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Pedalium murex: Preliminary Antioxidant activity screening by TLC-DPPH spot assay

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ABSTRACT: Background: *Pedalium murex* is a common medicinal plant in Indian system of Medicine used in the treatment of renal and other disorders, as they possess antioxidant activity. Various methods are adopted for the *In vitro* antioxidant study among which the most common, well established and reliable method is DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. It requires Spectrophotometer for reading color change to predict the activity. **Aim:** The study was aimed to evaluate the preliminary antioxidant activity and to screen the phytochemical constituents of *P. murex* extracts. **Method:** The phytochemical constituent of *P. murex* was extracted by Soxhlation using ethanol, chloroform and water as solvents. The antioxidant activity was evaluated by TLC-DPPH spot assay, done in TLC (thin layer chromatography) plate, which will be suitable as it can be done to screen out qualitatively and rapidly. Gallic acid (G/A) was used as the standard drug. Reduction of purple colour to the corresponding pale yellow hydrazine indicates the presence of the antioxidant activity. The Phytochemical constituents were screened as per standard methods. **Results:** The chloroform extract doesn't show antioxidant activity, where as ethanol extracts showed notable antioxidant activity. The *P. murex* leaf and fruits extracts significantly possessed Glycosides, Flavonoids, Phenols, Saponins, Tannins and Carbohydrates but Resin was absent in all the extracts. **Conclusion:** It could be concluded that the *P. murex* preliminarily passed for antioxidant activity.

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INTRODUCTIONS:

Pedalium murex (P. murex) is a medicinal herb used in both Ayurveda and Siddha, mostly for the renal disorders [1]. *P. murex* belongs to the sesame family, *Pedaliaceae*. It is found in different parts of the world such as tropical India, Pakistan, Srilanka, Mexico and africa. In India it is commonly found in Deccan and in some parts of Gujarat and in the coastal areas of southern India [2]. It is enerally called under the Hindi name "Gokhru or gokhar", in Sanskrit as "gaja -

daunstraka, gokshura or titta-gokshura" and in tamil called as Ananerinnil [3,4]. It is about 15 to 40 cm in height, having four angle spiny brownish colour fruits (1-2 cm) (Fig 1). The fruits are rich in flavonoids, sapogenin (diosgenin-0.06 %) and soluble proteins (20.14 mg/ym) [5].

Fig 1. *Pedalium murex* plant with flowers and fruits.

Traditionally, *P. murex* was utilized in various ways, either as a whole plant or individual plant parts or sometimes in different special preparations. The leaves are cooked and eaten as a vegetable. Leaves and branches, when briskly stirred in cold water yield thick mucilage similar to the white of a raw egg are reported to possess significant medicinal properties [6]. It has been reported that whole plant is used in the various urinary problems [7] and used in the treatment of urinary calculi and renal troubles by village folk of Rayalaseema [8]. Not only whole plant, different parts are used for various treatments. The fruits are reported to be traditionally used as demulcent, antispasmodic and aphrodisiac[9], Leaves are used to treat ulcers, dysuria, Bone fracture, diarrhea and in splenic enlargemen [10,11] and roots are used to treat leucorrhoea.

Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) readily combine and oxidize biomolecules such as carbohydrates, proteins and lipids and thus making them inactive with subsequent damage to cells, tissues and organs [12]. Therefore, antioxidants are vital substances which possess the ability to protect the body from damage caused by free radicals induced oxidative stress[13]. Generally, antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Medicinal plants processing various therapeutic activities may mostly possess antioxidant activity [14].

Various *in vitro* antioxidant test methods used are peroxyl radical scavenging (Oxygen Radical Absorbance capacity, ORAC); Total Radical-trapping Antioxidant Power (TRAP); metal reducing power (Ferric Reducing Antioxidant Power, FRAP); Cupric Reducing Antioxidant Power (CUPRAC); hydroxyl radical scavenging (deoxiribose assay); organic radical scavenging (2,2-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid), ABTS; 2,2-Diphenyl-1-picrylhydrazyl, DPPH); quantification of the products formed during the lipid peroxidation (ThiobarbituricAcid Reactive Substances, TRAPS); Low-density Lipoproteins (LDLs) oxidation, etc. [15] Among which DPPH is the simplest, convenient, reliable and most widely reported method for screening antioxidant activity in foods and many plant drugs [16]. In this assay, the purple chromogen radical DPPH is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 518 nm which is proportional to the antioxidant activity.

