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Evaluation of *in vitro* antioxidant activity of various extracts aerial parts of *Pavetta indica* (Linn)

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ABSTRACT

The aim of this study was to investigate in *vitro* antioxidant activities of various extracts of aerial parts of *Pavetta indica* . Evaluation of antioxidant properties by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, hydroxyl radical scavenging activity and iron chelating activity with reference standard rutin, ascorbate and EDTA respectively. The methanolic extract of *Pavetta indica* was found to more effective in the DPPH scavenging activity. The IC_{50} of the methanolic extract of *Pavetta indica* and rutin were found to be 210µg/ml and 480µg/ml respectively. An IC_{50} value was found that methanolic extract of *Pavetta indica* is more effective in scavenging hydroxyl radical activity and iron chelating activity than that of ethyl acetate and petroleum ether extract. But when compare to the all the three extracts with ascorbate and EDTA (standard), the methanolic extract of the *Pavetta indica* showed the similar result. It is concluded that a aerial parts of methanolic extract of *Pavetta indica* exhibits high antioxidant and free radical scavenging activities. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: Aerial parts of *Pavetta indica*, *In vitro* antioxidant, DPPH assay.

1. INTRODUCTION

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O^{2-}) and hydroxyl radicals (OH'), as well as nonfree- radical species such as hydrogen peroxide (H_2O_2) [1,2]. In living organisms various ROS can form in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionising radiation, certain pollutants, organic solvents, and pesticides [3]. Ethanomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

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]. *P. 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]. The plant is used for analgesic activity[10] and antidiabetic activity[11]. The 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.

2. MATERIALS AND METHODS

2.1. 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.

2.2. 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.

3. RESULTS AND DISCUSSION

3.1. Evaluation of antioxidant activity by *in vitro* techniques

3.1.1. DPPH photometric assay

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[19]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging\ activity\ (\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

3.1.2. Hydroxyl radical scavenging activity

This was assayed as described by Elizabeth and Rao (1990) [20]. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate – EDTA $-H_2O_2$ system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition

3.1.3. Iron chelating activity

The method of Benzie and strain (1996) [21] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

3.1.4. DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the

antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Pavetta indica* depicted in table 1. The IC_{50} values of the petroleum ether extract of *Pavetta indica* and Rutin were found to be 1175µg/ml and 480µg/ml respectively.

Table 1: Effect of Petroleum ether extract of *Pavetta indica*on DPPH assay

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Petroleum ether extract)	Standard (Rutin)
125	18.67 ± 0.064	18.85 ±0.076
250	24.23 ± 0.014	22.08 ±0.054
500	31.35 ± 0.018	52.21 ±0.022
1000	46.52 ± 0.042	69.83 ±0.014
	IC_{50} = 1175 µg/ml	**IC_{50} = 480 µg/ml**

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

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Pavetta indica* depicted in table 2. The IC_{50} values of the ethyl acetate extract of *Pavetta indica* and Rutin were found to be 1010µg/ml and 480µg/ml respectively.

Table - 2: Effect of Ethyl acetate extract of *Pavetta indica*on DPPH assay

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Ethyl acetate extract)	Standard (Rutin)
125	17.44 ± 0.023	18.85 ± 0.076
250	26.45 ± 0.034	22.08 ± 0.054
500	38.65 ± 0.021	52.21 ± 0.022
1000	50.08 ± 0.042	69.83 ± 0.014
	IC_{50} = 1010 µg/ml	**IC_{50} = 480 µg/ml**

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

The percentage of DPPH radical scavenging activity of methanolic extract of *Pavetta indica*depicted in Table 3. The IC_{50} values of the methanolic extract of *Pavetta indica*and Rutin was found to be 210µg/ml and 480µg/ml respectively.

Table - 3: Effect of Methanolic extract of *Pavetta indica*on DPPH assay

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Methanolic extract)	Standard (Rutin)
125	41.28 ± 0.024	18.85± 0.076
250	54.31 ± 0.053	22.08± 0.054
500	65.40 ± 0.076	52.21± 0.022
1000	72.22 ± 0.034	69.83± 0.014
	IC_{50} = 210 µg/ml	**IC_{50} = 480 µg/ml**

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

From the result indicated the IC_{50} values of methanolic extract of *Pavetta indica* exhibits significant antioxidant activity when compared to that standard Rutin. IC_{50} value of plant extract and Rutin was recorded as 210µg/ml and 480µg/ml respectively. But other two extracts showed lower activity when compared to that of methanolic extract of *Pavetta indica* and standard Rutin.

3.1.5. Hydroxyl radical scavenging activity

The percentage of Hydroxyl radical scavenging activity of petroleum ether extract of *Pavetta indica*presented in table 4. The petroleum ether extract of *Pavetta indica*was exhibited a maximum Hydroxyl radical scavenging activity of 48.89 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} values of the petroleum ether extract of *Pavetta indica* and ascorbate were found to be 1080µg/ml and 410µg/ml respectively.

