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Determination of *In -Vitro* Sunscreen Activity of *Pongamia Pinnata* (L.) Essential Oil

Madhavarao Buddepu[1*], Sabithadevi K[2], Vinnakoti Ashok[3], Madhira VNS Ramprasad[4]

[1]Vivimed Labs Ltd., Veeranag Towers, Habsiguda, Hyderabad, AP.
[2]Department of Biotechnology, Krishna College, Andhra University, Visakhapatnam
[3]Department of Microbiology, GITAM University, Rushikonda, Visakhapatnam-530045, INDIA
[4]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur

The present research work evaluates the sunrise protective factor (SPF) property of pongamol from *pongamia pinnata* seed (l) Fabaceae in the ultra violet region and its comparison with a well established standard sunscreen drugs P-amino benzoic acid (PABA) and AVOBENZNE. The dried seed of plant were extracted in soxhlet apparatus using hexane and esterifies with methanol. The extract was concentrated by evaporation of solvent and finally dried to get dry powdery form. Then 10 mg of the dry extract was dissolved in respective solvent and their absorption spectra were measured by using UV- visible spectrophotometer. Absorbance of different concentration of extracts i.e., 1ppm , 2ppm, 3ppm, 4ppm and 5ppm was read at 200-400nm and to study the maximum wavelength and area of UVA and UVB. The extract was found to be highly effective in the UVA region and the known standard drug PABA showed its protective action in the UVB and UVC regions and AVOBENZENE showed highly effective in the UVA region. The *pongamia pinnata* extract can be used to formulate highly effective sunscreen preparation as it will enhance and effectively contribute to the UV absorbing properties of a conventional sunscreen. It will also help in broadening the UV protection ability of the sunscreens along with the greatest advantage of avoiding adverse and undesired effects of synthetic sunscreen compounds.

Key words: Sunrise Protective Factor (SPF), UV Protection, Esterification, Photo Absorptive Property, Sunscreen,

INTRODUCTION

Many factors affect the formulation of sunscreen products. A product brief normally defines the SPF to be claimed, the degree of UVA protection and water-resistance required and whether it is to be a spray or a cream, a lotion or a gel. It should specify the materials to be used for the packaging components and for which markets the product is intended and gives a cost ceiling for the ingredients. *Pongamia pinnata* (L.) Pierre, Fabaceae, which is commonly called Karanja in Marathi and Hindi, Maktamala or Gaura in Sanskrit, Honge, Hulugala or Kanigemara in Kannada, and Indian Beech Nut Tree in English, is used in the Ayurveda and Siddha traditional medicine systems for the treatment of clinical lesions of skin and genitalia. It is a medium-sized glabrous tree which favourably grows in moist environmental conditions along sea coast or rivers all over India. It is native to humid and subtropical environments having annual rainfall between 500 and 2500 mm, the maximum temperature range suitable for growth being 27-38ºC and the minimum being 1-16ºC. It can grow on most soil types ranging from stony to sandy to clay soil and can be propagated either by seeds or by root suckers. It is distributed east wards in the littoral regions of southeastern Asia and Australia. The seeds and seed oil of Karanja have been used for treating various infectious diseases and inflammatory conditions like leprosy, leucoderma, lumbago and rheumatism. *P. pinnata* roots have been described as a remedy against fistulous sores, foul ulcers, gonorrhoea, urethritis, etc. The anti-inflammatory, ulcer protective and healing effects of alcoholic extract of *P. pinnata* root and seeds have been studied. Large amount of bioflavonoids are present in the flowers, which are used in diabetes, various skin diseases and renal disorders.

Ethanolic extract of *P. pinnata* root exhibits protective role in ischaemia-reperfusion injury and cerebro-vascular insufficiency. Extracts of the plant also possess significant anti-diarrhoeal, anti-plasmodial, anti-fungal and analgesic activities. *P. pinnata* has been found to contain a large number of furanoflavonoids, e.g., karanjin, pongapin, kanjone, pongamol and pongaglabrone along with arachidonic acids.

