Research Article

Phytochemical and *In Vitro* Sun Protection Factor Evaluation of *Peltophorum Pterocarpum* Leaf Extracts

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ABSTRACT

The medicinal plants are an important source of inexpensive and practical drugs for people throughout the world. Prolong exposure to UV radiation may initiate the production of reactive oxygen species, which causes oxidative injury and impairment of the antioxidant system. These injuries impair the metabolic pathways. Therefore the present study has been planned to evaluate *in vitro* flavonoid content and SPF of aqueous and ethanolic extracts of the *P. Pterocarpum.* The aqueous and ethanolic extracts were determined for flavonoid content, found to be 10mg/gm and 15mg/gm equivalent of quercetin. The cream and gel formulations were prepared for the extracts and tested for various parameters, further evaluated for Sun protection factor determination where the results were found to be 26.8, 34.7 and 9.70 for cream formulations followed by 15.6,16.8 and 100 for gel formulations of aqueous, ethanolic extracts compared to marketed product respectively. The ethanolic extract was more offering sun protection than aqueous extract and the responsible compounds were attributed to be flavonoids. Further phyto chemical screening is necessary to establish the phytochemical component responsible for the activity.

Keywords: *P. pterocarpum*, sunscreen, UV radiation.

INTRODUCTION

The medicinal plants are an important source of inexpensive and practical drugs for people throughout the world. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants[1]. Sunlight composed of various wavelengths ranging from ultraviolet light through infrared to visible light. The solar spectrum radiation of the sun is divided into five regions: Ultraviolet C or UV-C (from 100 nm to 290 nm), Ultraviolet B or UV-B (from 290 nm to 320 nm), Ultraviolet A or UV-A (from 320 nm to 400 nm), visible (from 380 nm to 780 nm) and infrared (from 780 o nm t106 nm)[2]. In winter high proportion of UV- radiation is reflected than in summer[3, 4]. Prolong exposure to UV radiation may initiate the production of reactive oxygen species, which causes oxidative injury and impairment of the antioxidant system. These injuries impair the metabolic pathways of the skin, which lead to photoaging, erythema, edema, sunburn, lines, wrinkles, photosensitivity, immunosuppression, DNA damage as well as skin cancer in most severe conditions[5].

UVA radiation reaches the deeper layer of the epidermis and dermis, provokes the premature aging (photoaging), of the skin by causing damage to the elastic and collagen fibers of the connective tissue of the skin[6]. UVB is responsible for the skin damage due to sunburn which brings about acute inflammation (sunburn) and intensification of photo-aging[6]. The most biologically damaging radiation UV-C being the shorter wavelength has been filtered out by the ozone layer. Sunscreens and sunblocks are the two chemicals that absorb or block UV rays of sunlight. Therefore sunscreen compounds are generally incorporated in many cosmetic formulations such as creams, lotions, moisturizers and other skin care products [7]. The main purpose of sunscreen is to protect the skin against UV-A and UV-B rays (sunburn and photoaging), conserve the moisture content of skin and its own natural oils, which may be lost during the exposure of solar radiation[8]. They also help in absorbing the portion of erythema on the skin caused by radiation energy of the sun. The Sun Protection Factor (SPF) is a numerical rating system to indicate the degree of protection provided by a sun care product like sunscreen[9]. SPF is defined as the ratio of minimal erythema dose (MED) of solar radiation measured in the presence and in the absence of a sunscreen agent[10].The MED is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce a minimal, perceptible erythema on the unprotected skin[11]. Most recently updated scientific method for evaluating the SPF of sunscreens has been developed by COLIPA (*The Comité de Liaison de la Parfumerie in Europe)* internationals.

