Investigation of *in vitro*Sunscreen Activity and Phytochemical Profile of *Flueggealeucopyrus* (Willd)

L.S. Perera[*], W.D. Ratnasooriya[**], R.N. Pathirana[**]

[*] School of Biomedical Science and Physiology, University of Wolverhampton, Wulfruna Street, Wolverhampton, WV1 1LY, UK.
[**]Department of Basic Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.

Abstract- This study was directed to evaluate the *in vitro* sunscreen potential of Sri Lankan medicinal plant - *Flueggealeucopyrus* (In Sinhala – Katupila, In Tamil – Mulluppulatti) (Family: Phyllanthaceae)using *in vitro* UV spectroscopic techniques and Mansur equation with 0.2 and 0.4 mg mL^{-1} methanolic extracts of the plant leaves. A methanol soluble fraction of Dermatone® (0.2 and 0.4 mg mL^{-1}) was used as the reference agent. The results revealed high absorbance levels of methanolic extract of the plant leaves at 290-320 nm (UVB) range and SPF (Sun Protection Factor) was determined as 14.63/ 27.04 respectively for the 0.2mg mL^{-1} and 0.4 mg mL^{-1} concentrations, which is a novel finding, while Dermatone® exhibited a SPF value of 14.03/ 25.32. It can be concluded that *F.leucopyrus* leaves have a high sun protection activity which is possibly mediated via antioxidant activity of existing phytochemicals; phenols, flavonoids, alkaloids and tannins which was revealed by the phytochemical screening.

Index Terms-antioxidants, *flueggealeucopyrus*, photo-protection, sun protection factor (SPF), sun protection, sunscreen, UV B rays

I. INTRODUCTION

Sunlight is the foundation of our existence. Since the beginning of the earth, it played a crucial part for the formation and evolution of life [1]. Sunlight is a part of the electromagnetic radiation which produced by sun. The fraction which reaches the earth can be divided into three categories, including ultraviolet radiation (UVR), visible (Vis) and infrared (IR) which occupies 6%, 52% and 42% respectively [2]. The stratospheric ozone layer absorbs the most energetic form of UVR before reaching the earth surface. This form is known as UVC (100 – 290 nm) [1], [3]. UVR which normally impact on the surface of earth include 5% of UVB (290 – 320 nm) and 95% UVA (320 – 400 nm) [1].

Human skin interacts with UVR in several ways. Initiation of cosmetic tanning is one of them [4]. Tanning by UVA does not involve with melanin production or photo-protection. Tanning by UVB provides photo-protection ability to the skin as it increases the melanin concentration [5]. This way, skin act as a natural sunscreen which protects the body from harmful UVR.

Further, as a major advantage of UV radiation, UVB induced of vitamin D production can be indicated. Over 90% of the vitamin D required for the human body is produced by a complex mechanism which involves bowel epithelium, liver, kidney and the skin [4]. Also, sunlight can be used as a treatment for some diseases and conditions (Heliotherapy) [6]. Some human skin diseases, such as atopic dermatitis and localized scleroderma, can be treated with Heliotherapy or artificial UV radiation (Phototherapy) [5]. Phototherapy has the ability to suppress multiple sclerosis independently of vitamin D synthesis [7]. Also it can be used to treat numerous diseases, including rickets [8], psoriasis [9], eczema [10] and neonatal jaundice [11].

UVB and UVA both have the ability to produce various free radicals in human skin cells. Nitric oxide (NO·), a gaseous free radical generate in skin by UVA exposure suppress the apoptosis in low concentrations [12]. It is also a good high blood pressure reliever and it indirectly influence on the transmittance of nerve signals [5]. NO· gas can be used as an antibacterial in burn injuries and chronic ulcers which indicate rapid wound healing properties [13], [14].

Excessive exposure to UVR can result acute and chronic conditions. In past few decades rate of skin cancer has been massively increased [15]. Free radicals which generates through UVB radiation reduces a considerable amount of the antioxidants present in skin. This decreases the ability of skin to protect itself from free radical generation [16] and it loses the fibrous tissue which can lead to wrinkling and photo-aging after a long term UVR exposure. This can lead to photodermatitis, actinic keratosis and skin cancer. According to the WHO Ultraviolet Radiation and the INTERSUN program, 2-3 million non-melanoma cancers and about 130,000 malignant melanomas are diagnosed annually. 66,000 deaths are recorded every year due to various types of skin cancer. [17].

