



## *Psidium guajava* L. (Common Guava) Peel, Pulp and Leaves as Natural Sources of Antioxidants, Antimicrobials and Photoprotective Agents for Development of Sun Protection Cosmeceuticals

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Sunscreens with natural ingredients for skin care become a new trend in cosmetology as it protects skin against photo-aging and dermatologic disorders. Therefore, the goal of this study was to evaluate the antioxidant, antimicrobial and photoprotective properties of methanolic extracts of leaves, peel and pulp of *Psidium guajava* L. to investigate their potentials to be used as sources of cosmeceutical ingredients for sun protection. Chemical constituents of the plant parts were extracted into methanol by maceration. Among the extracts, leaf extract exhibited higher DPPH free radical scavenging activity ( $IC_{50} = 89.56 \pm 0.97 \mu\text{g/mL}$ ), than that of peel and pulp extracts. The highest total phenolic content (TPC) and total flavonoid content (TFC) were found to be in the leaf extract. All extracts demonstrated antibacterial activity against the examined potential pathogens in cosmetic products. Among the extracts, leaf extract showed the highest photoprotective property with the sun protection factor (SPF) of  $30.38 \pm 0.22$  at 2 mg/mL indicating its potential to be used in the development of sunscreen formulations.

**Keywords:** Antioxidant, Antimicrobial, *Psidium guajava*, Sun protection factor.

### INTRODUCTION

Carcinogenesis in human skin including melanoma and non-melanoma become one of the most found cancer types among the world population as its rate of incidents is increasing rapidly day by day. More than 5 million new incidents have been reported annually in USA [1]. According to the World Health Organization (WHO), the mortality rate of people above 65,000 annually is estimated due to malignant carcinogenesis. 3% of melanoma cases reported in the USA out of the varieties of skin cancers. Unfortunately, it exhibits the 75% of the death count for skin carcinogenesis [2]. As a result, skin cancers become a major threat to human wellbeing around the world [3]. Approximately 10% of sun's entire light output contains ultraviolet radiation [4], which acts as one of the major contributors to skin carcinogenesis. Among the sub-regions of UV radiations, UV C ( $\lambda = 200\text{-}290 \text{ nm}$ ) radiation causes the maximum biological harm. Moreover, UV C radiation is significantly absorbed by the stratospheric ozone in the atmosphere and doesn't approach the earth's crust [5]. The majority of skin issues,

including melanoma and non-melanoma skin cancers, are caused by UV B ( $\lambda = 290\text{-}330 \text{ nm}$ ) radiation, which represents around 5% of all UV energy [6].

Since reducing excessive exposure to sun radiation would be difficult, a unique strategy of skin photoprotection by using sunscreens has been developed to protect the skin from harmful rays of the sun. The topical application of sunscreens, which contain chemical constituents with UV absorption, reflection or scattering of active molecules, is becoming the most common technique used to minimize the quantity of UV radiation that gets to the skin [7]. Several investigations have revealed that there is a decrease in many skin disorders and several melanomas in those who frequently use sunscreens.

Sun care products are categorized into three as products with minimal sunburn protection ( $\text{SPF} < 12$ ); moderate sunburn protection ( $12 < \text{SPF} < 30$ ); and high sunburn protection ( $\text{SPF} \geq 30$ ) based on their sun protection factor (SPF). The ratio of the minimal quantity of UV radiation necessary to generate minimum erythema on human skin coated with a sunscreen (protected skin) to the UV radiation needed to create a similar

erythema on exposed human skin (unprotected skin) is scientifically defined as the sun protection factor [8].

Antioxidants have now also been supplemented with photoprotective agents in sun care products as antioxidants can neutralize free radicals and prevent oxidative stress leading to aging [9]. A unique group of chemicals known as polyphenols has attracted a lot of attention as a powerful anti-aging agent with strong antioxidant effects [10]. Plants are rich in bioactive phytochemicals including phenols with antioxidant and photoprotective properties and therefore, are now being utilized as sources of herbal ingredients in the development of sunscreen formulations as they are cost-effective and considered to be safer than synthetic chemicals.

