EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 1
291-295
Year: 2016

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF AERIAL PARTS OF *PAVETTA INDICA* (LINN)

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Article Received on 04/11/2015 **Article Revised on 25/11/2015** **Article Accepted on 16/12/2015**

ABSTRACT
The antioxidant activity of various extracts of aerial parts of *Pavetta indica* (Linn) was investigated in various *in-vitro* methods. The antioxidant activity was evaluated by Total antioxidant activity (Phosphomolybdic acid method), FRAP assay with reference standard Ascorbate and total flavonoids content respectively. The methanolic extract of *Pavetta indica Linn*was found to more effective in the total antioxidant activity. The IC_{50} values of the methanolic extract of *Pavetta indica Linn* and ascorbate were found to be 215µg/ml and 410µg/ml respectively. The methanolic extract of *Pavetta indica* was found more effective in FRAP assay than that of petroleum ether and ethyl acetate extracts. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Pavetta indica* showed the better result. The methanolic extract of *Pavetta indica* contains high amount of flavonoids (3.896 ± 0.014) than that of other two extracts. All the above in vitro studies clearly indicate that the methanolic extract of *Pavetta indica* has a better antioxidant activity. These in vitro assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

KEYWORDS: Aerial parts of *Pavetta indica*, *Invitro* antioxidant, Total antioxidant activity, FRAP assay, Total flavonoids.

INTRODUCTION
Oxygen-derived free radicals such as super oxide anion and hydroxyl radical are cytotoxic and promote tissue injuries. Antioxidants act as a major defence against radical-mediated toxicity by protecting against the damages caused by free radicals[1]. The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress[2].Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties[3]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity. It is commonly recognized that antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutations and therefore, help prevent cancer or heart disease.

Pavetta indica Linn. belongs to the family Rubiaceae. It is widely distributed from the Andaman Islands, India and the north-western Himalayas to southern China and southwards throughout Malaysia to northern Australia. A shout bushy shrub 0.6-1.2 m high; bark thin, smooth, yellowish; young branches terete, glabrous. Leaves 7.5-15 by 2.5-6.3 cm, membranous, variable in shape and size, elliptic - oblong or elliptic - lanceolate, sometimes obovate - oblong, obtuse, acute or acuminate, glabrous on both sides, base tapering; main nerves 8-10 pairs; petioles 6-13 mm long; stipules connate, triangular, acute, thin, deciduous. Flowers white, odourous, in terminal sessile corymbose pubescent cymes; pedicles 4-6 mm long, densely pubescent; bracts broad, membranous, the lower copular; buds oblong- clavate. Calys densely pubescent, 3mm long; tube narrowly campanulate; teeth 1.25 mm long, triangular, acute, slightly reflexed at the tip. Corolla - tube 13 mm long; lobes 6-8 by 2.5 mm, linear - oblong, subacute. Style white, glabrous or nearly so; stigma green, narrowly clavate, puberulous. Fruit 6-14 mm diameter, glabose, black, smooth. The entire plant used medicinally as a bitter tonic, diuretic, inflammation, rheumatism, jaundice and ulcer[4]. In the indigenous system of medicine, it is reported that the decoction of the leaves are used to

relieve haemorrhoidal pain, as a lotion for nose, analgesic, antipyretic, appetizer and the ulceration of mouth[5,6]. In literature, it has been reported as an antibacterial, antiviral and antimalarial[7]. *Pavetta indica* leaves are used in the treatment of liver disease, pain from piles, urinary diseases and fever[8]. It is a medicinally important plant having antiinflammatory activities[9] *Pavetta indica* reported analgesic activity[10], antidiabetic activity[11], antimicrobial[12]activity of leaf extract of *P. indica*. Its root extract also have diuretic and purgative activity[13]. The leaves and roots are employed in the preparation of poultices for boils and itches; decoctions of leaves are used as a lotion for ulcerated nose and for heamorrhoids. Root is used for anticephalagic. Leaf is used in haemorrhoidol pain and ulcerated nose. Wood is used as antirheumatic. Fruits are used as anthelmintic[14-17]. The phytochemicals produced by the plants for their self protection have been demonstrated to protect human against a number of diseases. The leaves contain carbohydrate, glycosides, phytosterols, saponins, flavonoids and alkaloids. However, no data are available in the literature on the antioxidant activity of aerial parts of *Pavetta indica* (Linn). Therefore we undertook the present investigation to examine the antioxidant activities of various extract of aerial parts of *Pavetta indica* (Linn) through various *in vitro* models.

MATERIALS AND METHODS

Collection and Identification of Plant materials

The aerial parts of *Pavetta indica* (Linn), were collected form kalakkadu, Tirunelveli District , Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of *Pavetta indica* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[18] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of antioxidant activity by *in vitro* techniques

Total antioxidant activity (Phosphomolybdic acid method) [19]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999)[19]. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^{0}C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP assay[20]

A modified method of Benzie and Strain (1996)[20] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mM HCl and 20 mM $Fecl_3.6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3.6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Total flavonoids[21]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *Pavetta indica* presented in Table 1. The petroleum ether extract of *Pavetta indica* exhibited a maximum total antioxidant activity of 52.28 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 65.23 % at 1000 µg/ml. The IC_{50} values of the petroleum ether extract of *Pavetta indica* and ascorbate were found to be 290µg/ml and 410µg/ml respectively.

