Study of sunscreen activity of aqueous, methanol and acetone extracts of leaves of *Pongamia pinnata* (L.) Pierre, Fabaceae

Priyank A. Shenoy, Sachin S. Khot, Manisha C. Chavan, Jagruti V. Takawale, Sonia Singh
Alard College of Pharmacy, University of Pune, S.no:50, Marunje, Near Rajiv Gandhi Infotech Park, Pune – 57, Maharashtra, India

The present research work evaluates the photoabsorptive property of different extracts of the leaves of *Pongamia pinnata* (L.) Pierre, Fabaceae, in the ultraviolet region (200–400 nm) and its comparison with a well-established standard sunscreen drug, *p*-aminobenzoic acid (PABA). The shade-dried leaves of the plant were extracted in Soxhlet apparatus using three different solvents, i.e., water, methanol and acetone. The extracts were concentrated by evaporation of the solvent and finally dried to get dry extracts. Then, 20 mg of the dry extracts was dissolved in the respective solvents and their absorption spectra were measured using UV–visible spectrophotometer. Absorbance of different concentrations of the extracts, i.e., 5, 10, 15 and 20 mg/100 ml was read at their respective wavelengths (λ_{max}) of maximum absorption. The aqueous and methanol extracts were found to be highly effective in the UVB and moderately effective in the UVA region. Acetone extract was found to greatly absorb exclusively in the UVA region. The known standard drug PABA showed its protective action in the UVB and UVC regions with least effectiveness in the UVA region. The extracts of the leaves of the plant under study showed extremely good absorbance throughout the UV region including UVA region. The *P. pinnata* extract can be used to formulate highly effective sunscreen preparations 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 the adverse and undesired effects of synthetic sunscreen compounds.

Key words: Aqueous, broadening UV protection, methanol and acetone extracts, *p*-aminobenzoic acid, photoabsorptive property, *Pongamia pinnata*, sunscreen, UV region (200–400 nm)

INTRODUCTION

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 27–38°C and the minimum being 1–16°C. 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 eastwards in the littoral regions of southeastern Asia and Australia.[1] The seeds and seed oil of Karanja have been used for treating various infectious diseases and inflammatory conditions like leprosy, leucoderma, lumbago and rheumatism.[2] *P. pinnata* roots have been described as a remedy against fistulous sores, foul ulcers, gonorrhoea, urethritis, etc.[3] The anti-inflammatory, ulcer protective and healing effects of alcoholic extract of *P. pinnata* root[4-6] and seeds[7] have been studied. Large amount of bioflavonoids are present in the flowers, which are used in diabetes, various skin diseases and renal disorders.[8] Ethanolic extract of *P. pinnata* root exhibits protective role in ischaemia-reperfusion injury and cerebrovascular insufficiency.[9] 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 other simple flavonoids and lipids like arachidonic acid.[10,11]

Overexposure to UV radiation being the most important behavioral risk factor in development of skin cancer, the role of sunscreens and their photoprotective 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).[12] 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

Address for correspondence: Mr. Priyank A. Shenoy, B-708, Ojas Apartment, Sr.no.132/2, Baner-Pashan Link Road, Pashan, Pune – 411 021, Maharashtra, India. E-mail: priyank.shenoy@rediffmail.com
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and tanning, and the delayed type of long-term effects, including photoaging and photocarcinogenesis. 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.[13] 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.[14] When the DNA in skin absorbs UV radiation directly, it leads to characteristic mutations resulting in altered DNA.[15] As UVB is approximately 1000 times more effective than UVA in inducing erythema,[16] most sunscreens contain compounds which absorb radiations in the UVB region.

The present work was planned to study the photoabsorptive property of the various extracts of *P. pinnata*. Their activity was compared with that of a well-established standard sunscreen drug *p*-aminobenzoic acid (PABA). 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.

MATERIALS AND METHODS

The methodology adopted in the current study was based on the model for determination of anti-solar activity proposed by Patil *et al*.[12]

Collection and Authentication of Plant Specimen

Fresh leaves of *P. pinnata* were collected from a local Plant nursery (Vidya Bhushan Garden Developers and Maintenance, Aundh, Pune). The plant specimen (V. No.PRISPOP1) was identified and duly authenticated by T. Chakraborty, Scientist-D, Joint Director, Botanical Survey of India, Pune.

