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Evaluation of Phytochemical Constituents, Total Phenolic And Flavonoid Contents, And Antioxidant Activity of Exacum pedunculatum (L.)

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Abstract

Exacum pedunculatum (L.), a medicinal herb belonging to the family Gentianaceae, is traditionally used for the treatment of skin ailments, diabetes, and various inflammatory disorders. The present study aimed to investigate the phytochemical constituents and antioxidant potential of leaf extracts of E. pedunculatum prepared using solvents of different polarities, namely ethanol, methanol, acetone, and petroleum ether. Qualitative phytochemical screening revealed the presence of several bioactive compounds, including flavonoids, phenolics, saponins, tannins, coumarins, alkaloids, proteins, amino acids, and reducing sugars, with notable variations among the solvent extracts. Quantitative estimation demonstrated that methanol extract contained the highest total phenolic content (0.48 mg GAE g^{-1} DW), whereas ethanol extract exhibited the highest total flavonoid content (0.70 mg QE g^{-1} DW). Antioxidant activity was evaluated using the DPPH free radical scavenging assay. The methanolic extract showed a concentration-dependent antioxidant effect, with scavenging activity increasing from 21.65 ± 1.26% at 0.2 mg mL^{-1} to 75.36± 1.79% at 1.0 mg mL^{-1}. The extract exhibited a strong linear relationship between concentration and scavenging activity (R^2 = 0.9924) and an IC_{50} value of 0.564 mg mL^{-1}. Among all extracts, methanol exhibited the highest antioxidant activity, closely comparable to that of the standard antioxidant ascorbic acid (86.02 ± 1.25%). The findings indicate that E. pedunculatum is a promising natural source of antioxidant compounds and may serve as a potential candidate for pharmaceutical, nutraceutical, and food applications.

Keywords: Exacum pedunculatum, Phytochemical screening, Antioxidant activity, DPPH assay, Phenolic compounds, Flavonoids

1.Introduction

Medicinal plants remain an important source of bioactive compounds for the discovery of novel therapeutic agents and nutraceuticals. Secondary metabolites such as phenolic compounds, flavonoids, alkaloids, tannins, saponins, and terpenoids play essential roles in plant defense and exhibit a wide range

of biological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer properties (Zagoskina et al., 2023; Ren et al., 2026). Among these phytochemicals, phenolics and flavonoids have received considerable attention because of their remarkable ability to neutralize reactive oxygen species (ROS) and reduce oxidative stress, thereby contributing to the prevention of several chronic diseases.

Exacum pedunculatum (L.) belongs to the family Gentianaceae, is an endemic medicinal herb distributed primarily in the Eastern and Western Ghats of India, where it has been traditionally used for the treatment of skin diseases, diabetes, and other ailments. Despite its ethnomedicinal importance, the phytochemical composition and biological activities of this species remain inadequately investigated. Preliminary phytochemical investigations have reported the presence of alkaloids, flavonoids, glycosides, saponins, tannins, steroids, and triterpenoids in E. pedunculatum, indicating that the species possesses a diverse array of secondary metabolites with potential pharmacological significance (Ashwini & Majumdar, 2015). However, comprehensive quantitative evaluation of its phenolic and flavonoid contents together with antioxidant activity is still limited.

Phenolic compounds represent one of the largest classes of plant secondary metabolites and are recognized as major contributors to antioxidant activity because of their ability to donate hydrogen atoms or electrons, chelate transition metals, and inhibit lipid peroxidation. Flavonoids, a major subclass of phenolics, possess strong antioxidant potential in addition to anti-inflammatory, antimicrobial, antiviral, and cardioprotective activities. Numerous studies have demonstrated a positive correlation between total phenolic content, total flavonoid content, and free radical scavenging capacity, making these parameters valuable indicators for assessing the antioxidant potential of medicinal plants (Shen et al., 2022; Zagoskina et al., 2023).

Considering the limited scientific information available on E. pedunculatum, systematic evaluation of its phytochemical constituents and antioxidant properties is necessary. Therefore, the present study aimed to perform qualitative phytochemical screening, quantify the total phenolic and total flavonoid contents, and evaluate the antioxidant activity of different solvent extracts of E. pedunculatum. The findings are expected to provide scientific evidence supporting the traditional use of this medicinal plant and contribute to its potential application as a natural source of antioxidant compounds for pharmaceutical, nutraceutical, and food industries.

2. Materials and Methods

Figure 1. Habit of Exacum pedunculatum (L.)