The sunscreen should be capable of absorbing wavelength at the range of 280-450 nm, stable to withstand heat, light, and perspiration, should not be readily absorbed by the skin, protective, chemically inert, non-irritating, non-toxic[12]. There are several agents

available from both synthetic and natural sources with UV-filtering properties[13]. Synthetic UV filters are known to have potential toxicity in humans and also showed the ability to interfere only in selected pathways of multistage process of carcinogenesis[14]. Although most sunscreen products contain synthetic photoprotective agents of a high sun protection factor (SPF), in contrast, herbal botanical sunscreens are safe, widely accepted by consumers and also work in various ways, playing multiple roles in ameliorating the process of carcinogenesis[15]. Flavonoids such as quercetin, luteolin, and catechins are better antioxidants, were reported to be effective in UVA and UVB range[16]. Basic and applied research concerning sun protection has become a major concern.

Peltophorum pterocarpum (*DC.) Baker ex Heyne*, a traditional medicinal plant, geographically distributed in the regions of Australia, Bangladesh, Cambodia, India, Indonesia, Malaysia, Myanmar, Singapore, Srilanka, Thailand, Vietnam (www.worldagroforestry.org). The traditional healers use the leaves in the form of decoction for treating skin disorders[17]. The methanolic extract of flowers was studied for antimicrobial activity [18]. Leaves and flower extracts were studied for antioxidant activity[19].

The plant was studied for cytotoxic activity[20]. The leaf, bark, flower, and pod were studied for antiglycemic activity[21]. *In vitro,* antiurolithiatic studies were carried out on leaves[22]. Cycloisosativene, viridiflorol, Cordinol, valerenal from stem extracts[17], naringenin, ophioglonin, kaempferol, isorhamnetin, luteolin, chrysoeriol, quercetin,pachypodol from leaf extracts[23], β-Sitosterol, campesterol, stigmasterol[24], lupeol, naringenin-7-glucoside, (*E*, *E*)-terrestribisamide from flower extracts[20], lysine, valine, leucine, methionine. alanine from seed extracts[25], quercetin, rhamnetin, melanoxetin, meeting, and propelargonidin from fruit extracts[26], and linoleic acid, linolenic acid, oleic acid, palmitic acid from seed oil[27]. Review of the literature suggests that there were no scientific reports available on the plant *P. pterocarpum* for the determination of SPF value. Therefore the present study has been planned to evaluate *in vitro* flavonoid content and SPF of aqueous and ethanolic extracts of the *P. Pterocarpum.*

MATERIALS AND METHODS

The Plant material leaves of *Peltophorum pterocarpum* were collected in the month of January during the afternoon from the grounds of Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, and Vijayawada. The herbarium was prepared and the sample was authenticated by Dr. D.T. P. SatyanarayanaRaju, plant taxonomist, Department of Botany and Microbiology, AcharyaNagarjunaUniversity, Guntur, specimen no VIPW 09 was deposited in the Department of Pharmacognosy & Phytochemistry, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada. The photographs of the plant and leaves were depicted in fig 1 and fig 2. The photographs of dried leaves and leaf powder of *P. pterocarpum* were depicted in figure 3 and figure 4.

Figure 1: *Peltophorum pterocarpum*

Figure 2: Twig of *P.pterocarpum*

Figure 3: Dried leaves of *P.pterocarpum*

Figure 4: Dried leaf powder of *P. pterocarpum*

The powder was subjected to Soxhlet extraction by using distilled water and ethanol (Merck) as solvents. The crude extract was dried using a vacuum pump (Biotech) and weighed. The photographs displaying extraction using Soxhlet apparatus (JSGW) for the dried leaf powder of *Peltophorum pterocarpum* were given in figure 5 and extractive values were presented in table 3[28, 29, 30].

The aqueous and ethanolic extracts of the leaves of *Peltophorum pterocarpum* were screened for the phytochemical constituents according to the standard methods. The results were given in table 4[28, 29, 31].