Table 1: Total antioxidant activity of Petroleum ether extract of *Pavetta indica* by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	14.22 ± 0.079	26.87 ± 0.076
2	250	33.43 ± 0.053	30.30 ± 0.054
3	500	45.12 ± 0.021	60.64 ± 0.022
4	1000	52.28± 0.012	65.23 ± 0.014
		IC_{50} = 870 µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The percentage of total antioxidant activity of ethyl acetate extract of *Pavetta indica*presented in Table 2. The ethyl acetate extract of *Pavetta indica*exhibited a maximum total antioxidant activity of 57.33 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 65.23 % at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Pavetta indica* and ascorbate were found to be 680µg/ml and 410µg/m respectively.

Table 2: Total antioxidant activity of Ethyl acetate extract of *Pavetta indica* by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	12.65 ± 0.026	26.87 ± 0.076
2	250	31.85 ± 0.065	30.30 ± 0.054
3	500	44.81 ± 0.054	60.64 ± 0.022
4	1000	57.33± 0.034	65.23 ± 0.014
		IC_{50} = 680 µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The percentage of total antioxidant activity of methanolic extract of *Pavetta indica*presented in Table 3. The methanolic extract of *Pavetta indica* exhibited a maximum total antioxidant activity of 77.45 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 65.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Pavetta indica* and ascorbate were found to be 215µg/ml and 410µg/ml respectively.

Table 3: Total antioxidant activity of Methanolic extract of *Pavetta indica*by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	34.46 ± 0.022	26.87 ± 0.076
2	250	56.76 ± 0.042	30.30 ± 0.054
3	500	66.34 ± 0.052	60.64 ± 0.022
4	1000	77.45 ± 0.084	65.23 ± 0.014
		IC_{50} = 215 µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Based on the result showed the methanolic extract of *Pavetta indica* was found to more effective than petroleum ether and ethyl acetate extract. But when compare all the extracts with standard the methanolic extract of *Pavetta indica*was found strong antioxidant activity. The IC_{50} of the methanolic extract of *Pavetta indica* and Ascorbate were found to be 215µg/ml and 410µg/ml respectively.

FRAP assay

The antioxidant potential of *Pavetta indica* was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the petroleum ether extract of *Pavetta indica*and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 4. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 43.76% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 1200µg/ml and 50µg/ml respectively.

Table 4: Reducing ability of Pet. ether extract of *Pavetta indica* on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	19.23 ± 0.022	72.04 ± 0.014
2	250	26.32 ± 0.026	82.05 ± 0.034
3	500	37.86 ± 0.045	86.04 ± 0.026
4	1000	43.76 ± 0.048	98.07 ± 0.041
		IC_{50} = 1200 µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The reducing ability of the ethyl acetate extract of *Pavetta indica*and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 5. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 55.47% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 780µg/ml and 50µg/ml respectively.

Table 5: Reducing ability of Ethyl acetate extract of *Pavetta indica* on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	17.50 ± 0.065	72.04 ± 0.014
2	250	30.85 ± 0.054	82.05 ± 0.034
3	500	44.43 ± 0.034	86.04 ± 0.026
4	1000	55.47 ± 0.023	98.07 ± 0.041
		IC_{50} = 780 µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The reducing ability of the methanolic extract of *Pavetta indica*and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 6. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 76.22% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 215 µg/ml and 50µg/ml respectively.

Table 6: Reducing ability of Methanolic extract of *Pavetta indica* on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	37.96 ± 0.022	72.04 ± 0.014
2	250	53.32 ± 0.043	82.05 ± 0.034
3	500	65.28 ± 0.033	86.04 ± 0.026
4	1000	76.22 ± 0.028	98.07 ± 0.041
		IC_{50} = 215 µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Based on the above results indicated, the methanolic extract of *Pavetta indica*was found to most effective than that of petroleum ether & ethyl acetate extract. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Pavetta indica*showed the moderate result.

Total flavonoids

The total amount of flavonoids content of various extract of aerial parts of *Pavetta indica* was presented in Table 7.

Table 7: The flavonoids content of various extracts of aerial parts of *Pavetta indica*

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Pavetta indica*	0.216 ± 0.012
2	Ethyl acetate extract of *Pavetta indica*	1.415 ± 0.042
3	Methanolic extract of *Pavetta indica*	3.896 ± 0.014

*All values are expressed as mean ± SEM for three determinations

Based on the result the methanolic extract of *Pavetta indica* was found higher content of flavonoids than that of petroleum ether and ethyl acetate.

CONCLUSION

From the results obtained in the present study, it is concluded that a aerial parts of methanolic extract of *Pavetta indica* , which contains large amounts of flavonoids compounds, exhibits high antioxidant and free radical scavenging activities. So it can be concluded that these components might be involved in the antioxidant activity of *Pavetta indica* . Therefore, it is suggested that further work should be performed on the isolation and identification of the antioxidant components in *Pavetta indica.*

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