## High Potential of Herbal Plant Extracts for Skin Protection from Ultraviolet Radiation

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**Abstract:** This research is dedicated to exploring the photoprotective qualities of extracts from 10 different herbal plants to identify natural ultraviolet (UV) filtering agents suitable for use in cosmetics. The study pinpointed specific plant parts that showed promising photoprotective capabilities, including the fruits of Gardenia jasminoides L., the flowers of Chrysanthemum morifolium Ramat., as well as the leaves of Camellia sinensis L. and Moringa oleifera L. Among these, G. jasminoides extracts stood out for their superior photoprotection. Specifically, G. jasminoides led with an impressive in vitro SPF of  $40.8 \pm 0.2$ , with G. morifolium, G. sinensis, and G0. oleifera trailing closely. Hence, the extracts were added to the base of the lotion cream to evaluate the stability and photoprotective activity. Additionally, even though natural extracts cannot completely replace conventional UV filters, they have substantially decreased the reliance on physical or chemical G1. Thus, this study provides a strong foundation for natural antioxidants' status and potential use for G2.

**Keywords:** natural photoprotectors; photoprotective activity; antioxidant properties; anti-inflammatory; herbal sunscreens

## **■ INTRODUCTION**

The relentless quest for effective skin protection against the detrimental effects of ultraviolet (UV) radiation has become a cornerstone in dermatological research. As the body's largest organ, the skin serves as the primary shield against environmental aggressors, with its epidermis and dermis layers playing critical roles in defense mechanisms. The epidermis, primarily composed of keratinocytes and melanocytes, acts as a barrier against UV radiation through the production and distribution of melanin. Melanin, with its 2 forms—eumelanin and pheomelanin—varies in its ability to protect against UV-induced DNA damage, highlighting the importance of melanogenesis in skin defense [1-2]. Despite the protective functions of these cellular components,

prolonged exposure to UV radiation can cause harmful effects such as photoaging, DNA damage, and an elevated risk of skin cancers, which underscores the need to develop effective strategies for UV protection.

In the field of photoprotection, numerous synthetic sunscreen agents are available, providing diverse levels of UV protection. However, concerns regarding their potential limitations at the cellular level, skin irritability, and environmental impact have driven the search for alternative natural photoprotective agents [3]. Botanical extracts, rich in polyphenols, flavonoids, and other compounds, have emerged as promising candidates due to their antioxidant properties and mechanisms that extend beyond simple UV filtration. These natural agents not only absorb and scatter harmful radiation but also engage in cellular protective

mechanisms, such as DNA repair and immune system modulation, providing a multifaceted approach to photoprotection [4].

Although natural extracts are acknowledged in skincare formulations, their complete potential remains largely unexplored, especially regarding indigenous plant species in various regions. This gap is particularly evident in Vietnamese flora, which boasts a rich biodiversity yet is underexplored in photoprotective research. This study seeks to address the gap in understanding plant extracts' properties photoprotective and antioxidant investigating those derived from select Vietnamese plants. Specifically, this research focuses on extracts from Camellia sinensis L. (tea) leaves, Moringa oleifera L. (moringa) leaves, Chrysanthemum morifolium Ramat. (chrysanthemum) flowers, and Gardenia jasminoides Ellis. (gardenia) fruits. Ethanol is used as the solvent for preparing plant extracts, enabling the extraction of bioactive compounds that may contribute to their potential antioxidant and photoprotective effects. These extracts were evaluated for their antioxidant capacity and ability to protect against UV-induced damage. They were incorporated into a base formulation to assess their effectiveness and durability as photoprotective agents, using the sun protection factor (SPF) index under various conditions.