Figure 5: Soxhlation of *P. pterocarpum* leaf using water and ethanol

Quantitative determination of flavonoids

The extract (1.5 ml) was added to 1.5 ml of 2% methanolic $AlCl_3$ (Finar) solution. The mixture was vigorously shaken on a centrifuge (Lab India), for 5 minutes at 200 rpm and the absorbance was read at 367 nm after 10 minutes of incubation (Biotech). Quercetin (Sigma-Aldrich) was used as a standard for the calibration curve (figure 6). The assay was carried out in triplicate[32].The results were calculated by the given formula, data was produced in table 5 and figure 6.

$$C = c.V/m$$

C – Total phenolic compounds mg/g of plant extract;c – The concentration of standard established from the calibration curve mg/ml; V – The volume of extract in ml; m -The weight of pure plant extract

***In vitro* sun protection factor determination**

Formulation of herbal sunscreen cream

The oil phase of cream was prepared by heating the ingredients (cetostearyl alcohol, stearic acid, cetomacrogol-100, lanolin, and glycerin) at 75°C±2 with constant stirring using hot plate (JSGW) while, for the preparation of aqueous phase purified water was heated separately in 2000 ml capacity beaker at 80°C±2. To this methyl and propyl parabens were added, dissolved with occasional stirring and temperature was brought to 75°C±2. The two phases (oil and aqueous) were mixed together with vigorous stirring for about 1-2 minutes. Finally, the leaf extracts were added with constant stirring till a thick cream is formed. The temperature was further reduced to around 45°C using cold-water bath and stirring was discontinued. The cream was stored in a wide mouth airtight amber colored glass container and stored in cool dry place. The formula for the preparation of herbal sunscreen cream was given in table 1. The herbal sunscreen cream was prepared by using aqueous and ethanolic extracts and commercial herbal cream were displayed in figure 7 and figure 8[33].

Formulation of herbal sunscreen gel

To a few ml of water, methylparaben and propylparaben were added and dissolved completely. Carbopol 940 was added to the paraben solution and stirred well using mechanical stir. To this glycerine and triethanolamine were added and stirred. The extracts were added to the above mixture and stirred continuously until a uniform mixture was obtained. Sodium hydroxide was used to adjust the pH between 6.8 -7. The herbal sunscreen gel prepared by using aqueous and ethanolic extracts and commercial herbal gel were displayed in figure 9 and figure 10[34].

Table 1: Formulation of herbal sunscreen cream

S. No	Ingredients	Uses	Components (%w/w)
1.	Cetostearyl alcohol	Emulsifier	35
2.	Stearic acid	Emollient, Co-emulsifier	40
3.	Cetomacrogol-100	Emulsifier	9
4.	Lanolin	Emollient	50
5.	Glycerin	Humectant	156.6
6.	Methylparaben	Preservative	4
.7.	Propylparaben	Preservative	0.4
8.	Plant extract	Active ingredient	10
9.	Distilled water	Vehicle	6.95

Evaluation of herbal sunscreen cream and gel

Organoleptic Evaluation The color was observed visually against a dark background. Colour, appearance, and odor were evaluated manually. The results were given in table 6and table 7 for cream and gel formulations respectively[35,33,36].

pH measurement

1 g of cream was dispersed in 9 ml of distilled water to determine the pH at 27°C using the pH meter (Biotech) the results were given in table 6 and table 7[36,34,37].

Viscosity

Viscosity of the cream was found out using Ostwald viscometer. The results were given in table 6 and table 7 [32].

Consistency

The consistency of the product was evaluated manually. The results were given in table 6 and table 7 respectively for cream and gel formulations[36, 34, 37].

Grittiness

The cream was spread on the palm to find if any gritty particles are present. The result was given in table 6 [35, 33, 36].

Washability

The cream was applied on the hand and was washed by keeping the hand under running water. The result was given in table 6 [35,33,36].

Spreadability of cream formulation

The parallel plate method is a most widely used method for determining the spreadability of semisolid preparations. A modified laboratory apparatus was used to evaluate spreadability. The setup consists of two glass slides placed on a tripod stand on which excess of cream (3 g) was applied in between two glass slides. The upper slide is movable and the lower slide was firmly fixed to the stand. 100 g weight was placed on them for 5 minutes to compress the cream to a uniform thickness and the excess cream was scrapped off from the edges. Then 50 g weight was added to one side of the slide and the slide was pulled till it covers a distance of 10 cm. The time in seconds required to separate two glass slides by 10 cm was taken as a measure of spreadability. A shorter interval indicates better spreadability. Spreadability was calculated by using the formula given below. The results were given in table 6[35].

$$S=m.l/t$$

Where, S=Spreadability; m=Weight tied to upper glass slide;l=Length of glass slide; t= Time taken to separate them.