Guava, scientifically known as *Psidium guajava* L. (common guava) is a miniature tree from the family Myrtaceae. Guava is native to Mexico, South America and the Caribbean region and grown in several other territories with the climate varying in tropical and subtropical regions [11]. It is rich in nutrients and phytochemicals with different bioactivities including antimicrobials [12], antioxidants [13], antimalarial [14], anti-tussive [15], hepatoprotective [16], antigenotoxic [17], anti-mutagenic [18], anticancer [19], antidiabetic [20], anti-inflammatory [21], wound healing [22], cardiovascular [23], hypotensive [24], effects. Not only guava fruits but also other plant parts of guava (leaves, bark and roots) are known to be rich in phytochemicals with antidiarrheal, antidiabetic, antimicrobial, hepatoprotective [25], antihyperglycemic and analgesic activities [26], but less research has been conducted to explore their photoprotective properties and potential to be used in sun protection cosmeceuticals.

Therefore, the goal of this study was to assess the antioxidant, antibacterial, antifungal and photoprotective properties of methanolic extracts of leaves, peel and pulp of *Psidium guajava* L. to determine whether the plant parts could be used as natural sources of ingredients for the development of sunscreen formulations.

## EXPERIMENTAL

Methanol (99.8% assay), sodium carbonate (99.999%), sodium nitrite ( $\geq 99.0\%$ ), aluminium chloride (99%), ascorbic acid (99%), 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) and NaOH pellets ( $\geq 97.0\%$ ) were purchased from Sigma-Aldrich. Folin & Ciocalteu phenol reagent (2 N), butylated hydroxy toluene (99% assay), nutrient broth (Oxoid, UK), Muller Hinton agar (Oxoid, UK) and quercetin hydrate (95%) were purchased from Thermo-Fisher Scientific Inc. Gallic acid (98%) was purchased from S.D. Fine-Chem. Ltd, India. Sabouraud dextrose agar with chloramphenicol (Criterion, USA) was purchased from Amerigo Scientific. Amoxicillin (For injection, BP 600 mg) and clotrimazole powder was purchased from State Pharmaceuticals Corporation of Sri Lanka.

**Sample collection:** Freshly picked healthy leaves and medium size ripe fruits of guava were collected from Padukka, Western Province, Sri Lanka and plant authentication was done by the Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka.

**Sample preparation:** The leaves, deseeded pulp and peel were all individually rinsed and cleaned with distilled water. Guava leaves were dried after being exposed to air for 4 days and peel and pulp were dried in a hot air oven set at 40 °C for 3 days and 7 days, respectively. Dried materials were ground into a powder and sealed in sterile polythene bags.

**Cold extraction:** Guava leaves, pulp and peel (10 g each) were individually weighed using an electric balance (KERN EW 2200-2NM, Germany) and soaked in 99% methanol (120 mL) separately for 6 days at room temperature. All the extracts were filtered under gravity using Whatman filter papers and solvents were evaporated off by using a rotary evaporator at 36 °C at 30 rpm. The obtained extracts were stored at 4 °C until further usage.

**DPPH free radical scavenging activity:** The antioxidant potential of samples was determined by using the slightly modified *in vitro* DPPH free radical scavenging assay [27]. In brief, DPPH dissolved in methanol (40  $\mu$ L, 0.26 mg/mL) was introduced into 160  $\mu$ L of a concentration series of the samples and the standard, butylated hydroxytoluene (BHT) (250, 125, 62.5, 31.25, 15.63, 7.82, 3.91, 1.95  $\mu$ g/mL) and thoroughly mixed samples were stored in a dark environment for 15 min for incubation. The absorbance values of the samples at 517 nm were measured using a microplate reader (Thermo-Fisher Scientific). Methanol (200  $\mu$ L) was used as the blank solution and the control sample was prepared by mixing methanol (160  $\mu$ L) and DPPH (40  $\mu$ L). The percentage inhibition (%) was calculated by using eqn. 1:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

where  $A_{\text{sample}}$  and  $A_{\text{control}}$  are the absorbance of the sample or standard and the absorbance of the control, respectively. The concentration which showed 50% DPPH inhibition ( $IC_{50}$ ) was determined by the plot of percentage inhibition *versus* concentrations of the samples/standard.

