

Sun Protection Formulation Development from *Pyrrosia heterophylla* and *Pyrrosia lanceolata* LeavesS.M.A.K. Siriwardhana¹, G.A.J. Rathnasekara¹ and S.L.A. Gunawardana^{2*}¹Department of Pharmacy and Pharmaceutical Sciences, Faculty of Health Sciences, CINEC Campus, Malabe, Sri Lanka²Department of Pharmacy, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka*Corresponding author: gunawardana.shehara@gmail.com ORCID ID: <https://orcid.org/0000-0001-7415-8992>

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Abstract Excessive exposure to ultraviolet (UV) radiation can lead to detrimental effects on human skin, such as sunburn, premature aging, and an increased risk of skin cancer. Utilizing sun protection products is essential to mitigate these harmful effects. However, many of the sun protection products on the market contain synthetic chemicals, which may pose health risks with prolonged use. *Pyrrosia heterophylla* and *Pyrrosia lanceolata* are two ferns known for their traditional medicinal uses. It is used as a natural alternative for effective sun protection formulations in traditional medicine. Therefore, this study investigates the sunscreen activity of extracts from *P. heterophylla* and *P. lanceolata*, aiming to develop a natural and effective sun protection formulation. Plant leaves were collected and sequentially extracted into hexane, ethyl acetate and ethanol, and plant extracts with highest yield were used to prepare a concentration series of 156.25 to 5000 µg/mL. The SPF value of each extract was calculated, and toxicity was determined using brine shrimp lethality assay. The extract with the highest activity and the lowest toxicity was used for the development of herbal sunscreen. The cream was evaluated for its stability, pH, viscosity and spreadability. According to the results, ethanol extracts of both plants exhibit significant sun protection factor (SPF) values, with *P. heterophylla* showing the highest SPF of 41.99 ± 2.00 at 5000 µg/mL concentration, while *P. lanceolata* demonstrated an SPF value of 40.55 ± 2.00 at the same concentration. The lowest toxicity was observed for *P. heterophylla* with a LC_{50} value of 2677 µg/mL in brine shrimp assay which was chosen for sunscreen development. Stable cream formulations created, achieving SPF values exceeding 15, with a maximum SPF of 30.90 ± 0.2 at a concentration of 5000 µg/mL. These formulations maintain stable pH levels and exhibit favorable spreadability and viscosity. The results underscore the potential of herbal extracts from *P. heterophylla* and *P. lanceolata* in natural sunscreen applications, providing significant sun protection without adverse effects. This research contributes to the advancement of environmentally friendly skincare alternatives, highlighting the need for continued exploration and innovation in natural sun protection solutions.

Keywords: leaves, *Pyrrosia*, SPF, Sun protection, Traditional medicine, UV**Introduction**

Solar radiation, particularly ultraviolet (UV) radiation, is a major environmental stressor for the human skin. It can cause sunburn, premature aging, and skin cancer. UV radiation is classified into three types based on wavelength: UV_A (400-315 nm), UV_B (315-280 nm), and UV_C (280-100 nm) (Battistin et al., 2020). Among them UVB radiation is highly mutagenic and carcinogenic. To minimize the effects of UV radiation, the human body employs several mechanisms, such as DNA checkpointing and repair processes and skin pigmentation. These mechanisms help to identify and repair DNA damage that could lead to skin cancer, including melanoma. Skin pigmentation is

determined by the amount and type of melanin, which is responsible for the skin color (Lin & Fisher, 2007). The UV induced skin damage can be minimized by scavenging activity of melanin against Reactive Oxygen Species (ROS). However, these mechanisms are not always sufficient to prevent damage, thus the use of photoprotection, such as sunscreen, is necessary to prevent harmful effects on the skin caused by the sun (Sander et al., 2020).