#### EXPERIMENTAL SECTION

#### **Materials**

Ethanol and methanol were obtained from Chemsol (Vietnam). Sodium carbonate and dimethyl sulfoxide were purchased from Xilong (China). Folin-Ciocalteu reagent and gallic acid standard (99.5%) were obtained from Merck (Germany). DPPH free radical was supplied by Alfa Aesar (UK). Avobenzone, homosalate, quercetin, and ascorbic acid standards were procured from Sigma-Aldrich (USA). Plant materials were collected from Vietnam, i.e., *Cynara scolymus* L. leaves (artichoke), *C. sinensis* L. leaves (green tea), *M. oleifera* L. leaves (moringa), and *Lactuca indica* L. leaves (dandelion) from Bao Loc province. *G. jasminoides* Ellis. fruits (gardenia) from Can Tho province. *C. morifolium* Ramat. flowers (white chrysanthemum) were taken from Dong Nai

province. Other medicinal plants were supplied by Thaphaco Herbal Co., Ltd., i.e., *Garcinia mangostana* L. bark (mangosteen), *Kaempferia galanga* L. fruits (aromatic ginger), *Hibiscus rosa-sinensis* L. flowers (hibiscus), *Docynia indica* L. fruits (docynia), and *Spirulina platensis* L. leaves (spirulina).

#### Instrumentation

The study utilized a variety of laboratory equipment from reputable suppliers. These included a Rotavapor BUCHI R-210 rotary evaporator from Marshall Scientific (UK), a MA35 Moisture Analyzer from Chemsol (Vietnam), a GENESYS™ 30 Visible Spectrophotometer, and an Agilent 1100 HPLC system from Xilong (China). A homogenizer HG-15D from Daihan Scientific (South Korea) and a pH meter Starter 5000 from Ohaus (UK) were also employed. These instruments ensured accurate and reliable measurements throughout the research process.

#### **Procedure**

## Preparation of plant extracts

Collected plant materials were dried at 50 °C to achieve moisture content  $\leq$  12%, ground into powder, and stored at room temperature. Twenty grams of dried material were extracted twice with 96% ethanol (1:10 g/mL) at 70 °C for 60 min with continuous stirring. The liquid phase was filtered, and the solvent removed using a rotary evaporator.

## In vitro determination of the SPF

The SPF value of each extract was determined using the Mansur method [5] with adjustments for our conditions. Each extract (1 g) was dissolved in 100 mL of ethanol to obtain a concentration of 10,000 ppm, followed by 10 min of ultrasonication. Varying concentrations (1 to 5 mg/mL) were prepared from this stock solution. The SPF measurements were taken using a UV spectrophotometer at wavelengths from 290 to 320 nm at 5 nm intervals. Each concentration was measured in triplicate using a 1 cm quartz cell, with ethanol as the blank solution. The Eq. (1) was used to calculate the SPF;

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times A(\lambda)$$
 (1)

where EE ( $\lambda$ ): erythemal effect spectrum, I ( $\lambda$ ): solar intensity spectrum, Abs ( $\lambda$ ): absorbance of sunscreen product, and CF: correction factor (= 10).

# Quantification of total polyphenolic and flavonoids contents

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu technique [6]. Initially, the extracts were dissolved in 70% ethanol at a final concentration of 0.1% (w/v). Then, 0.4 mL of sodium carbonate solution (20% w/v) and 0.5 mL of Folin-Ciocalteu reagent (1 N) were added to 0.1 mL of the solution and incubated for 30 min at room temperature in the dark. The absorbance of the solutions was measured at 765 nm, with the gallic acid solution used as a standard. Results of TPC were expressed as mg of gallic acid equivalent/g of dry extract.

For the determination of total flavonoid content (TFC), the extracts were analyzed using a spectrophotometer following the method described by Pękal and Pyrzynska [7] with slight modifications. Initially, a mixture of methanol (1 mL), distilled water (3.5 mL), and 5% NaNO<sub>2</sub> solution (0.3 mL) was prepared, to which 0.2 mL of the extracts was added. After 5 min, 0.3 mL of 10% methanolic AlCl<sub>3</sub> solution was added, followed by incubation for 6 min. Subsequently, 1.7 mL of 1 M NaOH solution was added, and after 15 min, absorbance was measured at 510 nm.

