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Assessment of Phenolic Content and Photoprotective Potential of *Thespesia populnea, Cascabela thevetia,* and *Abutilon indicum* as Natural Ingredients for Herbal Sunscreen Development

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ABSTRACT

The present study aimed to develop herbal sunscreen formulations from flowers of *Thespesia populnea (L.) Sol. ex Corrêa* and *Cascabela thevetia* (L.) Lippold and leaves of *Abutilon indicum* (L.) Sweet. Total phenolic content was determined by the Folin-Ciocalteu method. The UV-filtering potency and the Sun Protection Factor (SPF) of the methanolic extracts of the above plants were subsequently determined. Thereafter, the extract was incorporated into the aqueous cream base at 25%, 50%, and 75% (w/w) and the SPF values of the prepared formulations were evaluated. The methanolic flower extract of *T. populnea* had the highest total phenolic content (244.814 mg Gallic Acid Equivalent (GAE)/g ± 0.005) and *A. indicum* had the intermediate total phenolic content (72.284 mg Gallic Acid Equivalent (GAE)/g ± 0.001); while the flower extract of *C. thevetia* had the lowest content (32.718 mg GAE/g ± 0.002). The initial SPF value of the crude flower extract of *T. populnea* (37.56 ± 0.33) showed minimal change after incorporation at 75% into the aqueous cream base. This 75% formulation surpassed the other two formulations and the commercial sunscreen (positive control) in terms of the higher SPF and broader spectrum of UV absorption. Its SPF value altered only slightly during the storage for 21 days in light conditions and was photostable. Preliminary observations demonstrated the appropriateness of *T. populnea* for the formulation of herbal sunscreens at the commercial stage.

Keywords: Herbal sunscreens, Photostability, Sun Protection Factor (SPF), UV radiation.

1. INTRODUCTION

The skin acts as a crucial organ within the integumentary system, working in conjunction with structures like hair, nails, and glands. Structurally, it is composed of two primary layers: the outermost epidermis and the underlying dermis[1]. Keratinocytes constitute the majority of cells within the epidermis. These cells store melanin, which functions as a 'natural sunscreen,' defending the skin from UV radiation[2]. A key contribution of ultraviolet radiation (UVR) to human health lies in its role in facilitating vitamin D production[3].

UV radiation can be subdivided into three types based on wavelength: UVC radiation (200–280 nm) is predominately filtered by the ozone layer in the stratosphere, UVB radiation (280–320 nm) is mainly absorbed by the epidermis, and UVA radiation has the longest wavelengths (320–400 nm) and penetrates deepest into the dermis, but it also interacts with the stratum corneum and epidermis[4]. UVA radiation damages skin cells and DNA, being responsible for photoaging and photocarcinogenesis. UVB radiation causes some changes, such as pigmentation and sunburn, as well as chronic changes, such as immune-suppression and photocarcinogenesis. Both UVA

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and UVB can cause sunburn, photo ageing, erythema and inflammation[5].

In order to prevent or reduce these harmful dermatological effects caused by UV radiation, various cosmeceutical formulations of chemicals that can absorb or reflect UV radiation have been introduced into the market[1]. These cosmeceutical formulations are known as sunscreens. Regular use of synthetic sunscreens has been reported to be associated with adverse skin effects, including irritation and allergic reactions[6]. Some organic filters degrade under UV light and form free radicals that may harm the skin, while para-aminobenzoic acid can trigger photoallergic and photosensitivity reactions. In addition, studies report that UV-activated inorganic filters like titanium dioxide can produce reactive oxygen species, potentially causing cytotoxic, genotoxic effects and even cell death in certain human cells[7].

The use of sunscreens is essential to prevent skin damage; therefore, several studies aimed at creating new safe and effective formulas by discovering new plant species[8]. Herbal sunscreens effectively protect the skin from the damaging effects of UV rays. Herbal-based sunscreens can help reduce the side effects associated with synthetic sunscreens[6,9].