Sun Protection Factor (SPF) measures the effectiveness of sun screening products in protecting the skin. According to the skin cancer foundation the individuals with intensive outdoor exposure are recommended to use sunscreen



products with SPF value 30 or higher. The higher the SPF value, the greater the protection. SPF values range from 2 to 50 + are classified as low (2-11), medium (12-29), high (30-50), and very high (50+). SPF of 15 prevents about 93% of UVB rays, while an SPF of 30 prevents about 97% of UVB rays (Wolf et al., 2001). However, the side effects such as skin irritations, burning sensations, development of acne associated with conventional sunscreen products and their ingredients underscore the importance of considering alternative options for sun protection (Chavda et al., 2023). Thus, increasing demand has been growing towards the natural sun screening agents. Natural herbs tend to be gentler on the skin, reducing the likelihood of irritation and allergic responses (da Silva Fernandes et al., 2015; Kulkarni et al., 2014). The phytochemicals present in plants such as flavonoids, have shown to possess photoprotective and antioxidant properties that can protect against UV radiation. Moreover, some (Kulkarni et al., 2014).

Pyrrosia belongs to genus fern and family *Polypodiaceae* and the class *Polypodiopsida*. It consists of 10 subgroups, informally divided based on species characteristics. *Pyrrosia* is characterized by a short creeping rhizome, leaf-bearing *phyllopodia* with lateral branch buds, and leaf-bearing vertical branches with arrested apical growth (Hovenkamp, 1986). In Sri Lanka, *Pyrrosia* species such as *Pyrrosia heterophylla*, *Pyrrosia lanceolata*, *Pyrrosia ceylanica*, *Pyrrosia porosa*, *Pyrrosia pannosa*, and *Pyrrosia gardneri* are used in traditional medicine. Different plant parts are used to prepare oil and the leaves are used to stop capillary hemorrhages and eczema (Ranil et al., 2006). The *Pyrrosia* plant is known for its diverse range of bioactive compounds, including

triterpenoids, flavonoids, xanthenes, essential oils, and steroids. These compounds have been found to use for have multiple therapeutic properties, including sun protection, antitussive, antibacterial, antiviral, anti-inflammatory, diuretic, antioxidant, immune-boosting and kidney protection (Chen LiJun et al., 2011). Therefore, this study mainly focused on evaluating the sun protective action of the plant leaves of *Pyrrosia heterophylla* and *Pyrrosia lanceolata* and development of a herbal sunscreen formulation.

Methodology

Sample collection and authentication

Plant samples of *P. heterophylla* (L.) M.G. Price was collected from Balangoda in Rathnapura district and *P. lanceolata* (L.) Farw. was collected from Warakapola in Kegalle district. The plant samples were authenticated at the National Botanic Garden, National Herbarium, Peradeniya, and voucher specimens were deposited (Tag no, ARA 001, ARA 002).

Extraction method (Sequential extraction)

Fresh plant leaves were collected, cleaned and dried in the oven at 40 °C. The dried powder samples of *P. heterophylla* and *P. lanceolata* were sequentially extracted following the order of hexane, ethyl acetate and ethanol. The filtrate was rotary evaporated to obtain the crude dried extracts. The weight of the resulting extract was measured, and the percentage yield of the extract was determined using the following formula:

$$\text{Yield Percentage} = \frac{(\text{Weight of the extract})}{(\text{Weight of the dry plant powder})} \times 100\%$$

Further analysis was conducted on the plant extract with the highest yield percentage, based on the results obtained.

Measurement of ultraviolet (UV) radiation absorbance in plant extract.

A double dilution series of the plant extracts ranging from 5000 µg/mL to 156.25 µg/mL was

prepared using 70% ethanol. The UV absorbance of plant extracts was measured at 5 nm intervals between wavelengths of 290-320 nm using a UV spectrophotometer. The experiment was performed in triplicate and 70% ethanol solution was used as the blank. The results were recorded, and SPF values were calculated using Mansur's equation (Mansur et al., 1986).

