Journal of Cardiovascular Disease Research
ISSN: 0975-3583,0976-2833 VOL 15, ISSUE 1, 2024

Phytochemical Profiling and In Vitro Evaluation of the Antioxidant Potential of *Pedalium murex* Leaf Extracts

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Abstract

Introduction Plants are rich sources of various medicinal components that can be used to treat a variety of health issues. One such plant is *Pedalium murex,* which is used in treatment of disorders of urinary systems like gonorrhea, dysuria, and incontinence of urine. The fruits of this plant are rich in polyphenolics and glycosides and due to high polyphenolics present in this plant it is necessary to investigate its antioxidative activity.

Materials and methods Preparation of ethanolic extract of *Pedalium murex* was prepared by Maceration technique, where leaves are ground, placed in a container. The menstruum is poured until the material is covered completely . The content is stirred and shaken periodically. The micelle is separated by filtration and separated from the menstruum by evaporation on top of a

water bath. Antioxidant activity was measured in terms of radical scavenging capacity using the DPPH (2,2-Diphenyl Picryl-Hydrazyl) method, which involves stable radicals.

Results As for antioxidant activity, at a high concentration the extract has increased capacity to scavenge free radicals and has 70% more radical inhibition when compared to the standard, having 40% inhibition. The qualitative phytochemical screening shows the presence of four groups, which explains the medicinal and antioxidant properties of *Pedalium murex*. These groups include carbohydrates, glycosides, quinones and saponins.

Conclusion Phytochemicals were derived from leafy extract of *Pedalium murex* and its antioxidant activity was confirmed using DPPH assay and at higher concentrations, performed better than the current standard.

Keywords Antioxidant activity, DPPH assay, In vitro, *Pedalium murex*, Phytochemicals

Introduction

One of the most priceless natural resources for humans and animals since their existence are plants. They provide the most basic but valuable necessities for living including food, oxygen, residence, construction and ecosystem balancing support (1). Additionally, all parts of plants are rich sources of various medicinal components that can be used to treat a variety of health issues. One such plant that is said to have various medicinal benefits is *Pedalium murex,* which is currently being studied extensively, both in vivo and in vitro for its properties. It is a member of the sesame family, Pedaliaceae, found in different parts of the world such as tropical Africa, Srilanka, India, Mexico and Pakistan. In India, it occurs mainly in the Western and Coromandel coasts as a weed of waste places and is generally called under the Hindi name “Gokhru” and in Sanskrit as "gaja-daunstraka” (2). An infusion or extract prepared from the different parts of the plant in cold water is used as demulcent, diuretic and also found to be used in the treatment of disorders of urinary systems such as gonorrhea, dysuria, incontinence of urine and vice versa (3).

A decoction of the fruits was found to be effective as demulcent, diuretic, antispasmodic and aphrodisiac. The decoction of the root can also be used as an antibiliary. Studies have revealed that *P. murex* is a source of medicinally active compounds and has various pharmacological effects, hence, the plant is used to find new therapeutic uses (4). An infusion from leaves and stems was reported to be used in the treatment of gonorrhea and dysurea. In the past, several

flavonoids have been isolated from the leaves and flowers of this specific plant are rich in polyphenolics (flavonoids and phenolics), glycosides like sapogenin and soluble proteins (4). Phytochemically the plant is popular for the presence of a considerable amount of diosgenin and vanillin. They are regarded as an important source and useful starting materials for synthesizing steroidal contraceptive drugs and isatin alkaloids. Quercetin, ursolic acid, caffeic acid, amino acids (glycine, histidine, tyrosine, threonine, aspartic acid and glutamic acid) and classes of fatty acids are some other phytochemicals reported to be found in the plant extract (5).

An antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate (6). Fruits, teas, vegetables, cereals, and medicinal plants, which are rich sources of natural antioxidants, are used not only for the prevention and treatment of various diseases caused by oxidative damage, but also for improving the shelf life of food products. They have received great attention since they are effective free radical scavengers, by donating hydrogen to highly reactive radicals, thereby preventing further radical formation (7).

Human bodies possess enzymatic and non-enzymatic antioxidative mechanisms and minimize the generation of reactive oxygen species (8). Thus, it is essential to develop and utilize effective natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases. Epidemiological studies suggest that increased consumption of fruits are important dietary sources of antioxidant polyphenols to humans associated with a lower risk of degenerative diseases, therefore they play an important role in health care (9). Due to high polyphenolics present in this plant it is duly necessary to investigate its antioxidative activity by comparing with the standard compound.

Materials and method

Collection of sample and preparation of ethanol of extract

The leaves of plants were collected from the botanical garden in Chennai, Tamilnadu, India. The

identity of the plant material was confirmed by a botanist. The leaf extract was prepared using Maceration technique. This is an extraction procedure where the leaves are coarsely powdered and placed inside a container. The menstruum is poured on top until completely covering the drug material. The container is then closed and kept for three days. The content is stirred periodically and shaken from time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration. Subsequently, the micelle is then separated from the menstruum by evaporation on top of a water bath (10).