UVA initiate the mass production of ROS (reactive oxygen species) and RNS (reactive nitrogen species). Hydroxyl ions which generate through a procedure called “Fenton reaction” can damage DNA massively. Various types of oxidative DNA lesions including altered DNA bases, single and double stranded breaks and DNA + protein crosslinks can be provoked by ROS and RNS due to the high diffusibility of DNA [3]. Cells that bear the DNA which cannot be recovered by base excision repair (BER) and nuclear excision repair (NER) go through apoptosis to prevent the spreading of the mutation. But some cells can escapes the mechanism and this can lead to malignancies [18].

To minimize these adverse effects caused by UVR, dermatologists recommend using a broadspectrum sunscreen which has a higher SPF.The term Sun Protection Factor (SPF)

which appears as a numberis a universal laboratory measure for the ability of photo-protection[19]. A perfect sunscreen should provide an even protection against a range of solar radiation including UVB and UVA [20]. It should also have "aesthetically pleasing composition and acceptable sensory and tactile profile that enhances user's compliance"[20], [21]. Ideally the sunscreen must be 100% photo-stable and dispel the absorbed energy without any harmful effects to the body. It should not penetrate the skin and reach cell's DNA and it should not produce free radicals in the skin[22] or any discomfort to the skin. In a cosmetic perspective, a sunscreen should be non-comedogenic, hypoallergenic and cosmetically elegant. These characteristics of an ideal sunscreen do not appear altogether in a single sunscreen that is in use today. Thereforeit is in high demand for a sunscreen which covers majority of these requirements [20], [21].

There are two types of sunscreens; organic and inorganic.Organic sunscreens are normally an aromatic compound consist with a carbonyl group. These structures protect the skin by absorbing low wavelengths of UVR[23]. Para-amino benzoic acid (PABA), cinnamates, salicilates, octocrylene, benzophenone and avobenzone are widely used organic sunscreens [23], [24]. Inorganic Sunscreenshave the ability to scatter and reflect the UVR. Minerals such as Titanium dioxide (TiO_2) and Zinc oxide (ZnO) are popular choices for inorganic sunscreens[23].

Both organic and inorganic sunscreens produce negative effects such as allergic reactions, staining the clothes, contact and/or irritant dermatitis, hypersensitivity, whitening effect, melanomas, skin cancers and they have the ability to reduce the production of vitamin D significantly [21], [23], [25]. And another point of particular important is that the DNA damage caused by TiO_2 (Titanium dioxide) when exposed to the sunlight. It has been proved that TiO_2 cause major damages to the cellular DNA. These damages can lead to mutations which will eventually turn into carcinogens[22].

Many studies have noted the opposing effects of currently available sunscreen substances [25]–[28]. Currently, there is a high demand for new substances which can be used as broad spectrum sunscreens. Just as much as every cosmetic product, it should be non-comedogenic, safe and consumer friendly [25]. Considering above requirements, choosing natural substances extracted from plants might lead to the novel findings of photo-protective agents. Plants have adaptations against damaging UVR such as producing antioxidants[19], [29]. Therefore, choosing plants which are rich sources of original biomoleculesfor antioxidant properties might give the best results as sun protective agents [19].

The main aim of this study is to investigate the sunscreen potential of leaves of *F. leucopyrus* (family Phyllanthaceae) which normally grows in the dry zone of Sri Lanka where the environmental conditions are hazardous and is also rich on antioxidant activity [30], [31]. The other objective is to investigate its phytochemical profile. Biomedically, the leaves are shown to promote wound healing [32] and possess anticancer [33], [34], aquaretic[35], antimicrobial[32], [36], antiproliferative and antioxidant activities [37].

II. MATERIALS AND METHODS

Collection of plant leaves

All leaves were collected from trees that are directly subjected to the sunlight from Madampe (North Western province, 7.4972° N, 79.8413° E)and Kelaniya (Western province 6.9520° N, 79.9186° E) in Sri Lanka during March 2016.

