

METHOD

Preparation of plant extracts

Plant materials were cut into small pieces and extracted thrice with the suitable solvent by agitating. The extracts were then concentrated under vacuum using a rotary evaporator. *Piper betle* ethyl acetate extract, *Alpinia galangal* ethanolic extract, Green tea extract, *Punica granatum* ethanolic extract and juice, *Centella asiatica* ethanolic extract, *Phyllanthus emblica* ethanolic extract, *Alpinia calcarata* ethanolic extract and *Aloe vera* juice were prepared.

Preparation of 0.02% ethanolic solution of plant extracts

The extracts and cream purchased from the market (0.10g) were taken and dissolved in 5 ml of pure ethanol and transferred into 10 ml volumetric flask. By adding more pure ethanol, the solution was made up to the mark. The contents were mixed well and the solution was filtered. First 5 ml solution was discarded and the second 5 ml solution was collected. To prepare 0.02% solution, 1 ml was taken from the initial solution using a 1 ml pipette and transferred into a 50ml volumetric flask. Pure ethanol was added to make the solution up to the mark. Thereafter, absorbance values of each aliquot prepared were determined from 290 to 320 nm, at 5nm intervals, taking pure ethanol as blank, using UV-Visible spectrophotometer (U-2910 spectrophotometer). Standard solution of the sun protection cream purchased from the market was prepared to compare the results with plant extracts as well as the plant based gel developed in the lab.

Preparation of 0.1% ethanolic solution of essential oil

A portion of 0.1ml of sample was taken in a 10 ml volumetric flask, dissolved in 40% ethanolic solution and made up to the mark. From this 0.1% was prepared by taking 1ml and diluting to 10 ml with 40% ethanolic solution. Thereafter, absorbance values of each aliquot prepared was determined as mentioned above.

Preparation of the plant based sunscreen gel

P.betel extract (0.43g), *A.galanga* extract (1.52g), Green tea extract (1.52g), *P.emblica* extract (0.57g), *C.asiatica* extract (0.75g), *P.granatum* extract (15.00g), *A.calcarata* extract (5.00g) 10 ml of Cinnamon bark oil and 5 ml of Citronella oil were taken in a beaker and 10 ml of pure ethanol was also added to dissolve the extracts. Mixture was stirred to get a homogeneous mixture. A portion of 15 ml of Aloe juice was taken in a separate beaker, methyl paraban was

added to it and stirred well. After methyl paraban dissolved, mixture was heated up to 50 deg Celsius. Carbopol Ultrez was slowly added to the mixture. This mixture was stirred again and transferred in to the mixture of extracts. Final mixture was stirred well until it started to gel. An ethanolic solution of the gel (0.02%) was prepared for the UV absorbance test as mentioned above.

Testing of radical scavenging activity of the prepared gel employing DPPH method

The antioxidant activity was determined by DPPH. scavenging assay as described by Navarro et al (1993) with some modifications (Ordonez et al., 2006). In this assay, known concentrations (0 - 160 µg/ml) of methanolic solution of gel and butylated hydroxyl toluene (BHT) were placed in different test tubes, and the volumes made up to 1.5 ml by adding methanol. Volume of 3 ml of a methanolic solution of DPPH. (4 mg/100 mL in MeOH) were added to each of these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 5 min. and the absorbance was measured at λ 517 nm. Control tubes were prepared as above by adding methanol instead of test solution. Butylated hydroxyl toluene(BHT) was used as positive control.

Results and Discussion

Sun protection factor

In vitro sun protection factor values were determined using the method and the equation stated above. Individual SPF values are shown in table 2.1 & 2.2

Table 2.1
SPF of plant extracts

Plant extract	SPF Value
<i>P.betel</i>	5.17
<i>A.galanga</i>	13.96
Green tea	7.83
<i>P.emblica</i>	5.50
<i>C.asiatica</i>	6.51

Table 2.2
SPF of plant extract & Cream

Plant extract	SPF Value
<i>P.granatum</i>	6.76
Cinnamon bark oil	28.40
Citronella oil	20.91
<i>A.calcarata</i>	14.67
<i>A.vera juice</i>	0.19
Purchased Cream	20.48

Calculation of the sun protection factor of the gel

Calculation of the sun protection factor of the plant based gel is given below. Absorbance curve of the gel is also shown figure 1.