The Sun Protection Factor (SPF) is the most widely accepted method for evaluating the photoprotective effectiveness of sunscreens, and it is considered the primary information on sunscreen labels. SPF exclusively measures protection against sunburns occur by UVB, so the protective effect, in terms of absorption or transmission percentages, relates solely to preventing the development of erythema[10,11]. The qualities of a good sunscreen are widely considered to include a high SPF rating, comprehensive protection against both UVA and UVB rays, and the ability to remain stable when exposed to light[8]. There is no single, universally accepted classification system for SPF values; however, some sources categorize sunscreen products as minimal (SPF < 15), moderate (SPF 15-29), or high (SPF ≥ 30)[12]. WHO recommends to apply a broad-spectrum sunscreen of SPF 15+ liberally and re-apply every two hours, or after working, swimming, playing or exercising outdoor. The goal of sunscreen formulation is to block UV rays and increase the protection from UV rays[13].

Located in the equatorial belt, Sri Lanka receives a high level of solar radiation year-round. This intense sun exposure makes its population highly susceptible to skin damage caused by UV rays[14]. For adequate sun protection, individuals in tropical or subtropical regions are advised to exclusively use broad-spectrum sunscreens with an SPF of 30 or higher[15]. The American Academy of Dermatology (AAD) has published guidelines recommending sunscreen products that have an SPF of 30 or higher and broadspectrum coverage[16].

Secondary metabolites, including flavonoids and phenols, are crucial for protecting plants from UV radiation. They act as a natural "sunscreen" by absorbing UV radiation and also serve as potent antioxidants to neutralize the free radicals generated by UV exposure[17]. The accumulation of these compounds in plant is considered a primary defence mechanism[18].

Medicinal plants contain a variety of therapeutic compounds that are effective in treating different illnesses in both humans and animals[17]. The present study is aimed to develop herbal sunscreens using three medicinal plants available in Sri Lanka; *Thespesia populnea (L.) Sol. ex Corrêa*, *Cascabela thevetia* (L.) Lippold and of *Abutilon indicum* (L.) Sweet.

Thespesia populnea (L.) Sol. ex Corrêa, previously known as *Hibiscus populnea*, is an evergreen tree belonging to the family Malvaceae. The various parts of the plant like roots, bark, leaves, flowers and fruits are known to possess different pharmacological properties[19,20]. The *T. populnea* plant has a long history of medicinal use, and modern studies have validated its effectiveness in treating a wide range of ailments. This includes its traditional use for skin infections, inflammation, and bruises, as well as its reported pharmacological activities, including potential anticancer, antimicrobial, anti-inflammatory, and antioxidant properties[20].

A. indicum is a member of the Malvaceae family, has a history of traditional medicinal

applications. Traditionally, its leaves have been used to treat ulcers and as a warm compress for pain relief. A decoction of the leaves is also employed to manage toothaches, tender gums, and inflammation of the bladder. The plant is further recognized for its various other properties, such as being an effective laxative, expectorant, diuretic, astringent, analgesic, anti-inflammatory, anthelmintic, demulcent, and aphrodisiac[21,22].

C. thevetia also known as yellow oleander, is a small tree from the Apocynaceae family that thrives in tropical and subtropical climates. Its native range includes Central and South America, and it is also commonly found in various Asian countries like India and Sri Lanka. This plant is recognizable by its green foliage and distinctive yellow or orange-yellow, trumpet-shaped flowers[23]. Historically and in the present day, *C. thevetia* is a valuable medicinal plant used in traditional and alternative medicine, as well as in the pharmaceutical industry[24,25]. Its traditional and experimental studies indicate medicinal relevance, including anticancer potential[24]. The present study aims to formulate and develop herbal sunscreen creams using aqueous methanolic extracts of *Thespesia populnea, Abutilon indicum*, and *Cascabela thevetia*.

2. MATERIALS AND METHODOLOGY

2.1 Plant Material and Preparation of extract

Flowers of *T. populnea* and leaves of *A. indicum* were collected from Ketewela (7.6148° N, 80.6516° E), Matale District - Central Province of Sri Lanka and Flowers of *C. thevetia* were collected from Nallur (9.6744° N, 80.0298° E), Jaffna District - Northern Province of Sri Lanka in 2024 (shown in Figure. 1). These plants were confirmed with the herbarium samples kept in the Department of Chemistry, Faculty of Science, University of Jaffna.