Methodology for antioxidant activity

The antioxidant activity of the sample was measured in terms of radical scavenging capacity using the DPPH (2,2-Diphenyl Picryl-Hydrazyl) method, which involves stable radicals. 0.004g of DPPH was dissolved in 100 mL of ethanol to prepare a 0.004% DPPH solution The test samples were prepared at different volumes. For the blank, 2 mL of distilled water was added and for the standard, 1.9 mL of distilled water and 100 µL of ascorbic acid solution was added. Test samples were added to the corresponding test tubes and 2 mL of the prepared DPPH solution to each test tube. After incubating the mixture in the dark for 30 min at room temperature, the absorbance was measured using a spectrophotometer at 517 nm.

Results

Figure 1 shows the initial reaction in micro centrifuge tubes for DPPH assay where different concentrations of the extract are being tested. C (Control) is the negative control, containing the DPPH radical without any plant extract. S1 to S4 represents increasing concentrations of the *Pedalium murex* leaf extract. S1 and S2 show little change from the control, suggesting the concentration of antioxidants is low to neutralize the radicals. S3 shows a slight shift towards a brownish tint. S4 shows the most successful and proves that at this concentration, the extract has a high capacity to scavenge free radicals. The last tube refers to the standard - ascorbic acid.

Figure 2 represents the qualitative chemical analysis of the *Pedalium murex* extract. The

variation in color across the tubes suggests different concentrations of extracts being tested for phenolic density. Tube 1 shows a pale yellow color, suggesting a low concentration of phenols. Tube 2 is the strongest positive result. The dark blue-green color indicates high concentration of phenolic compounds, thereby providing direct evidence that *Pedalium murex* is rich in these bioactive molecules. Tubes 3, 4, and 5 show varying shades of brownish-orange, showing different subclasses of phenols. The darker orange color in Tube 5 suggests the presence of tannins and specific quinones.

Figure 3 provides the quantitative data for the plant's antioxidant potency. There is a clear upward trend from sample S1 to S4, which demonstrates that as the concentration of the *Pedalium murex* extract increases, its antioxidant power also increases. Sample S4 shows the highest level of activity, achieving roughly 70% inhibition. This matches the results seen in the DPPH assay where the corresponding tube showed the most significant color change.The standard reference (ascorbic acid) shows roughly 40% inhibition, providing a baseline to compare the effectiveness of the samples.

Table 1 shows the results of the qualitative phytochemical screening. It shows the presence of four phytochemical groups, which explains the medicinal and antioxidant properties of *Pedalium murex*. Carbohydrates are primary metabolites that serve as the structural backbone for complex molecules like glycosides. Glycosides are known for their pharmacological effects such as supporting heart health and natural diuretics. Quinones are aromatic compounds associated with pigments in plants, possessing antimicrobial and antioxidant properties, contributing to the scavenging activity seen in DPPH results. Saponins are known for their soap-like foaming characteristics and are known for their ability to lower cholesterol and boost the immune system.