The major disadvantage of DPPH assay is dependence on a spectrophotometer. To overcome this drawback, the colorimetric determination of the antioxidant activity using a scanner and freely available Image J software was developed. In this new method, the mixtures of solutions of DPPH and standard antioxidants or extracts of common medicinal herbs were dropped onto TLC plates, after an incubation period. The spot images were evaluated with Image J software to determine CSC50 values, the sample concentrations providing 50% colour reduction, which were very similar with the SC50 values obtained with spectrophotometric method [17-19]. By this same way, for a preliminary qualitative and screening of antioxidant study, TLC-DPPH spot method can be used for rapid estimation.

MATERIALS AND METHODS:

Chemicals and reagents:

DPPH and Gallic acid were procured from S.D. Fine Chemical, Mumbai. All other chemicals and reagents

used in this research work were of analytical grade and were procured from authorized dealer

Pant collection and authentication:

Pedalium murex plants were collected from the banks of Ousteri lake, Puducherry, by following the good collection practice. *Pedalium murex* plants was identified and authenticated by plant taxonomist from French Institute, Puducherry.

Table 1. Phytochemical screenings of ethanolic and aqueous extract of fruit and leaf of *Pedalium murex*.

Sl. No	Phyto chemical constituents	Ethanol Extracts		Aqueous Extracts	
		Fruits	Leaf	Fruits	Leaf
1	Alkaloids	+	+	+	-
2	Glycosides	+	+	+	+
3	Flavonoids	+	+	+	+
4	Steroids	+	+	-	-
5	Phenols	+	+	+	+
6	Saponins	+	+	+	+
7	Tannins	+	+	+	+
8	Proteins	+	-	-	+
9	Carbohydrates	+	+	+	+
10	Resins	-	-	-	-

(-) absent, (+) present.

Preparation of extracts:

Whole plant (W/P) and various parts of *Pedalium murex* plants namely, fruits (F), leaves (L), stem (S) and root (R) are separated and was shade dried. After drying the parts were powdered separately with the cutter mill. The powder was then passed through sieves no: 60. Those fine powders were stored in a well closed container and taken for the extraction. About 20 g powder of whole plant, fruits, leaves; stem and root were subjected to extraction with chloroform, ethanol and water by soxhlation and degoction method respectively. Chloroform and ethanolic extracts were dried with rotary vacuum dryer and the aqueous extracts were freeze dried, and the extracts were stored in a well closed container.

Preliminary Qualitative antioxidant screening by TLC-DPPH spot method:

For the preliminary qualitative study, TLC-DPPH spot method [20] was used with modification. About 50 mg of various extracts ($CHCl_3$, Et-OH and Water extract) are dissolved in the 10 ml of respective solvent in a 10 ml standard flask. Prepared solution was sonicated using a bath sonicator for 10 min to have complete solubility. About 20 µl of each samples are placed on to the TLC plate (Readymade -Sigma-Aldrich) using micro pipette. After drying 20 µl of 1mM DPPH in methanol solution was dropped over the sample spot. About 20 µl of 1mM DPPH in methanol solution was placed separately without sample as the control colour. Gallic acid (G/A) is used as the standard. Reduction of purple colour to the corresponding pale yellow hydrazine, indicates the presence of the antioxidant activity. After an incubation period of half an hour in dark, the colour comparison was made visually and basic antioxidant activity was studied and reported.

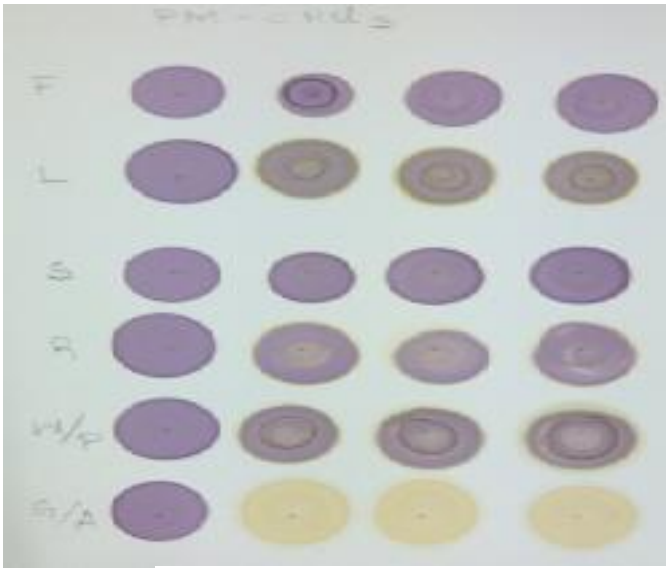

Fig 2. TLC-DPPH spot assay plates of chloroform extracts of various parts of *P. murex*.