The plant materials were washed with running water to remove dust and then shade-dried at 30 °C for two weeks. Dried plant materials were cut into small pieces and they were powdered using a domestic grinder. Then the plant materials (15 g) were extracted in 200 mL of 70% methanol-water. The extract was evaporated into dryness with the use of a rotary evaporator.

2.2 Determination of the total phenolic content (TPC) of the crude extracts

Total phenolic content of each crude extracts was determined in triplicate by the Folin– Ciocalteu procedures according to the method adopted from Gangwar *et al*.[26] with some alterations. A volume of 0.5 mL of crude extract solution (1 mg/mL) in 70% methanol-water was mixed with 10% of Folin-Ciocalteu reagent (2.5 mL). The resulting solution was mixed and incubated for 5 minutes. Thereafter, 7.5% (W/V) of Na_2CO_3 (2.5 mL) was added and the reaction mixture was diluted to 10 mL by adding distilled water, followed by incubation at room temperature in the dark for 2 hours.

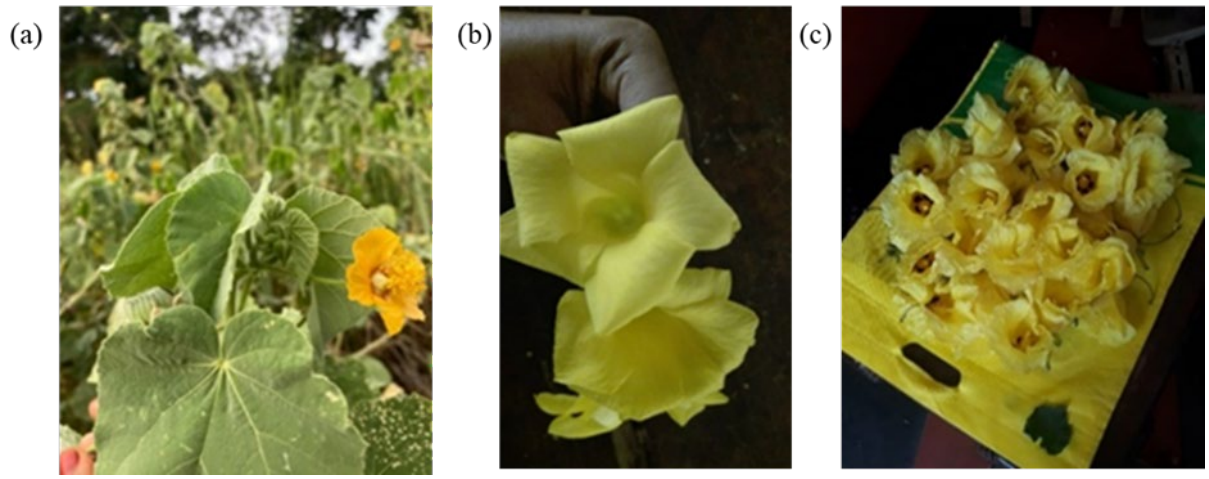

Figure 1. (a) Leaves of *Abutilon indicum* (b) Flowers of *Cascabela thevetia* (c) Flowers of *Thespesia populnea*

The absorbance was measured at 765 nm using a UV-visible spectrophotometer in the Department of Chemistry, Faculty of Science, University of Jaffna. TPC values were determined from a calibration curve prepared with a series of gallic acid standards (15.6, 31.3, 62.5, 125, 250 and 500 µg/mL). Distilled water used as the blank. Total phenolic content was expressed as µg Gallic acid equivalent/mg using the equation obtained from the calibration curve for Gallic acid (R^2 = 0.9924). Data were expressed as mean±SD (mean ± standard deviation) of three replicates.

2.3 Qualitative analysis for flavonoid of the crude extracts

The qualitative tests for the detection of flavonoid compounds were carried out for all the crude extracts (1 mg/mL) as per the standard methods[14,27].

