentic, and antitumor properties (Li, *et al*., 2018) thus lending scientific support to the plant's ethnomedicinal uses. Therefore, in this research, we have a plan to investigate the sun protection factor of herbal sunscreen creams from the various extracts of stem of *Mansonia gagei* J.R. Drumm (Kalamet).

Botanical Description

Family name : Sterculiaceae
Botanical name : *Mansonia gagei* J.R. Drumm (Kress *et al.*,2003)
Myanmar name : Kalamet
Part used : Stem
Distribution : Myanmar, India, tropical West Africa

Figure 1. Photographs of plant, stem and powder of *Mansonia gagei* J.R. Drumm (Kalamet)

Materials and Methods

Sample Collection

The sample of *Mansonia gagei* J.R. Drumm (Kalamet) stem was collected from Kaunghmudaw Pagoda Market, Sagaing Region, in June 2023. The dried sample was made into powder by using a grinding machine. The dried powder sample was stored in an airtight container to prevent moisture changes and other contaminations. The dried powdered samples were used to investigate their chemical and biological activities. The identification of the sample was conducted with the Department of Biology at Sagaing University of Education.

Preliminary Phytochemical Tests of *M. gagei* (Kalamet) Stem

A preliminary phytochemical tests of the selected sample were carried out using standard methods (Harborne, 1984, M-Tin-Wa, 1972 and Marini-Bettoto *et al.,* 1981). The results are shown in Table 1.

Preparation of Extracts of *M. gagei* (Kalamet) Stem

The dried powdered sample (10 g) was percolated with ethanol (200 mL) for three days and then filtered. The same procedure was repeated three times. The combined filtrate was concentrated in a water bath. The dried filtrate was transferred to a weighed porcelain basin and

evaporated to dryness in a water bath to obtain ethanol extract. For water extract, a dried powdered sample (10 g) was boiled with distilled water (200 mL) for 1 h. The extract was then filtered, transferred to a weighed porcelain basin, and evaporated to dryness in the water bath. The two extracts were stored in a desiccator containing dry silica gel prior to use in each experiment.

Preparation of Herbal Sunscreen Creams from *M. gagei* (Kalamet) Stem

The oil phase of cream was prepared by heating the ingredients (coconut oil, stearic acid, lanolin, and glycerin) at 75±2 ˚C with constant stirring using a hot plate, while for the preparation of the aqueous phase, purified water was heated separately in a 50 mL capacity beaker at 80±2 ˚C. Methylparaben and propyl paraben were added, to the above mixture and dissolved with occasional stirring, at 75±2 ˚C. The two phases (oil and aqueous) were mixed together with vigorous stirring for about 1-2 min. Finally, the stem extracts (water and ethanol) were individually added with constant stirring until a thick cream was formed. The temperature was further reduced to around 45 ˚C using a cold-water bath. The cream was collected in a wide-mouthed airtight glass container, and stored in a dry place. And then, the prepared herbal cream were characterized by instrumental methods (Mamillapalli *et al*., 2018).

Determination of Physicochemical Parameters of Prepared Herbal Sunscreen Creams

Prepared herbal sunscreen creams were evaluated for physicochemical characteristics (color, odor, appearance, homogeneity, consistency, washability, and pH) by visual and instrumental methods. The viscosity of the prepared herbal sunscreen creams was measured using a rotor spin type (L-4) at 250 rpm in an ATAGO viscometer at the Physical Research Room, Department of Chemistry, University of Yangon. The viscosity value of the prepared creams was recorded directly from the instrument display.

Determination of Skin Irritancy Test

For the skin irritancy test, we selected 10 volunteers who met the following inclusion criteria: age ≥ 18 years old, physically and mentally healthy, skin free from lesions, and no history of allergy. All volunteers provided informed consent. According to the skin patch test description, the student volunteers were divided into two groups. Group A volunteers were applied cream-H_2O, and Group B volunteers were applied cream-EtOH. Firstly, a marked area (5 cm^2) on the right-hand dorsal surface and their left-hand dorsal surface were used as control. The cream was applied to the specified area at twice (between 9:00-12:00 am and 1:00–3:00 pm) a day for one week. Irritancy, erythema, and edema were checked if any, for regular intervals up to 24 h and reported (Hiremath *et al*., 2008).