Figure 1 - Initial reaction for a DPPH assay in different concentrations

Figure 2 - Presence of phenolic compounds

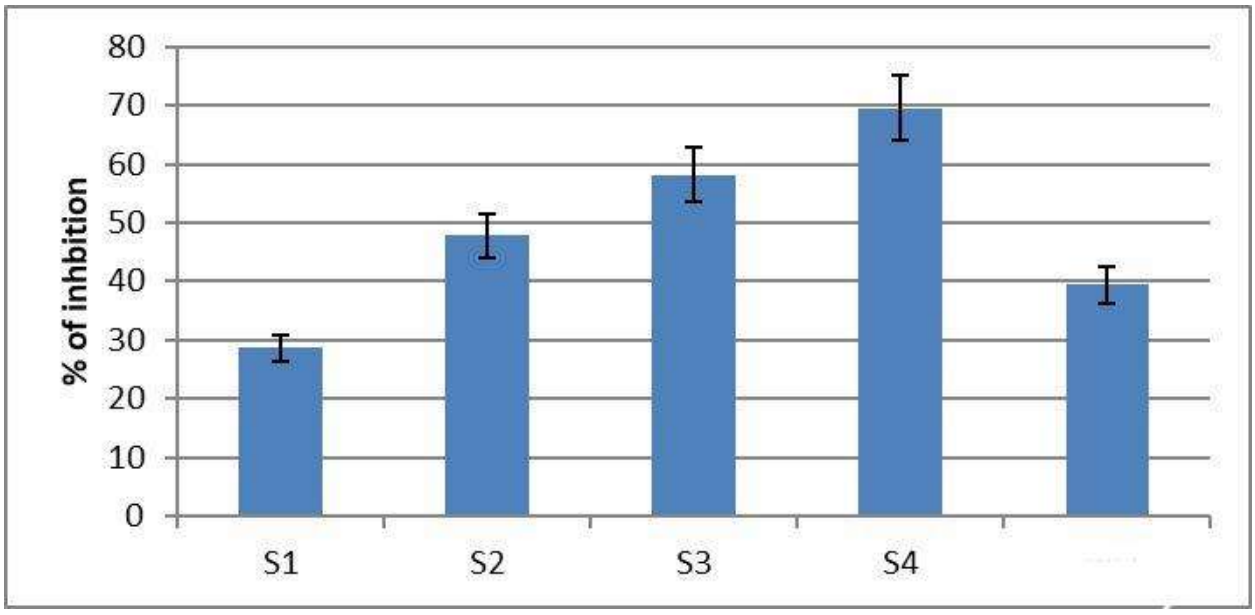

Figure 3 - Percentage Inhibition of ethanolic extract of *Pedalium murex*

COMPOUNDS	PRESENCE
Carbohydrates	+
Glycosides	+
Quinones	+
Saponins	+

Table 1 - Presence of various compounds in ethanolic extract of *Pedalium murex*

Discussion

From the results obtained above, we have confirmed the successful synthesis of leaf extract *Pedalium murex*, and observed the presence of various phytochemical groups such as carbohydrates, glycosides, quinones and saponins. We have also tested its antioxidant activity.

In an in vivo study conducted on rats, it was noted that methanolic extract dosed rats have shown efficacy to overcome the oxidative stress caused by hepatic injury and decreased efficacy of glutathione catalase, glutathione reductase, peroxidase antioxidant enzymes and superoxide

dismutase (11). The results of the studies have revealed that different alcoholic extract fractions of these plants have very high contents of phenolic components that are very effective anti-oxidants for human health (12).

Another study that compared the phenolic compounds and antioxidant efficacy between the leaf and fruit of the plant extract, it was noted that leafy stem aqueous extract contained significantly more phenolics, flavonoids and tannins than the fruit extract. With respect to antioxidant activity, it was noted that aqueous extract of the leafy stem exhibited a higher DPPH scavenging activity than the fruit. However, the extract's ability to scavenge DPPH was less marked as compared to ascorbic acid (4).

In another recent study, it was reported that 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 ethyl acetate extract. The highest total phenolics and flavonoids content were found in the 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 (13).

A study that screened the phytochemicals of *P. murex* fruit extract, revealed the presence of alkaloids, saponins, tannins, flavonoids, sugar, glycosides, phenols and sterols. The total phenolic content determined using linear regression equation was found to be 27.1± 0.72 mg/g equivalent of gallic acid while the flavonoid content was expressed as 17.6 ± 0.79 mg/g equivalent of quercetin (13).

Apart from antioxidant activity, the plant extract has also been studied for its anti ulcer activity and concluded that pretreatment with aqueous extract of leaves of *Pedalium murex* at a dose of 200 mg/kg in a single schedule and 100 mg/kg for 15 and 30 days treatment annihilated alterations and elevated the level of glutathione (14). Similarly, a study conducted on male wistar albino rats noted that when the ethanolic fruit extract of *P. murex* was delivered to ethylene glycol intoxicated rats, there were reverted levels of the liver and kidney markers to near normal levels protecting liver and renal tissues from damage and also prevent the crystal retention in tissues (15).

This plant extract has been praised for its anti-urolithiatic potential, as proven in a study that concluded that there was significant result ($p < 0.05$) observed in 1% ethyl acetate extract (300 mg) treated group than cystone treated group. From the histological study, reduced renal damage and glomerular development were observed. *P. murex* extract enhances the reducing activity on struvite crystal and prevents the crystal formation both *in-vitro* and *in-vivo* (3). Additionally, the plant extract has been studied for its antibacterial activity, where it was noticed that ZnO-NPs and ZnO-NSs derived from *P. murex*, were tested against both gram positive (*B. subtilis*, *S. aureus*) as well as gram negative bacteria (*P. mirabilis*, *S. typhi*) by modified disc diffusion method and it showed important antibacterial activity against *P. mirabilis* and *S. typhi* (16).

Conclusion

From the above results obtained, it can be concluded from this study that we have successfully identified the phytochemicals present in the leafy extract of *P. murex* such as carbohydrates, glycosides, quinones and saponins. Additionally we have also studied its antioxidant property against the standard where it can be noted that at concentration, the extract has a high capacity to scavenge free radicals, achieving roughly 70% inhibition whereas the standard showed 40% inhibition.

Acknowledgement:
We express our sincere gratitude to Saveetha Institute of Technical and Medical Sciences for their constant support and encouragement.

Conflict of Interest:
Nil.

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