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Phytochemical determination and antioxidant potential of whole aerial parts of *Pedalium murex* L.

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Abstract

The current study assesses the phytochemical content and antioxidant capacity of the entire aerial portions of *Pedalium murex* L. (Family: Pedaliaceae), a historically significant medicinal plant. We used solvents with different polarities, like water, methanol, chloroform, and n-hexane, to extract the material. Qualitative phytochemical screening showed that there were sugars, glycosides, flavonoids, terpenoids, and phytosterols, but no alkaloids or saponins. GC-MS investigation revealed multiple bioactive chemicals, including n-hexadecanoic acid, octadecanoic acid, phytol, and stigmasterol, suggesting considerable pharmacological potential. The DPPH assay was used to test for antioxidant activity. It showed that the activity depended on the concentration, with the chloroform extract showing the most activity ($IC_{50} = 89.08 \mu\text{g/ml}$). The results underscore the medicinal significance of *P. murex* L. and validate its conventional application as a source of natural antioxidants and bioactive substances.

Keywords: *Pedalium murex* L., Phytochemical determination, Antioxidant activity, GC-MS investigation

Introduction

Medicinal plants have been an important aspect of traditional medicine for a long time, especially in places like India where ethnomedicine is a big part of everyday life. Medicinal plants are full of bioactive chemicals such as alkaloids, flavonoids, phenolics, and terpenoids that make them useful for medicine (Sharma, 1992; Nadkarni, 1976) ^[16, 13]. In recent years, scientists have become more interested in plant-based chemicals since they work well, are cheap, and have few adverse effects compared to synthetic pharmaceuticals (Patwardhan *et al.*, 2005; Newman & Cragg, 2020) ^[15, 14, 4]. Oxidative stress, which happens when there are too many free radicals and not enough antioxidants, is a big cause of many long-term illnesses, such as cancer, diabetes, heart problems, and diseases that cause the brain to lose function (Gupta *et al.*, 2013; Aggarwal *et al.*, 2013) ^[7, 1]. Natural antioxidants obtained from medicinal plants are essential in counteracting free radicals and safeguarding cellular integrity. Consequently, the identification and assessment of plant-derived antioxidants have emerged as a significant domain of pharmacological study (Isman, 2006; Dixon, 2001) ^[18, 6]. *Pedalium murex* L., a member of the Pedaliaceae family, is a well-known medicinal plant that grows in dry and semi-dry areas of India. It has been traditionally employed for the treatment of urinary diseases, reproductive issues, inflammation, and microbiological infections (Kumar *et al.*, 2006; Cunningham, 2001) ^[11, 5]. Although it has been widely used in traditional medicine, there is still not enough scientific evidence to fully support its phytochemical makeup and antioxidant properties.

Materials and methods

Collection of plant materials

Whole aerial part of *P. murex* were collected from Karauli district, Rajasthan. Those were washed with running tap water, shade dried and ground to make coarse powder and stored in refrigerator for further use.

Extraction

The collected parts were extracted in various solvents. For this, 1 g of plant material was dipped into 10 ml of solvent (water, methanol, chloroform and n-hexane).

Those were filtered and solvents were evaporated. Dry extract yield was noted down.

Qualitative determination of phytochemicals

The extracts were determined for the presence of various primary and secondary metabolites using various standard methods such as-

- **Carbohydrate test (Fehling's test):** Extract was treated with Fehling's solution; formation of brick-red precipitate indicated presence of carbohydrates.
- **Protein test (Ninhydrin test):** Extract was heated with ninhydrin reagent; development of violet/purple colour confirmed proteins.
- **Glycosides test (Sulphuric acid test):** Extract was treated with concentrated H_2SO_4 ; colour change indicated presence of glycosides.
- **Flavonoid test (Lead acetate test):** Addition of lead acetate produced yellow precipitate, confirming flavonoids.
- **Phenol test ($FeCl_3$ test):** Ferric chloride was added to extract; formation of blue/green colour indicated phenolic compounds.
- **Alkaloid test (Mayer's and Dragendorff's tests):** Addition of Mayer's or Dragendorff's reagent resulted in precipitate formation, indicating alkaloids.
- **Saponin test (Foam test):** Extract was shaken with water; stable foam formation indicated presence of saponins.
- **Terpenoid test (Salkowski's test):** Extract was treated with chloroform and H_2SO_4 ; reddish-brown coloration indicated terpenoids.
- **Phytosterol test (Liebermann-Burchard test):** Extract was treated with acetic anhydride and H_2SO_4 ; green/blue colour confirmed phytosterols.