Determination of Sun Protection Factor (SPF)

The photoprotective activity of tested samples was measured by the determination of sun protection factor (SPF). The tests were measured at the Pharmaceutical Research Laboratory, Department of Biotechnology Research (DBR), Kyaukse Township, Mandalay Region, Myanmar. Sample (2 mg) was diluted in 1 mL of methanol to obtain a concentration of 2 mg/mL. Spectrophotometric scanning was performed at wavelengths between 290 and 320 nm, with intervals of 5 nm, using a 96-well microplate reader. The SPF value was obtained according to the equation developed by? (Mansur *et al*., 1986).

$$SPF = CF \times EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where, CF = correction factor (= 10),

EE (λ) = erythmogenic effect of radiation with wavelength λ,

I (λ) = solar intensity spectrum,

Abs (λ) = spectrophotometric absorbance values of sunscreen product at wavelength λ (Mansur *et al*., 1986)

EE x I = constant is determined by (Sayre *et al*., 1979)

Screening of Antibacterial Activity of Prepared Herbal Sunscreen Creams

The antibacterial activity of prepared herbal sunscreen creams of *M. gagei* were determined against the gram-positive bacteria *Staphylococcus aureus*, was used as the tested microorganism for this experiment (Schlegel and Zaborosch, 1993). The tests were screened at the Pharmaceutical Research Laboratory, Department of Biotechnology Research (DBR), Kyaukse Township, Mandalay Region, Myanmar.

The tested microorganism was inoculated in Muller Hinton broth at 37 ˚C for overnight. On the next day, the overnight broth culture was diluted with normal saline to obtain the OD_{600} at 0.08 to 0.1 with an approximate cell density of 1.5 x 10^8 CFU/mL. Muller Hinton agar plates were prepared and sterilized by autoclaving at 121 ˚C for 15 min. The broth inoculums were evenly spread out with sterile cotton swabs on the Muller Hinton agar plates to obtain the uniform inoculums. After the plate was inoculated, 8 mm diameter wells were made on the agar medium by using a sterile cork borer. Each 50 µL (0.2 g/mL) of samples was introduced into each well labelled. Chloramphenicol (30 µg/well) was used as the positive control. Then, the plates were placed in an incubator at 37 ˚C for 16 to 18 h. After incubation, the plates were examined, and zone diameters of complete inhibition were measured and recorded to the nearest millimeter.

Results and Discussion

The sample of *M. gagei* (Kalamet) stem was cleaned and dried at room temperature for two weeks. The dried sample was made into powder by using a grinding machine. The dried powdered sample was stored in an airtight container to prevent moisture changes and other contaminations. The dried powdered sample was used to investigate for their chemical and biological activities. *M. gagei* stem was rich in organic constituents such as α-amino acids, glycosides, phenolic compounds, saponins, carbohydrates, and flavonoids, except alkaloids, reducing sugar, starch, steroids, and tannins.

Table 1. Results of Phytochemical Constituents of *M. gagei* (Kalamet) Stem

No.	Test	Extract	Test reagent	Observation	Result
1	alkaloids	1 % HCl	Dragendorff's reagent	no orange ppt.	–
			Mayer's reagent	no white ppt.	–
			Wagner's reagent	no reddish brown ppt.	–
2	α-Amino acids	H_2O	Ninhydrin reagent	violet colour	+

No.	Test	Extract	Test reagent	Observation	Result
3	carbohydrates	H_2O	10 % α-Naphthol, conc. H_2SO_4	red ring	+
4	flavonoids	EtOH	Mg turning, conc. HCl	pink colour	+
5	glycosides	H_2O	10 % Lead acetate solution	white ppt.	+
6	phenolic compounds	EtOH	1 % $FeCl_3$ solution	dark blue colour	+
7	reducing sugars	H_2O	Benedict's solution	no brick-red ppt.	–
8	saponins	H_2O	Distilled water	frothing	+
9	starch	H_2O	1 % Iodine solution	no blackish blue	–
10	steroids	PE	Acetic anhydride, conc. H_2SO_4	no greenish blue	–
11	tannins	H_2O	1 % Gelatin	no white ppt.	–

(+) = presence, (−) = absence, (ppt.) = precipitate

Preparation of Herbal Sunscreen Creams from *M. gagei* (Kalamet) Stem

The herbal sunscreen creams were prepared by using the watery and ethanol extract of the stem of *M. gagei*. The ingredients used in the formulation were described as in Table 2.

Table 2. Composition of Prepared Herbal Sunscreen Creams of *M. gagei*

No.	Ingredients	Components (% w/w)
1.	stearic acid	40
2.	lanolin	50
3.	glycerin	156.6
4.	coconut oil	35
5.	methylparaben	4
6.	propylparaben	0.4
7.	plant extract	10
8.	distilled water	6.95

Determination of some Physicochemical Parameters

Prepared herbal sunscreen creams were evaluated for their physicochemical characteristics: color, odor, appearance, homogeneity, consistency, washability, viscosity, and pH. The results of various tests for the evaluation parameters of herbal sunscreen cream formulations using watery and ethanol extracts indicated that it exists in yellowish brown and pale brown colors with a pleasant odor and smooth appearance with homogeneity and good

consistency. Homogeneity was confirmed by visual appearance and touch. All the prepared creams were found to have good homogeneity.

