

# Methanolic Leaf Extract of *Ipomoea Pes-caprae* Possesses *In Vitro* Sun Screen Activity

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**Abstract:** Currently, there is an unprecedented demand for safe, cheap and efficacious topical herbal sunscreen formulations. With this in mind, we investigated the sun screen potential of the leaves of a common beach creeper, *Ipomoea pes-caprae* (Family: Convolvulaceae) *in vitro* using a well recognized UV spectroscopic technique and Mansur equation. Sun Protection Factor (SPF) was evaluated (which is an index of sun protection activity) in a methanolic extract of the leaves (concentration: 2.0 mg/mL) and also of methanol soluble fraction of Dermatone® (reference drug concentration; 2.0 mg/mL), which is a well known sun screen cream. The results showed that leaf extract exhibited three absorbance peaks between 200 – 320nm wave lengths: 3.0 at UV C region, 1.67 at UV B region and 1.2 at UV A region. SPF value of the leaf extract was  $15.39 \pm 0.89$  [mean  $\pm$  SEM] whilst the corresponding value for Dermatone® was  $25.05 \pm 0.33$ . Phytochemical analysis revealed the presence of flavonoids, tannins and phenolics and diterpenes. It is concluded that methanolic leaf extract of *Ipomoea pes-caprae* possesses marked sun screen activity which is possibly mediated via antioxidant activity. Strong possibility exists to develop a safe, effective and affordable sun screen from leaves of this plant

**Key words:** *Ipomoea pes-caprae*, sunscreen, sun protection factor, photoprotection, antioxidant

## 1. Introduction

Uses of topical sunscreen formulations are desirable to minimize deleterious effects to human skin and hair caused by overexposure to UV rays emitted by the sun. In fact, sun screen use begins in the early 20<sup>th</sup> century [1]. There are three main categories of UV rays: UVC (200-280nm), UVB (280-320nm) and UVA (320-400nm) [2,3]. Of these, UV-B rays (commonly known as burning rays) is the most biologically effective UV

radiation and overexposure of these rays is mainly responsible for producing injurious effects of the skin and hair [2]. These injurious effects are basically of two types. Acute effects such as irritations, inflammation, pain, erythema (sun burn), hyperpigmentation (tanning), development of brown and red spots, hyperplasia and local immune suppression [2,3,4,5,6,7]. Photoaging/premature ageing of skin (rough texture, sagging, dry and lathery appearance and wrinkling), photocarcinogenesis, degenerative changes in fibrous tissue and blood vessels of the skin are the chronic effects [1,2,8,9,10].

Currently, there are several sunscreen formulations available in the market in the form of creams, gels, oils, ointments, lotions, balms, butters and waxes [1,2,6,10,11]. Basically, all these sunscreens function either by absorbing or reflecting or scattering sun's rays [11,12,13], and fall into two main types, synthetic and natural. Synthetic sun screens are generally efficacious, fast acting and provide broad spectrum protection from solar UV rays [6,11,12,13,14], but, are relatively expensive and induce unacceptable side effects [6,7,10,11,14]. Conversely, herbal sunscreens are relatively inexpensive, safer and user friendly [6,7,10,13,14]. However, there is a need for development of novel plant based sun screens which are much cheaper and have higher activity.

In this regard, we have started a research programme to investigate *in vitro* sun screening activity [in terms of sun protection factor (SPF)] of Sri Lankan herbs. As of now, we have evaluated the *in vitro* sun protection activity of Sri Lankan orthodox black tea made from leaves of *Camellia sinensis* [15] and four salt marshy plants, *Suaeda monoica*, *Suaeda maritima*, *Halosarcica indica* [16] and *Salicornia brachiata* [17].

In this study, we report, for the first time, *in vitro* sunscreen potential of leaves of *Ipomoea pes-caprae* plant (Bintamburu in Sinhala and Adambu, adappangodi in Tamil), a member of Family:

Convolvulaceae by evaluating the SPF value using UV spectroscopic technique and Mansur equation [10,16,18]. This plant was selected since we thought that it may have marked sunscreen activity as it thrives, well in very stressful coastal environment where it is continuously exposed to sun's rays [19,20,21] and, its leaves contain large quantities of flavonoids and vitamin C [20,21,22], possessing strong antioxidant activity [20,21,22,23]: which is linked with sunscreen activity [2,3,10]. Additionally, leaf extracts of *Ipomoea pes-caprae* is shown to possess collagenase inhibitory activity [20] which can play a vital role in suppressing wrinkle formation, which is one of the chronic effects of overexposure to UV B rays [1,2,8,9,10].

*Ipomoea pes-caprae* is a pan tropical evergreen perennial fast growing beach coastal creeper with heart shaped, succulent thick smooth leaves without teeth, having numerous prostrate fleshy, glabrous and cylindrical stems growing along the ground and rooting at nodes. Its flowers are regular, bisexual large and funnel shaped [19,20,21].