Preliminary phytochemical analysis:

The extracts which showed notable antioxidant activity (Ethanolic and aqueous extract of fruits and leaf of *Pedalium murex*) were tested for various Phytoconstituents by standard procedures [21]. They are generally tested for the presence of Alkaloids, Flavonoids, Tannins, Phenols, Cardiac glycosides, Triterpenes, Steroids and Saponins.

RESULTS AND DISCUSSION:

Preliminary qualitative Antioxidant screening:

Preliminary antioxidant screening of various parts of *Pedalium murex* showed that chloroform extract of all parts had no notable colour reduction (Fig 2). The ethanolic extract of leaf, fruit and whole plant showed good anti oxidant activity (Fig 3). The aqueous extract of leaf and whole plant showed good colour reduction and fruit showed a very slight colour drop (Fig 4).

Preliminary phytochemical analysis:

The preliminary phytochemical study revealed that all extracts possess Glycosides, Flavonoids, Phenols, Saponins, Tannins and Carbohydrates, as evident from Table 1. The aqueous extracts were lack of Steroids, where as aqueous leaf extracts was lack of Alkaloids. The resin was absent in all extracts.

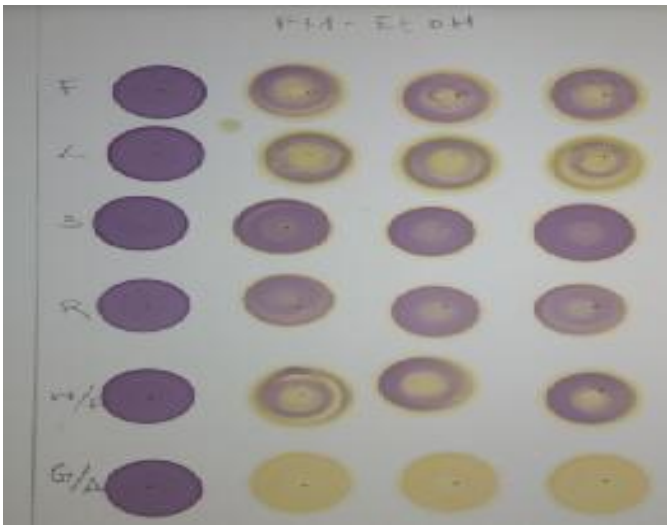

Fig 3. TLC-DPPH spot assay plates of ethanol extracts of various parts of *P. murex*.

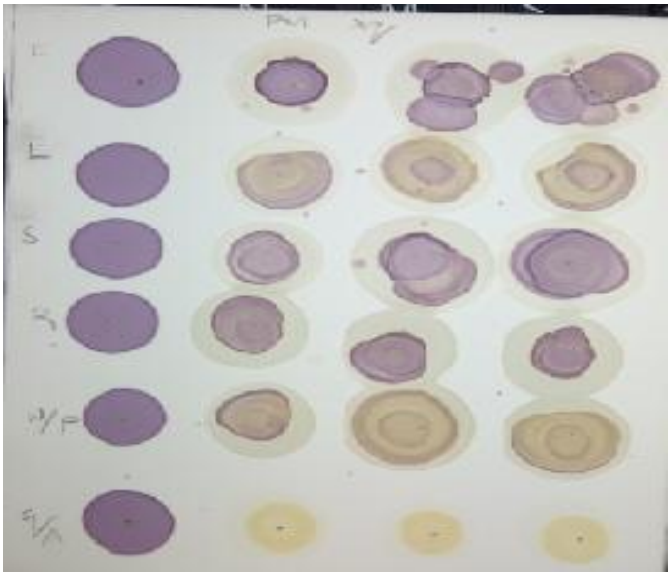

Fig 4. TLC-DPPH spot assay plates of aqueous extracts of various parts of *P. murex*.

CONCLUSION:

In Preliminary Qualitative antioxidant screening by TLC-DPPH spot method, the ethanolic extract of leaf, fruit and whole plant showed good anti oxidant activity and in the aqueous extracts, leaf and fruit showed good antioxidant activity than fruit extract. Moreover this TLC-DPPH spot method will be an ideal and convenient for basic, Preliminary Qualitative antioxidant screening studies. With this preliminary data the quantitative estimation of the DPPH antioxidant assay shall be done with the UV spectrophotometer for the selected candidates. Thus it could be preliminarily concluded that *Pedalium murex* might be possessed significant antioxidant activity, which shall be useful in safe management of several diseases, as mentioned in Folker medicine.

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