2.1. Collection and Identification of Plant Material

Leaves of Exacum pedunculatum were collected from their natural habitats in Amravati district, Maharashtra, India. The plant specimens were authenticated using regional floras (Cooke, 1967; Kamble & Pradhan, 1988; Dhore & Joshi, 1998; Dhore, 2002; Naik, 1998; Singh & Karthikeyan, 2001) and confirmed by local botanical experts.

2.2. Preparation of Plant Extracts

For extraction, 50 g of powdered leaf material was separately macerated with 250 mL of ethanol, methanol, acetone, and petroleum ether for 72 h at room temperature under dark conditions. The mixtures were intermittently shaken and filtered through Whatman No. 1 filter paper. The filtrates were concentrated and stored at 4°C until further analysis.

2.3. Phytochemical Screening

Qualitative phytochemical analysis of different solvent extracts was carried out using standard procedures described by Harborne, (1998) and Kokate, (1994). The extracts were screened for alkaloids, flavonoids, tannins, phenolics, coumarins, proteins, amino acids, reducing sugars, fixed oils, and saponins using specific chemical tests.

2.4. Determination of total phenolic contents (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method (Singleton et al., 1999). Briefly, 10 µl of extract was mixed with Folin reagent and sodium carbonate solution and incubated in the dark for 30 min. Absorbance was measured at 750 nm using an ELISA reader. Gallic acid was used as standard and results were expressed as mg gallic acid equivalents (GAE)/g extract (Fig 2).

2.5. Determination of total flavonoid contents (TFC)

Total flavonoid content was estimated using the aluminium chloride colorimetric method (Zhishen et al., 1999). Quercetin was used as the standard compound (Fig.4) and absorbance was recorded at 510 nm. The flavonoid content was expressed as mg quercetin equivalents (QE)/g extract.

2.6. Determination of Antioxidant Activity (DPPH Assay)

The antioxidant activity of different extracts was evaluated using the DPPH free radical scavenging assay described by Shimada et al., (1992). The reaction mixture consisted of 10 µl extract and 190 µl of 0.1 mM DPPH solution. The mixture was incubated at 37 °C for 5 min and absorbance was measured at 517 nm. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \left(\frac{\text{Ac} - \text{As}}{\text{Ac}}\right) \times 100$$

where Ac is the absorbance of control and As is the absorbance of sample.

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Linear regression analysis was performed to determine the relationship between extract concentration and DPPH radical scavenging activity, and the coefficient of determination (R^2) was calculated. The IC_{50} values were estimated from the regression equation.

3. Results

3.1. Phytochemical Analysis

Preliminary phytochemical screening of ethanol, methanol, acetone, and petroleum ether extracts of E. pedunculatum revealed notable variations in the distribution of secondary metabolites depending on the extraction solvent. Ethanol and methanol extracts exhibited the richest phytochemical profiles, showing positive results for alkaloids, carbohydrates, tannins, phenols, proteins and amino acids, saponins, flavonoids, coumarins, and glycosides. This indicates that polar solvents are more effective in extracting a broad range of bioactive constituents from the plant material. The acetone extract tested positive for carbohydrates, phenols, saponins, flavonoids, anthocyanins, and fixed oils and fats but was negative for alkaloids, tannins, proteins and amino acids, coumarins, and glycosides. Notably, anthocyanins and fixed oils and fats were detected only in the acetone extract among the solvents tested. The petroleum ether extract displayed the least diverse phytochemical composition, yielding positive results only for coumarins while testing negative for alkaloids, carbohydrates, tannins, phenols, flavonoids, proteins and amino acids, saponins, anthocyanins, glycosides, and fixed oils and fats.

Overall, the findings suggest that ethanol and methanol are the most suitable solvents for extracting a wide spectrum of phytoconstituents from E. pedunculatum, whereas acetone selectively extracts certain compounds such as anthocyanins and fixed oils, and petroleum ether is comparatively less efficient for phytochemical extraction. These observations are in agreement with previous reports on species of the genus Exacum. Skrzypczak-Pietraszek, (2015) highlighted that members of this genus are rich in diverse phytochemicals, including phenolic compounds, flavonoids, xanthones, alkaloids, and other bioactive metabolites with pharmacological significance. Similarly, Paulsamy and Jeeshna, (2011) reported the occurrence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds in Exacum bicolor, supporting the present findings. Phytochemical investigations by Vinayaka et al., (2016) also confirmed the presence of major secondary metabolites such as flavonoids, tannins, and phenols in medicinally important Exacum species collected from the central Western Ghats.