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

Where:

CF = Correction Factor

EE = Erythral effect spectrum

I = Solar intensity spectrum

Abs = Absorbance of sample

Brine shrimp lethality assay

The plant extracts which exhibited higher sun protection activity were tested for toxicity using Brine Shrimp lethality assay. A tank containing

artificial sea water and brine shrimp eggs was prepared and left to aerate for 24 hours under an illuminated light. After 24 hours, the hatched nauplii were collected and 10 of them were transferred to each test tube containing 5 mL of various concentrations (5000, 2500, 1250, 625, 312.5, 156.25 µg/mL) of plant extracts. After 24 hours the number of surviving nauplii was counted and the percentage of nauplii mortality was determined for each concentration after 24 hours. The lethality percentage of the nauplii was then calculated using the equation provided (Richardson & Hamilton, 1990).

$$\text{Percentage motility \%} = \frac{\text{Number of dead nauplii}}{\text{Total Number of nauplii in the control}} \times 100\%$$

Selection of the dose of the plant extract

The dose of the plant extract in the sunscreen formulation was determined using the SPF values of the plant extracts and the LC₅₀ values obtained from toxicology assays.

Development of the primary formulation

Several cold cream base formulations were developed mixing different ratios of Tween 80, water, and castor oil (Table 1). Initially, the required amounts of Tween 80 and castor oil were mixed according to the predetermined ratios and water was added dropwise. Subsequently, the mixture was heated to a specified temperature to facilitate thorough mixing of all components. After achieving homogeneity, the mixture was allowed to cool to room temperature while continuously stirring to ensure uniformity. Finally, the cooled formulation was transferred into suitable containers for storage and stability testing. Over a 15-day period, observations were meticulously recorded on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th, and 15th days to assess the stability of these formulations. Based on the results obtained, the most stable base formulations were selected for further development (Gunawardana et al., 2025).

Table 1. Test samples and their corresponding chemical weights used for primary formulation

Test No.	Tween 80 (g)	Water (g)	Castor oil (g)
01	30	30	40
02	30	40	30
03	40	30	30
04	20	30	50
05	20	20	60
06	25	35	40
07	20	50	30

Development of the secondary formulation

The stable primary base formulation selected was further enhanced by incorporating appropriate amounts of emulsifying agent 01, 02 and 03 and adding Tween 20 instead of Tween 80 (Table 2). The oil phase was prepared by blending castor oil, selected emulsifying agents (01, 02 or 03) and Tween 20 in a porcelain dish. This mixture was then heated in a water bath at 70 °C until all ingredients were completely melted and homogenized. Meanwhile, the aqueous phase was prepared separately by suspending the plant extract in distilled water and heating it to 70 °C. Gradually, the water phase was added drop wise to the oil phase while maintaining continuous stirring at 600 rpm. Subsequently, the mixture was allowed to cool

to room temperature with continuous stirring. Finally, just before transferring the finished product to a clean, dry ointment jar, fragrance and

preservative were added, and the resulting mixture was thoroughly stirred.

Table 2. Test samples and the weights of excipients used for secondary formulation

Sample No	Water (g)	Castor Oil (g)	Tween 20 (g)	Emulsifying agent 01 (g)	Emulsifying agent 02 (g)	Emulsifying agent 03 (g)
1	6	10	1	2.857	—	—
2	6.5	10.5	0.5	1.5	—	1.5
3	7.5	9.5	0.5	1.5	—	1
4	5	9	1	—	2	1.5
5	6.842	8.421	0.526	—	1.052	2.105
6	6.335	10.559	0.621	1.863	—	1.863
7	6.219	9.146	0.609	1.219	—	1.829
8	6.219	9.146	0.609	1.219	—	0.609
9	6.41	9.43	0.628	1.886	—	0.628
10	11.57	4.21	1.05	—	5.0	3.15

The formulations were stored at room temperature and observed for stability over 30 days, after which the most stable formulation was selected. This stable formulation was then infused separately with both plant extractions (*P. heterophylla* and *P. lanceolata*), as well as with a combination of both plant extractions.

SPF test for cream formulation

One gram (1 g) of the cream formulation was weighed and dissolved in ethanol (99%) in a 100 mL volumetric flask and mixed well until all the creams were dissolved. The solution was then filtered through Whatman No.1 filter paper, and the filtrate was collected after rejecting the first few milliliters. An aliquot of 5 mL of filtrate was placed in a 50 mL volumetric flask and filled with ethanol (99%) to the mark. The resulting solution was filtered, and the same steps were repeated. Then, the diluted solution was taken into a 25 mL volumetric flask and topped up with ethanol. The SPF value was measured according to the method mentioned above in 2.3.