2.3.1 Alkaline reagent test: All three plant crude extracts (1 mL) were taken in various test tubes and few drops of NaOH were mixed in all the test tubes respectively. The appearance of intense yellow color which turned to colorless upon the addition of diluted HCl was an indication of the presence of flavonoids.

2.3.2 Lead acetate test: Additionally, the crude extracts (1 mL) were treated with a few drops of lead acetate solution and the formation of a yellow-colored precipitate was an indication of the presence of flavonoids.

2.4 Evaluation of UV-filtering potential of the crude extract

Following the methodology of Napagoda *et al*.[28] the UV-filtering potential of the crude extracts was evaluated and the sun protection factor (SPF) was subsequently calculated *via* the Mansur equation[29]. In brief, the UV absorption of the extracts (at a concentration of 1 mg/mL) were measured between 260–400 nm at 5 nm intervals using a UV-visible spectrophotometer. The UV absorbance values between 290–320 nm were substituted in the Mansur equation to calculate the SPF. (The experiment was conducted triplicate). Table (1) suggests that the extract is capable of absorbing UV radiation more effectively.

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda)$$

where: EE(λ) – erythemal effect spectrum; I(λ) – solar intensity spectrum; Abs(λ) – absorbance of sunscreen product; CF – correction factor (=10), (The values of EE x I are constants)

2.5 Formulation of herbal sunscreens and evaluation of UV-filtering potential

The methanolic extracts (i.e. the residues obtained after solvent evaporation) of *T. populnea*, *A. indicum* and *C. thevetia* were separately mixed with an aqueous cream base (that comprised cetostearyl alcohol, white soft paraffin wax, and sodium lauryl sulphate) at concentrations 25%, 50%, 75% (w/w) to prepare sunscreen formulations. This process resulted in three distinct sunscreen formulations for each plant extract. The UV filtering capability of each formulation was determined as mentioned above. All the formulations were exposed to direct sunlight for 21 days and UV absorption was measured on 7th, 14th, and 21st day and subsequently, the SPF values were calculated. The aqueous cream base was employed as the negative control, while a commercially available sunscreen product containing *Aloe,* micronized ZnO and TiO_2 and benzophenone as active ingredients was used as the positive control.

Statistical analysis

All experiments were performed in triplicate, and the results were expressed as mean ± SD.

3. RESULTS AND DISCUSSION

3.1 Determination of the total phenolic content of the crude extract

The Folin-Ciocalteu reagent was used to estimate the total phenolic content of crude aqueous methanolic extract of *T. populnea*, *A. indicum* and *C. thevetia* expressed as Gallic Acid Equivalents (GAE). For the assay, 0.5 mL of each extract (1 mg/mL) was used. The Figure 2 explains the crude extracts UV absorption in the wave length range from 260 to 400 nm.

Table 1. Comparison of Total Phenolic Content

Plant extracts	TPC (µg/mL)	Mean value	TPC in mg GAE/g
Thespesia populnea	244.814	0.210	244.814±0.005
Abutilon indicum	72.284	0.568	72.284 ±0.001
Cascabela thevetia	32.718	0.127	32.718 ±0.002

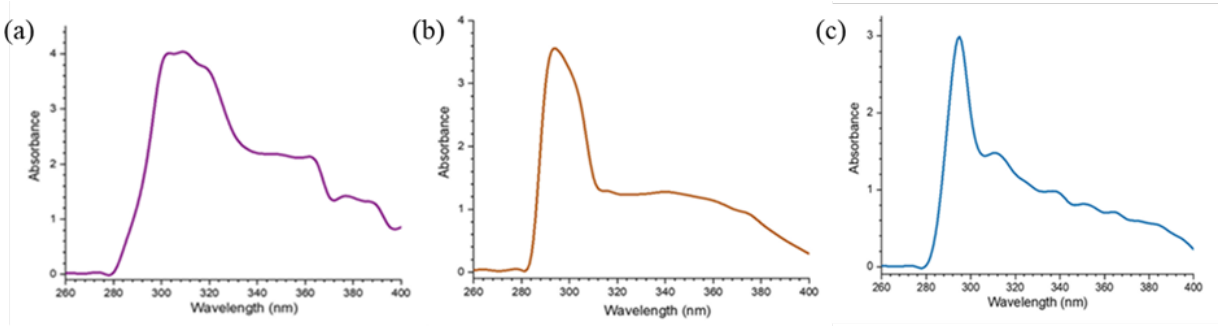

Figure 2. Absorption of UV radiation between 260–400 nm by crude extract of (a) *T. populnea*, (b) *A. indicum*, (c) *C. thevetia*

