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Existence of phenolics, flavonoids compounds and its antioxidant activity of *Pedalium murex* L. plant

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Abstract
Pedalium murex (L.) has long been treating as a medicinal plant for various diseases like disorders of urinary systems, renal injury and other problems and also it contains an abundance of phytochemical compounds. Therefore, it is important to screen the total phenolics (TP) and flavonoids content (TF), its potent antioxidant capacities using standard methods to find out the novel antioxidant supplements to food and used in pharmaceutical industries. The whole plant of *P. murex* L. was used to prepare the Aqueous extract (AQEP) and ethyl acetate extract (EAEP) was adopted with boiling and Soxhlet extraction methods respectively. Furthermost, it has been screened for the presence of phenolic and flavonoid compounds and its antioxidant capability. The presence of phytocompounds was increasing the antioxidant properties to terminate free radicals in the body. It was determined by UV method, DPPH for free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl) and 2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), respectively. From the results, EAEP has highest phenolic (283.16±7.18 mg GAE/g extract), flavonoid (521.00±1.44 mg GAE/g extract) than AQEP. Ethyl acetate extract (IC_{50}6.07±1.94) has more scavenging activity than aqueous extract (IC_{50}10.09±1.68) compared to positive control Rutin and BHT. From the results, we concluded that EAEP has support as medicine for treating of infectious diseases and also to eradicate free radicals to protect the human from various diseases due to the presence of high content of phytocomponents.

Keywords: *Pedalium murex* L., radical scavenging activity, free radicals, phenolic, flavonoid

Introduction
Since ancient period, medicinal plants are basic sources for the remedies of nearly 87% of diseases in human and it was studied for their antioxidant activity [1-3]. Intake of natural antioxidant through food which reduces the risk of cardiovascular diseases, age-related disorders, atherosclerosis, neurodegenerative diseases, cancer, inflammation [4] and also it needs some interface with free radicals and co-factors [5, 6]. Free radicals are extremely active atoms or molecules with unbalancing valence electron which is takes part in human body by involvement in the energy creating process, immunization process, the signalling process and the cell growth and due to high activity it may cause various cell and tissue disorders with damage or alteration of DNA structure. Finally, it leads to cell fatality, abnormal in a gene, cancer, heart attack, atherosclerosis, diabetes, malaria, hypertension, rheumatoid arthritis, cancer and neurodegenerative disorder this may be reduced with the help of plant properties. [7] According to the recent survey, nearly 3000 species of plants have medicinal values which were used by 80% of peoples in developed and developing countries for curable and incurable diseases as primary health care [8, 3]. Currently, the researcher involved in the plant based treatment due to the lower risk factor, good pharmacological properties and low expenditure. Based on the scientific commodities, antioxidants have the capacity to scavenge free radicals and decrease oxidative damage due to destructive process and in medicinal plants it is present in the form of raw extracts or chemical composition [9-11]. Due to the side effects of synthetic antioxidant such as Butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) act as carcinogens and form liver injure and microbial contamination, to overcome this we search some natural product as antioxidant from medicinal plants [12-15]. Though, there is no wide scale research work has to be done on the extracts of *P.murex* L. the plant till date. This work should have the baseline of the plant that can be used for various ailments.
From the investigation, there is an urgent need for the development of novel and efficient constituent to treat untreatable diseases without any complicated side effects. The health conditions will be improved by antioxidants capacity of phenolic and flavonoids compounds in the plant. The phenolic compounds are the central ingredients for dietary applications and it

includes polyphenols (hydrolyzable and condensed tannins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and flavonoids it may reduce the coronary heart disease, death in cancer and protective in several health-related properties [16]. A phytochemical screening method is an important step to explore antioxidant properties present in the plants to cure several ailments in human as dietary food. Based on an earlier study regarding the antioxidant effect, the extracts from *P. murex* L. have been established as free radical scavengers. Still, there has been none other immense work carried out on the antioxidant properties in the whole plant of *P. murex* (L.) [17].