Table 4: Hydroxyl radical scavenging activity of Petroleum ether extract of *Pavetta indica*

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Petroleum ether extract)	Standard (Ascorbate)
125	21.38 ± 0.032	26.87 ± 0.076
250	35.34 ± 0.014	30.30 ± 0.054
500	42.36 ± 0.058	60.64 ± 0.022
1000	48.89 ± 0.034	75.23 ± 0.014
	IC_{50} = 1080 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of hydroxyl radical scavenging activity of ethyl acetate extract of *Pavetta indica*presented in table 5. The ethyl acetate extract of *Pavetta indica*was exhibited a maximum hydroxyl radical scavenging activity of 53.82 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Pavetta indica*and ascorbate were found to be 780µg/ml and 410µg/ml respectively.

Table - 5: Hydroxyl radical scavenging activity of Ethyl acetate extract of *Pavetta indica*

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Ethyl acetate extract)	Standard (Ascorbate)
125	24.27± 0.016	26.87 ± 0.076
250	35.32± 0.043	30.30 ± 0.054
500	46.24± 0.072	60.64 ± 0.022
1000	53.82± 0.029	75.23 ± 0.014
	IC_{50} = 780 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of hydroxyl radical scavenging activity of methanolic extract of *Pavetta indica*presented in table 6. The methanolic extract of *Pavetta indica*was exhibited a maximum hydroxyl radical scavenging activity of 89.64 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Pavetta indica*and ascorbate were found to be 225µg/ml and 410µg/ml respectively.

Table - 6: Hydroxyl radical scavenging activity of Methanolic extract of *Pavetta indica*

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Methanolic extract)	Standard (Ascorbate)
125	36.14 ± 0.062	26.87 ± 0.076
250	53.18 ± 0.032	30.30 ± 0.054
500	64.36 ± 0.053	60.64 ± 0.022
1000	69.98 ± 0.071	75.23 ± 0.014
	IC_{50} = 225 µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the above result the methanolic extract of *Pavetta indica*(IC_{50} = 225 µg/ml) was found more effective than that of standard (IC_{50} = 410 µg/ml). The methanolic extract of *Pavetta indica*was found to more effective than that of petroleum ether and ethyl acetate extracts.

3.1.6. Iron chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components [22].

Table - 7: Effect of Pet. ether extract of *Pavetta indica* on Iron-chelating method

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Pet. ether extract)	Standard (EDTA)
125	22.20 ± 0.032	58.68 ± 0.010
250	29.76 ± 0.035	65.87 ± 0.018
500	36.64 ± 0.022	83.83 ± 0.012
1000	51.55 ± 0.046	97.90 ± 0.019
	IC_{50} = 970 µg/ml	**IC_{50} = 65 µg/ml**

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

Table - 8: Effect of Ethyl acetate extract of *Pavetta indica*on Iron-chelating method

Concentration (µg/ml)	% of activity (±SEM)*	
	Sample (Ethyl acetate extract)	Standard (EDTA)
125	17.22 ± 0.024	58.68± 0.007
250	29.54 ± 0.036	65.87± 0.018
500	50.62 ± 0.069	83.83± 0.012
1000	67.87 ± 0.041	97.90± 0.019
	IC_{50} =510 µg/ml	**IC_{50} = 65 µg/ml**

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

Iron binding capacity of the petroleum ether extract of *Pavetta indica* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 7. The IC_{50} value of plant extract and EDTA was recorded as 970µg/ml and 65µg/ml respectively.

Iron binding capacity of the ethyl acetate extract of *Pavetta indica*and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 8. The IC_{50} value of plant extract and EDTA was recorded as 510µg/ml and 65µg/ml respectively.

Iron binding capacity of the methanolic extract of *Pavetta indica* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 9. The IC_{50} value of plant extract and EDTA was recorded as 130µg/ml and 65µg/ml respectively.

Table - 9: Effect of Methanolic extract of *Pavetta indica* on Iron-chelating method

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Methanolic extract)	Standard (EDTA)
125	49.65 ± 0.022	58.68± 0.007
250	60.35 ± 0.047	65.87± 0.018
500	73.39 ± 0.048	83.83± 0.012
1000	79.18 ± 0.016	97.90± 0.019
	IC_{50} = 130 µg/ml	**IC_{50} = 65 µg/ml**

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

From the result indicated the IC_{50} value of methanolic extract was found more effective than that of EDTA as a standard. Based on the above results indicated, the methanolic extract of *Pavetta indica* exhibited significant antioxidant activity was comparable to that of petroleum ether & ethyl acetate extracts of *Pavetta indica* .

4. CONCLUSION

From the results obtained in the present study, it is concluded that a aerial parts of methanolic extract of *Pavetta indica* exhibited significant antioxidant activity was comparable to that of petroleum ether & ethyl acetate extracts of *Pavetta indica.* These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the in vivo antioxidant activity of this extract needs to be assessed prior to clinical use.

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