GC-MS study

The methanolic samples of both plants were subjected to determine phytochemical constituents. The samples were sent to Accuphysem Analytics, Jaipur for characterization.

DPPH Free radical scavenging activity

For determination of DPPH radical scavenging potential of the extracted samples 1,1-diphenyl 2-picryl-hydrazil (DPPH) method was applied. The mixing of 100 μ l sample was done in 3.9 ml taken from 0.1 mM DPPH (methanolic) solution. Then blend was subjected to vortex and left for incubation in the dark for 30 min. Its OD (Optical Density) was calculated at 515 nm while methanol was used as blank. The radical scavenging activity was determined by the ratio

$$= (Ab_{\text{control}} - Ab_{\text{sample}} / Ab_{\text{control}}) \times 100$$

Where Ab_{control} is presenting the absorbance of the DPPH solution and absorbance of the DPPH solution with sample is denoted by Ab_{sample} .

Linear plot of concentration versus % inhibition was plotted and by this IC_{50} values were determined. The antioxidant potential of each extract was showed in form of IC_{50} (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of inhibition curve.

Results

Extraction of the plant material

Pedaliu murex L. showed maximum extraction yield in methanol (0.435 g/5 g), followed by aqueous (0.169 g/5 g), chloroform (0.08 g/5 g), and n-hexane (0.025 g/5 g). Polar solvent extracts (methanol and water) were dry, while non-polar extracts were sticky in nature. These findings indicate that polar solvents are more efficient in extracting phytoconstituents from the plant species.



Fig 1: Collection and Processing of plant material.

Table 1: Properties of the extracts.

Sample	solvent	Extractive value (mg/g.dw)	Consistency
<i>Pedaliu murex</i> L.	N hexane	0.005	Sticky
	Chloroform	0.016	Sticky
	Methanol	0.087	Dry
	water	0.0338	Dry

Phytochemical test

In *Pedaliom murex* L., phytochemical analysis demonstrated the presence of carbohydrates in all extracts. Proteins and glycosides were detected only in the aqueous extract, whereas flavonoids were observed exclusively in the

methanolic extract. Terpenoids were present in chloroform extract, and phytosterols were detected in all solvent extracts. Phenolic compounds were either absent or present in negligible amounts. Alkaloids and saponins were not detected in any extract.



Fig 2: Qualitative phytochemical test of *Pedaliom murex* L.

Table 2: Qualitative phytochemical test for *Pedaliom murex* L.

Phytochemical test	water		chloroform		n-Hexane		methanol	
	O	R	O	R	O	R	O	R
Protein	Yellow	+	Green transparent	-	Transparent	-	Green	-
Glycosides	Red brown	+	Blue	-	Brown	-	Blue	-
Carbohydrate	Blue (ketose sugar)	+	Red brown (Aldose sugar)	+	Red brown (Aldose sugar)	+	Red brown (Aldose sugar)	+
Flavonoid	Brown	-	Transparent yellow	-	Transparent	-	yellow	+
Phenol's	Brown	-	Yellow	-	Yellow	-	Brown	-
Alkaloid's	Brown	-	Green transparent	-	Transparent	-	Green	-
	Yellow	-	Green	-	Yellow transparent	-	Red brown	-
Saponins	Yellow	-	Brown transparent	-	Yellow transparent	-	Transparent	-
Terpenoids	Yellow	-	Grey	+	Transparent	-	Green	-
Phytosterols	Brown ring	+	Green ring	+	Light green ring	+	Green ring	+

*O- Observation; R- Results

GC-MS studies

GC-MS study of *Pedaliom murex* L. extracts identified a variety of bioactive chemicals, including fatty acids, terpenoids, alcohols, esters, and phytosterols, recognized for their notable pharmacological activities.