Selection of the stable formulation

Centrifugation Stability Test

Cream formulations were subjected to centrifugation at 5000 rpm for 5 minutes to evaluate

stability, particularly for phase separation (Franco-Gil et al., 2024).

Short-term Stability Test

Cream formulations were visually examined over 14 days at three distinct temperatures (8 ± 2 °C, 28 ± 5 °C, and 40 ± 2 °C) to detect potential instability issues. Various physical alterations including creaming, sedimentation, flocculation, coalescence, and phase separation were monitored (Franco-Gil et al., 2024).

Long-term Stability Test

Cream formulations underwent visual scrutiny at three different temperatures (8 ± 2 °C, 28 ± 5 °C, and 40 ± 2 °C) for 90 days to assess stability under diverse thermal conditions. Physical changes such as creaming, sedimentation, flocculation, coalescence, and phase separation were observed (Franco-Gil et al., 2024).

Determination of pH

The pH of stable medicated cream formulations was determined by dissolving 1 g in 50 mL of distilled water. pH measurements were conducted thrice at room temperature (28 ± 5 °C) using a digital pH meter, with the average reading recorded. This

method assessed the acidity or basicity of the cream formulations (Franco-Gil et al., 2024).

Determination of Spreadability

A 0.5 g portion of the cream was spread on a pre-marked 2 cm diameter circle on a glass plate by placing a second glass plate, along with a 0.5 g weight on the top of the cream placed on the circle for 5 minutes. The diameter of the spread circle was measured, and the test was repeated thrice at 37.2 °C, with average values calculated (Senarathne et al., 2025).

Spreadability of the cream was determined using the equation:

$$S = m \times L/t$$

Where:

S – Spreadability

m – Weight applied on upper glass slide

L – Diameter

t – Time

Determination of Viscosity

Viscosity of prepared stable medicinal cream formulation was measured using a Brookfield viscometer-DVE at 5 rpm with spindle number 28.

Results

Percentage yield of the extracts

According to the results the highest yield was given by the ethanol extracts while the hexane and methanol showed lower yield percentages (Table 3). Therefore, the ethanol extract with the highest yield percentage was chosen for further investigations.

Table 3. Yield values of plant extracts of *P. heterophylla* and *P. lanceolata*

Solvent	<i>P. heterophylla</i>	<i>P. lanceolata</i>
Hexane	9%	5%
Ethyl acetate	4%	7%
Ethanol	37%	67%

SPF values of *P. heterophylla* and *P. lanceolata* extracts

Among the ethanolic extracts the extract of *P. heterophylla* exhibited the highest average SPF value of 41.99 ± 2.00 at the concentration of 5000 µg/mL. Notably, even at a low concentration of 312.5 µg/mL, the plant extracts demonstrated a SPF value exceeding 15, thereby indicating its substantial sun protecting activity. Moreover, *P. lanceolata* extracts with a concentration of 2500 µg/mL or higher showed a SPF value greater than 15 while the highest SPF value of 40.55 ± 2.00 was obtained at the concentration of 5000 µg/mL (Table 4).

Table 4. SPF test results of *P. heterophylla* and *P. lanceolata*

Concentration	SPF value of <i>P. heterophylla</i>	SPF value of <i>P. lanceolata</i>
5000 µg/mL	41.99 ± 2.00	40.55 ± 2.00
2500 µg/mL	40.07 ± 2.00	25.58 ± 2.00
1250 µg/mL	38.87 ± 2.00	13.77 ± 2.00
625 µg/mL	31.23 ± 2.00	6.57 ± 1.00
312.50 µg/mL	15.38 ± 2.00	3.38 ± 0.20
156.25 µg/mL	7.45 ± 2.00	1.25 ± 0.20

Toxicity test results (Brine shrimp assay)

The toxicity of the samples was tested at different concentrations, ranging from 156.25 to 5000 µg/mL. According to the results, the LC₅₀ value for *P. heterophylla* and *P. lanceolata* extracts was 2677 µg/mL and 2463 µg/mL respectively (Table 5).