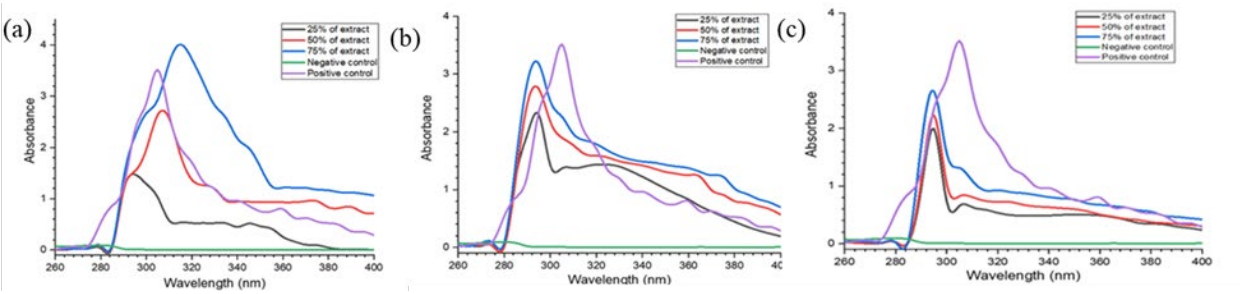

Figure 3. Absorption of UV radiation between 260–400 nm by sunscreen formulations of (a) *T. populnea*, (b) *A. indicum*, (c) *C. thevetia* in day 21

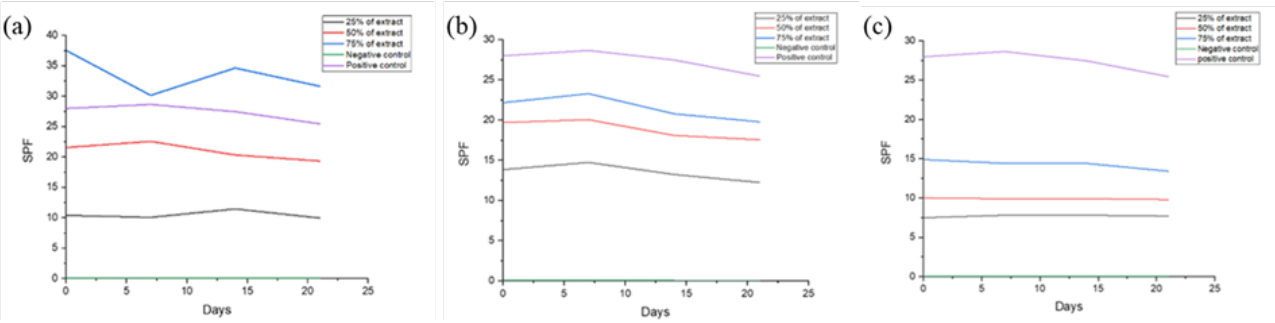

Figure 4. Variation of SPF at different time intervals in herbal sunscreen formulations developed from (a) *T. populnea* (b) *A. indicum* (c) *C. thevetia* (n=3).

The amount was calculated using the linear regression equation of the standard curve of gallic acid ($y = 0.00415x + 0.05951$, $R^2 = 0.9924$).

3.2 Evaluation of UV-filtering potential of the crude extract

In accordance with the findings of Napagoda *et al.*[14], the aqueous methanolic extract exhibited widespread UV absorption, particularly notable in the 280–340 nm range, encompassing both UV-B and UV-A regions. When comparing the SPF values of the three plant crude extracts, *T. populnea* (37.56 ± 0.33) showed the maximum protection against UV radiation. SPF values of *A. indicum* and *C. thevetia* were 25.29 ± 0.207, 17.43 ± 0.24 respectively.