Viscosity governs many properties of the product, such as the spreadability and pourability of the product from the container. The viscosities of the prepared creams were found to be in the range of >5000 cP, which indicated that the cream was easily spreadable by small amounts of shear. The pH of creams was determined to examine the possible side effects of acidic or alkaline pH, which can lead to irritation of the skin. Acidic or alkaline pH may cause irritation to the skin and influence the rate of hydration of polymers. The cream in general has a pH of 6-9 for sunscreen (Donglikar and Deore, 2017). The pH values were found to be 6.4 (cream-H_2O) and 6.6 (cream-EtOH).

Results of Skin Irritation Test

Safety testing is essential before raw materials or end products can be sold to consumers. Therefore, in this test, selected 10 volunteers who provided informed consent and were divided into two groups. One group is used for cream-H_2O and another is for cream-EtOH. During the assessment, the prepared herbal sunscreen creams indicated that they did not irritate the skin.

Determination of SPF value

The well-known UV Vis spectrophotometric method can be used for the determination of SPF values in many cosmetic formulations. The efficiency of the sunscreen products depends on the sun protection factor (SPF) value. The higher the SPF, the more protection a sunscreen offers against UV light. The calculated SPF values of prepared herbal sunscreen were found to be 15.17 (cream-H_2O), 17.91 (cream-EtOH), and 34.48 for commercial sunscreen. The prepared cream with ethanolic extract offered more sun protection than the aqueous extract compared with the commercial sunscreen, which may be attributed to the presence of a high flavonoid content. The SPF values of each prepared creams were calculated by applying the Mansur equation, e.g., for the prepared herbal sunscreen cream-EtOH, as given in Tables 3, 4, and Figure 2.

Table 3. Calculation of the Sun Protection Factor Value of the Prepared Herbal Sunscreen Cream-EtOH of *M. gagei*

Sample	Wavelength	Absorbance	EE x I	Abs x EE x I
cream-EtOH of *M. gagei*	290	2.98±0.16	0.0150	0.0447
	295	2.35±0.08	0.0817	0.1919
	300	2.01±0.09	0.2874	0.5777
	305	1.80±0.09	0.3278	0.5900
	310	1.46±0.10	0.1864	0.2721
	315	1.18±0.05	0.0839	0.0990
	320	0.83±0.09	0.0180	0.0149
				1.791±0.65

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda)$$

$$= 10 \times 1.791$$

$$= \mathbf{17.91}$$

Table 4. The Sun Protection Factor Values of the Prepared Herbal Sunscreen Creams

No.	Prepared Sample	SPF	UV protection rate (%)
1	cream-H_2O	15.17	93
2	cream-EtOH	17.91	94
3	commercial Sunscreen	34.48	97

*Sunblock 27 from Nature Republic (Korea)
SPF(Reference): 2-12 = minimum sun protective activity, 12-30 = moderate sun protective activity
≥ 30 = high sun protective activity, ≥ 50 = very high sun protective activity (Ratnasooriya *et al*., 2014)

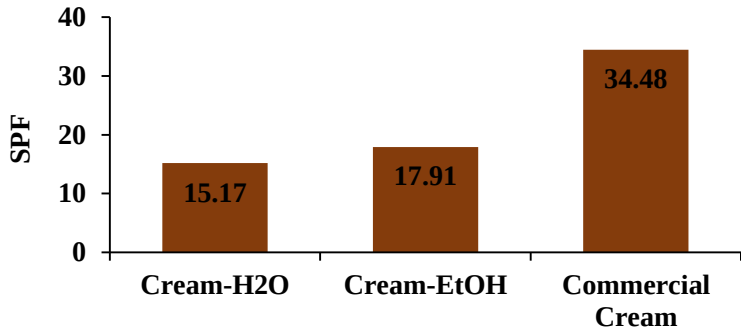

Figure 2. Comparison of SPF values of the prepared herbal sunscreen creams of *M. gagei*

Screening of Antibacterial Activity of Prepared Herbal Sunscreen Creams

In this present work, the antibacterial activity of prepared herbal sunscreen creams **(**H_2O, EtOH) obtained from the stem of *M. gagei* was investigated on *S. aureus* by the agar- well diffusion method. The measurable zone diameter, including the well diameter, shows the degree of antibacterial activity. The well diameter is 8 mm in this experiment. The larger the zone diameter, the more activity there is in the test organisms. The results of antibacterial activity were shown in Table 5 and Figure 3. It was found to have moderate antibacterial activity in both prepared creams (inhibition zone diameter = 11~14 mm).