AR grade chemicals of Research-Lab brand were procured from a local chemical dealer (New Neeta Chemicals, Pune, India).

Preparation of Extracts and Dilutions

Fresh leaves of *P. pinnata* were collected and shade dried at room temperature. Dried leaves were powdered mechanically through mesh sieve. One hundred grams of freshly powdered leaves was evenly packed in soxhlet apparatus and the extraction was done with double distilled water (aqueous extraction). Then, the liquid extract was evaporated at low temperature and concentrated. Finally, the extract was completely dried. Then, using this dry aqueous extract, solutions of different concentrations, i.e., 5 mg/100 ml, 10 mg/100 ml, 15 mg/100 ml and 20 mg/100 ml were prepared in double distilled water. The obtained extract solutions were finally filtered using Whatmann filter paper to remove any gross plant debris that could have accompanied the original extract. Similarly, extraction of further 100 g of shade-dried and powdered leaves of the plant was carried out using methanol and acetone as solvents separately. The extracts thus obtained were evaporated, concentrated and dried. The dry methanol extract and dry acetone extract were used to prepare solutions of different concentrations as above, using the respective solvents to make up the solutions. Finally, these extract solutions were filtered. Different concentrations of PABA were prepared by dissolving accurately weighed quantity of PABA in double distilled water to give 5, 10, 15 and 20 mg% w/v standard solutions.

Absorbance and Spectra Measurement

Spectra and absorbance of the extracts were 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.

RESULTS

Aqueous extract [Figure 1] was found to be most effective in UVC region. It showed two peaks of maximum absorbance at λ_{max} 232.4 nm with absorbance of ~1.99 and λ_{max} 269.2 nm with absorbance of ~1.65. In the UVC region, plateau of high absorbance was seen in the range of wavelength 255–275 nm. The extract was found to be highly effective throughout the UVB region with approximately constant absorbance of ~1.25 in the range 300–320 nm. The extract showed moderate absorbance in the UVA region with absorbance declining gradually from 335 to 400 nm.

Similar to the aqueous extract, the methanol extract [Figure 2] also exhibited maximum protective action in the UVC region. It exhibited two peaks, one at λ_{max} 233.8 nm with absorbance of ~2.3 and the other at λ_{max} 271 nm with absorbance of ~1.9. It showed a high plateau absorbance in the wavelength range of 255–275 nm in the UVA region as in the case of aqueous extract. Methanol extract showed extremely good, plateau

like constant absorbance in the UVB region in the wavelength range of 295–320 nm, with absorbance around ~1.7. It showed moderate absorbance in the UVA region with absorbance decreasing in the long wave region.

Acetone extract [Figure 3] was found to absorb exclusively in the UVA region with almost no absorbance in the UVB and C regions. It showed extremely good and uniform absorbance in the whole UVA region. The wavelength of maximum absorbance was found to be λ_{max} 337.9 nm with absorbance ~2. The absorbance was initially found to exhibit a steep rise in the wavelength range 320–335 nm; further, the absorbance gradually decreased in the range 335–370 nm and, finally, a moderate rise in the absorbance was seen again while moving towards the longer wavelength end of the region.

Standard drug PABA [Figure 4] 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 two wavelengths, i.e., λ_{max} 244 nm with absorbance ~2.98 and λ_{max} 299.8 nm with absorbance ~2.75.

The results of the preliminary phytochemical screening of the leaf extracts showed that glycosides, anthocyanins, polyphenols and flavonoids are present in the plant leaves.

DISCUSSION

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. Aqueous extract and methanol extract were found to be more effective in the UVB region with lesser effectiveness in the UVA region. High effectiveness of the acetone extract in

