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Invitro antioxidant activities of methanolic extract of whole plant of *Pedalium murex* (Linn.)

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ABSTRACT
In vitro antioxidant activities of methanol extract of tuberous root of Ipomoea digitata (Linn.) was investigated. The free radical scavenging activity to evaluate by DPPH (2, 2-diphenyl -1- picryl hydrazyl) method, superoxide anion scavenging and iron chelating activity. DPPH radical scavenging activity of Methanolic extract and reference standard Rutin IC_{50} values was found to be 240 µg/ml and 480 µg/ml, superoxide anion scavenging activity of methanolic extract and reference standard Quercetin IC_{50} values was found to be 99 µg/ml and 60 µg/ml and iron chelating activity of methanolic extract and reference standard EDTA IC_{50} values was found to be 440 µg/ml and 65 µg/ml respectively. The above result of possess good an antioxidant activity when compare to the above all standard.

Keywords: Antioxidant, Ipomoea digitata, DPPH method, Superoxide anion scavenging activity, Iron chelating activity.

INTRODUCTION
Radical reactions are also important in the development of chronic diseases that are life limiting like cancers, hypertension and cardiac infarction, atherosclerosis, rheumatism and also in cataract[1]. Naturals antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienbols) ascorbic acid and carotenoids. The use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value[2]. **e**pidemiological and in vitro studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological system[3-5].

Pedalium murex L. (Pedaliaceae) is a diffuse, more or less succulent herb found near the sea coast of south India(6). The fruits as well as the leaves and stems produced milk mucilage when agitated, and it is recommended as a treatment for gonorrhea(7). An infusion or extract prepared from leaves is diuretic and demulcent, useful in treating disorders of the urinary system such as ardor urine, dysuria, spermatorrhoea, and incontinence of urine. As an emmenagogue, the juice is used in puerperal diseases and also to promote lochial discharge(8). The mucilage from leaves and young shoots is used as an aphrodisiac in seminal debility(9). The petroleum ether extract of *P.murex* is effective against Japanese

encephalitis vector culex (10). The aqueous extract of the whole plant has been found to possess analgesic and anti-inflammatory properties (11). Extensive phytochemical investigations on the plant have revealed the presence of Pedalitin and Pedalin (major flavanoids) along with Diosmetin, Dinatin, Dinatin-7-glucoronide, Quercetin, Quercimeritin, and Quercetin-7- glucorhamnoside (12). Triterpenoids such as α- amyrin acetate, Rubusic acid, ursolic acid, and lupeol acetate are reported (13) Steroids such as β-sitosterol (14). Sapogenins (15) and Diosgenin (16). have also been reported. Lipids (17). Phenolic acids such as caffeic acid, ferulic acid, protocathechic acid, and vanillic acid 9, and amino acids such as aspartic acid, glutamic acid, and histidine are other phytoconstituents present in P. murex (18). Although the plant contains several phytoconstituents, they have not been evaluated for their pharmacological activities in detail. Since no scientific data are available on the plant. Therefore in the present work attempt has been made to study the antioxidant effect of fruits of *Pedalium murex* in CCl4-intoxicated experimental rats. It has been hypothesized that one of the principal causes of CCl4 –induced liver injury is LPO by free radical derivatives of CCl4. Thus the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl4 –induced Hepatopathy (19).

MATERIAL AND METHODS

Collection and Identification of Plant materials

The tuberous root of *Pedalium murex* (Linn), were collected form Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The tuberous root of *Pedalium murex* (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 methanol by hot continuous percolation method in Soxhlet apparatus [20] for 24 hrs. The extract was 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

DPPH photometric assay [21]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[14]. 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.

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

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

Superoxide radical scavenging activity [22]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[15]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Iron chelating activity [23]

The method of Benzie and strain (1996) [23] 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.

RESULT AND DISCUSSION:

DPPH METHOD

The percentage of DPPH radical scavenging activity of methanolic extract of *Pedalium murex* presented in Table 1. The methanolic extract of *Pedalium murex* exhibited a maximum DPPH scavenging activity of 59.80% at 1000 µg/ml whereas for Rutin (standard) was found to be 71.74% at 1000 µg/ml. The IC_{50} of the methanol extract of *Pedalium murex* and Rutin were found to be 260 µg/ml and 440µg/ml respectively

Table 1: Effect of Methanolic extract of tuberous root of *Pedalium murex* (Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	41.02±0.022	19.65 ± 0.086
2	250	54.07± 0.04	29.03 ± 0.084
3	500	56.35±0.016	45.81 ± 0.062
4	1000	59.80±0.015	71.74 ± 0.054
		IC_{50} = 260µg/ml	**IC_{50} = 440µg/ml**

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

SUPER OXIDE METHOD

The percentage of Superoxide anion scavenging activity of methanolic extract of *Pedalium murex* presented in Table 2. The methanolic extract of *Pedalium murex* exhibited a maximum Superoxide anion scavenging activity of 91.16% at 1000 µg/ml whereas for Quercetin (standard) was found to be 97.01% at 1000 µg/ml. The IC_{50} of the methanolic extract of *Pedalium murex* and Quercetin were found to be 93µg/ml and 66µg/ml respectively

Table 2: Effect of Methanolic extract tuberous root of *Pedalium murex* (Linn) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	43.02±0.020	75.71 ± 0.004
2	250	72.22±0.024	82.51 ± 0.071
3	500	85.23±0.041	89.86 ± 0.029
4	1000	91.16±0.024	97.01 ± 0.016
		IC_{50} = 93µg/ml	**IC_{50} = 66µg/ml**

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

IRON CHELATING METHOD

The percentage of iron chelating activity of methanolic extract of *Pedalium murex* presented in Table 3. The methanolic extract of *Pedalium murex* exhibited a maximum iron chelating activity of 84.42% at 1000 µg/ml whereas for EDTA (standard) was found to be 93.36% at 1000 µg/ml. The IC_{50} of the methanolic extract of *Pedalium murex* and EDTA were found to be 420µg/ml and 69µg/ml respectively.

Table 3: Effect of Methanolic extract of *Pedalium murex* (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Methanolic extract)	Standard (EDTA)
1	125	31.65±0.042	54.44 ± 0.002
2	250	41.34±0.063	71.83 ± 0.014
3	500	64.50±0.059	89.43 ± 0.016
4	1000	84.42±0.035	93.36 ± 0.015
		IC_{50} = 420µg/ml	**IC_{50} = 69µg/ml**

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

The results of the above investigation indicated that the methanolic extract of tuberous root of Ipomoea digitata (Linn) showed significant antioxidant activity was found in DPPH method, superoxide anion scavenging activity and Iron chelating method when compared to the reference standard Rutin, Quercetin and EDTA respectively.

CONCLUSION

The results of the above investigation indicated that the methanolic extract of tuberous root of *Pedalium murex* Linn) showed significant antioxidant activity. However, Phytochemical screening of extract showed presence of terpenoids and flavonoids. So it can be concluded that these components might be involved in the antioxidant activity of *Pedalium murex*

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