Identification and authentication

Leaves were identified and authenticated at the Department of Plant Sciences, University of Colombo, Sri Lanka. A voucher specimen (K001) is deposited at British College of Applied Studies, Colombo 06, Sri Lanka.

Preparation of the methanolic extract

Leaves were plucked and thoroughly washed in running tap water. Drained leaves were air dried and 300 g were separated. These 300 g samples was separated into three batches (100 g x 3) and oven dried at 40°C for 8 days until a constant weight was obtained.The dried leaves were cut into small pieces (approximately 2-3 mm^2 pieces) and 20g were macerated for 10 days in 200 ml of methanol (Sigma Aldrich Co, St. Louis, USA).They were stored in airtight containers in a cool dark place during the maceration process. The resulting dark green color extract was then filtered through the double layered muslin cloth. Then the filtrate was evaporated up to 100 mL at 65°C using a laboratory water bathand then was freeze dried. The solid product of the sample was re-dissolved in methanol to prepare 0.4 mg mL^{-1} and 0.2 mg mL^{-1} concentrations in quintuplets (x5). Same concentrations were made for the Dermatone® (reference drug).

Spectroscopic evaluation of SPF

Absorbance of UV radiation by the extracts of *F.leucopyrus* and Dermatone® were measured by a UV spectrophotometer at 290, 295, 300, 305, 310, 315 and 320 nm (UV-B) after an equilibration time of 1 hour at Zoology department ofUniversity of Colombo, Sri Lanka. 1 cm quarts cells were used and the whole procedure was done at room temperature (25°C) with five minute intervals. Cuvettes were washed withmethanol and cleaned by soft paper towels before adding a new sample. Every sample was loaded in to the cuvette by using a 1000 µl pipette. New pipette tip was introduced to each sample and the used were discarded. Methanol was used as the blank. All the data was recorded.

SPF values were determined using the Mansur equation which was proposed by J.S. Mansur in 1986 where EE (λ) = erythemal effect spectrum, I (λ) = solar intensity spectrum, Abs (λ) = absorbance of sunscreen product, CF = correction factor (=10). EE x I is a constant and predetermined by R. M. Sayre in 1979[25], [38]–[40].

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

Statistical analysis
Statistical analysis was done by comparing both*F.leucopyrus* and Dermatone®samples by Mann-Whitney U-test.

Phytochemical analysis of F.leucopyrusmethanolic extract
In order to identify the color changes of the reactions in phytochemical testing, chlorophyll was removed from the extract. [41]. After removing chlorophyll, the aqueous extract was turned into alight brown color. This was subjected to qualitative analysis for alkaloids, saponins, flavonoids, tannins, phenols, amino acids/ proteins and phytosterols[42].

III. RESULTS

The UV absorption spectrum of methanolic leaf extract of *F. leucopyrus* is shown in Figure 1. As shown, both concentrations of *F. leucopyrus* extract exhibited wide range of high absorbance between 290 – 320 nm. A similar trend of absorbance was evident with the two concentrations of Dermatone®.

As shown in Table 1, lower concentration of *F. leucopyrus* leaf extract exhibited a SPF value of 14.629±0.0192 and the high concentration SPF value of 27.042±0.0056. Corresponding values for the lower and higher concentrations ofDermatone®was 14.025±0.0313 and 25.315±0.0055respectively. SPF values of F. leucopyrus leaf extract and Dermatone® were not statistically significant. Furthermore, sun protection activity (in terms of SPF value) of *F. leucopyrus* and Dermatone® appears to be concentration related (dependent).

Table 1: Sun Protection Factor (SPF) of methanolic leaf extract of *F. leucopyrus* and Dermatone®

Methanolic Extract Tested	Concentration (mg/mL)	SPF value (±SEM)
F. leucopyrus	0.2	14.629±0.0192
	0.4	27.042±0.0056
Dermatone®	0.2	14.025±0.0313
	0.4	25.315±0.0055

Photochemical analysis of *F.leucopyrus* revealed the presence of phenols, flavonoids, alkaloids, amino acids, phytosterols and tannins.Saponins were absent in the extract.