In *Pedaliom murex* L., GC-MS analysis identified several major compounds, with ethane, 1-chloro-1-fluoro- (26.83%), isopropyl alcohol (20.93%), and 6-octadecenoic acid (20.47%) as the predominant constituents. Other significant compounds included n-hexadecanoic acid (9.36%), diethyl phthalate (5.16%), octadecanoic acid (3.44%), and stigmasterol (3.44%). Additionally, compounds such as phytol (0.75%), neophytadiene,

hexadecane, and alpha-Hydroxy stearic acid were also detected in smaller amounts. The presence of fatty acids, phytosterols, and terpenoid compounds indicates the potential of *P. murex* L. for antioxidant, anti-inflammatory, and pharmacological activities.

Pedaliom murex L. exhibited a predominance of fatty acids and alcohol-derived compounds. The fact that the plant has phytol, n-hexadecanoic acid, octadecanoic acid, and stigmasterol in them shows that the plant could be useful for medicine. Overall, GC-MS profiling shows that the plant species are rich in physiologically active chemicals, which backs up their traditional medicinal uses and antioxidant qualities.

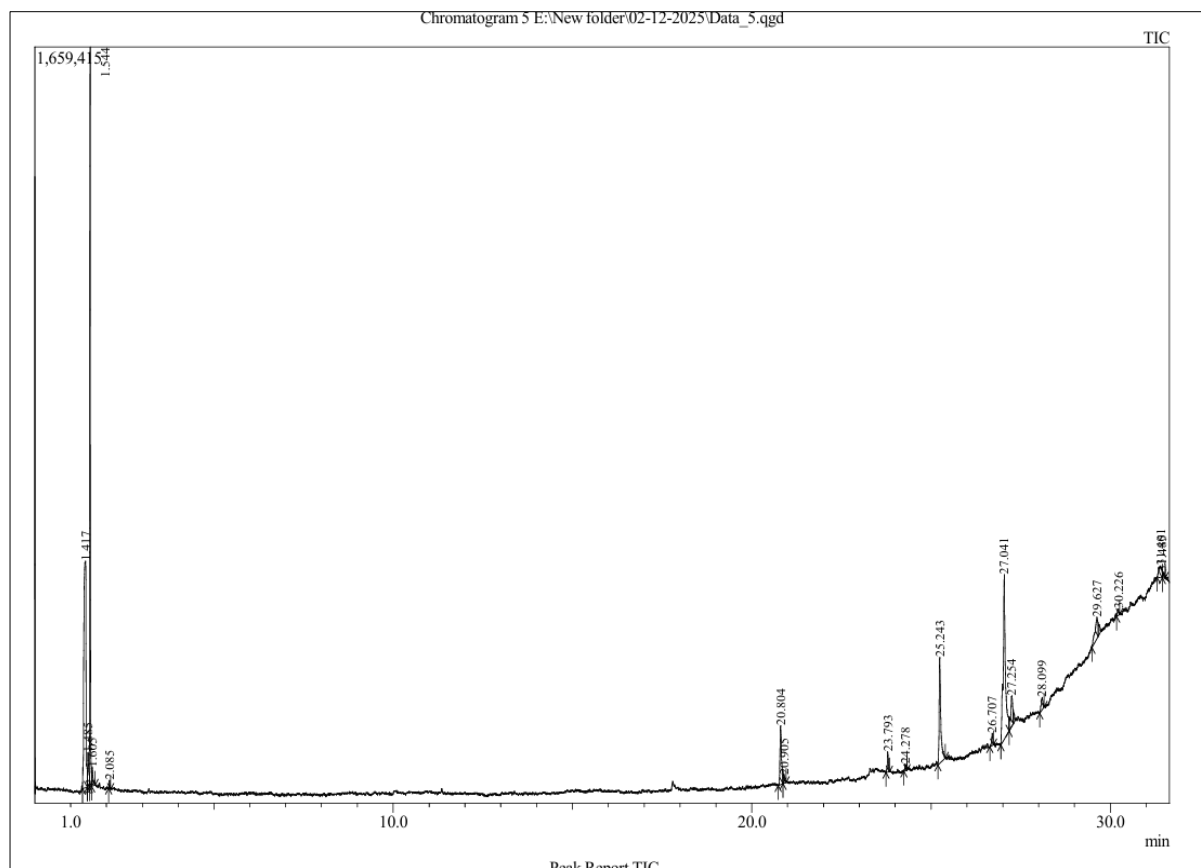


Fig 3: GCMS chromatograph of *P. murex* L.