Pedalium murex L. is commonly called "Gokhru" belongs to the sesame family Pedaliaceae and distributed in India, Pakistan, Sri Lanka and tropical Africa. It is highly medicinally important plant and its fruits contain high amount of polyphenolics (flavonoids and phenolics), soluble proteins (20.14 mg/g) and glycosides like sapogenin (diosgenin-0.06%) [17-19]. Due to the presence of high polyphenolics content in this plant, it has useful effects on renal injury and also for the treatment of disorders in urinary systems such as dysuria, gonorrhoea, and incontinence of urine. Therefore, in the current work proposed to explore the presence of phenolic, flavonoids content, antioxidant profile using aqueous and ethyl acetate extract from *P.murex* L. whole plant parts.

Materials and Methods

Collection and authentification of plant

P. murex (L.) plant was collected from Thanjavur district, Tamil Nadu, India and its identification (Voucher number is RHPM SR 001) taxonomically done by the director Rev Dr. S. John Britto SJ, in the Rapinat Herbarium and Centre for Molecular Systematic at St. Joseph College (Autonomous), Tiruchirapalli, Tamil Nadu, India.

Extract preparation

P. murex L. plant was thoroughly washed under tap water then finally with distilled water after that it was dried on dry paper at room temperature to obtain reliable weight, later ground to fine powder using a blender which passed through 20 m mesh cloth for coarse powder.

Aqueous Extraction

Using hot extraction, we have to add 50 g of plant material with 250 ml of Millipore water and it kept for 24 hrs at room temperature after that it was boiled for 15 min on low flame. Then the filtrate was filtered through four layers of muslin cloth to obtain the filtrate. Finally, the extract was preserved at 4ºC for further requirements [17].

Solvent extraction

The coarse powder of *P.murex* L. was extracted with 1:2 w/v of ethyl acetate using Soxhlet apparatus it was concentrated at 45 °C using rotary vacuum evaporator under reduced pressure to become thick paste by placing on a water bath at 4 ºC in an air tight glass bottle for the entire study. The yield of extract was 15.1% w/w in terms of dried material [20].

Determination of total phenolic content

For the determination of total phenolic content in *P. murex* L. plant, the Foline- Ciocalteu reagent (FCR) method was followed by Singleton *et al*., [21]. Briefly, 10 ml of Foline-Ciocalteu reagent (FCR) and 6% Na_2CO_3 solution was mixed in distilled water. Each sample of aqueous and ethyl acetate extracts were mixed in 4 ml of methanol and aliquots were taken each of 200 ml was kept in separate tubes. Then, 10% FCR of 1.5 ml was added to every tube and incubated for 5 min in the dark place. After that, 1.5 ml of 6% sodium carbonate was taken and added to each tube and it was shaken by hand. Finally, it was incubated for 2 hrs in the dark place and absorbance was measured by UV visible spectroscopy at 760 nm wavelength. For the gallic acid standard solution, the same procedure was carried out and the calibration curve was ready from different concentrations of gallic acid and it was expressed as mg gallic acid equivalent (mg GAE)/g extract).

Determination of total flavonoids content

By the colorimetric method, with some modifications, TFC was determined by Shen *et al*., 2009 [22]. Aliquots (0.5 ml) of extracts were transferred into 15 ml tubes made of polypropylene containing 2 ml double distilled water and 0.15 ml of 5% of sodium nitrite. After 5 min of incubation, add 10% of 0.15 ml Aluminium chloride Hexahydrate solution, and then it was allowed for another 5 min to stand, then 1 ml of 1 mol L−1 sodium hydroxide was mixed and incubated for 15 min. At 415 nm, the absorbance was taken and the results were calculated from a standard rutin curve expressed as rutin equivalents (mg RE/g dry weight).

Antioxidant Activity

DPPH radical scavenging activity

The free radical scavenging activity of the two extracts such as aqueous and ethyl acetate, butylated hydroxytoluene (BHT) and rutin was measured in terms of free radical scavenging react with stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) described by Stanojevic *et al*., 2009 [23]. By measuring, the degree of alteration from purple to yellow colour for the measurement of scavenging potential of plant extracts of *P. murex* (L.). Aliquots of aqueous and ethyl acetate extracts solutions (1 mg/ml) were taken to 3 ml volume with methanol then add freshly prepared 0.15 ml of DPPH solution, stirred and left for 30 min at room temperature in dark place. For the test activity, the DPPH solution kept as control and methanol as blank solution and absorbance was measured at 517 nm using spectrometer. Scavenging free radicals was calculated by

Scavenging activity (%)

= [(Absorbance control – Absorbance sample/Absorbance control)] × 100

Whereas, Abs control is the absorbance of DPPH radical and methanol; Abs sample is the absorbance of DPPH radical and Sample extracts/standard.