IV. DISCUSSION

During the past few decades, knowledge of adverse effects of UVR on human skin has significantly increased.To prevent these adverse effects of solar radiation, dermatologists recommend using a broad spectrum sunscreen with a SPF above 15 as a part of the "Photo-protection strategy" [20]. As photo-protection became important, US Food and Drug Administration (FDA) changed the status of sunscreens to "Over the counter" (OTC) drugs[21]. Commercially available sunscreens which are composed of organic and inorganic UV filters have opposing side effects too and they are relatively expensive[25]. Majority of these products increase the risk of skin cancers than preventing them. Some compounds interact with vitamin D production and cause contact sensitivity and hives, while others cause irritants, allergies and skin whitening[23], [24]. Therefore formulations of herbal non-comedogenic, cheap and safe sunscreens are in high demand. Sunscreening potential of an agent is evaluated in terms of SPF value [21]. Higher the SPF value greater is the sunscreening activity [43]. SPF values are determined both by *in vivo* and *in vitro* techniques. *In vivo* studies have revealed that sunscreens with SPF values of 15, 30 and 60 absorb 93.3%, 96.7% and 98.3% of erythemogenic UVR respectively[43]

This study investigated the sun protection activity of *F.leucopyrus*(Willd) *in vitro* by UV spectroscopic method (290 – 320 nm). This method allows the determination of sun protection factor which is the numerical value for the efficacy of sunscreen. Using UV spectroscopic method and Mansur equation is a quick, reliable, inexpensive and widely used method for evaluating sun protection activity*in vitro*.Previous studieshave also proved the reliability of this spectroscopic method. [25], [38], [39]. In contrast generally, *in vivo* techniques are expensive, time consuming, troublesome, produce variable results and also involve ethical issues [25].

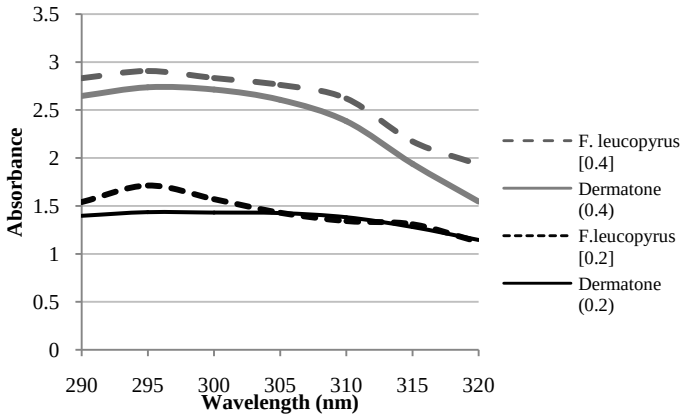

Figure 1: Absorption spectrum of methanolic leaf extract of F.leucopyrus and Dermatone®

The results of this *in vitro* study showed, for the first time, that methanolic leaf extract of Sri Lankan version of *F. leucopyrus* has marked sunscreening activity; 0.2 mg/mL, SPF value 14.629 and 0.4 mg/mL, SPF value 27.042. This was comparable to the SPF values of Dermatone®, reference agent; 0.2 mg/mL, SPF value 14.025 and 0.4 mg/mL, SPF value 25.315. Thus, the SPF values of *F. leucopyrus* even at low concentrations are above the threshold value of a good sunscreen; dermatologists strongly recommend to use topical sunscreens having SPF value of 15 or more, preferably year around to minimize harmful effects resulting from overexposure to sun's UV rays particularly UV B rays [21], [44], [45]. Further, the leaf extract of *F. leucopyrus* exhibited a wide range of absorbance (between 290 -320 nm) with a mild peak at 295 nm (in the UV B region). This will enhance the sunscreening potential of *F. leucopyrus* leaf extract further. Wider the range of absorbance of a sunscreen agent/ formulation higher would be its effectiveness in inhibiting

sunburns (erythema) [43], [46]. Experimentally,*F. leucopyrus* leaves have been shown to possess strong anticancer properties [34], [47] and it is used in Sri Lankan traditional medicine to treat cancer [47]. This property should undoubtedly increase the potential of *F. leucopyrus* leaves as a sunscreen since some sunprotectives are known to induce skin cancers/ melanomas [22].