Table 5: Results of Inhibition Zone Diameter of Prepared Herbal Sunscreen Creams

Microorganism	Inhibition Zone Diameter (mm)			
	70 % EtOH	Kalamet Cream-H_2O	Kalamet Cream-EtOH	Chloramphenicol
Staphylococcus aureus	0	11	14	22

Well size = 8 mm, 10-12 mm = weak activity, 13-18 mm = high activity, >18 mm = very high activity

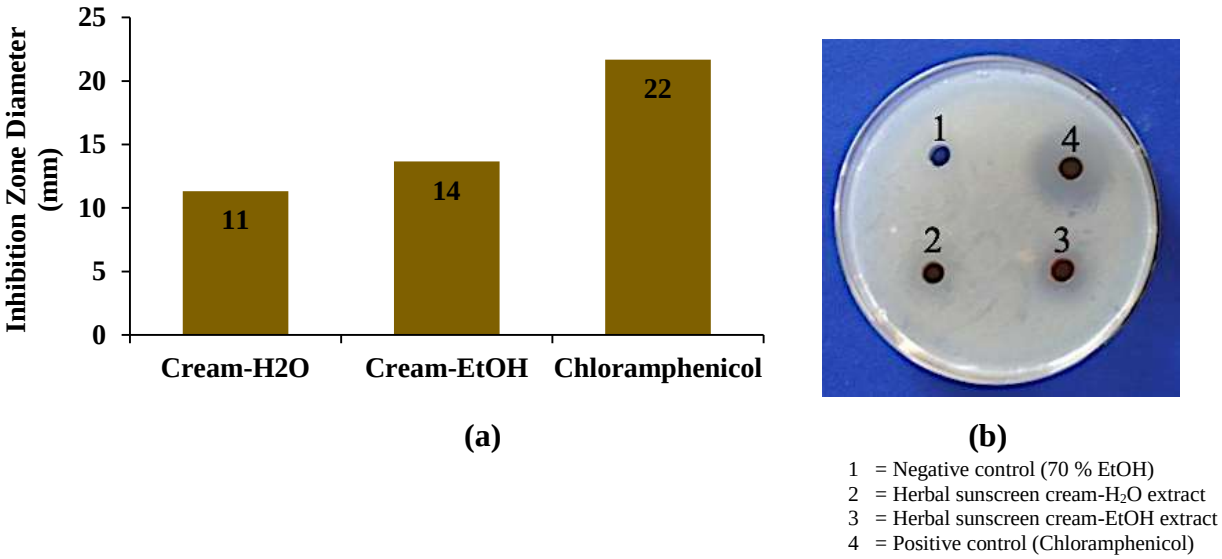

Figure 3. (a) A bar graph of antibacterial activity and (b) culture plates showing zone of inhibition of prepared herbal sunscreen creams against *S. aureus*

Conclusion

In this study, the investigation of phytochemical constituents, preparation of herbal sunscreen creams, physicochemical parameters, skin irritancy test, SPF values, and antibacterial activity from the stem of *M. gagei* (Kalamet) were reported. The results of preliminary phytochemical screening of different crude extracts revealed the presence of α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, and saponins. However, alkaloids, reducing sugars, starch, steroids, and tannins were found to be absent. The results of various tests for the evaluation parameters of herbal sunscreen creams using water and ethanol extracts indicated that they exist in yellowish-brown and pale brown colors with a pleasant odor and smooth appearance with homogeneity and good consistency. They were easily washable with a spread ability time of 5 sec, the viscosity in both creams were >5000 cP, and the pH values were found to be within a permissible range. The skin irritation results for all volunteers in this study showed that they do not irritate the skin. According to mild SPF results, they can be used on normal skin to prevent sunburns. It will also help in broadening the UV protection ability of conventional sunscreen formulations. From this results of antibacterial activity test, it was found that the moderate antimicrobial activity with inhibition zone diameters between 11~14 mm against *S. aureus*. Therefore, the selected sample may be useful as a related disease to alleviate itches. It can be concluded that the present research might hopefully bring advancements in the treatment of sunburns caused by exposure to UV rays. Further, the study reveals that UV spectroscopy is the most rapid, acceptable, and reproducible method for evaluating the efficacy of herbal sunscreens.