Overexposure to UV radiation being the most important behavioral risk factor in development of skin cancer, the role of sunscreens and their photo protective strategies against these harmful rays are very important. UV rays are divided into the following regions: ultraviolet C (UVC 200-290 nm), ultraviolet B (UVB 290-320 nm), and ultraviolet A (UVA 320-400 nm). UVA is further divided into UVA II (320-340 nm) or short wave UVA, and UVA I (340-400 nm) or long-wave UVA. The adverse reactions to sunrays in normal, healthy skin are classified into two types: the immediate type of sunburn and tanning, and the delayed type of long-term effects, including photo aging and photo carcinogenesis. UVC has not been a major factor in causing human cancers because it is sufficiently absorbed by the ozone layer itself. However, both UVA and UVB radiation from the sunlight reach the earth in abundant quantities. In addition to causing cancer of the skin, UV rays have also been estimated to be a factor causing melanoma of the eye. Although UV rays constitute only a percentage of the sun's total radiant energy, they are known to have highly adverse effects on human skin, including photoaging and cutaneous burns. When the DNA in skin absorbs UV radiation directly, it leads to characteristic mutations resulting in altered DNA.

Corresponding Author:
Madhavarao Buddepu, Vivimed Labs Ltd., Veeranag Towers, Habsiguda, Hyderabad, AP.
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As UVB is approximately 1000 times more effective than UVA in inducing erythema, most sunscreens contain compounds which absorb radiations in the UVB region.

The present work was planned to study the photo absorptive property of the oil extract of *P. pinnata seed*. Their activity was compared with that of a well-established standard sunscreen drug *p*-aminobenzoic acid (PABA) and AVOBENZENE. It has become very important to study the sunscreen activity of herbal drugs, so as to avoid various adverse effects of synthetic chemical sunscreens like aminobenzoic acids and derivatives, anthranilates, benzophenones, cinnamates, salicylates and inorganic sunscreens like titanium dioxide and zinc oxide. The therapeutic properties of *P. pinnata* are very well recorded in the texts of traditional Indian medicines, Siddha and Ayurveda. The plant and its various parts have been used in many indications since earlier times. However, the sunscreen activity of the plant has not been reported till date. This forms the basis for selection of the plant for the study of its sunscreen activity. The methodology adopted in the current study was based on the model for determination of anti-solar activity proposed by Patil *et al.*

Collection and Authentication of Plant Specimen:

Fresh leaves of *P. pinnata* seeds were collected from a local herbal products department in hydra bad. The seeds were identified and duly authenticated by Dr. Srimannarayana proposer in natural product department IICT Hyderabad AR grade chemicals of Research-Lab brand were procured from a local chemical dealer.

Preparation of Extracts and Dilutions:

Dried seed of *P. pinnata* was collected and grounded in to powder form. One hundred grams of powdered material was evenly packed in soxhlet apparatus and the extraction was done with hexane then, the hexane extract was evaporated at low temperature and concentrated. Finally, the extract was completely removed solvent and get in oily form. Then, this oily extract was washed with methanol solution and esterified by using sulfuric acid and kept for some time without disturbing for settling of compound and filtrated by using watsman filter paper and re crystallized with ethanol. The recrystalised compound was dried at low temperature. Then using this dried extract was analyzed by UV of different concentrations, i.e., 1ppm, 2ppm, 3ppm, 45ppm, and 5ppm were prepared in methanol. The obtained extract solutions were finally filtered using Whatmann filter paper. Different concentrations of PABA and AVOBENZENE were prepared by dissolving accurately weighed quantity of PABA in methanol give1ppm, 2ppm, 3ppm, 4ppm and 5ppm standard solutions.

Absorbance and Spectra Measurement:

Spectra and absorbance of the extracts was measured using JASCO V-600 double beam UV-visible spectrophotometer. Ultraviolet absorption spectra of individual extracts and standard were measured in 1 cm quartz cell using "Spectra Measurement" mode, employing reference cell containing respective pure solvents. Absorbance of different concentrations of extracts and standard were measured in "Fixed Wavelength Measurement" mode of the instrument.

RESULT

Pongamol extract was found to be most effective in UVA region. It showed two peaks of maximum absorbance at λ_{max} 350nm with absorbance of ~0.0760,0.155,0.224,0.295,0.369 and area of UVA is 2.050, 3.957, 5.515, 7.132, 8.934 at respective concentration 1ppm, 2ppm, 3ppm, 4ppm, 5ppm, The extract was found to be highly effective throughout the UVB region with approximately constant absorbance of 0.423 at 5ppm in the range 300-320 nm. It showed extremely good uniform absorbance in the whole UVA resign.