The results are further supported by studies on related taxa. Mothana et al., (2006), working with Exacum affine, documented the presence of several phytochemical classes, including phenolic

constituents and flavonoids, which were associated with promising biological activities. Likewise, Ashwini et al., (2015) identified numerous bioactive compounds in Exacum bicolor through phytochemical screening and GC–MS analysis and linked these constituents with antioxidant, thrombolytic, and anti-inflammatory properties. Pharmacognostic and phytochemical evaluation by Rajisha and Fernandes, (2020) also confirmed the occurrence of alkaloids, flavonoids, tannins, saponins, and glycosides in Exacum bicolor, reinforcing the consistency of phytochemical patterns within the genus.

Table 1. Qualitative phytochemical screening of different solvent extracts of Exacum pedunculatum.

S. No.	Sample Tested for	Name of Test	Ethanol	Methanol	Acetone	P.E.
1.	Alkaloids	Hager's Test	+	+	-	-
		Dragendorff's	+	+	-	-
		Mayer's Test	+	+	-	-
		Wagner's Test	+	+	-	-
2.	Carbohydrates	Fehling's Test	+	+	+	-
3.	Tannins	10% NaOH Test	+	+	-	-
4.	Phenols	FCR test	+	+	+	-
5.	Proteins and Amino Acids	Ninhydrin Test	+	+	-	-
6.	Saponins	Foam Test	+	+	+	-
7.	Flavonoids	$AlCl_3$ Method	+	+	+	-
		Lead acetate test	+	+	+	-
8.	Anthocyanin	HCL Test	-	-	+	-
9.	Coumarin	NaOH Test	+	+	-	+
10.	Glycoside	Keller Killani test	+	+	-	-
11.	Fixed Oils &Fats	Spot test	-	-	+	-

3.2. The total phenolic contents (TPC)

The total phenolic content (TPC) of E. pedunculatum varied among the different solvent extracts and was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW). The methanolic extract exhibited the highest TPC (0.477 ± 0.044 mg GAE g^{-1} DW), followed by the ethanolic extract (0.379 ± 0.036 mg GAE g^{-1} DW) and the acetone extract (0.323 ± 0.024 mg GAE g^{-1} DW). In contrast, no detectable phenolic content was observed in the petroleum ether extract. These results indicate that polar solvents, particularly methanol, are more effective in extracting phenolic compounds from E. pedunculatum than non-polar solvents such as petroleum ether. The higher phenolic content

observed in the methanolic extract may be attributed to the greater solubility of phenolic compounds in polar solvents.

Figure 2. Calibration curve of gallic acid used for total phenolic content estimation.

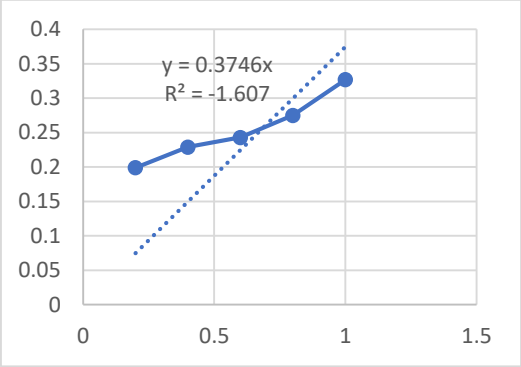

Figure 3. Total phenolic content of different solvent extracts of Exacum pedunculatum.

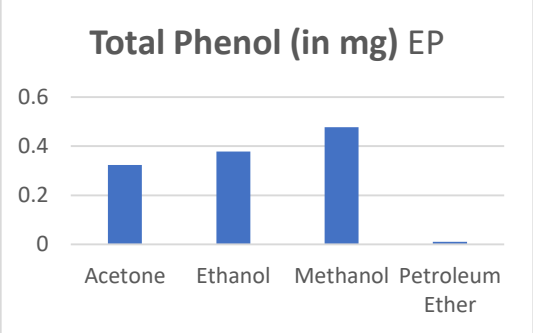

Figure 4. Calibration curve of quercetin used for total flavonoid content estimation

3.3. The total flavonoid contents (TFC)

The total flavonoid content (TFC) of E. pedunculatum, expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE g^{-1} DW), differed among the solvent extracts. The ethanolic extract exhibited the highest flavonoid content (0.693 ± 0.141 mg QE g^{-1} DW), followed by the methanolic extract (0.458 ± 0.076 mg QE g^{-1} DW) and the acetone extract (0.294 ± 0.125 mg QE g^{-1} DW). No detectable flavonoid content was observed in the petroleum ether extract. These findings suggest that ethanol is the most effective solvent for extracting flavonoids from E. pedunculatum, while the non-polar petroleum ether is ineffective for flavonoid extraction under the conditions employed in this study. Ethanol exhibited superior extraction efficiency for flavonoids, suggesting that solvent polarity plays a critical role in flavonoid recovery.