Ethnomedicinally, *Ipomoea pes-caprae* is claimed to treat rheumatism, arthritis, inflammation, pain, headache, skin disorders, ulcers, vomiting, colonic disorders, diarrhoea, hemorrhoids, bronchitis, fever, diabetic, gonorrhoea, cancer or general weakness [20,21,22,23]. In addition, it is used as an aphrodisiac, as an agent to delay ageing process and to treat dermatitis caused by stings of jelly fish and portugese man-of-war [22,23]. Moreover, experimental studies have shown that *Ipomoea pes-caprae* possess antiinflammatory, antibacterial, antinociceptive, antioxidant, antipyretic, hypoglycemic, insulogenic, hepatoprotective, anticancer, antispasmodic and platelet aggregation inhibitory activities [20,21,22,23,24,25]

## 2. Materials and methods

### Collection and identification of the plant

*Ipomoea pes-caprae* plants were collected from Kalutara beach (geographical coordinates; 6°34'34" North, 79°57'57" east), Western province of Sri Lanka in November, 2015. The plants were identified and authenticated by Prof. A. Seneviratne, Department of Plant Sciences, University of Colombo, Sri Lanka. Voucher specimens of leaves of *Ipomoea pes-caprae* (BLCS/Pharm/03) were deposited in pharmaceutical laboratory, Department of Pharmacy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defense University, Werahara, Sri Lanka for future references.

### Preparation of methanolic extract of *Ipomoea pes-caprae*

The leaves were removed from the plants, washed twice in running tap water and were oven dried at 40°C for 2 weeks, until a constant weight was obtained. The dried leaves were then powdered (coarse) using a domestic blender. Sixty grams of the powder was macerated for 7 days in 100 mL of distilled methanol (Sigma-Aldrich Company, St. Louis, U.S.A). The resulting dark green coloured extract was filtered through double layered of muslin cloth and the filtrate was evaporated to dryness in a water bath. The yield was 11.04%. The green coloured product was stored in tied air bottle at 4°C until use.

### Phytochemical analysis of *Ipomoea pes-caprae* methanolic extract

Methanolic extract of *Ipomoea pes-caprae* was subjected to standard qualitative analysis for alkaloids (using Meyer's test, Wagner's test and Dragandoff's test), tannins and phenols (FeCl<sub>3</sub> test), flavonoids (HCl and magnesium turnings test), glycosides (Keller-killiani test) and diterpenes (Cu(CH<sub>3</sub>COO)<sub>2</sub> test)

### In vitro evaluation of sun protection factor of methanolic extract of *Ipomoea Pes-Caprae*

The solid product of *Ipomoea pes-caprae* obtained following evaporation of the methanolic extract was redissolved in methanol (ACS reagent, 99.8% purity from Sigma-Aldrich) to prepare a solution of 2.0 mg/mL. In addition, Dermatone® was dissolved in methanol to obtain a solution of 2.0 mg/mL. Absorbance of UV radiation by the methanol extracts of *Ipomoea pes-caprae* and Dermatone® was determined (at 23°C with an equilibration time of 1 hr) in 1 cm quartz cells, in triplicate, using a UH 5300 Hitachi spectrophotometer from 290 to 320 nm, at 5 min intervals taking methanol as the blank. SPF values were then determined using the Mansur equation (7,12,16) given below.

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

Where EE –erythema effect spectrum; I –Solar intensity spectrum; Abs –Absorbance of sunscreen product; CF –correction factor (=10). The values of EE X I are constant and predetermined.

### Statistical analysis

The results are depicted as mean  $\pm$ SEM. Statistical comparison was made using  $X^2$  test. Significance was set at  $p < 0.05$ .

### 3. Results

The UV phytochemical analysis revealed the presence of flavonoids, tannins and phenols and diterpenes whilst alkaloids, saponins and glycosides were absent.

The UV absorption spectrum of methanolic leaf extract of *Ipomoea pes-caprae* is shown in Figure 1. As shown, one high absorbance peak (3.0) was evident in UV C region (200-280 nm), one mild absorbance peak (1.67) and UV B region [280-320nm] and one low absorbance peak (1.2) in UV A [320-400 nm] region. Further, methanolic leaf extract showed absorbance between 1.32 to 1.64 between wave lengths 290-320 nm whilst Dermatone®, reference agent, exhibited absorbance between 2.05 and 2.61 at the same wave lengths. SPF value determined for methanolic leaf extract was  $15.39 \pm 0.89$  and for Dermatone® was  $25.05 \pm 0.33$ . Further, the SPF value of reference agent, Dermatone® was significantly ( $p < 0.05$ ) higher than the leaf extract (by 66%).