Table 5. Toxicity and LC₅₀ value of the plant extracts

<i>P. heterophylla</i>			<i>P. lenceolata</i>	
Concentration	Percentage Mortality	LC ₅₀	Percentage Mortality	LC ₅₀
156.25 µg/ml	0	2677 µg/mL	0	2463 µg/mL
312.5 µg/ml	0		0	
625 µg/ml	0		0	
125 µg/ml	10		0	
2500 µg/ml	40		10	
5000 µg/ml	90		20	

Development of the Topical Sunscreen formulation

Depending on the results of the toxicity assay and SPF values *P. heterophylla* was selected as the least toxic and highest active extract. Three formulations were developed incorporating the doses 2500, 1250 and 625 µg/mL into the cream base separately.

*Formulation development**Stability evaluation of the primary emulsion*

According to the results of the stability test of the primary formulation sample 4 which contained Tween 80: Water: Castor oil (20: 30: 50) was found to be stable after 15 days. Therefore, sample number 4 was used for the development of the secondary formulation.

Stability evaluation of the secondary emulsion

Samples were evaluated for short-term stability by storing them at three different temperatures: 8 ± 2 °C, 28 ± 5 °C, and 40 ± 2 °C for 14 days. Among the samples tested samples 2,5,6,9 and 10 were found to be stable while the phase separation was observed in the other formulations.

SPF test results for cream formulation

Based on Table 6, the negative control displayed an SPF value of 2.95 ± 0.2 . Conversely, the inclusion of the extract in the cream resulted in SPF values surpassing 15, reaching a peak of 30.90 ± 0.2 at a concentration of 5000 µg/mL. The lowest SPF

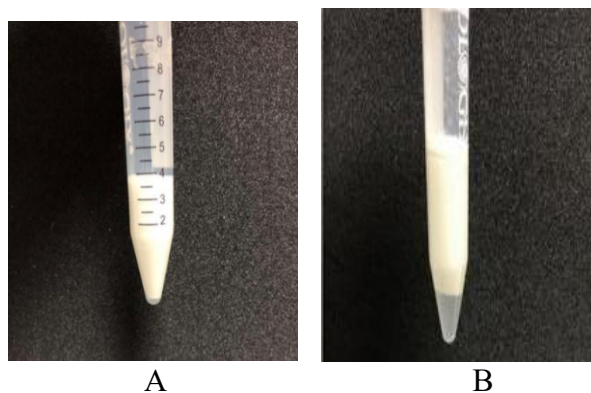
value recorded was 23.60 ± 1.0 , observed at a concentration of 625 µg/mL.

Table 6. SPF value of the cream formulations containing different concentrations of the plant extracts

Concentration of plant extract	SPF Value
Without extract	2.95 ± 0.2
5000 µg/mL	30.90 ± 0.2
2500 µg/mL	28.59 ± 0.2
1250 µg/mL	26.40 ± 1.0
625 µg/mL	23.60 ± 1.0

Stability tests results

Only Sample 10, which incorporates 11.57g of water, 4.21g of castor oil, 1.05g of Tween 20, 5.0g of emulsifying agent 02, and 3.15g of emulsifying agent 03 was found to be stable, while phase separation was observed in the other samples (Figure 1).

**Figure 1:** (A) Stable formulation without phase separation (B) formulations which exhibited phase separation

pH test results

According to the results obtained from Table 02, formulation 10 yielded pH values ranging from 4.5 to 6 across all storage times (1 day, 3 days, 7 days, 14 days, 21 days, and 28 days). This falls within the typical range observed for numerous skincare products.

Spreadability test results

The formulation showed a mean diameter of 5.6 cm withing 300 s under a weight of 500 g. while the spreadability was determined as 9.334 cm².

Viscosity evaluation

The viscosity of the formulation was determined as 36150 cP (Table 7).

Table 7. Viscosity of the developed formulation

Description	Value
Viscosity, cP	36150
Torque, %	71.1
Spindle no	28
Temperature °C	29
Speed, rpm	10

Discussion

The pursuit of natural and effective sun protection solutions has gained considerable attraction in recent years, prompted by growing awareness of the potential risks associated with conventional sunscreen ingredients. This study explores the efficacy of herbal extracts derived from *P. heterophylla* and *P. lanceolata* as potential candidates for natural sunscreen formulations. The findings highlight not only their significant sun protective properties but also their potential to address the limitations inherent in traditional sunscreens.