As UVB facilitate the production of free radicals such as OH·, O2·and HOO·[48], [49], it can reduces a considerable amount of the antioxidants present in skin[1], [16], [49]. Producing high levels of antioxidants is an adaptation of plants to the damaging UVR[19]. In order to find herbal photo-protective agents, research has been focused on their antioxidant activity[50]. Antioxidants are claimed to confer sun protection [51] and some sunscreen are formulated with antioxidants like Vitamin E [44]. High antioxidant properties of *F.leucopyrus*was revealed using inhibition of DPPH radical scavenging and 2-deoxy-D-ribose degradation assay[37]which could explain the photo-protective abilities of this plant.Antioxidant activities of plants are mediated via phyto-constituents such as phenolics; flavonoids/ tannins/ alkaloids and vitamin C[52]–[55]. This study revealed the presence of phenols, flavonoids and tannins in the methanolic extraction of *F.leucopyrus* along with phytosterols, amino acids and alkaloids. Thus the sunscreening activity of *F. leucopyrus* is likely to be mediated via synergistic antioxidant activities of phenolics, flavonoids and tannins. Presence of these phyto-constituents and the antioxidant properties of *F.leucopyrus*,shown by previous studies [37], might contribute to the high sun protective properties found on this study.

V. CONCLUSION

In conclusion this study shows for the first time, considerable *in vitro* sunscreen activity of the methanolic leaf extract of Sri Lankan variety of *F. leucopyrus*. This activity together with its capacity to absorb wide range of UV rays, anticancer, antioxidant properties, makes it an ideal candidate to develop a novel, cheap, efficacious and cosmetically elegant sunscreen formulation.

ACKNOWLEDGEMENT

The author acknowledges the contribution of everyone who guided and encouraged throughout this study; in particularly, Prof. W.D. Ratnasooriya and Prof. R.N. Pathirana and Ms. Surangi Fernando who provided their insight and expertise. Special thanks go to Dr.Inoka Perera,Dr.SujeewaHettihewa, Dr.CharithaGoonasekara and Mr. Chamara De Zoysafor sharing their pearls of wisdom that greatly assisted this research.

REFERENCES

[1] R. J. B. T.G. Polefka, T.A. Meyer, P.P. Agin, “Effects of solar radiation on the skin.,” *J. Cosmet. dermatologu*, vol. 11, no. 2, pp. 134–143, 2011.

[2] M. Ichihashi, H. Ando, M. Yoshida, Y. Niki, and M. Matsui, “Photoaging of the skin,” *anti aging Med.*, vol. 6, no. 6, pp. 46–59, 2009.

[3] A. Svobodová and J. Vostálová, “Solar radiation induced skin damage: review of protective and preventive options.,” *Int. J. Radiat. Biol.*, vol. 86, no. 12, pp. 999–1030, 2010.

[4] M. K. B. Bogh, “Vitamin D production after UVB: aspects of UV-related and personal factors.,” *Scand. J. Clin. Lab. Invest. Suppl.*, vol. 243, no. Suppl 243, pp. 24–31, 2012.

[5] A. Juzeniene and J. Moan, “Beneficial effects of UV radiation other than via vitamin D production,” *Dermatoendocrinol.*, vol. 4, no. 2, pp. 109–117, 2012.

[6] R. Roelandts, “The history of phototherapy: Something new under the sun?,” *J. Am. Acad. Dermatol.*, vol. 46, no. 6, pp. 926–930, 2002.

[7] P. Sundström and J. Salzer, “Vitamin D and multiple sclerosis-from epidemiology to prevention,” *Acta Neurol. Scand.*, vol. 132, no. S199, pp. 56–61, 2015.

[8] M. F. Holick, “Resurrection of vitamin D and rickets,” *J. Clin. Invest.*, vol. 116, no. 8, pp. 2062–2072, 2006.

[9] F. Almutawa, N. Alnomair, Y. Wang, I. Hamzavi, and H. W. Lim, “Systematic review of UV-based therapy for psoriasis,” *Am. J. Clin. Dermatol.*, vol. 14, no. 2, pp. 87–109, 2013.

[10] T. Gambichler, N. Othlinghaus, N. S. Tomi, T. Holland-Letz, S. Boms, M. Skrygan, P. Altmeyer, and A. Kreuter, “Medium-dose ultraviolet (UV) A1 vs. narrowband UVB phototherapy in atopic eczema: A randomized crossover study,” *Br. J. Dermatol.*, vol. 160, no. 3, pp. 652–658, 2009.