## **Evaluation of antioxidant activity**

The antioxidant activity was evaluated using the DPPH radical scavenging assay method described by Sharma and Bhat [8]. The optical density value at wavelength 517 nm was measured. The antioxidant activity was calculated using the Eq. (2);

Antioxidant (%) = 
$$\frac{A_b - (A_s - A_c)}{A_b} \times 100\%$$
 (2)

where A<sub>c</sub>: Absorbance of the color sample, A<sub>s</sub>: Absorbance of the measured sample, and A<sub>b</sub>: Absorbance of the blank sample.

## Cell culture and cytotoxicity evaluation

Cytotoxicity of concentrated plant extracts was evaluated using RAW 264.7 cell lines with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

(MTT) assay [9]. Cells were seeded in 96-well plates at  $2\times10^4$  cells/well and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5% CO<sub>2</sub> for 24 h. Extracts were applied at 50, 100, 200, 400, and 800 µg/mL concentrations and incubated for 48 h. Post incubation, cells were washed with phosphate buffer solution (PBS) and treated with 10 µL of 5 mg/mL MTT solution per well for 4 h. The media and excess MTT were then removed, and 100 µL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 540 nm using an ELISA reader.

## In vitro anti-inflammatory evaluation

The impact of 4 plant extracts on NO secretion was assessed using a method similar to that of Joo et al. [9]. First, 7 cells were cultured in DMEM medium with 10% FBS until the 3<sup>rd</sup> passage, then seeded onto 96-well plates at  $2 \times 10^4$  cells/well. After 24 h, cells were treated with an extract at a suitable concentration (chosen to maintain cell viability above 80% based on toxicity assessment results) and incubated at 37 °C with 5% CO<sub>2</sub>. After 1 h post-extract treatment, lipopolysaccharide (LPS) was added at a final concentration of 1 µg/mL. Negative control (untreated cells), positive control (treated with ibuprofen and LPS), and an LPS-only control was included. After 24 h of incubation, NO secretion was measured by adding 50 µL of Griess reagent solution to each well, followed by agitation on a horizontal shaker for 10 min. Absorbance was then read at 540 nm using an ELISA reader. If air bubbles formed, they were punctured to ensure accurate readings.

## Formulation of sunscreen lotion

Lotion bases were created using emulsification. Emulsifiers enable stable and smooth lotion formation by combining lipophilic components in the oil phase with hydrophilic components in the water phase. In preparation, phase D was added to phase A and stirred at 300 rpm for 10 min. Both phases A and B were heated to 70 °C and homogenized at 5000 rpm for 5 min. After cooling to 40 °C, phase C was added, and the mixture was stirred for another 10 min at 300 rpm. Fragrance and preservatives were then incorporated with an additional

Phase **INCI** Name F1 (%) F2 (%) F3 (%) F4 (%) A Agua 60 60 60 60 Disodium EDTA 0.1 0.1 0.1 0.1 В Glyceryl stearate and PEG-100 stearate 3 3 3 3 Cetostearyl alcohol 2 2 2 2 Stearic acid 1 1 1 1 Butyrospermum parkii (Shea) Butter 1.5 1.5 1.5 1.5 Triethylhexanoin 6 6 6 6 C Chrysanthemum flower extract 1 Gardenia fruit extract 1 Moringa leaf extract 1 Green tea leaf extract 1 Water 20 20 20 20 D Propandiol 4 4 4 4 Xanthan gum clear 0.3 0.3 0.3 0.3 Ε Phenoxyethanol 0.8 0.8 0.8 0.8

0.3

Table 1. Lotion base formula

5 min of stirring. Formulations with varying phytochemical compositions have been developed, adhering to regulatory guidelines. The ingredients are detailed in Table 1.

