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RESEARCH ARTICLE

EVALUATION OF *IN VITRO* ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *Pithecellobium dulce* BENTH FRUIT PEEL

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ABSTRACT

Pithecellobium dulce (*Kodukkapuli* in Tamil and *Vilayati babul* in Hindi) is a small to medium-sized spiny tree cultivated throughout the plains of India. Its bark and leaves possess astringent property, and leaves have emollient, abortifacient and antidiabetic properties. The antioxidant and antibacterial potential of the fruit peel *Pithecellobium dulce* was assessed and the results revealed significant activity in the ethyl acetate, methanolic and aqueous extracts of fruit peel.

INTRODUCTION

Plants have been a valuable source of natural products for maintaining human health since long time. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated for better understanding of their properties, safety and efficiency (Ellof, 1998). Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals results in the onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis as well as degenerative processes associated with aging (Halliwell and Gutteridge, 1984). Organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau *et al.,* 2002). As an integral part of the human diet, vegetables, fruits, seeds, tea, wines and juices have received much attention, since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular disease, stroke and certain types of cancer. These protective effects have partly been ascribed to the antioxidant properties. Among flavanone aglycons, naringenin, hesperetin, eriodictyol and isosakuranetin are the most common, but they are present in much smaller quantities than glycosides. Citrus flavonoids, especially hesperidin, have shown a wide range of therapeutical properties such as anti-inflammatory, anti-hypertensive, diuretic, analgesic and hypolipidemic activities (Inga *et al.,* 2007). Many studies suggest that endogenous antioxidants or exogenous antioxidants from diet can function as free radical scavengers and improve human health. In this regard, consumption of a variety of plant foods may provide additional health benefits (Mojzisova *et al.,* 2001; Connor *et al.,* 2002).

The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists (Kafaru, 1994). Owing to their popular use as remedies for many infectious diseases, search for plants containing antimicrobial substances are frequent (Betoni *et al.,* 2006). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Lewis and Ausubel, 2006). There are several reports on the antimicrobial activity of different herbal extracts (Bonjar, 2004; de Boer *et al.,* 2005). According to WHO, medicinal plants would be the best source for obtaining variety of drugs (Santos *et al.,* 1995). These evidences contribute to support and quantify the importance of screening natural products.

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Pithecellobium dulce Benth (Family: *Leguminosae*) is a small to medium-sized, ever green, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as 'vilayati babul' in Hindi and 'kodukkapuli'in Tamil (Anonymous, 1969). The bark of the plant is reported to be used as astringent in dysentery, febrifuge and is useful in dermatitis and eye inflammation. Roots have been reported to possess estrogenic activity (Saxena and Singal, 1998). The fruits have been shown to have anti-inflammatory activity (Bhargvakrishna *et al.,* 1970) and leaves have been reported to be a folk remedy for ear ache, leprosy, peptic ulcer, tooth ache, and venereal disease. It also acts as astringent, emollient, abortifacient, antidiabetic, anodyne and larvicidic in folk medicines. The bark of the plant is reported to be used as an astringent for dysentery, febrifuge and it is also useful in dermatitis and eye inflammation and also posses antivenomous activity (Pithayanukul *et al.,* 2005). Studies on free radical-scavenging properties (Sugumaran *et al.,* 2008) and antimycobacterial activity of leaves were recently reported (Shanmugakumar *et al.,* 2006). It is evident that the plant has great potentials in treating a number of ailments where free radicals have been reported to be the major factors contributing to the disorders (Arouma, 1998). The objective of this study is to evaluate the antioxidant and antibacterial activity of the extracts of *Pithecellobium dulce* fruit peel.

MATERIALS AND METHODS

Plant materials and extraction: The plant *Pithecellobium dulce* was collected from the Namakkal district, Tamil Nadu. The plant was taxonomically identified and authenticated by Botanical survey of India, Coimbatore (Tamil Nadu) and a voucher specimen was deposited in our laboratory for future reference. Around 100 g of the fruit peel was dried in shade, pulverized by a mechanical grinder and passed through a 40-mesh sieve to get a fine powder and stored in an airtight container. The dried powder (25 g) was extracted with petroleum ether (60-80 °C) to remove wax and then extracted with ethyl acetate, 80% methanol and water sequentially in a soxhlet apparatus. The solvents from various extracts were then concentrated in rotary evaporator at reduced pressure below 40 °C.

Chemicals: DPPH (1,1-diphenyl-2-picryl-hydrazyl), butylated hydroxy toluene (BHT) and catechol were purchased from Sigma, St. Louis, MO, USA. All other chemicals and reagents were of analytical grade.