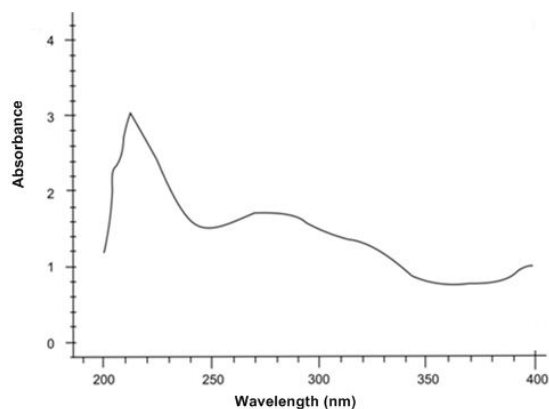


Figure 1. Absorption spectrum of methanolic leaf extract of *Ipomoea pes-caprae*

### 4. Discussion

This study evaluated the *in vitro* sun protective activity (in terms of SPF value) of the leaves of the common beach creeper, *Ipomoea pes-caprae* using spectroscopic technique and Mansur equation [10,16,18]. This bioassay is the most widely used reliable, validated, quick *in vitro* technique to assess the sun protection potential of both natural and synthetic products/ formulations [3,6,10,15]. Further, the experimental conditions and setups

used were similar to those used in several other investigations [6,7] and by us [16,17].

The results convincingly showed, for the first time that, 2.0 mg/mL methanolic extract of leaves of *Ipomoea pes-caprae* possess marked sunscreen activity, with a SPF value of 15.39, which is above the threshold value of a good sunscreen: dermatologist strongly recommend to use topical sunscreens having SPF value 15 or more, preferably year round to minimize harmful effects resulting from over exposure to suns UV rays, particularly UV B rays [11,27]. Further, it is of interest to note that in SPF ratings, SPF values 2-12, 12-30 and  $>30$  are considered as having minimum, moderate and high sun screen activities respectively [8]. The leaf extract displayed a wide range of absorbance (between 280-320 nm) with a mild peak at 282 nm (in the UV B region). This is yet another beneficial feature of leaf extract of *Ipomoea pes-caprae* as a sunscreen: wider the range of absorbance of a sunscreen formulation higher would be its effectiveness in impairing sun burns [3,5,6,7].

The SPF value reported in this study is almost identical to the SPF value (SPF 15.55) reported for *Suaeda monoica*, a Sri Lankan halophyte, growing under similar conditions [16]. On the other hand two other Sri Lankan halophytes, *Suaeda maritima* (SPF value = 10.84) and *Halosarcia indica* (SPF value = 8.63) reported to have lower SPF values (respectively by 41% and 67%) than *Ipomoea Pes-Caprae* reported in this study [16]. In contrast, *Salicornia brachiata*, yet another Sri Lankan halophyte is shown to possess a SPF value almost double (SPF value 30.84) [17] to *Ipomoea pes-caprae* shown in this study. Such a variation in SPF values amongst these plants (16,17) and present study is unexpected, as these plants are found in similar stressful environmental conditions (such as high temperature, strong wind, exposure to high level of UV B rays) and usually, high content of UV absorbing compounds have been shown in plants growing under these conditions: but may be attributed to difference in the nature and quantities of phytoconstituents present amongst the different plant species [16,17,20,21,22,23].

It is now well established that free radicals are actively involved in UV induced pathogenesis of skin damages [23]. Moreover, it is known that antioxidants by quenching free radicals (anti radical effects) can suppress UV – induced skin damages and thereby conferring sun protection [2,3,10]. Several studies have shown powerful antioxidant activity in extract of leaves of *Ipomoea pes-caprae* [20,21,22,23] and also presence of flavonoids and vitamin C [20,21,22]. Flavonoids and vitamin C are strong antioxidants [20,21,22,23]. This study too showed the presence

of Flavonoids, tannins and phenolics and diterpenes. It is now known that even diterpenes can function as antioxidants [28,29]. Thus, it is likely that sun protection activity of leaves of *Ipomoea pes-caprae* are mediated by synergistic antioxidative activity of the before mentioned phytoconstituents. It is of interest to note that chromatic hydroxyl groups are very important for antioxidative effects of these phytoconstituents [28].

Tannins are shown to possess hyaluronidase inhibitory activity [30] and tannins were present in *Ipomoea pes-caprae* leaf extract in this study. Further, *Ipomoea pes-caprae* leaves shown to have collagenase inhibitory activity [20]. Since both hyaluronic acid and collagen play a vital role in maintaining structural integrity of human skin [31]. These two inhibitory activities would undoubtedly impair wrinkling of skin and thereby add value to *Ipomoea pes-caprae* leaf extract as a potent sun screen.

In conclusion, this study showed for first time marked *in vitro* sun screen activity of methanolic leaf extract of common beach plant *Ipomoea pes-caprae* and it may be possible to develop a novel cheap, efficacious and user friendly sunscreen formulation from this plant. Further, it appears worthwhile to investigate sun protective activity of other beach plants as well.

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## 6. Reference

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