Fragrance

## **Evaluation of sunscreen lotions**

**Physical parameters, pH, and viscosity.** The sunscreen lotions were evaluated for texture, color, and homogeneity upon application. The pH of the developed batches was determined using a pH meter Ohaus Starter 5000 Instrument. Viscosity was measured using a rotary viscometer (ST-2020L) with spindles SPL1 to SPL4.

**Stability studies.** The stability of lotions was assessed through centrifugation at 5000 rpm for 30 min, which checked for phase separation, and accelerated stability tests at 45 °C to monitor changes in appearance and SPF value. Additionally, photostability was tested under solar irradiation at periodic intervals. All samples were evaluated for appearance and SPF over 28 days. Any signs of phase separation or liquid phase emergence were considered indicative of instability.

*In vitro* SPF determination of the lotions. About 1.0 g of the sample was diluted suitably with ethanol. The SPF of the solution was calculated using the Eq. (1) [5].

#### RESULTS AND DISCUSSION

0.3

0.3

0.3

#### In Vitro Determination of the SPF

Based on Fig. 1, the extraction yields of G. jasminoides and M. oleifera were the highest, at  $30.2 \pm 1.2\%$ and  $23.8 \pm 1.1\%$ respectively. remaining raw materials exhibited extraction efficiencies ranging from 10% to 25%. Specifically, hard materials such as K. galanga and G. mangostana yielded than 20%  $(19.7 \pm 1.0\%)$ and  $18.1 \pm 1.1\%$ respectively). Overall, the inherent properties of hard and sharp materials, including their surface area, accessibility, binding forces, resistance to mechanical forces, and compatibility with extraction methods, can contribute to lower extraction efficiency compared to softer or more easily extractable materials [10].

All extracts and standards were measured for SPF at a 2000  $\mu$ g/mL concentration. These values were notably approximately comparable to those of the two commercial UV filters used as standard reference substances, which are homosalate (SPF 42.4  $\pm$  0.2) and avobenzone (SPF 38.8  $\pm$  0.2). Additionally, Fig. 1 showed that *G. jasminoides* exhibited the highest photoprotective potential (SPF 40.8  $\pm$  0.2), followed by

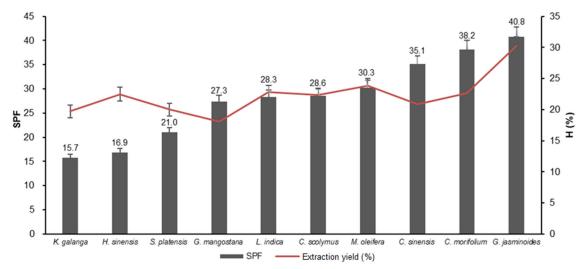


Fig 1. Extraction efficiency and SPF value of ethanol extracts

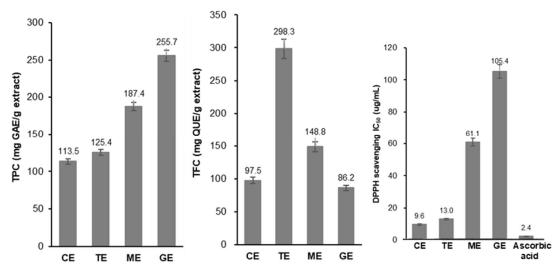


Fig 2. TPC, TFC, and DPPH radical scavenging IC<sub>50</sub> values of 4 extract samples

C. morifolium, M. oleifera, and C. sinensis, all of which have SPF values greater than 30. C. scolymus, L. indica, S. platensis, and G. mangostana have SPF values ranging from 20 to 30. K. galanga and H. sinensis, on the other hand, all have SPF values less than 20. Consequently, the four most potent extracts, i.e., G. jasminoides, C. morifolium, M. oleifera, and C. sinensis, were further subjected to detailed investigations to determine their suitability as components of herbal sunscreens.