Bacterial strains: To study the antibacterial activity of various extracts of *Pithecellobium dulce* fruit peal, the strains of bacteria were obtained form Microbiology Laboratory at PSG Hospitals, Coimbatore. The selected bacteria included *Staphylococcus epidermis, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Pseudomonas putida* and *Proteus vulgaris.*

Antioxidant activity

Determination of total phenolic content: Total phenol content was determined by the method adapted from Singleton and Rossi (1965) with some modifications using the Folin-Ciocalteu reagent. 1 ml of the extract was mixed with 1 ml of Folin-Ciocalteu's phenol reagent (1:10). After 3 min, 1 ml of saturated sodium carbonate (35%) was added to the mixture and it was made up to 10 ml by adding deionised distilled water. The mixture was kept for 90 min at room temperature in the dark. The absorbance was measured at 725 nm against the blank. The total phenolic content is expressed as mg of Catechol equivalents (CE) per g of dry extract.

Determination of DPPH free radical scavenging activity: The scavenging effect of the extracts on DPPH radicals was determined according to the method adapted from Shimada *et al.* (1992). Various concentrations of sample (1.5 ml) were mixed with 3 ml of 200 μM DPPH solution. The mixture was shaken vigorously and allowed to stand for 40 min, and the absorbance was measured at 517 nm, using butylated hydroxy toluene (BHT) as the standard. The percentage of inhibition was calculated according to the formula:

$[(A_{control} - A_{sample})/A_{control}] \times 100.$

IC_{50} values were determined by linear regression.

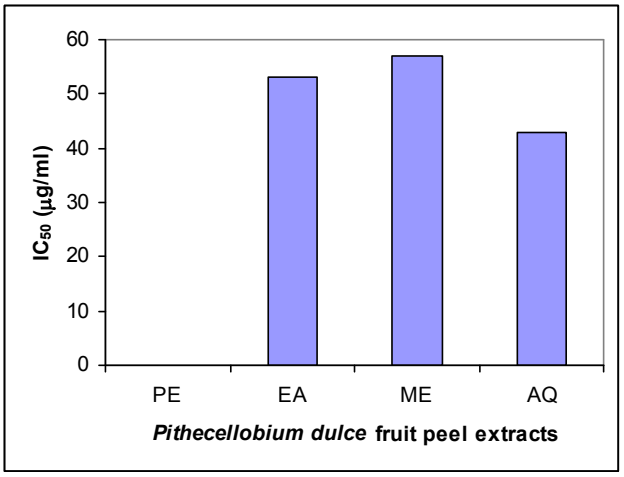
Determination of Antibacterial activity by disc diffusion method: The *in vitro* antibacterial activity of the sample solutions was studied by disc diffusion method. Plates were prepared by pouring 20 ml of sterile nutrient agar (Hi-media) into sterile Petri dishes and were inoculated with a loopful broth culture of each organism. Sterile discs (6 mm dia) impregnated with 20 μl (1.5 mg/disc) of various extracts dissolved in dimethyl sulphoxide (DMSO) were air-dried and placed on the agar plates. The plates were incubated at 37 °C for 24 h. Control studies with polymixin-B and rifampicin 30 μg were used as antibacterial standard drugs. The control experiments with solvent DMSO were done concurrently (Umadevi *et al.,* 2003). The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antibacterial activity. All data regarding antibacterial activity are the average of triplicate analyses.

RESULTS AND DISCUSSION

Antioxidant activity

Total phenolic content: The total phenolic content of the various extracts of *Pithecellobium dulce* fruit peel is given in Table 1. The aqueous, ethyl acetate and methanolic extracts of the fruit peel showed higher levels of total phenolic contents than the petroleum ether extract. It is well known that plant phenolic compounds are responsible for effective free radical scavenging and antioxidant activities (Velioglu *et al.,* 1998). Reactive oxygen species (ROS) are continuously produced during normal physiological events and are removed by antioxidant defence mechanisms (Halliwell *et al.,* 1992). Under pathological conditions, ROS are overproduced and results in lipid peroxidation and oxidative stress (El-Habit *et al.,* 2000). Antioxidant-based drugs or formulations are used to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals (Cooper *et al.,* 2002).