**Total phenolic content (TPC):** The total phenolic content (TPC) of the methanolic extracts of guava leaves, pulp and peel was assessed using the slightly modified Folin-Ciocalteu technique [28] and as reference, gallic acid was used. Samples (1000 g/mL, 0.1 mL) were added to deionize water (7.9 mL) separately. Folin-Ciocalteu reagent (0.5 mL) was introduced to each sample mixture. Sodium carbonate solution (1.5 mL, 200 g/L) was added 3 min later. Then the samples were agitated, kept 2 h duration at room temperature and then, using a UV visible spectrophotometer (Orion Aqua Mate 8000, Thermo Scientific), absorbance at 760 nm was measured. A blank solution was prepared by replacing the sample with 0.1 mL of methanol. Using the standard curve of gallic acid ( $R^2 = 0.9946$ ), the TPC was determined and reported as mg of gallic acid equivalent (GAE) per gram of dry weight of the plant extract.

**Total flavonoid content (TFC):** Total flavonoid content (TFC) of all samples was determined by a slightly modified  $AlCl_3$  colorimetric method [29] and quercetin was used as the reference standard. Sample/standard (1.0 mL) was added to a mixture of distilled water (4.0 mL) and  $NaNO_2$  (0.3 mL, 5%) and allowed to stand for 5 min at ambient conditions. Next,

$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  (0.3 mL, 10%) was added to the mixture and allowed to stand for another 6 min under ambient conditions. In last step, NaOH (2 mL, 1 N) was added and the sample solution was diluted with distilled water (10 mL) and absorbance at 510 nm was measured. By replacing 1.0 mL of methanol for the sample, a blank solution was prepared. The standard curve of absorbance *versus* concentration ( $R^2 = 0.9825$ ) of quercetin was used to determine TFC of each sample and reported as mg of quercetin equivalent (QE) per gram of dry weight of the plant extract.

**Antimicrobial activity:** Antimicrobial activity of guava leaf, pulp and peel extracts were evaluated by agar well diffusion method [30] against the bacterial cultures of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027) and a fungal culture *Candida albicans* (ATCC 10231), commonly found pathogenic microorganisms in cosmeceuticals. The media utilized for bacterial and fungi cultures were Muller Hinton Agar and Sabouraud Dextrose agar with chloramphenicol, respectively. Agar plates were swabbed with sterile cotton swabs by using bacterial and fungal suspensions from a culture that represents 0.5 McFarland standard. A well was prepared with a diameter of 8 mm, punched aseptically with a sterilized cork borer and 100  $\mu\text{L}$  of antimicrobial agent or extract solution (2000 g/mL) was added to the well and plates were incubated for 24 h at 37  $^\circ\text{C}$  in an incubator. Amoxicillin and clotrimazole were used as positive controls for bacterial and fungal cultures, respectively. The zone of inhibition was measured to the nearest millimeter along two axes and the mean was computed and reported.

***in vitro* Sun protection factor (SPF):** The samples and a reference sunscreen were dissolved in methanol to prepare solutions of 2.0 mg/mL concentration for the determination of the *in vitro* sun protection factor (SPF). The absorbance of the reference sunscreen and the samples was measured after every intervals of 5 min between wavelengths of 290-320 nm. Methanol was used as the blank solution. The Mansur equation [31] was used to calculate the *in vitro* SPFs for all samples as well as the SPF of the reference sunscreen:

$$\text{SPF} = \text{CF} \times \sum_{320}^{290} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda) \quad (2)$$

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

The  $\text{EE} \times \text{I}$  values are constants which were predetermined and listed in Table-1.