## **Biological Activity**

The comprehensive evaluation of the biological activity of the four selected extracts involves an intricate analysis of their antioxidant capacity, TPC, TFC, and SPF

determination, all factors in assessing their potential as photoprotective agents, summarized in Fig. 2. Compounds abundant in conjugated structures, notably phenolics and flavonoids with benzene rings, exhibit heightened UV absorption capabilities due to the intricate  $\pi$ - $\pi$  and p- $\pi$  conjugation facilitated by their structural components [11]. According to experimental data, the highest TPC was 255.7  $\pm$  3.4 mg GAE/g extract of *G. jasminoides* extract (GE), and the highest TFC was 298.3  $\pm$  3.4 mg QUE/g extract of *C. sinensis* extract (TE).

Polyphenols, characterized by their condensed aromatic rings and multiple hydroxyl groups, stand out for their exceptional ability to absorb UV radiation across both the UV-A and UV-B spectra [12].

Consequently, formulations enriched with plant extracts boasting high polyphenol content offer superior photoprotective properties, outperforming those relying solely on synthetic filters [13]. Moreover, the presence of flavonoids and antioxidant properties further amplifies the photoprotective potential of these extracts. Extracts with elevated TPC, TFC, and antioxidant properties demonstrate enhanced UV radiation absorption, leading to elevated SPF values.

By analyzing the experimental data, it can be seen that two extracts of *C. morifolium* (CE) and *M. oleifera* (ME) extracts had the highest antioxidant capacity. Their IC<sub>50</sub> was  $9.6 \pm 0.3$  and  $13.1 \pm 0.4 \,\mu\text{g/mL}$ , respectively. It became apparent that each extract possesses distinct characteristics. GE showcases the highest TPC, while TE exhibit the highest TFC, and CE demonstrates remarkable antioxidant activity. These variations can be attributed to nuanced differences in chemical structures, sizes, and the number of hydroxyl groups within each compound, all of which influence their respective reaction mechanisms [12-13].

It is essential to highlight that while a high TPC indicates rich polyphenolic content, it does not always correspond to superior DPPH activity. Structural disparities among polyphenols significantly impact their interaction with the DPPH radical, emphasizing the importance of structural considerations and hydroxyl group accessibility in determining DPPH activity.

## **Absorbance Spectrum**

Avobenzone and homosalate were selected as positive controls. As depicted in Fig. 3, avobenzone exhibits significant absorption in the UV-A region (320–400 nm), whereas homosalate demonstrates high absorption in the UV-B region (290–320 nm). This observation aligns with existing evidence and research [14-15]. Most extracts displayed a high absorption spectrum in the UV-B region, so homosalate was chosen as the positive control.

In addition, the extracts' absorption spectra varied based on each plant's distinct properties. For the CE, HPLC chromatograms revealed typical UV absorptions obtained with a water PDA detector, with maximum absorptions observed at 252–268 nm and 333–348 nm for flavones or at 243 and 326–327 nm for caffeoylquinic acid derivatives such as 3,5-dicaffeoylquinic acid and 1,3-dicaffeoyl-epi-quinic acid [16]. Furthermore, as shown in Fig. 3, the UV spectrum of CE closely resembles that of homosalate, partially substantiating why it exhibits the highest SPF when incorporated into sunscreen lotion.

In the case of the GE, spectral data from all peaks were accumulated in the range of 200–500 nm, with UV-vis chromatograms recorded at 325 nm for hydroxycinnamic acid derivatives, 440 nm for crocetin derivatives, and 254 nm for iridoid glycosides and rutin. Specifically, caffeoylquinic acids, 3,5-dicaffeoylquinic acids ( $\lambda_{max} = 325$  nm), and rutin ( $\lambda_{max} = 254$  nm), as well