Table 2: Formulation of herbal sunscreen gel

S. No	Ingredients	Quantity	Uses
1.	Plant extract	1%	Sunscreen
2.	Carbopol 940	25	Gelling agent
3.	Methyl paraben	0.1%	Preservative
4.	Propyl paraben	0.1%	Preservative
5.	Triethanolamine	2%	Neutraliser
6.	Propylene glycol(glycerine)	2%	Humectant
7.	Water	Qty sufficient	Vehicle

Spreadability of gel formulation: Two glass plates were selected. The gel was spread on one side of the slide and the other slide is placed on top of it like a sandwich. The slides are fixed to allow the upper slide to slip off. The time required for the slide to get separated is calculated using the formula. The results were given in table 7[33, 36].

$$S=m.l/t$$

Where, S= Spreadability; l= length of the glass plate, m=Weight tied to upper plate; T= Time taken for the two plates to get separated

Determination of SPF of herbal sunscreen cream formulation

1.0 gm of cream formulation and commercial cream was weighed, transferred to 100 ml volumetric flask, diluted to volume with ethanol and water (40:60), and then ultrasonication for 5minutes, after that filtered through Whatman No. filter paper and filtrate was collected by rejecting the first 10 ml of the filtrate. 5.0 ml of the aliquot was taken in 50 ml volumetric flask and diluted to volume with ethanol and water (40:60). Subsequently, 5.0 ml of the aliquot was transferred to 25 ml volumetric flask and the volume completed with ethanol and water (40:60). The absorbance values of each aliquot prepared were determined from 290 nm to 320 nm at the 5nm interval, using ethanol and distilled water (40:60) solution as a blank. The readings were taken in triplicate and the determinations were made at each point. The obtained absorbance values of 290 nm to 320 nm were multiplied with the respective EE (λ) values. Their summation was taken and multiplied with the correction factor (10) to obtain the SPF values. Data were expressed as ± standard error mean. The results of the screening of UV protective activity were given in table 8[38].

$$SPF_{Spectrophotometric=} CF*320\sum 290\ EE(\lambda)*I(\lambda)*abs(\lambda)$$

Where, CF= Correction factor; EE (λ) = Erythmogenic effect of radiation with wavelength λ, abs (λ) = spectrophotometric absorbance values at wavelength λ; The values of EE×I are constant.

Determination of SPF of herbal sunscreen gel formulation

50 g of the gel was taken and was dissolved in isopropanol and water at 50:50 ratios. The sample was taken in 1cm quartz cuvettes and was placed in the UV spectrophotometry (Lab India) and the spectrum was recorded from 400-200 nm. The same nm was used to find the spectrum of the extracts. The results of the screening of UV protective activity were given in table 9[39, 40, 41].

$$SPFSpectrophotometric= CF*320\sum 290\ EE\ (\lambda)*I\ (\lambda)*abs(\lambda)$$

Where, C.F=Correction factor; E.E= erythemal effect spectrum; I= Solar intensity spectrum, the higher the SPF factor, the greater the efficiency of the product.

RESULTS AND DISCUSSION

The results of extraction, qualitative screening, quantitative determination and *In vitro* sun protection factor activity for cream and gel formulations of *Peltophorum pterocarpum* leaf extracts were presented as below.

Phytochemical study

Extraction

Table 3: Extractive values of AQLPP & ELPP

S. No.	Yield of AQLPP (% w/w)	Yield of ELPP (% w/w)
1.	10	13.33

Note: AQLPP, ELPP- Aqueous extract of leaves of *Peltophorum pterocarpum,* ethanolic extract of leaves of *Peltophorum pterocarpum.*

The percentage yield of extractive value was found to be 10 % w/w.