[11] M. J. Maisels, B. Ch, A. F. Mcdonagh, and D. Ph, “Phototherapy for Neonatal Jaundice,” *Lancet*, vol. 295, no. 7651, pp. 825–826, 1970.

[12] R. Weller, “Nitric oxide: A key mediator in cutaneous physiology,” *Clin. Exp. Dermatol.*, vol. 28, no. 5, pp. 511–514, 2003.

[13] A. B. Shekhter, V. A. Serezhenkov, T. G. Rudenko, A. V. Pekshev, and A. F. Vanin, “Beneficial effect of gaseous nitric oxide on the healing of skin wounds,” *Nitric Oxide - Biol. Chem.*, vol. 12, no. 4, pp. 210–219, 2005.

[14] A. Ghaffari, C. C. Miller, B. McMullin, and A. Ghahary, “Potential application of gaseous nitric oxide as a topical antimicrobial agent,” *Nitric Oxide - Biol. Chem.*, vol. 14, no. 1, pp. 21–29, 2006.

[15] Y. Matsumura and H. N. Ananthaswamy, “Toxic effects of ultraviolet radiation on the skin,” *Toxicol. Appl. Pharmacol.*, vol. 195, no. 3, pp. 298–308, 2004.

[16] A. Svobodova, D. Walterova, and J. Vostalova, “Ultraviolet light induced alteration to the skin.,” *Biomed. Pap. Med. Fac. Univ. Palack??, Olomouc, Czechoslov.*, vol. 150, no. 1, pp. 25–38, 2006.

[17] WHO, “WHO | World Health Organization,” *WHO*, 2016.

[18] G.-Y. Liou and P. Storz, “Reactive oxygen species in cancer.,” *Free Radic. Res.*, vol. 44, no. 5, pp. 479–96, 2010.