DPPH free radical scavenging activity: Being a stable free radical, DPPH is regularly used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, but upon reduction, with an



PE – Petroleum ether extract; EA – Ethyl acetate extract; ME – Methanolic extract; AQ – Aqueous extract

Fig. 1. IC_{50} values for dpph free radical scavenging activity of *pithecellobium dulce* fruit peel

antioxidant, its absorbance decreases due to the formation of its non-radical form DPPH-H (Blois, 1985). Thus, the radical scavenging activity in the presence of a hydrogen-donating antioxidant can be monitored by a decrease in the absorbance of DPPH solution. The free radical scavenging effects of *Pithecellobium dulce* fruit peel and BHT are given in Table 2. The extracts had significant scavenging effects on the DPPH radical. The positive DPPH test suggests that the samples are free radical scavengers. The IC_{50} values of the extracts in the DPPH free radical scavenging assay are illustrated in the Fig 1.

scavenging activity. Phenolic compounds may contribute directly to the antioxidative action (Hatano *et al.,* 1989). These results indicate a strong relationship between total phenolic contents and radical scavenging activity, suggesting that phenolic compounds are responsible for the antioxidative properties of *Pithecellobium dulce* fruit peel extracts. Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants (Rice-Ecans *et al., 1995)*. Similarly, Shahidi and Naczk (1995) reported that naturally occurring phenolic compounds exhibit antioxidative activity. Thus, the therapeutic properties of *Pithecellobium dulce* fruit peel extracts may be possibly attributed to the phenolic compounds present.

Antibacterial activity

Phytoconstituents present in plants, namely flavonoids, alkaloids and triterpenoids are producing exhilarating opportunity for the expansion of modern chemotherapies against wide tang of microorganisms (Cowan, 1999). Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al.,* 1998). Many reports are available on the antibacterial properties of plants (Samy and Ignacimuthu, 2000; Palombo and Semple, 2001). In the present study, the ethyl acetate of *Pithecellobium dulce* fruit peel was found to be effective against *S. epidermis, E. coli, K. pneumonia, S. aureus, E. faecalis, P. aeruginosa* and *P. putida*, while the methanolic extract was active against *K. pneumonia, S. aureus* and *P. putida* (Table 3). The aqueous extract was found to be effective against *K. pneumonia* and *S. aureus* only, while petroleum ether extract was active only against *P. Putida.*

Table 1. Total phenolic content (µg/ml) of the extracts of *Pithecellobium dulce* fruit peel

Extract	Petroleum ether	Ethyl acetate	Methanol	Aqueous
Pithecellobium dulce fruit peal	5.20 ± 0.15	45.00 ± 1.35	45.00 ± 1.35	47.50 ± 1.45

Values are expressed as mean ± SD (n=3) as Catechol equivalents (CE).

Table 2. DPPH free radical scavenging assay of *pithecellobium dulce* fruit peel

Extract	% Inhibition			
	Petroleum ether	Ethyl acetate	Methanol	Aqueous
Pithecellobium dulce fruit peel (1 mg/ml)	0.98 ± 0.03	88.29 ± 2.65	96.49 ± 2.90	87.39 ± 2.60

Values are expressed as mean ± SD (n=3)

Table 3. Antibacterial activity of *Pithecellobium dulce* fruit peel

Extracts	Zone of inhibition (mm dia)					
	Petroleum ether	Ethyl acetate	Methanol	Aqueous	Standards	
					PM	RF
Staphylococcus epidermis	-	10	-	-	30	18
Escherichia coli	-	9	-	-	28	11
Klebsiella pneumonia	-	13	18	14	28	30
Staphylococcus aureus	-	12	11	11	38	25
Enterococcus faecalis	-	10	-	-	35	30
Pseudomonas aeruginosa	-	9	-	-	10	18
Pseudomonas putida	8	9	9	-	36	10
Proteus vulgaris	-	-	-	-	24	4

Values are expressed as mean (n=3) PM – Polymyxin – B; RF – Rifampicin

These results indicate that *Pithecellobium dulce* fruit peel extracts, particularly the methanolic, ethyl acetate and aqueous extracts, exhibit the ability to quench DPPH radical, suggesting that the extracts are good antioxidants with radical

None of the extract was found to be active against *Proteus vulgaris*. Thus the ethyl acetate extract of *Pithecellobium dulce* fruit peel showed good antibacterial activity against most of the tested bacteria, suggesting the presence of

antibacterial agent in the plant. Considering the total phenolic contents and DPPH free radical scavenging activities, the ethyl acetate, methanolic and aqueous extracts of *Pithecellobium dulce* fruit peel showed higher levels compared to the petroleum ether extract. The ethyl acetate extract showed higher antibacterial activity than the other activities. Antioxidants that retard the oxidation process may additionally exhibit antimicrobial activity (Puupponen-Pimia *et al.,* 2001). Thus there is correlation between the antibacterial activity and the antioxidant activity of the *Pithecellobium dulce* fruit peel extracts.

Conclusion

The results of present study reveals that ethyl acetate, methanolic and aqueous extracts of fruit peel of *Pithecellobium dulce* were found to have antioxidant by virtue of their phenolic contents and DPPH free radical scavenging, while the ethyl acetate extract is found to have better antibacterial activities than the other extracts.

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