ABTS radical scavenging assay

The standard procedure for the measurement of ABTS was carried out following the method of shah *et al*., 2014 [24]. For the analysis, mix the stock solution which contains 7 mM ABTS solution and 2.45 mM potassium persulfate solution and then allowed for 12 h at room temperature in the dark for the reaction. After that, it was diluted with 1 ml ABTS solution with 50% ethanol to obtain an absorbance of 734 nm using the spectrophotometer. For each assay, ABTS solution was freshly prepared. For the measurement of activity, 1.0 ml aqueous and ethyl acetate extracts (1 mg/ml) was mixed to react with 1 ml of the ABTS solution after 7 min it was measured the absorbance at 734 nm. The ABTS scavenging

capacity of the extracts was compared with that of BHT and Rutin.
Calculate the percentage of inhibition,

ABTS radical scavenging activity (%)

= [(Abs control - Abs sample)/Abs control] × 100

Where, Abs control is the absorbance of ABTS radical + methanol

Abs sample is the absorbance of ABTS radical + Sample extracts/standard.

Superoxide radical scavenging activity

Superoxide radical scavenging assay was determined by the methods of Beauchamp and Fridovich (1971) [25] with slight modifications. All the solutions were prepared with 0.2 M phosphate buffer (pH 7.4). Then the extracts of aqueous and ethyl acetate were mixed with 3 mL of buffer solution (pH 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM nitro blue tetrazolium. The reaction solution was observed with two 30 W fluorescent lamps for about 20 min and the absorbance was measured at 560 nm and compared with BHT.

Superoxide radical scavenging activity (%)

= (1− abs of sample / abs of control) × 100

Control = Reaction mixture without any sample

Results

Total Phenolic and flavonoids contents

The total phenolic content of the aqueous and ethyl acetate extracts, were calculated from the calibration curve (R^2 = 0.998), was 181.32±7.47 and 283.16±7.18 gallic acid equivalents/g, and the total flavonoid content was estimated from (R^2 = 0.999) was 197.15±4.30 and 521.00±1.44 rutin equivalents/g. The best content was observed in EAEP than AQEP are represented in Table 1.

Table 1: Quantification of Total phenolics and Flavonoid contents

Extracts	Total phenolics (mg GAE/g extract)	Flavonoids (mg RE/g extract)
Aqueous	181.32±7.47	197.15±4.30
Ethyl acetate	283.16±7.18	521.00±1.44

Values are mean of triplicate determination (n=3) ± standard deviation; GAE - gallic acid equivalents; RE - Rutin equivalents

***In - vitro* antioxidant activity**

ABTS radical scavenging activity

The ABTS radical scavenging activity in extracts of *P. murex* L. was carried out by using 2, 2 azobis-(3-ethylbenzothiozoline-6-sulphonic acid). The best activity in ABTS assay was shown in EAEP 13829.41±1591.51 (µM TE/g extract) than AQEP which has low activity 21307.15±751.00 (µM TE/g extract) represented in Table 2.

Table 2: *In-vitro* Antioxidant assays (ABTS, DPPH and Superoxide radical scavenging activity)

Extracts	$ABTS^{\cdot+}$ (µM TE/g extract)	DPPH IC_{50} (µg/mL extract)	Superoxide radical Scavenging assay IC_{50} (µg/mL extract)
Ethyl acetate	21307.15±751.00	13.07±1.01	6.07±1.94
Aqueous	13829.41±1591.51	18.75±2.00	10.09±1.68
BHT	60002.65±509.42	5.05±0.51	3.11±1.5
Rutin	43220.96±7307.70	4.24±0.40	4.15±0.3

Values are mean of triplicate determination (n=3) ± standard deviation; TE – Trolox Equivalents; BHT – Butylated hydroxytoluene

DPPH radical scavenging activity

From the analysis, IC_{50} values of DPPH scavenging radicals for the AQEP and EAEP were 18.75±2.00 and 13.07±1.01µg/ml, respectively (Table 2). Moreover, the antioxidant potential of extracts was found to be low ($P < 0.05$) than those of BHT and rutin. Finally, it was revealed that EAEP has prominent antioxidant activity than AQEP because the IC_{50} values are irreversible to antioxidant activity that is the lower value of IC_{50} which increase the antioxidant properties of plants. The presence of phenolic compounds (containing phenolic hydroxyls) is mainly found in both two fractions that have high antiradical properties of these extracts.