Table 3: Phytoconstituents identified in aerial part of *P. murex* L. by GC-MS study.

Peak#	R. Time	I. Time	F. Time	Area	Area%	Height	Height%	A/H	Compound Name
1	1.417	1.330	1.470	2122177	26.83	497009	15.17	4.27	Ethane, 1-chloro-1-fluoro-
2	1.485	1.470	1.520	143763	1.82	75678	2.31	1.90	2-Propanone, 1-methoxy-
3	1.544	1.520	1.585	1655619	20.93	1622859	49.54	1.02	Isopropyl Alcohol
4	1.605	1.585	1.680	98586	1.25	39990	1.22	2.47	Acetic acid, methyl ester
5	2.085	2.065	2.120	26793	0.34	17896	0.55	1.50	Propane, 2,2-dimethoxy-
6	20.804	20.740	20.875	408209	5.16	129601	3.96	3.15	Diethyl Phthalate
7	20.905	20.875	20.955	53779	0.68	17883	0.55	3.01	Hexadecane
8	23.793	23.745	23.840	91540	1.16	44925	1.37	2.04	Neophytadiene
9	24.278	24.245	24.330	34959	0.44	15171	0.46	2.30	Neophytadiene
10	25.243	25.190	25.395	740444	9.36	232473	7.10	3.19	n-Hexadecanoic acid
11	26.707	26.640	26.740	59710	0.75	26728	0.82	2.23	Phytol
12	27.041	26.945	27.175	1619318	20.47	361476	11.04	4.48	6-Octadecenoic acid (Z)-
13	27.254	27.175	27.295	271710	3.44	66771	2.04	4.07	Octadecanoic acid
14	28.099	28.030	28.150	96969	1.23	26989	0.82	3.59	1-Hexadecanol, 2-methyl-
15	29.627	29.485	29.665	272037	3.44	47298	1.44	5.75	Stigmasterol
16	30.226	30.175	30.255	38244	0.48	14053	0.43	2.72	2-Methyltetracosane
17	31.401	31.300	31.455	143976	1.82	26014	0.79	5.53	alpha-Hydroxy stearic acid
18	31.485	31.455	31.530	31776	0.40	12789	0.39	2.48	Fumaric acid, Penta decyl 3-pentyl ester

Antioxidant assay

The DPPH free radical scavenging experiment demonstrated that all extracts from the plant had antioxidant activity that clearly increased with concentration. The chloroform extract had the most activity, with an IC_{50} value of 89.08 $\mu\text{g/ml}$.

The aqueous and methanolic extracts showed similar levels of activity, with IC_{50} values of 98.38 $\mu\text{g/ml}$ and 98.91 $\mu\text{g/ml}$, respectively. The hexane extract has the least antioxidant activity ($IC_{50} = 100.67 \mu\text{g/ml}$).

Table 4: DPPH free radicals scavenging activity of *P. murex* L. extracts.

Solvents	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	60 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	$IC_{50} \mu\text{g/ml}$
Methanol	43.68 $\pm$ 0.14	44.86 $\pm$ 0.21	45.44 $\pm$ 0.01	49.37 $\pm$ 0.16	49.99 $\pm$ 0.11	98.91 $\pm$ 1.59
Chloroform	30.86 $\pm$ 0.19	31.4 $\pm$ 0.26	44.28 $\pm$ 0.05	49.93 $\pm$ 0.07	50.9 $\pm$ 0.03	89.08 $\pm$ 0.15
Water	35.29 $\pm$ 0.08	36.44 $\pm$ 0.12	41.52 $\pm$ 0.05	48.72 $\pm$ 0.14	49.32 $\pm$ 0.03	98.38 $\pm$ 0.24
Hexane	36.52 $\pm$ 0.05	43.61 $\pm$ 0.02	44.28 $\pm$ 0.11	45.98 $\pm$ 0.10	49.82 $\pm$ 0.19	100.67 $\pm$ 1.25