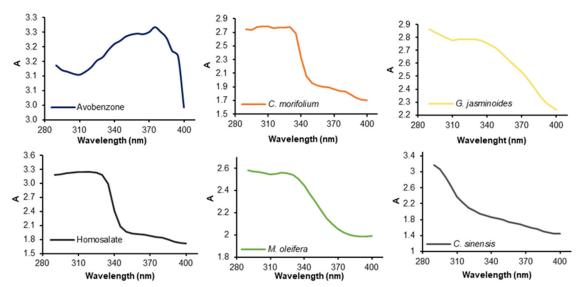


Fig 3. UV absorbance of standards and extracts

as crocetin derivatives, exhibit characteristic absorption bands at 440 and 464 nm with a shoulder around 415 nm [17]. This finding aligns with previous studies, suggesting that the chromophore structure is crocetin. Corresponding to Fig. 3, the UV absorption spectrum of GE is also broader compared to that of other extracts. For the ME, the highest peaks observed at wavelengths lower than 347 nm correspond to phenolic acids and derivatives of caffeic, *p*-coumaric, or ferulic acid [18], consistent with previous findings.

Meanwhile, in the case of the TE, Liu et al. [19] found that catechins and caffeine exhibit high sensitivity at 280 nm. In comparison, the maximum absorption wavelength of organic acids is approximately 320 nm, and that of flavonols is 360 nm [19]. However, the UV absorption of organic acids and flavonols at 280 nm is relatively higher and more stable. Considering this comprehensively, the wavelength of 280 nm can effectively absorb, consistent with the research results of Marzuki et al. [20]. This explains why the UV spectrum of green tea still tends to increase after 290 nm.

## In Vitro Anti-inflammatory Evaluation

UV rays are a significant cause of skin inflammation, leading to redness and irritation, underscoring the importance of sun protection in skincare [21]. The integration of anti-inflammatory properties into sunscreen formulations enhances effectiveness by blocking harmful UV rays and mitigating the

inflammatory responses provoked by UV exposure [22]. When incorporated into sunscreens, botanical extracts have anti-inflammatory properties and serve a dual purpose: functioning as physical or chemical UV blockers and actively mitigating skin inflammation triggered by UV rays [23]. This renders them potentially superior to conventional UV filters such as avobenzone and homosalate. These extracts not only prevent sunburn and protect against UV damage but also alleviate conditions such as dermatitis exacerbated by sunlight, adding a therapeutic dimension to sun protection products.

The evaluation of the toxic potential from TE, CE, ME, and GE on RAW264.7 cells indicated that at concentrations of 25, 50, and 100 µg/mL, cell viability remained high ( $\geq$  87%), with no significant difference compared to control groups (cell survival with 1 µg/ml LPS and DMSO  $\geq$  91%). However, cells treated with the commercial anti-inflammatory drug ibuprofen at 20 nM experienced approximately 20% cell death, demonstrating that the extracts are not significantly toxic to RAW264.7 cells.

Fig. 4 showed that cells stimulated with 1  $\mu$ g/mL LPS experienced inflammation and a notable increase in NO concentration, with a 23% increase compared to untreated cells. However, treatment with ibuprofen did not significantly inhibit NO levels in LPS-treated cells, showing only a 6% decrease. Additionally, the impact of the DMSO solvent at a concentration equivalent to

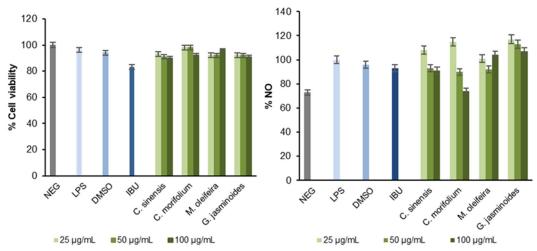


Fig 4. Cell viability and anti-inflammatory activity of the extracts

 $100\,\mu g/mL$  of the extract showed slight inhibition of NO concentration. These findings suggest that LPS increased nitrite concentration. At the same time, both ibuprofen and DMSO decreased NO secretion in RAW 264.7 cells, indicating that the reduction in nitrite concentration was independent of cytotoxicity from the test substances.