Table 3 Absorbance of the gel in the wave length range of 290-400 nm.

Wave length/ nm	Absorbance	EE x l	Absorbance x EE x l
290	3.1030	0.0150	0.0465
295	3.0373	0.0817	0.2481
300	2.948	0.2847	0.8473
305	2.639	0.3278	0.8651
310	2.023	0.1864	0.3771
315	1.221	0.0839	0.1024
320	0.652	0.0180	0.0117

$$SPF_{in vitro} = CF \sum_{290}^{320} EE \times l \times Abs$$

$$CF = \frac{1}{Abs_{290}}$$

$$SPF_{in vitro} = 10 \times 2.4983$$

$$SPF_{in vitro} = 24.98$$

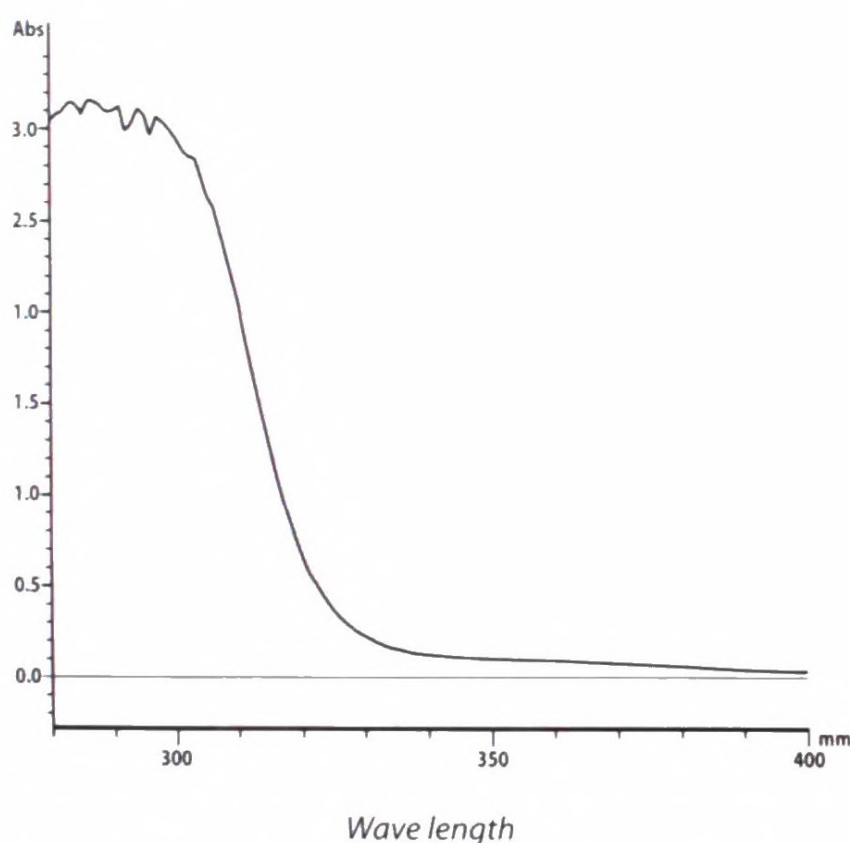


Figure.1 UV absorption curve of the standard solution (0.02%) of the gel with in the wave length range of 290-400nm

Anti oxidant property of the gel

Absorbance values of the aliquots prepared in methanol were recorded at λ 517nm. Percentage of radical scavenging activity (RSA) of the gel and BHT were calculated using the general equation shown below.

$$\text{Percentage of RSA} = \frac{(A_0 - A_s)}{A_0} \times 100$$

Where A_0 is the absorbance of the control and A_s is the absorbance of the sample at λ 517 nm. IC50 values denote the concentration of sample required to scavenge 50% DPPH. Free radicals.