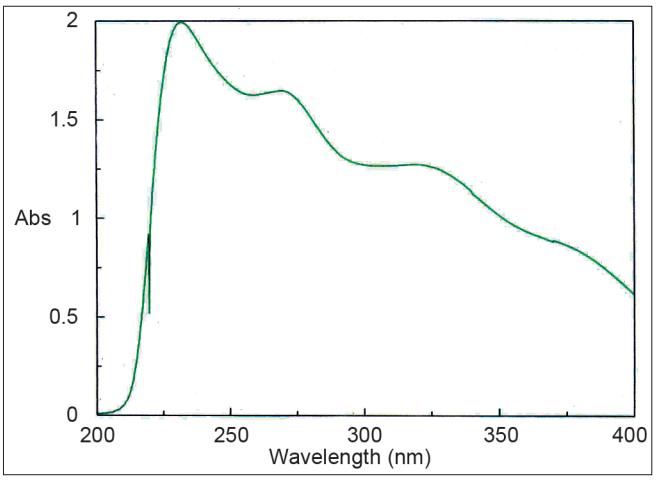

Figure 1: Ultraviolet spectrum of aqueous extract of *P. pinnata* leaves

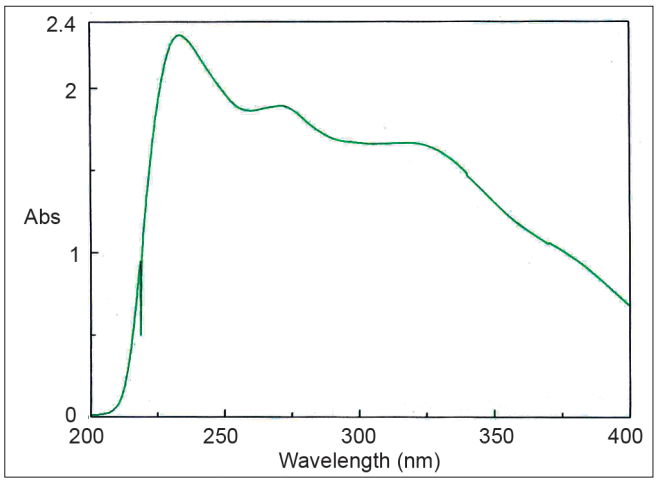

Figure 2: Ultraviolet spectrum of methanol extract of *P. pinnata* leaves

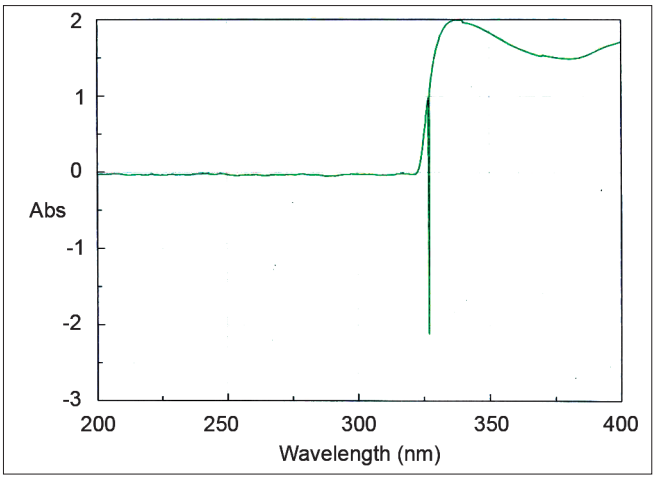

Figure 3: Ultraviolet spectrum of acetone extract of *P. pinnata* leaves

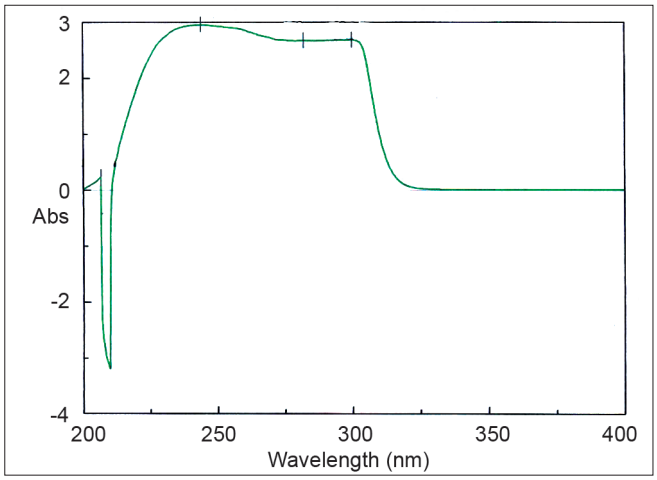

Figure 4: Ultraviolet spectrum of PABA

the UVA region may be due to its lesser polarity as compared to the water and methanol, thus extracting greater amounts of the nonpolar photoabsorptive compounds from the plant leaves. 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, the acetone extract was found to be highly effective absorbent throughout the UVA region. As primarily apparent from the absorbance of various extracts and standard as given in the Tables 1–4, and their comparison in Figure 5, at respective wavelengths of maximum absorbance, on an average, aqueous extract was found to absorb 67.9% of the UV radiation as that absorbed by the standard drug PABA. Methanol extract was found to absorb an astonishing 88.5% of radiation as that absorbed by the pure standard. However, acetone extract was found to absorb only 45%.