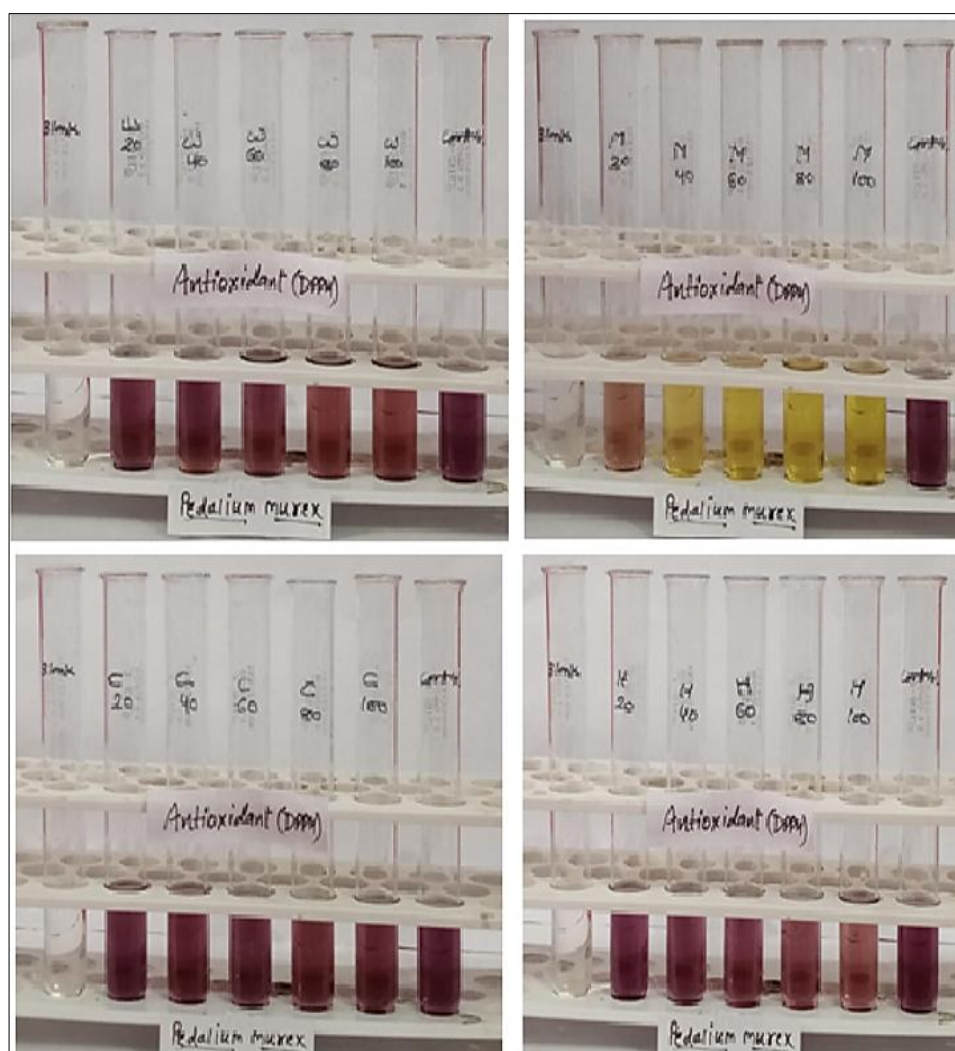
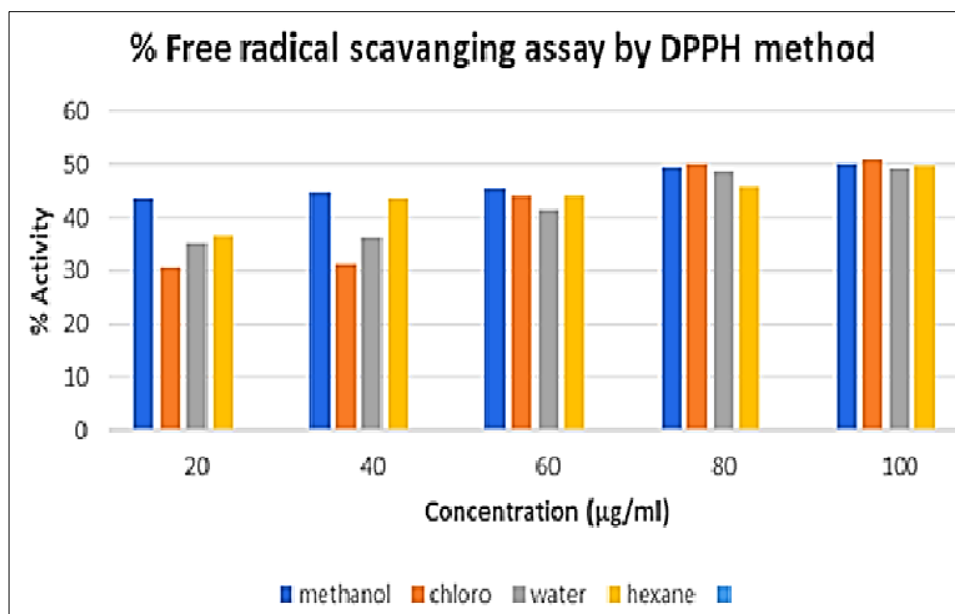