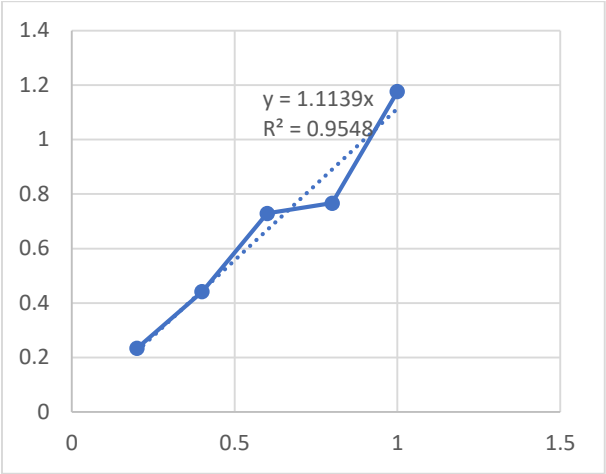

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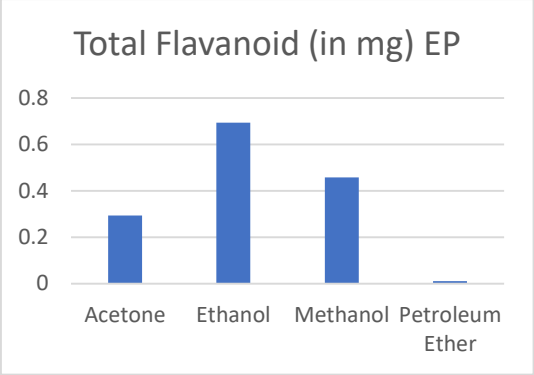
Figure 5. Total flavonoid content of different solvent extracts of Exacum pedunculatum.



3.4. Antioxidant Potential of Exacum pedunculatum

The DPPH radical scavenging activity of E. pedunculatum varied considerably among the different solvent extracts, indicating differences in their antioxidant potential. The methanolic extract exhibited the highest radical scavenging activity (83.247 ± 0.544%), demonstrating superior antioxidant capacity. This was followed by the acetone extract (55.67 ± 0.878%), while the ethanolic extract showed moderate activity (32.268 ± 1.223%). The petroleum ether extract displayed the lowest DPPH radical scavenging activity (10.206 ± 1.754%). These findings suggest that methanol is the most effective solvent for extracting antioxidant constituents from E. pedunculatum, whereas petroleum ether extracts possess comparatively weak free radical scavenging activity under the experimental conditions.

The antioxidant activity of the methanolic extract of E. pedunculatum was evaluated using the DPPH free radical scavenging assay at concentrations ranging from 0.2 to 1.0 mg. The extract exhibited a concentration-dependent increase in radical scavenging activity, with values of 21.253 ± 2.331%, 42.339 ± 1.656%, 59.326 ± 2.475%, 63.026 ± 2.699%, and 75.362 ± 1.784% at 0.2, 0.4, 0.6, 0.8, and 1.0 mg, respectively. Linear regression analysis yielded the equation $y = 64.453x + 13.59$ with a coefficient of determination ($R^2 = 0.9444$), indicating a strong positive relationship between extract concentration and DPPH radical scavenging activity. The calculated IC_{50} value was 0.564 mg, suggesting that the methanolic extract possesses appreciable antioxidant potential by effectively scavenging free radicals in a dose-dependent manner.

The present study demonstrated that the methanolic extract of E. pedunculatum exhibited the highest DPPH radical scavenging activity, indicating superior antioxidant potential compared to the other solvent extracts. The enhanced antioxidant activity of the methanolic extract may be attributed to its greater ability to extract phenolic compounds and other bioactive secondary metabolites that are known to neutralize free radicals. The concentration-dependent increase in DPPH radical scavenging activity and the relatively low IC_{50} value further support the effectiveness of the methanolic extract as a natural antioxidant source.