Qualitative preliminary phytochemical screening

Table 4: Qualitative phytochemical screening of aqueous and alcoholic extracts of *Peltophorum pterocarpum leaves*

S. No.	Phytochemical	AQLPP	ELPP
1.	Alkaloids	+	+
2.	Tannins & Flavonoids	+	+
3.	Saponins	+	+
4.	Steroids	+	_
5.	Triterpens	+	+

Note: AQLPP, ELPP- Aqueous extract of leaves of *Peltophorum pterocarpum,* ethanolic extract of leaves of *Peltophorum pterocarpum.*

The qualitative preliminary phytochemical screening of aqueous and alcoholic leaf extracts of *Peltophorum pterocarpum* revealed the presence of alkaloids, tannins, and flavonoids, saponins, steroids, and triterpenoids respectively.

Quantitative Determination

Table 5: Quantitative Phytochemical screening of aqueous and alcoholic extracts of *Peltophorum pterocarpum* leaves

S. No.	Phytochemical	AQLPP	ELPP
1.	Flavanoids (mg/g)	10±0.1	15±0.05

Note: Values represented mean ± S.D. of three parallel measurements. **AQLPPP, ELPP**- aqueous extract of *Peltophorum pterocarpum*, methanolic extract of leaves of *Peltophorum pterocarpum.*

The total flavonoid content of AQLPP was found to be 10 mg/g equivalent of quercetin and 15 mg/g equivalent of quercetin for ELPP respectively. The results indicate that good amount of flavonoids were present in the ethanolic extract than aqueous extract which may account for sunscreen protection activity.

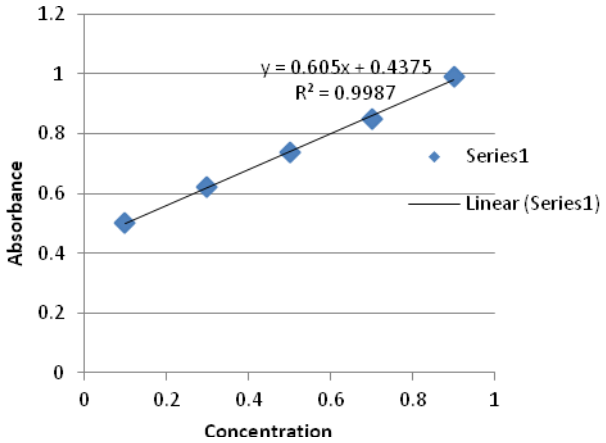

Figure 6: Standard calibration curve of Quercetin for flavonoids

Evaluation parameters of herbal sunscreen cream

Figure 7: Cream formulation of AQLPP, ELPP

Figure 8: Marketed Sunscreen cream

Table 6: Evaluation parameters of herbal sunscreen cream

S. No.	Parameters	Observation AQLPP	Observation ELPP
1.	Colour	Brownish black	Greenish white
2.	Odour	Pleasant	Pleasant
3.	Appearance	Smooth	Smooth
4.	pH	6.42	6.59
5.	Viscosity	9.00	9.12
6.	Consistency	Good	Good
7.	Grittiness	No gritty particles	No gritty particles
8.	Washability	Easiliy washable	Easiliy washable
9.	Spreadability	7 sec	5.3 sec

Note: AQLPP, ELPP- Aqueous extract of leaves of *Peltophorum pterocarpum,* ethanolic extract of leaves of *Peltophorum pterocarpum.*

The results of various tests for evaluation parameters for herbal sunscreen cream formulation using AQLPP indicate that it exists in brownish black color with a pleasant odor and smooth appearance. The pH value was found to be 6.42, viscosity 9.00, has good consistency, no gritty particles were present, was easily washable with a spreadability time of 7 sec.

The results of evaluation parameters for herbal sunscreen cream formulation using ELPP were found to be greenish white color, had a pleasant odor, smooth appearance, pH 6.59, viscosity 9.12, with good consistency, had no gritty particles, was easily washable and the spreadability value was 5.3 sec.