The substantial SPF values obtained from the extracts of *P. heterophylla* and *P. lanceolata* indicate their efficacy in attenuating UV radiation, surpassing the recommended threshold for effective sun protection (Bernard et al., 2014). This suggests that these herbal extracts possess inherent photoprotective properties comparable to or even surpassing those of synthetic counterparts. Such

efficacy is crucial in ensuring adequate protection against harmful UV radiation, thereby mitigating the risk of sunburn, premature aging, and skin cancer (D'Orazio et al., 2013).

Additionally, formulating stable creams incorporating these herbal extracts enhances their practical utility in skincare formulations. Stability is paramount in skincare products to ensure consistent efficacy and safety over time (Sirsat et al., 2022). The reference standards Sri Lanka Standards 743:2014 (SLS 743:2014) provides the specifications for skin creams and lotions. The result of the present study indicates that pH of the skin cream was within the specified range 5.5 – 8.0, spreadability and viscosity were also appropriate for a skin cream (Gunawardana et al., 2025). The stability test confirmed that the formulation is suitable for long term storage with no phase separation, change in color or odour. A study done by Donglikar & Deore, 2017 have shown that sunscreen formulations developed using the quercetin, curcumin and resveratrol have shown a pH range of 6-9 and viscosity in the range of 28000-32000 cp. These characteristics are comparable to the findings of the present study, suggesting that the current formulation possesses similar stability and consistency profiles, thereby reinforcing the suitability of using natural bioactive compounds in the development of effective and cosmetically acceptable sunscreen products.

Furthermore, the absence of adverse effects observed in toxicity assays presents a significant advantage of herbal sunscreens. Unlike conventional sunscreens, which may contain potentially harmful chemicals, herbal extracts offer a natural and safer alternative. Recent studies have shown that synthetic sunscreen agents such as oxybenzone, octinoxate, amino benzoic acid have been associated with adverse effects including skin irritation, allergic reactions, endocrine disruption etc. In contrast, herbal sunscreens tend to be non-toxic, non-irritant, and are generally better tolerated by the skin. This makes them especially appealing for individuals with sensitive skin or those seeking long-term sun protection with fewer side effects. Importantly, the shift towards herbal alternatives also addresses growing environmental concerns, as many synthetic agents have been found to negatively impact aquatic ecosystems, particularly coral reefs. Therefore, herbal sunscreens not only offer effective protection but also align with the

increasing demand for safer and more environmentally sustainable skincare solutions (Mansuri et al., 2021).

However, despite the promising findings of this study, further research is warranted to fully exploit the potential of herbal sunscreen. Optimization of formulations, exploration of additional herbal extracts, and comprehensive safety assessments are necessary steps in advancing this field. Additionally, regulatory considerations and market acceptance will play pivotal roles in the widespread adoption of herbal sunscreens. The exploration of herbal extracts from *Pyrrosia heterophylla* and *Pyrrosia lanceolata* represents a significant step towards the development of natural and effective sun protection solutions. These extracts exhibit substantial sun protective properties, formulation compatibility, and safety, making them promising candidates for use in sunscreen products. By harnessing the power of nature, herbal sunscreens offer a compelling alternative to conventional sunscreens, meeting consumer demand for safer, more sustainable skincare options.

Conclusion

In conclusion, the utilization of herbal extracts from *Pyrrosia heterophylla* and *Pyrrosia lanceolata* holds promise for the development of natural sunscreen formulations. These extracts demonstrated significant sun protective properties and were successfully formulated into stable creams with SPF values exceeding 15. The absence of adverse effects in toxicity assays further supports their safety and suitability for use in skincare products. By harnessing the power of nature, herbal sunscreens offer consumers an effective and environmentally friendly alternative to conventional sunscreens. Continued research and innovation in this field are essential for advancing the development of natural sun protection solutions and promoting healthier skincare practices.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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