3.3 Development of herbal sunscreens and evaluation of UV-filtering potential

Optimal sunscreen requires high SPF for strong UVB protection, broad-spectrum coverage against UVA and UVB rays, and photostability to maintain effectiveness under sunlight[3]. The colour intensity of sunscreen formulations varied depending on the percentage of the extract incorporated in the formulation.

The sunscreen formulations and a positive control were exposed to direct sunlight for 21 days. Figure 3 illustrates the UV absorbance pattern versus wavelength of sunscreen formulations on day 21. In general, all three formulations exhibited the highest UV absorbance in the UV-B range, similar to the original crude extract.

3.4 Variation of SPF values in the sunscreen formulations

We calculated the SPF values on days 7, 14, and 21 employing UV-absorbance data in Mansur equation. Figure 4 illustrates how the SPF values changed over these time intervals (n=3). The initial SPF values of the formulations consisting of 25%, 50% and 75% of *T. populnea* were observed as 10.39 ± 0.06, 21.55 ± 0.32 and 37.53 ± 0.21 respectively. Interestingly the SPF values of *A. indicum* and *C. thevetia* formulations hardly changed during the monitored time period. In the case of *A. indicum,* the initial SPF values were recorded as 13.82 ± 0.14, 19.68 ± 0.15 and 22.15 ± 0.21 for formulations consisting of 25%, 50% and 75% of crude extract respectively. In the case of *C. thevetia,* the initial SPF values were recorded as 7.48 ± 0.05, 9.99 ± 0.29 and 14.89 ± 0.18 for formulations consisting of 25%, 50% and 75% of crude extract respectively. The higher SPF value calculated from the absorbance data for the positive control was 27.98 ± 0.21 although it was mentioned as SPF of 30+[26].

The results showed that the aqueous methanolic extract of *T. populnea* contained a high content of phenols (244.814±0.005 mg GAE/g) suggesting that the extract is capable of absorbing UV radiation more effectively. 75% (w/w) *T. populena* sunscreen formulations demonstrated a higher capacity for UV absorption compared to the commercial sunscreen product (positive control). The cream base used for the formulations, which served as the negative control, showed negligible UV absorbance. This confirms that the significant UV absorption observed in the herbal formulations is due to the active ingredients and not the base itself. Of all the sample concentrations examined, 75% (w/w) *T. populena* demonstrates the highest level of absorbance.

Despite their benefits, botanical ingredients are rarely used at high concentrations in cosmetic formulations due to stability issues, irritation risk, and adverse effects on product sensory properties[30]. Our observations demonstrated that the formulation comprised of 75% (w/w) extract showed a specific level of protection against UV radiation: high for *T. populnea* due to its SPF value 37.53 ± 0.21, moderate for *A. indicum* due its SPF value of 22.15 ± 0.21, and minimal for *C. thevetia* due to its SPF value 14.89 ± 0.18. The analysis has shown that a sunscreen with a 75% (w/w) concentration of *T. populnea* could indeed serve as a highly protective alternative to commercially produced sunscreens.

4. CONCLUSION

This study successfully formulated and developed herbal sunscreen creams using aqueous methanolic extracts of *T. populnea, A. indicum,* and *C. thevetia* at varying concentrations. Results indicated that extracts at 75% (w/w) concentration of *T. populnea* and *A. indicum* were shown the most promise for sunscreen formulations. Likely due to their high phenolic content, which facilitates effective UV radiation absorption. Among the 75% (w/w) concentration formulations, *T. populnea* (37.53 ± 0.21) exhibited the highest Sun Protection Factor (SPF) and total phenolic content (244.814±0.0045), demonstrating strong and broad-spectrum UV-filtering capabilities. Furthermore, comparison of SPF values of the three plant crude extracts showed that *T. populnea* exhibited the highest protection against UV radiation. These findings suggest that the aqueous methanolic extract of *T. populnea* holds

significant potential for developing commercially valuable herbal skincare products.

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