Fig 4: Antioxidant potential of leaves of *P. murex* L. by DPPH assay.

Discussion

The findings of this study unequivocally indicate that *Pedalium murex* L. possesses a varied array of phytochemicals, albeit their distribution is contingent upon the solvent employed for extraction. Methanol and aqueous extracts exhibited elevated extractive yields, suggesting that polar solvents are more efficacious in extracting bioactive

chemicals. This finding corroborates prior research indicating that phenolic and flavonoid chemicals exhibit greater solubility in polar solvents (Gupta *et al.*, 2013; Singh *et al.*, 2010) [17, 7]. Qualitative phytochemical screening showed that all extracts had carbohydrates, but only the aqueous extract had proteins and glycosides. Flavonoids were only found in the methanolic extract, which shows

how important solvent polarity is for extracting phytochemicals. The lack of alkaloids and saponins indicates a comparatively selective phytochemical profile in relation to other therapeutic plants (Biswas *et al.*, 2002; Mondal *et al.*, 2009) ^[12, 3].

GC-MS analysis elucidated the chemical composition of *P. murex*, revealing various biologically active chemicals, including n-hexadecanoic acid, octadecanoic acid, phytol, and stigmasterol. These substances are recognized for their antioxidant, anti-inflammatory, antibacterial, and anticancer characteristics (Newman & Cragg, 2020; Cragg & Newman, 2013) ^[14, 4]. The prevalence of fatty acids and alcohol-derived substances reinforces the plant's medicinal potential. The DPPH experiment indicated that the antioxidant activity increased with concentration, which means that the radical scavenging activity also increased. The chloroform extract showed the most antioxidant activity of all the extracts, which suggests that it contains moderately polar bioactive chemicals that are responsible for scavenging free radicals. Other medicinal plants have shown similar results, where the polarity of the solvent has a big effect on the antioxidant capacity (Kalita *et al.*, 2013; Ali *et al.*, 2008) ^[10, 2]. But *P. murex*'s overall antioxidant activity was only moderate when compared to other plants that are well-known for having a lot of antioxidants. This indicates that its therapeutic efficiency may be augmented when utilized in conjunction with other medicinal plants or via the isolation of active chemicals (Patwardhan *et al.*, 2005; Kala, 2005) ^[15, 9].

Conclusion

The current investigation validates that *Pedaliium murex* L. is a significant source of bioactive phytochemicals with considerable pharmacological potential. The identification of substances such as fatty acids, phytosterols, and terpenoids using GC-MS analysis corroborates its conventional medical applications. The antioxidant activity seen in different extracts shows even more how it could help fight disorders related to oxidative stress. The antioxidant activity was moderate, but the study lays a strong groundwork for more research on how to isolate, purify, and characterize beneficial molecules. Future research concentrating on *in vivo* assessment and clinical validation is essential to comprehensively investigate its medicinal uses. In general, *P. murex* L. looks like a good natural resource for making plant-based drugs and nutraceuticals.

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