Evaluation parameters of herbal sunscreen gel

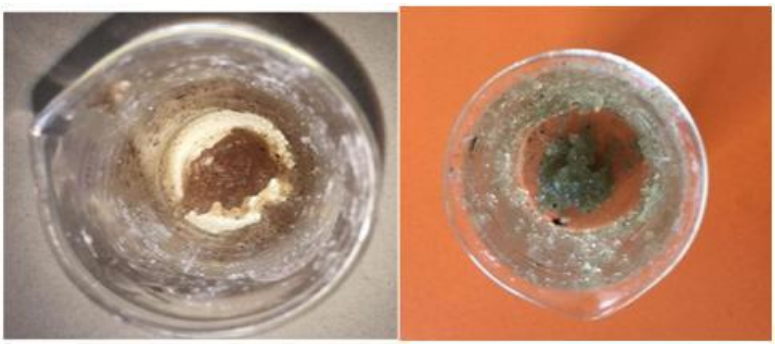

Figure 9: Gel formulation of AQLPP, ELPP

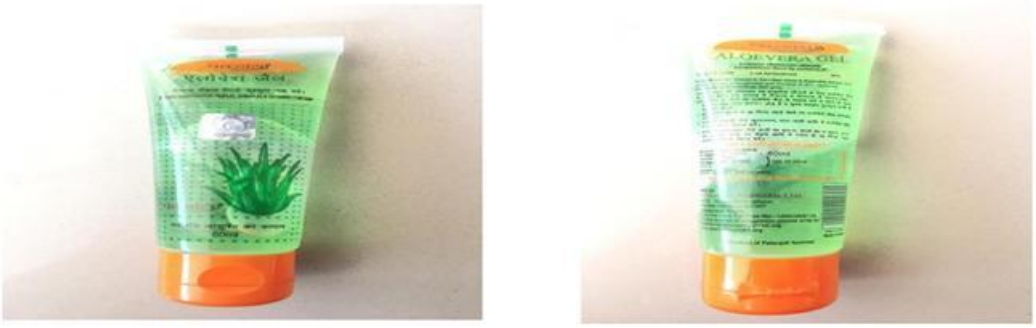

Figure 10: Marketed Sunscreen Gel

Table 7: Evaluation parameters of herbal Sunscreen Gel

S.No.	Parameters	Observation AQLPP	Observation ELPP
1.	Colour	Dark brown	Greenish white
2.	Odour	Pleasant	Pleasant
3.	Appearance	Smooth	Smooth
4.	pH	6.80	6.82
5.	Viscosity	8.47	8.54
6.	Consistency	Good	Good
7.	Grittiness	No gritty particles	No gritty particles
8.	Washability	Easiliy washable	Easiliy washable
9.	Spreadability	5sec	4.3sec

Note: AQLPP, ELPP- Aqueous extract of leaves of *Peltophorum pterocarpum,* ethanolic extract of leaves of *Peltophorum pterocarpum.*

The results of various tests for evaluation parameters of herbal sunscreen gel formulation using AQLPP indicate that it exists in dark brown color with a pleasant odor and smooth appearance. The pH value was found to be 6.80, viscosity 8.47, had good consistency, no gritty particles were present, was easily washable with a spreadability time of 5 sec.

The results of herbal sunscreen gel formulation using ELPP indicate that it had greenish white color with a pleasant odor and smooth appearance. The pH value was found to be 6.82, viscosity 8.54, had good consistency, no gritty particles were present, was easily washable with a spreadability time of 4.3 sec.