Wavelength (nm)	EE $\times$ I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

EE = erythemal effect spectrum; I = solar intensity spectrum

**Statistical analysis:** Each experiment was conducted in triplicate and the mean  $\pm$  standard deviation was reported.

## RESULTS AND DISCUSSION

**DPPH free radical scavenging activity:** Phytochemicals with the strong capability for scavenging free radicals may prevent or decrease oxidation of other molecules in cells [33]. Therefore, the DPPH free radical scavenging activity of methanolic extracts of guava leaf, peel and pulp was investigated to determine the antioxidant potential of the extracts. The delocalization of the spare electron provides the DPPH free radical a deep violet colour at a maximum wavelength of 517 nm [34]. As a result of the DPPH free radical (violet) being scavenged by an antioxidant, DPPH is reduced to the corresponding hydrazine, DPPH-H (yellow) and percent DPPH inhibition can be determined by using spectrophotometry in order to assess the anti-oxidant potential of the compound being tested.

The percent inhibition of DPPH with the concentration of methanolic extracts of the samples and standard BHT is depicted in Fig. 1, while the corresponding  $\text{IC}_{50}$  values are shown in Table-2. According to the results, all the extracts exhibited DPPH free radical scavenging activity and among the extracts, the guava leaf extract showed the highest DPPH free radical scavenging activity (50% inhibition at 517 nm) with the lowest  $\text{IC}_{50}$  value ( $89.56 \pm 0.97 \mu\text{g/mL}$ ) and the pulp extract possessed the lowest antioxidant potential ( $\text{IC}_{50} = 119.72 \pm 0.55 \mu\text{g/mL}$ ).

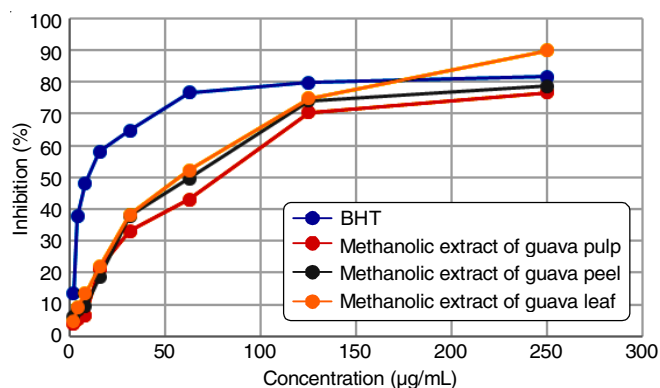


Fig. 1. DPPH free radical scavenging activity (Inhibition%) of methanolic extract of guava leaves, peel, pulp and BHT. BHT was used as the standard

Sample	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ ) $\pm$ SD
Guava leaf	$89.56 \pm 0.97$
Guava peel	$106.20 \pm 0.74$
Guava pulp	$119.72 \pm 0.55$
BHT	$12.30 \pm 0.79$

Shanthirasekaram *et al.* [35] reported that methanolic extracts of two varieties of guava (*Psidium guajava*-common guava and *Psidium guajava*-Pubudu) leaves showed radical scavenging activity with  $\text{IC}_{50}$  of  $192.89 \pm 0.07$  ppm and  $267.10 \pm 0.28$  ppm, respectively. Further, Venkatachalam *et al.* [36] reported that the DPPH scavenging activity of methanolic extract

and aqueous extract of guava leaves was  $42.33 \pm 0.76$  mg/g ascorbic acid equivalent and  $39.33 \pm 0.29$  mg/g ascorbic acid equivalent, respectively. Milani *et al.* [37] found a higher antioxidant activity ( $IC_{50}$  of  $19.80 \mu\text{g mL}^{-1}$ ) in the *Psidium guajava* agro-industrial byproduct extract. The study conducted by Lee *et al.* [38] showed that essential oil (EO) of guava leaves had an antioxidant potential with  $IC_{50}$  of  $460.37 \pm 1.33 \mu\text{g/mL}$ .