Among the four extracts tested, CE demonstrated the most effective inhibition of NO secretion in RAW264.7 cells treated with LPS at two concentrations of 100 and 50  $\mu$ g/mL, reducing NO secretion by 27% and 13%, respectively, surpassing the performance of commercial drugs. Other extracts showed no significant inhibition of NO secretion. Specifically, TE and GE at lower

concentrations (25  $\mu g/mL$  for TE and both 25 and 50  $\mu g/mL$  for GE) did not effectively inhibit NO secretion.

#### **Extracts in Lotions**

Incorporating the extract into sunscreen introduces a unique aspect due to the extract's distinct coloration, which varies from green to yellow-orange. This coloration is directly imparted to the sunscreen base, with the color intensity deepening as the concentration of the extract increases, as shown in Table 2. This is a result of the higher content of active ingredients present. In contrast, CE maintains a milky white appearance, not affecting the base cream's color.

**Table 2.** Content of the extracts in lotions

Sample	1%	2%	3%	4%	5%
C. morifolium Ramat. flowers extract					
G. jasminoides Ellis. fruit extract					
M. oleifera L. leaves extract					
C. sinensis L. leaves extract					

To effectively blend the concentrated extract into the cream, it is advised to first pre-disperse the extract in water. This is particularly important as the concentration increases, requiring longer stirring times to ensure the extract is evenly distributed throughout the cream. However, when the concentration exceeds 5%, it becomes difficult to achieve an even dispersion, and the intense color may not be aesthetically pleasing to all consumers. Therefore, it is recommended to gradually incorporate the concentrated extract into the base cream, keeping the concentration of active ingredients between 1% and 5%. This approach is applicable for extracts from CE, ME, TE, and GE, each contributing its unique benefits and hues to the sunscreen formula.

## **Physicochemical Evaluation**

The pH of the samples rapidly decreased with increasing concentration. At 4% and 5% concentrations, most extracts exhibited a pH around or below 5, with ME and GE showing the lowest pH values at  $4.9 \pm 0.1$  and  $4.9 \pm 0.1$ , respectively. This acidity was attributed to the presence of phenol radicals in natural compounds, with acidity directly linked to extract concentration, potentially causing a burning sensation and irritation with high usage. Consequently, further investigation was conducted at 1%, 2%, and 3% concentrations.

Apart from evaluating the SPF values of the tested plant extracts, another notable observation pertains to the viscosity variation observed with increasing concentration. Viscosity is a critical factor in cosmetic emulsions, directly affecting product appearance and stability. The product in this study necessitated a specific consistency, neither overly liquid nor too thick. Natural extracts may contain various compounds such as polyphenols, flavonoids, phenolic acids, catechins, and caffeine, which could interact with the emulsifier or other emulsion ingredients, impacting stability and consistency. These interactions may disrupt the emulsion structure, leading to reduced stability and a more liquid-like consistency. This could be attributed to interactions between TE extract compounds and the emulsifier, weakening the emulsion's structural integrity. Besides, CE may contain natural polysaccharides that enhance the bonding between molecules in the compatible matrix, thereby increasing the viscosity. Fig. 5

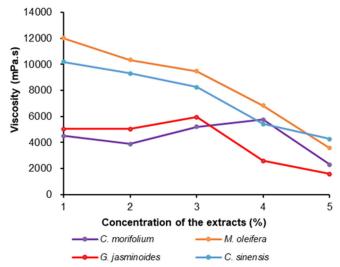


Fig 5. Viscosity of sunscreen lotion from 4 extract samples

showed a sudden decrease in viscosity occurred when a large amount of extract was added to reach a 3% concentration, affecting sensory test results and reducing stability. An appropriate 3% concentration of extract in lotion was advisable to ensure a more comprehensive investigation.