Determination of SPF of herbal sunscreen cream

Table 8: Determination of SPF of herbal sunscreen cream

S. No.	F	λ E.E*I	290 0.015	295 0.081	300 0.287	305 0.327	310 0.186	315 0.083	320 0.018	∑ 1	SPF
1.	ACLP	A E.E*I*A*CF	0.236 0.035	0.228 0.186	0.220 0.632	0.196 0.642	0.182 0.339	0.155 0.129	0.132 0.023	1.349 1.988	26.8
2.	ECLP	A E.E*I*A*CF	0.268 0.040	0.260 0.212	0.246 0.707	0.234 0.767	0.205 0.382	0.171 0.143	0.144 0.025	1.528 2.277	34.7
3.	MKPT	A E.E*I*A*CF	0.139 0.020	0.131 0.107	0.123 0.353	0.118 0.385	0.111 0.206	0.105 0.087	0.099 0.017	0.826 1.175	9.70

Note: F- Type of formulation; A- Spectrophotometric absorbance values at wavelength λ, E.E*I- Erythmogenic effect of radiation with wavelength; The values of EE×I are constant; CF=Correction factor; ACLP- Cream formulation using

aqueous extract of leaves of *Peltophorum pterocarpum,* ECLP - Cream formulation using ethanolic extract of leaves of *Peltophorum pterocarpum;* MKPT- Marketed cream formulation product

The SPF value of ACLP was found to be 26.8, ECLP was34.7. A marketed sunscreen cream formulation was used as a standard, SPF was found to be 9.70.The results of SPF value determination of herbal sunscreen cream indicate that ethanolic extract of the plant possesses higher SPF value 34.7 than aqueous extract 26.8 and standard marketed product. The flavonoid content of ECLP was also found to be more. Therefore, it suggests that ECLP shows sunscreen protective activity which may be attributed to the presence of high flavonoid content.

Determination of SPF of herbal sunscreen gel

Table 9: Determination of SPF of herbal sunscreen gel

S. No.	F	λ E.E*I	290 0.015	295 0.081	300 0.287	305 0.327	310 0.186	315 0.083	320 0.018	∑ 1	SPF
1.	AGLP	A E.E*I*A*CF	0.189 0.028	0.172 0.140	0.155 0.445	0.147 0.481	0.139 0.259	0.130 0.108	0.122 0.021	1.054 1.482	15.6
2.	EGLP	A E.E*I*A*CF	0.196 0.029	0.179 0.146	0.162 0.465	0.153 0.501	0.144 0.268	0.135 0.112	0.126 0.022	1.095 1.543	16.8
3.	MKPT	A E.E*I*A*CF	0.469 0.070	0.453 0.370	0.438 1.258	0.227 0.744	0.412 0.767	0.399 0.333	0.386 0.069	2.789 3.611	100

Note: F-Type of formulation; A- Spectrophotometric absorbance values at wavelength λ,E.E*I- Erythmogenic effect of radiation with wavelength; The values of EE×I are constant; CF=Correction factor; AGLP – Gel formulation using aqueous extract of leaves of *Peltophorum pterocarpum,* EGLP- Gel formulation using ethanolic extract of leaves of *Peltophorum pterocarpum;* MKPT- Marketed gel formulation product.

The SPF value of AGLP was found to be 15.6, EGLP was 16.8. A marketed sunscreen gel formulation was used as a standard, SPF was found to be 100.The results of SPF value determination of herbal sunscreen gel indicate that ethanolic extract of the plant possesses higher SPF value 16.8than aqueous extract 15.6. The SPF of the standard marketed product was found to be very high 100. Both AGLP and EGLP showed sunscreen protection activity. The flavonoid content of ELPP was also found to be more. Therefore, it suggests that both ECLP and EGLP showed sunscreen protective activity which may be attributed to the presence of high flavonoid content.

CONCLUSION

Plants contain various substances, especially the flavonoids, which are often good for skin care, having no harmful effects. Therefore, good combination of phytoconstituents, which can produce best possible effects on the skin, is necessary. The study provided sensible data to conclude that the cream and gel formulations prepared from the leaf extracts of the plant *Peltophorum pterocarpum* (*DC.) Baker ex Heyne* possesses the rich amount of flavonoids, capable of protecting the skin from the harmful effects of UV rays. Hence efforts should be made to commercially produce herbal sunscreen cream and gel formulations' using the plant extracts.

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