These findings are consistent with previous studies on species of the genus Exacum. Ashwini and Majumdar, (2015) reported significant in vitro antioxidant activity in Exacum bicolor, accompanied by appreciable levels of phenolic and flavonoid compounds, suggesting a strong relationship between phytochemical content and antioxidant capacity. Similarly, Ashwini et al., (2015) demonstrated that extracts of Exacum bicolor possessed considerable free radical scavenging activity and identified several bioactive constituents through GC–MS analysis that may contribute to these pharmacological properties. The comprehensive review by Skrzypczak-Pietraszek, (2015) also emphasized that Exacum species are rich in phenolics, flavonoids, xanthones, and other secondary metabolites with recognized antioxidant and therapeutic potential.

Further support is provided by the work of Fernandes, (2022), who reported notable antioxidant activity in Exacum bicolor using in vitro assays and highlighted the medicinal significance of its phytochemical constituents. Likewise, Rajisha and Fernandes, (2022) observed substantial free radical scavenging and anti-inflammatory activities in Exacum bicolor, reinforcing the view that members of the genus possess biologically active compounds capable of mitigating oxidative stress. The antioxidant activity observed in the present investigation therefore agrees well with earlier reports and suggests that E. pedunculatum may serve as a promising natural source of antioxidant molecules. The correlation between its phytochemical composition and antioxidant performance underscores its potential for future pharmacological and nutraceutical applications.

Figure 6. DPPH radical scavenging activity of different solvent extracts of Exacum pedunculatum.
(A:Ethanol, B: Methanol, C: Acetone, D: Petroleum Ether)

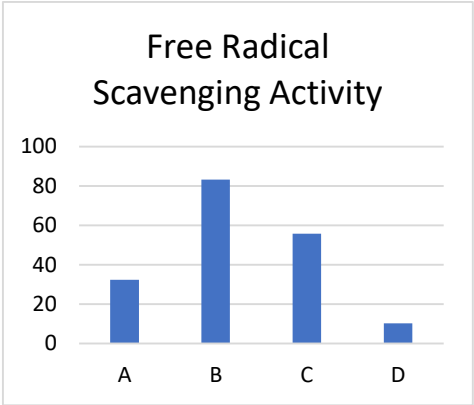
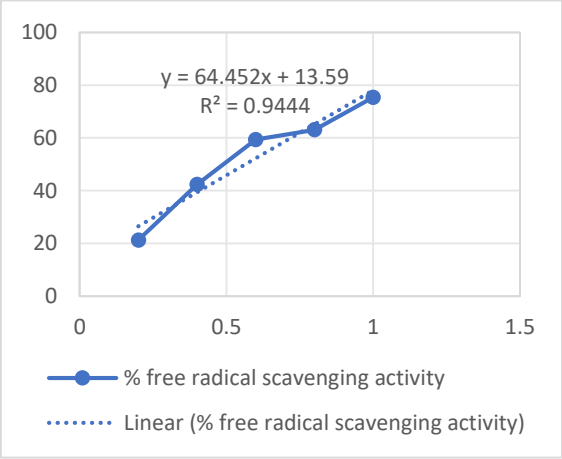


Table 2. DPPH radical scavenging activity of the methanolic extract of Exacum pedunculatum

S. No.	Conc (in mg)	% free radical scavenging activity	Y equation	R^2 value	IC_{50} (in mg)
1	0.2	21.253±2.331	y = 64.453x + 13.59	R^2= 0.9444	0.564
2	0.4	42.339±1.656			
3	0.6	59.326±2.475			
4	0.8	63.026±2.699			
5	1.0	75.362 ±1.784			

Figure 7. Concentration-dependent DPPH radical scavenging activity of the methanolic extract.



5. Conclusion

The present study demonstrated that E. pedunculatum contains a diverse array of phytochemically active constituents, including alkaloids, flavonoids, phenolics, tannins, saponins, coumarins, and glycosides. Methanolic and ethanolic extracts exhibited the richest phytochemical profiles, indicating the effectiveness of polar solvents for the extraction of bioactive compounds. The methanolic extract showed the highest total phenolic content and DPPH radical scavenging activity, whereas the ethanolic extract possessed the highest total flavonoid content. The strong antioxidant activity observed may be attributed to the presence of phenolic and flavonoid compounds. These findings support the traditional medicinal use of E. pedunculatum and highlight its potential as a natural source of antioxidants for pharmaceutical.

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