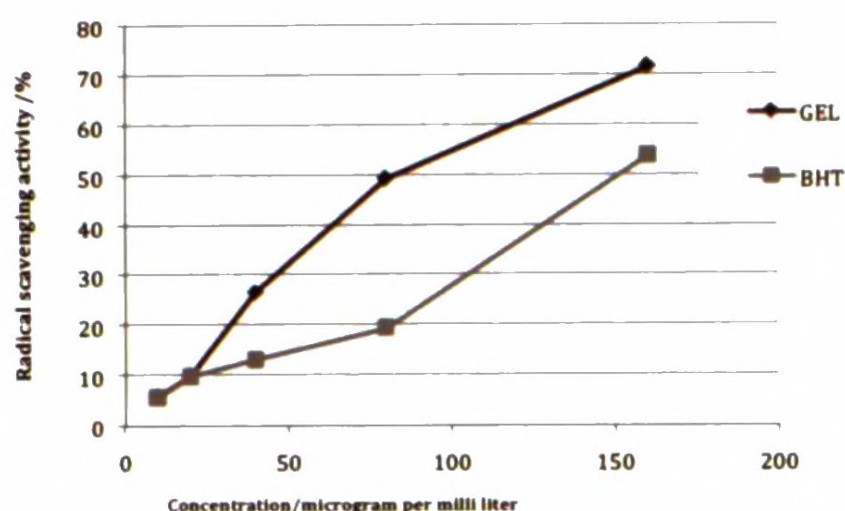


Fig .2 RSA versus concentration curve

IC50 values

IC50 value of gel = 82.44 μ g/ml

IC50 value of BHT=151.05 μ g/ml

Conclusion

The gel that was prepared using plant extracts and essential oils as UV absorbers, absorbed mostly the UV B (280- 320 nm) region, which is more harmful than UV A region. By increasing the amount of Alpinia extract which absorb in both regions the gel can be improved further. Sun protection factor of the gel is higher than the value of the sun screen cream which was purchased from the market. Therefore this plant based gel is more effective than the cream. Since the gel contains natural plant extracts, side effects can be less. IC50 value of gel is 82.44 μ g /ml whereas IC50 value of BHT is 151.05 μ g/ml. Therefore the gel is more effective in scavenging radicals compared to BHT. As the plant based gel shows higher value for sun protection factor compared to the cream, and has very good antioxidant property, it can be used for topical applications. Since the gel also contains Citronella oil as an active ingredient, it can also act as mosquito repellent during day times. However the performance of this gel has to be assessed through *in vivo* method which is performed on human volunteers

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All intellect and no feeling can be characteristic of the arch criminal and all feeling and no intellect exemplifies the harmless idiot. But when intellect and feeling are perfectly balanced, then we get the superlative actor.

Charlie Chaplin in his autobiography

The Yasuni National Park, Ecuador.



Giant Kapok trees with sprawling buttress roots soar like Roman columns straight into the canopy, their bifurcating branches draped with orchids and bromeliads that sustain entire communities of insects, amphibians, birds and mammals. Strangler figs coil around their trunks in a tightening embrace. There is so much life here that tiny killifish are wriggling in a shallow puddle created by animal tracks. A turn down a slope and the forest is studded with bizarre looking Socratea trees commonly called walking palms, with four foot high stilt roots that allow the trees to shift location slightly in a quest for light and nutrients. It is one of the untold millions of evolutionary adaptations unfolding all around the rain forest.

Scott Wallace in the National Geographic Magazine January 2013

Friendship is like a river; it flows around rocks; adapts itself to valleys and mountains, occasionally turns into a pool until the hollow in the ground is full and it can continue on its way. Just as the river never forgets that its goal is the sea, so friendship never forgets that its only reason for existing is to love other people.

Paulo Coelho in: The manuscript found in Accra.