**Total phenolic and total flavonoid contents:** The TPC and TFC of methanolic extracts of *Psidium guajava* L. leaves, pulp and peel are presented in Table-3. Total phenolic content (TPC) of guava leaves, pulp and peel methanolic extracts were calculated from the calibration curve of gallic acid ( $y = 0.0011x + 0.0282$ ,  $R^2 = 0.9946$ ) and expressed in mg of gallic acid equivalents (GAE)/g of the dry weight of the plant extract. Several researchers have investigated the TPC of both guava leaves and fruit and found that they are rich in phenolic compounds. Noriham & Zahidah [39] reported that pink guava leaf extract ( $368.61 \pm 25.85$  mg GAE/100 g dry extracts) contained a significantly higher quantity of phenolic constituents than that of guava seed extracts ( $79.03 \pm 3.48$  mg GAE/100 g dry extracts). Also, guava leaf extract had a higher flavonoid amount than that of the guava seed extract. A study conducted by Melo *et al.* [40] revealed that there is a correlation between water amount used in the extraction solvent and total phenolic content. According to the study, increasing the water content in the extraction solvent enhances the amount of TPC in the guava leaf extract.

TABLE-3  
TPC AND TFC OF METHANOLIC EXTRACTS OF  
GUAVA LEAVES, PULP AND PEEL (n = 3)

Sample	TPC in mg of GAE per g of the dry weight of the plant extract $\pm$ SD	TFC in mg of QE per g of the dry weight of the plant extract $\pm$ SD
Guava leaves	$662.24 \pm 1.97$	$18.63 \pm 0.74$
Guava pulp	$105.58 \pm 1.59$	$5.64 \pm 0.16$
Guava peel	$211.03 \pm 2.09$	$8.26 \pm 0.20$

The TFC of methanol extracts of guava leaves, pulp and peel was calculated from the calibration curve of quercetin ( $y = 0.0441x - 0.118$ ,  $R^2 = 0.9825$ ) and reported in quercetin equivalents (QE)/g of the dry weight of the plant extract. Among the extracts, the methanolic extract of guava leaves was the richest in TPC ( $662.24 \pm 1.97$  mg of GAE per g of dry weight of plant extract) and TFC ( $18.63 \pm 0.74$  mg of QE per g of dry weight of plant extract). TPC and TFC in the methanolic extracts were in the order of leaves > peel > pulp. In comparison to the TPC, the TFC of extracts was significantly lower. Shanthirasekaram

*et al.* [35] reported that the TFC in all the tested varieties of guava including getta pera, ambul pera, costorican, apple guava, kanthi, pubudu and common guava varied from  $25.25 \pm 0.13$  mg QE/g to  $34.23 \pm 0.05$  mg QE/g. Seo *et al.* [41] analyzed the flavonoid content of water, ethanolic and methanolic extracts of *P. guajava* L. leaves. According to this study, the TFC of water and ethanolic extracts of leaves was higher (50 mg of QE/g, 50 mg of QE/g) than that of guava leaf extract in methanol (35 mg of QE/g).

**Antimicrobial activity:** As reported in numerous studies *Psidium guajava* L. possesses antibacterial properties thus, can be applied to combat the problem of disease resistance [40,42]. Antimicrobial activities of methanolic extracts of guava leaves, pulp and peel against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027) and *Candida albicans* (ATCC 10231) the potential bacterial and fungal pathogens in cosmetics are tabulated in Table-4.

Guava peel and pulp extracts exhibited better antibacterial activity against *Staphylococcus aureus* ( $18 \pm 1.00$  mm,  $18 \pm 1.04$  mm) and *Escherichia coli* ( $16 \pm 0.50$  mm,  $16 \pm 1.26$  mm). *Pseudomonas aeruginosa* was much more susceptible to the antibacterial effects of guava pulp extract, with an inhibition zone of  $21.00 \pm 1.76$  mm. In contrast to guava pulp extract, which exhibited no antifungal activity against *Candida albicans*, guava peel extract demonstrated the maximum antifungal activity with an inhibition zone of  $16.00 \pm 2.62$  mm.