Acknowledgements

The authors would like to express their profound gratitude to the Department of Higher Education, Ministry of Education, Myanmar for providing of the opportunity to do this research, Thanks are also extended to Dr Khin Hnin Yee (Pro-Rector), and Dr Cho Kyi Than (Pro-Rector), Sagaing University of Education for their kind encouragement and nice suggestions for this research paper.

References

DeFilippss, R. A. and G. A. Krupnick. (2018). "The Medicinal Plants of Myanmar". *Phytokeys*, vol. 102, pp. 1-341.

Donglikar, M. M. and S. L. Deore. (2017). "Development and Evaluation of Herbal Sunscreen". *Pharmacgn*, vol. 9, pp. 83-97.

Hanrahan, J. R. (2012). "Sunscreens". *Australian Prescriber*, vol. 35(5), pp. 148-151.

Harborne, J. B. (1984). *Phytochemical Methods, A Guidde to Modern Techniques of Plant Analysis*. New York: 2nd Ed., Chapman and Hall, pp. 120-126.

Hiremath, S. S. P., F. S. Dasankoppa, A. Nadaf, V. G. Jamakandi, J. S. Mulla, S. A. Sreenivas, H. N. Sholapur, A. Ahmed and N. G. Nanjunda Swamy. (2008). "Formulation and Evaluation of a Novel in Situ Gum Based Ophthalmic Drug Delivery Syste of Linezolid". *Sci Pharm*., vol. 76, pp. 515-532.

Kress, W. J., R. A. Defilipps, E. Farr and Yin Yin Kyi. (2003). *A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar*. Department of Systematic Biology-Botany National Museum of Natural History, Washington DC.

Kubitzki, K. and C. Bayer. (2013). "The Families and Genera of Vascular Plants". *Springer-Verlag Berlin Heidelberg*, pp. 130-135.

Li, T., D. Zhang, Thaung Naing Oo, Myint Myint San, Aye Mya Mon, Pyae Phyo Hein, Y. Wang, C. Lu and X. Yang. (2018). "Investigation of Traditional Myanmar Medicinal Plants". *Evidence-Based Complementary and Alternative Medicine*, vol. 2018, pp. 1-13.

M-Tin-Wa. (1972). "Phytochemical Screening Methods and Procedures". *Phytochemical Bulletin of Botanica Society of America*, vol. 5(3), pp. 4-10.

Mamillapalli, V., P. L. Khantamneni, S. Koleti, K. Ghanta, H. M. Yakkali, K. M. Puli, and G. Kolusu. (2018). "Phytochemical and in Vitro Sun Protection Factor Protection Factor Evaluation of Peltophorum Leaf Extracts". *Int. J. Pharm. Sci. Rev. Res*., vol. 48(2), pp. 49-58.

Mansur, J. S., M. N. R. Breder, M. C. A. Mansur and R. D. Azulay. (1986). "Determinação Do Fator De Proteção Solar Por Espectrofotometria". *An. Bras. Dermatol*., vol. 61, pp. 121-124.

Marini-Bettolo, G. B., M. Nicolettic, and M. Patamia. (1981). "Plant Screening by Chemical and Chromatographic Procedure Under Field Conditions". *J. Choursomato*., vol. 45, pp. 121-123.

Nishina, A., A. Miura, M. Goto, K. Terakado, D. Sato, H. Kimura, Y. Hirai, H. Sato and Nyunt Phay. (2018)."Mansonone E from *Mansonia Gagei* Inhibited α-MSH-Induced Melanogenesis in B16 Cells by Inhibiting CREB Expression and Phosphorylation in the PI3K/Akt Pathway". *Biol Pharm Bull*, vol. 41, pp. 770-776.

Pandey, A., M. K. Yadav and P. Yadav. (2021). "Formation of Cosmetic from Medicinal Plants". *International Journal of Pharmaceutical Research and Applications*, vol. 6(5), pp. 842-847.

Ratnasooriya, W. D., J. R. A. C. Jayakody, S. R. D. Rosa and C. D.T. Ratnasooriya. (2014). "In *Vitro* Sun Screening Activity of Sri Lankan Orthodox Black Tea (Camellia sinensis linn)". *World Journal of Pharmaceutical Sciences*, vol. 1, pp. 144-148.

Sayre, R. M., P. P. Agin, G. J., Le Vee and E. Marlowe. (1979) "A Comparison of in *Vivo* and in *Vitro* Testing of Sunscreen Formulas". *Photochem. Photobiol*., vol. 29(3), pp. 559-566.

Schlegel, H. G. and C. Zaborosch. (1993). *General Microbiology*. Cambridge: 7th Edition University Press.