Standard drug PABA was found to show its protective action in the UVB and C regions only, with no effectiveness in the UVA region. It showed very high and plateau absorbance in the UVB region. It showed high absorbance at, i.e., λ_{max} 288 nm with absorbance ~0.616 at 5ppm concentration and area of UVB is 0.506 and area of UVA is nil.

AVOBENZENE was found to absorb exclusively in the UVA resign with almost no absorbance in the UVB resign the wavelength of maximum absorbance was found to be λ_{max} 358 nm with absorbance ~ 0.581 and area of UVA is 4.059 and area of UVB is nil.

Drug concentration	Absorbance of Pongamol	Absorbance of PABA	Absorbance of AVOBENZENE
1ppm	0.085	0.127	0.114
2ppm	0.173	0.265	0.230
3ppm	0.253	0.393	0.332
4ppm	0.337	0.510	0.476
5ppm	0.508	0.616	0.581

Compound name	λ_{max}	UV Resign	Area
Pongamol	350nm	UVA(320-400nm)	2.050
PABA	288nm	UVB(290-320nm)	0.101
AVOBENZENE	357nm	UVA(320-400nm)	4.059

DISSCUSION

Formulations of sunscreen mostly contain compounds which are very effective in absorbing UVB radiation and thus provide no protection against UVA radiation. Although the UVA rays are less energetic and less erythemagenic than UVB rays, UVA rays penetrate the skin more deeply than the UVB rays and are hence capable of causing damage to the deeper portions of the skin tissue. Also, UVA rays magnify the damage caused by the UVB rays to the skin tissue. So, protection against the UVA rays is very important as well. High effectiveness of the pongamol extract in the UVA region may be due to its lesser polarity as compared to the other products, thus extracting greater amounts of the nonpolar photoabsorptive compounds from the plant seed. As compared to the standard chemical sunscreen PABA which showed absorbance only in the UVB region with no effect at all in the UVA region, but the AVOBENZENE was found to be highly effective absorbent throughout the UVA region than seed extract. As primarily apparent from the absorbance of extract and standard as given in the table, and their comparison in figre5 at respective wavelengths of maximum absorbance, area of UV region. When compared with standard drug AVOBENZENE the studied compound show 80% UVA absorbance properties.

This sunscreen property of the pongamol compound may be attributed to the presence of some naturally occurring photoabsorptive compounds like flavonoids which are produced by the plants that are subject to extraordinary amounts of solar radiation, in order to protect especially sensitive parts from damage. These compounds are produced by the plants as they have experienced a high degree of natural selective pressure in evolutionary terms. Natural substances extracted from plants like green tea polyphenols, *Aloe barbadencis* and aromatic compounds isolated from lichens have been considered as potential sunscreen resources on similar grounds. Earlier phytochemical examinations of *P. pinnata* have reported the presence of furano flavones, furano flavonols, chromeno flavones, flavones, furano diketones and flavonoid glucosides. It is also a well-established fact that most medicinal plants are rich in phenolic compounds and bio-flavonoids that have excellent antioxidant property. Ability of the components of the extract to scavenge free radicals formed by the action of UV rays, i.e., its antioxidant property in conjunction with the UV absorbing property renders its usage as a highly effective sunscreen, as natural antioxidants preventing free radical damage, and thus preventing wrinkles, premature skin aging, sun spots and skin cancer. As the extracts of the seed of the plant studied in the current paper showed good absorbance throughout the UV region, the photo absorptive compounds in the seed can be isolated and purified and can be used to formulate highly effective sunscreen preparations.

CONCLUSION

As pongamol and karanjin extracts were found to be extremely good absorbents of the UV rays in the UVA and B regions, we can positively conclude that the seed of *P. pinnata* contain such photoabsorptive compounds which when put together in a single herbal formulation like ointments, lotions or creams can give rise to an extremely effective sunscreen preparation showing its protective action throughout the broad ultraviolet region. The plant oil extracts can be used along with other established standard drugs, as it will enhance and effectively contribute to the UV absorbing properties of the sunscreen. It will also help in broadening the UV protection ability of the conventional sunscreen formulations. Considering the distress of the patients suffering from skin cancers along with the adverse effects and associated deficits of the synthetic sunscreen compounds currently used, it is the need of the time to seek out various herbal plants which would exhibit prophylactic utility when formulated into efficacious sunscreen formulations.

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