#### **Evaluation of SPF of the Sunscreen Lotions**

Fig. 6 showed that CE had the highest SPF value  $(36.4 \pm 0.1)$  among the tested extracts. It was followed by GE (28.1  $\pm$  0.1), ME (26.5  $\pm$  0.1), and TE (22.1  $\pm$  0.1). At 1, 2, and 3 mg/mL concentrations, the SPF index fell below 10, suggesting that these extracts cannot be used as standalone sunscreens without additional UV filters. Sometimes, sunscreen formulations that use only synthetic UV filters do not achieve as high an SPF as those combined with extracts with photoprotective activity [24]. Besides, although sunscreens containing only extracts cannot provide as high an SPF as those with synthetic UV filters, these natural UV filters can partially reduce dependence on these chemical compounds. This may explain why, despite the lack of officially approved natural commercial UV filters, there is an increasing number of commercial sunscreen products containing plant extracts on the market [25]. While synthetic sunscreens cannot be entirely replaced by those with natural UV filters immediately, the information provided in this study could serve as an essential starting point for research aimed at developing more effective sunscreens.

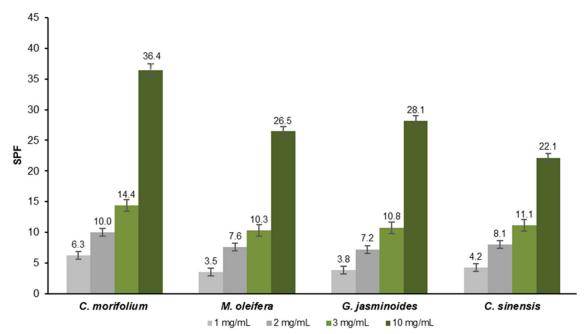


Fig 6. SPF value of herbal sunscreen lotions with different concentrations

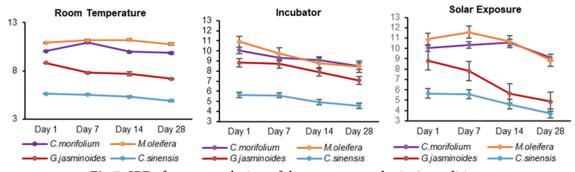


Fig 7. SPF of sunscreen lotion of the extract samples in 3 conditions

## **Evaluation of Stability**

A focused study on 3% extract concentrations was initiated to further understand the formulations' longevity and performance, particularly given the 5% extract's unsuitability due to its high acidity, dark coloration, and poor skin absorption despite offering a higher SPF. The stability of these 3% extract formulations was monitored over 28 days under 3 different environmental conditions, examining their pH, SPF, and organoleptic properties at specified intervals. Notably, while the overall physical characteristics of the samples, including texture, color, and viscosity, remained constant, direct sunlight exposure resulted in a slight pH increase, hinting at the degradation of bioactive components under

UV light and heat. The fluctuation observed in Fig. 7 showed SPF values across the study period could be tied to plastic containers, which may absorb UV radiation and thus reduce the interaction between UV rays and the sunscreen samples. This absorption potentially led to a minor reduction in SPF efficacy. To mitigate such effects and preserve the formulations' integrity, storing the products in dark-colored bottles away from direct sunlight is recommended. Despite these observations, the formulations showed relative stability over the 28-day trial. However, the slight changes noted necessitate further long-term studies to comprehensively evaluate the products' shelf-life and ensure their efficacy and safety for consumer use.

#### CONCLUSION

This study explored the photoprotective activity of 10 herbal extracts commonly found in Vietnam. Among these, GE demonstrated the highest SPF, highlighting its potential as an effective photoprotective agent. Further research concentrated on 4 specific extracts, i.e., CE, GE, ME, and TE. The findings revealed that both GE and CE extracts posse antioxidant and anti-inflammatory activities and maintain a high and stable SPF index. These extracts exhibited significant photoprotective effects and proved stable under various conditions.

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## **■ CONFLICT OF INTEREST**

The authors have no conflict of interest.

#### AUTHOR CONTRIBUTIONS

Phuong Yen Dang, Uyen Khanh Nguyen Tran, Nhu Quynh Trung Nguyen, and Thanh Truc Tran conducted the experiment. Tien Xuan Le, Phuong Yen Dang wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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