[19] M. Hupel, N. Poupart, and E. Ar Gall, “Development of a new in vitro method to evaluate the photoprotective sunscreen activity of plant extracts against high UV-B radiation,” *Talanta*, vol. 86, no. 1, pp. 362–371, 2011.
[20] A. Shehzad, S. Khan, and I. Hussain, “Sunscreens : some practical issues,” *J. Pakistan Assoc. Dermatologists*, vol. 24, no. 1, pp. 1–3, 2014.
[21] A. Abraham and S. Kaimal, “Sunscreens,” *Indian J. Dermatology, Venereol. Leprol.*, vol. 77, no. 2, p. 238, 2011.
[22] N. Serpone, D. Dondi, and A. Albini, “Inorganic and organic UV filters: Their role and efficacy in sunscreens and suncare products,” *Inorganica Chim. Acta*, vol. 360, no. 3, pp. 794–802, 2007.
[23] R. Rai and C. R. Srinivas, “Photoprotection.,” *Indian J. Dermatol. Venereol. Leprol.*, vol. 73, no. 2, pp. 73–9, 2007.
[24] K. L. Granger and P. R. Brown, “the Chemistry and Hplc Analysis of Chemical Sunscreen Filters in Sunscreens and Cosmetics,” vol. 24, no. 19, pp. 2895–2924, 2001.
[25] W. D. Ratnasooriya, R. N. Pathirana, A. S. Dissanayake, B. L. C. Samanmali, and P. K. Desman, “Evaluation of invitro sun screen activities of salt marshy plants Suaeda monoica , Suaeda maritima and Halosarcia indica,” vol. 5, no. 2, pp. 15–20, 2016.
[26] M. S. Díaz-Cruz and D. Barceló, “Chemical analysis and ecotoxicological effects of organic UV-absorbing compounds in aquatic ecosystems,” *TrAC - Trends Anal. Chem.*, vol. 28, no. 6, pp. 708–717, 2009.
[27] M. Silvia Díaz-Cruz, M. Llorca, D. Barceló, and D. Barceló, “Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment,” *TrAC - Trends Anal. Chem.*, vol. 27, no. 10, pp. 873–887, 2008.
[28] D. L. Giokas, A. Salvador, and A. Chisvert, “UV filters: From sunscreens to human body and the environment,” *TrAC - Trends Anal. Chem.*, vol. 26, no. 5, pp. 360–374, 2007.
[29] M. Ali, R. Enayatifard, M. Khalili, and M. Ghaffarloo, “Correlation between Sun Protection Factor and Antioxidant Activity , Phenol and Flavonoid Contents of some Medicinal Plants,” vol. 13, no. May 2013, pp. 1041–1047, 2014.
[30] G. H. Krause, C. Schmude, H. Garden, O. Y. Koroleva, and K. Winter, “Effects of Solar Ultraviolet Radiation on the Potential Efficiency of Photosystem II in Leaves of Tropical Plants1,” *Plant Physiol.*, vol. 121, no. December, pp. 1349–1358, 1999.
[31] G. H. Krause, A. Gallé, R. Gademann, and K. Winter, “Capacity of protection against ultraviolet radiation in sun and shade leaves of tropical forest plants,” *Funct. Plant Biol.*, vol. 30, no. 5, pp. 533–542, 2003.
[32] T. Dudhamal, S. Gupta, V. Mahanta, and A. Ajmeer, “Katupila Securinega leucopyrus as a potential option for diabetic wound management,” *J. Ayurveda Integr. Med.*, vol. 5, no. 1, p. 60, 2014.
[33] A. S. Mendis, I. Thabrew, S. R. Samarakoon, and K. H. Tennekoon, “Modulation of expression of heat shock proteins and apoptosis by Flueggea leucopyrus (Willd) decoction in three breast cancer phenotypes,” *BMC Complement. Altern. Med.*, vol. 15, no. 1, p. 404, 2015.
[34] S. D. E. Fernando, W. S. Karunaratne, D. B. M. Wickramarathne, W. A. R. T. Wickramarachchi, E. W. M. A. Ekanayake, and V. Thevanesam, “Flueggea Leucopyrus (Katupila) Showing Anti-Tumor Activity Against Plant Tumors Using Agrobacterium,” vol. 18, p. 2014, 2014.
[35] N. U. Ellepola, S. A. Deraniyagala, W. D. Ratnasooriya, and K. Perera, “Aqueous extract of flueggea leucopyrus increases urine output in rats,” *Trop. J. Pharm. Res.*, vol. 14, no. 1, pp. 95–101, 2015.
[36] L. M. Bakshu, A. J. Ram, and R. R. V. Raju, “Antimicrobial activity of Securinega leucopyrus,” *Fitoterapia*, vol. 72, no. 8, pp. 930–933, 2001.
[37] P. Soysa, I. S. De Silva, and J. Wijayabandara, “Evaluation of antioxidant and antiproliferative activity of Flueggea leucopyrus Willd (katupila).,” *BMC Complement. Altern. Med.*, vol. 14, no. 1, p. 274, 2014.
[38] E. A. Dutra, D. Almança, E. R. M. Kedor-, M. Inês, and R. Miritello, “Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry,” *Brazilian J. Pharm. Sci.*, vol. 40, no. 3, pp. 381–385, 2004.
[39] C. Malsawmtluangi, D. K. Nath, I. Jamatia, E. Z. Lianhimgthangi, and L. Pachuau, “Determination of Sun Protection Factor (SPF) number of some aqueous herbal extracts,” *J. Appl. Pharm. Sci.*, vol. 3, no. 9, pp. 150–151, 2013.
[40] R. M. Sayre, P. P. Agin, G. J. LeVee, and E. Marlowe, “A COMPARISON OF IN VIVO AND IN VITRO TESTING OF SUNSCREENING FORMULAS,” *Photochem. Photobiol.*, vol. 29, no. 3, pp. 559–566, Mar. 1979.
[41] P. J. Houghton and A. Raman, *Laboratory handbook for the fractionation of natural extracts*. Chapman & Hall, 1998.
[42] N. R. Farnsworth, “Biological and Phytochemical Screening of Plants,” *J. Pharm. Sci.*, vol. 55, no. 3, pp. 225–276, Mar. 1966.
[43] D. Reinau, U. Osterwalder, E. Stockfleth, and C. Surber, “The meaning and implication of sun protection factor,” *Br. J. Dermatol.*, vol. 173, no. 5, p. 1345, 2015.
[44] J. Kockler, M. Oelgemöller, S. Robertson, and B. D. Glass, “Photostability of sunscreens,” *J. Photochem. Photobiol. C Photochem. Rev.*, vol. 13, no. 1, pp. 91–110, 2012.
[45] L. Araújo, S. Curty, A. Moreira, A. Rossi, N. Raposo, U. Vaz, and H. Polonini, “Development of Broad-Spectrum Natural Sunscreens using Combinations of five Plant Species,” vol. 8, no. 2, pp. 144–148, 2016.
[46] L. Mbanga, M. Mulenga, P. T. Mpiana, K. Bokolo, M. Mumbwa, and K. Mvingu, “Determination of Sun Protection Factor (SPF) of Some Body Creams and Lotions Marketed in Kinshasa by Ultraviolet Spectrophotometry,” *Int. J. Adv. Res. Chem. Sci.*, vol. 1, no. 8, pp. 7–13, 2014.
[47] S. R. Samarakoon, S. B. Kotigala, I. Gammana-Liyanage, I. Thabrew, K. H. Tennekoon, A. Siriwardana,