This sunscreen property of the leaf extracts 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* extract 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 furanoflavones, furanoflavonols, chromenoflavones, flavones, furanodiketones and flavonoid glucosides. It is also a well-established fact that most medicinal plants are rich in phenolic compounds and bioflavonoids that have excellent antioxidant property.[17] 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 leaves of the plant studied in the current paper showed good absorbance throughout the UV region, the photoabsorptive compounds in the leaves can be isolated and purified and can be used to formulate highly effective sunscreen preparations.

CONCLUSION

As aqueous and methanol extracts were found to be extremely good absorbents of the UV rays in the UVB and C regions and the acetone extract was found to be highly effective in UVA region, we can positively conclude that the leaves of *P. pinnata* contain such photoabsorptive compounds which when put together in a single herbal formulation like ointments, lotions or creams can give rise

Table 1: Absorbances of aqueous extract of *P. pinnata*

Concentration	Absorbance	CV
5 mg/100 ml	0.7290±0.00028	0.0692
10 mg/100 ml	1.2764±0.0012	0.1647
15 mg/100 ml	1.8161±0.0015	0.1465
20 mg/100 ml	2.2552±0.0015	0.1186

Absorbance values were expressed as mean±SEM; Number of times of absorbance taken for each individual concentration; n=4, CV - coefficient of variation

Table 2: Absorbances of methanol extract of *P. pinnata*

Concentration	Absorbance	CV
5 mg/100 ml	0.9768±0.00057	0.0998
10 mg/100 ml	1.8820±0.00028	0.0259
15 mg/100 ml	2.4649±0.0039	0.2748
20 mg/100 ml	2.5942±0.0047	0.3201

Absorbance values were expressed as mean±SEM; Number of times of absorbance taken for each individual concentration; n=4

Table 3: Absorbances of acetone extract of *P. pinnata*

Concentration	Absorbance	CV
5 mg/100 ml	0.4443±0.00011	0.0381
10 mg/100 ml	0.8289±0.00034	0.0675
15 mg/100 ml	1.2000±0.00023	0.0361
20 mg/100 ml	1.5611±0.0024	0.2739

Absorbance values were expressed as mean±SEM; Number of times of absorbance taken for each individual concentration; n=4

Table 4: Absorbances of PABA

Concentration	Absorbance	CV
5 mg/100 ml	1.0162±0.00011	0.0235
10 mg/100 ml	2.5537±0.0125	0.8481
15 mg/100 ml	2.6582±0.0102	0.6642
20 mg/100 ml	2.7216±0.0025	0.1665

Absorbance values were expressed as mean±SEM; Number of times of absorbance taken for each individual concentration; n=4

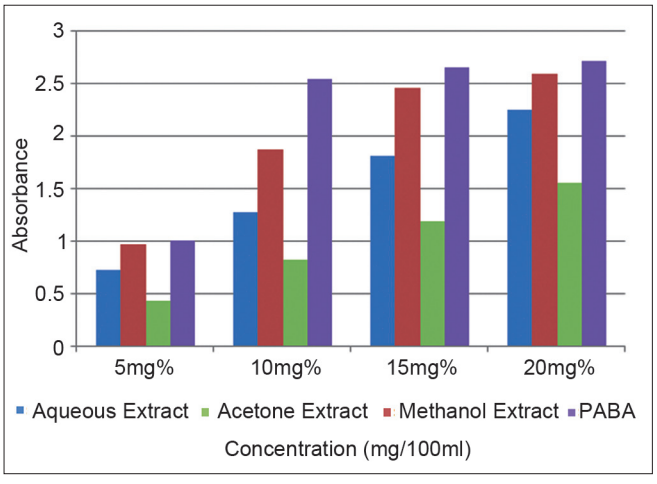

Figure 5: Comparison of absorbances of various extracts with PABA

to an extremely effective sunscreen preparation showing its protective action throughout the broad ultraviolet region. The plant 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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