Superoxide radical scavenging activity

The superoxide radical scavenging effect of two extracts was compared with the BHT and Rutin. The IC_{50} values of AQEP and EAEP were represented in (Table 2). When compared to BHT and Rutin; the superoxide scavenging activity of the extracts was found to be low ($P < 0.05$). Even though this, two extracts (10.09±1.68and 6.07±1.94 µg/ml respectively) behave as powerful superoxide scavengers that may consist of therapeutic use against oxidative stress.

Discussion

In recent years, we look back to the natural way of remedies for different types of diseases using medicinal plants because it was safe, affordable and its availability. Based on the literature survey, we analyzed the whole plant characteristic of *Pedalium murex* L. as the natural antioxidant for infectious diseases caused by microbes. The biological activities of medicinal plants mainly depend on the phytoconstituents basically phenolic and flavonoids compounds [26]. Phenolic compounds have redox properties, which is a class of antioxidants includes polyphenols [27, 28] used for the termination of free radicals due to the presence of hydroxyl groups and also it could be used as a beginning for rapid screening of antioxidant activity.

Other compound flavonoids include flavones, flavanols and condensed tannins are secondary metabolites and it has antioxidant activity by the existence of free OH groups, particularly 3-OH for the prevention of oxidative stress [28, 29] which may responsible for DPPH, ABTs activities. It consists of a polyphenolic compounds which are a large group of secondary metabolites, contains benzopyrone structure, and helps the plants to produce them in multiple ways [30, 31] and it has the properties of a mitochondrial adhesion inhibitor, antimicrobial agent, an antiarthritic agent, an antiulcer agent, an antiangiogenic agent and an anticancer agent [31, 32]. It was observed from our work, the aqueous and ethyl acetate has more or less equally effective solvent but slightly EAEP has efficient than AQEP. The effectiveness of this plant has been

well recognized in preceding studies but it was a slight difference that EAEP has more or less equal antioxidant property with AQEP so we indicate it is best as compared to previously reported work [18].

Antioxidant was used to scavenge the reactive oxygen species or act as defence mechanisms by fighting against free radicals produced in our body. DPPH method was used to measure the electron donor capacity in natural products and for free radicals scavenging from our body by the addition of radical species that decolourize DPPH solution. The potentialities and concentration of the plant extracts were finding out based on the degree of colour changes during the reaction and also the free radicals scavenging properties. Based on the results, the aqueous and ethyl acetate extracts has the property to scavenge the free radicals related to the phenolic content but EAEP slightly higher than AQEP. It was noted that the Phytoconstituents present in the plant extracts was responsible for donating a hydrogen bond to free radicals to scavenge the damage from our body. Superoxide radicals have the properties of reactive oxygen species as the major biological source. It is a weedy oxidant which gives the tremendous increase of treacherous hydroxyl radical that damage our body mechanisms. From our research, the AQEP has the capacities of scavenging superoxide free radicals slightly than the EAEP extract [33].

Conclusion

An increasing requisite for natural additives as the food it has been shifted from synthetic to natural antioxidants. The total phenolics, flavonoids and antioxidant activity were measured in the aqueous and ethyl acetate extracts of *P. murex* L. using standard methods and its best activity was found in EAEP. The highest TPC and TFC were found in this plant and it is correlated with higher antioxidant activity. It may contain more non-phenolic compounds or possess phenolic compounds that contain a smaller number of active groups than the other solvents. Therefore, from overall work, it was indicated that *P. murex* L. has proper extraction and it could serve as a remedy against free-radical-associated with oxidative damage. The toxicity of medicinal plant extracts is further investigated, used as diet as antioxidant sources would be placed in safe limits for human health.

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