and P. B. Galhena, “Cytotoxic and apoptotic effect of the decoction of the aerial parts of Flueggea leucopyrus on human endometrial carcinoma (AN3CA) cells,” *Trop. J. Pharm. Res.*, vol. 13, no. 6, pp. 873–880, 2014.

[48] V. T. Natarajan, P. Ganju, A. Ramkumar, R. Grover, and R. S. Gokhale, “Multifaceted pathways protect human skin from UV radiation.,” *Nat. Chem. Biol.*, vol. 10, no. 7, pp. 542–51, 2014.

[49] K. Jung, M. Seifert, T. Herrling, and J. Fuchs, “UV-generated free radicals (FR) in skin: Their prevention by sunscreens and their induction by self-tanning agents,” *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, vol. 69, no. 5, pp. 1423–1428, 2008.

[50] R. Korać and K. Khambholja, “Potential of herbs in skin protection from ultraviolet radiation,” *Pharmacogn. Rev.*, vol. 5, no. 10, p. 164, 2011.

[51] Y. Wu, M. S. Matsui, J. Z. S. Chen, X. Jin, C. M. Shu, G. Y. Jin, G. H. Dong, Y. K. Wang, X. H. Gao, H. D. Chen, and Y. H. Li, “Antioxidants add protection to a broad-spectrum sunscreen,” *Clin. Exp. Dermatol.*, vol. 36, no. 2, pp. 178–187, 2011.

[52] M. Kopjar, M. Orsolic, and V. Pilizota, “Anthocyanins, phenols, and antioxidant activity of sour cherry puree extracts and their stability during storage,” *Int. J. Food Prop.*, vol. 17, no. 6, pp. 1393–1405, 2014.

[53] A. Czyzowska, E. Klewicka, E. Pogorzelski, and A. Nowak, “Polyphenols, vitamin C and antioxidant activity in wines from Rosa canina L. and Rosa rugosa Thunb.,” *J. Food Compos. Anal.*, vol. 39, pp. 62–68, 2015.

[54] H. S. Chung and J. C. Shin, “Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (Oryza sativa cv. Heugjinjubyeo),” *Food Chem.*, vol. 104, no. 4, pp. 1670–1677, 2007.

[55] W. H. Wong, W. X. Lee, R. N. Ramanan, L. H. Tee, K. W. Kong, C. M. Galanakis, J. Sun, and K. N. Prasad, “Two level half factorial design for the extraction of phenolics, flavonoids and antioxidants recovery from palm kernel by-product,” *Ind. Crops Prod.*, vol. 63, pp. 238–248, 2015.

AUTHORS

First Author – L.S. Perera, University of Wolverhampton, UK (lumzi.perera@gmail.com)
Second Author – Prof. W.D. Ratnasooriya, Kotelawala Defence University, Sri Lanka. (rmedchem@gmail.com)
Third Author – Prof. R.N. Pathirana,Kotelawala Defence University, Sri Lanka. (rmedchem@gmail.com).

Correspondence Author – L.S. Perera, University of Wolverhampton, UK (lumzi.perera@gmail.com) +94715674953