**in vitro Sun protection factor (SPF):** Mansur's approach [31] is commonly used to calculate the *in vitro* SPF of a given substance to evaluate its photoprotective property. Sun protection factors of methanolic extracts of guava leaves, pulp, peel and the reference sunscreen are listed in Table-5. Numerous chemicals are used in sunscreens to block, scatter, or absorb the sun's damaging rays. When the photoprotective properties of guava peel and pulp extracts were compared with that of the leaf extract, the methanolic leaf extract displayed a greater level

TABLE-5  
SPF VALUE OF METHANOLIC EXTRACT OF GUAVA  
LEAVES, PULP, PEEL AND THE REFERENCE COMMERCIAL  
SUNSCREEN AT 2.0 mg/mL CONCENTRATION

Sample	SPF value $\pm$ SD
Reference sunscreen	$39.18 \pm 0.56$
Guava leaves	$30.38 \pm 0.22$
Guava peel	$20.59 \pm 0.49$
Guava pulp	$16.24 \pm 0.67$

Data are expressed in mean  $\pm$  SD (n = 3).

TABLE-4  
ZONE OF INHIBITION OF THE GROWTH OF BACTERIAL AND FUNGAL BY  
METHANOLIC EXTRACTS OF GUAVA LEAVES, PULP AND PEEL (2000  $\mu\text{g/mL}$ ) (n = 3)

Sample/Culture	Zone of inhibition (mm) $\pm$ SD			
	<i>Staphylococcus aureus</i> (ATCC 25923)	<i>Escherichia coli</i> (ATCC 25922)	<i>Pseudomonas aeruginosa</i> (ATCC 9027)	<i>Candida albicans</i> (ATCC 10231)
Guava leaf	$14.00 \pm 1.00$	$13.00 \pm 0.76$	$20.00 \pm 0.57$	$14.00 \pm 1.50$
Guava pulp	$18.00 \pm 1.04$	$16.00 \pm 1.26$	$21.00 \pm 1.76$	No inhibition
Guava peel	$18.00 \pm 1.00$	$16.00 \pm 0.50$	$17.00 \pm 1.32$	$16.00 \pm 2.62$
Amoxicillin (positive control for bacteria)	$58.00 \pm 0.70$	$38.00 \pm 0.56$	$22.00 \pm 1.00$	No inhibition
Clotrimazole (positive control for fungi)	No inhibition	No inhibition	No inhibition	$26.00 \pm 1.50$

of photoprotection (SPF = 30.38 ± 0.22) than the methanol extracts of peel (SPF = 20.59 ± 0.49) and the pulp (SPF = 16.24 ± 0.67). The results indicated that the methanolic extract of guava leaf is a potential source of photoprotective agents with high SPF values of above 30, while chemical constituents in guava peel and pulp extracts exhibited a moderate photoprotective ability against UV radiations.

## Conclusion

The findings of this study revealed that the methanolic extract of guava leaves is rich in chemical constituents with DPPH free radical scavenging activity than that of guava pulp and peel extracts, indicating its higher antioxidant activity. Among the extracts of plant parts tested there are significantly high levels of phenolic compounds including flavonoids in guava leaves. In conformity with the results, guava peel, pulp and leaves are abundant in bioactive chemicals having antibacterial potential against the tested bacteria and fungus. The results further revealed that chemical constituents in guava leaves exhibited photoprotective properties with a high sun protection factor (SPF) value of above 30 while chemical constituents in guava pulp exhibited a moderate photoprotective ability against UV radiations. Based on the results it can be concluded that peel, pulp and mainly the leaves of *Psidium guajava* L. can be used as natural sources of ingredients for the development of sun protection cosmetics as they are rich in chemical constituents with both UV-absorption properties and antioxidant activity and further research should be carried out to isolate and identify bioactive phytochemicals present in it.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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