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Phytochemical determination evaluation of antioxidant potential of different parts of in *Xanthium Strumarium*

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Abstract
Xanthium strumarium L. is a medicinally important plant which is widely used in traditional healthcare system for treatment of various ailments like infections, inflammation and respiratory disorders. The present study was planned to evaluate the phytochemical composition and antioxidant potential of different parts of the plant i.e. roots, stems, leaves and flowers using aqueous, methanolic and petroleum ether extracts. The extractive values showed significant variation depending on the plant part and solvent used. Methanolic extracts showed maximum extraction efficiency especially from stem (384 mg/g dry weight) and leaf (352 mg/g dry weight). Preliminary phytochemical screening revealed the presence of wide range of primary and secondary metabolites such as carbohydrates, proteins, tannins, alkaloids, flavonoids, phytosterols, saponins and terpenoids. Methanol was found to be the best solvent for extraction. Quantitative analysis revealed organ-specific differences in the distribution of metabolites. Leaves had highest concentrations of starch (21.82 ± 1.18 mg/g dw), total soluble sugars (16.88 ± 0.38 mg/g dw), ascorbic acid (1.23 ± 0.16 mg/g dw), chlorophyll (3.11 ± 0.08 mg/g dw), carotenoids (1.92 ± 0.11 mg/g dw), phenols (1.11 ± 0.11 mg/g GAE) and phytosterols (2.27 ± 0.11 mg/g dw), whereas flowers had highest protein content (20.91 ± 0.72 mg/g dw). The antioxidant activity determined by DPPH free radical scavenging assay showed a concentration dependent increase in all the extracts. Among the tested samples, leaf and flower extracts showed the highest antioxidant potential with maximum scavenging activity of aqueous leaf extract (28.74%) and methanolic flower extract (28.25%) at 250µg/mL. These results suggest that *Xanthium strumarium* is a rich source of bioactive phytochemicals and has a significant antioxidant potential mainly in foliar and floral tissues. These results are in favour of the traditional medicinal use of the plant and also provide evidence for its potential application in pharmaceutical, nutraceutical and natural antioxidant fields.

Keywords: *Xanthium strumarium*, phytochemicals, antioxidant activity, DPPH assay, phenolic compounds, flavonoids, medicinal plants

Introduction

Medicinal plants have long been recognized as valuable sources of bioactive compounds with significant therapeutic potential. The growing demand for natural products in healthcare, pharmaceuticals, and nutraceutical industries has stimulated extensive research on medicinal plants as alternatives to synthetic drugs. Plant-derived secondary metabolites such as phenolics, flavonoids, alkaloids, tannins, terpenoids, and saponins exhibit a broad spectrum of biological activities including antioxidant, antimicrobial, anti-inflammatory, anticancer, and hepatoprotective effects. Consequently, the exploration of medicinal plants for their phytochemical composition and biological activities remains an important area of scientific investigation (Ahvazi *et al.*, 2012) [1].

Among the diverse medicinal plants used in traditional medicine, *Xanthium strumarium* L. (family Asteraceae), commonly known as cocklebur, occupies a prominent position due to its wide distribution and extensive ethnomedicinal applications. The plant is an annual herbaceous species commonly found in tropical, subtropical, and temperate regions throughout the world. In India, it grows abundantly along roadsides, wastelands, agricultural fields, and riverbanks. Various parts of the plant, including roots, stems, leaves, flowers, and fruits, have been utilized in traditional systems of medicine for the treatment of fever, malaria, skin infections, bronchitis, sinusitis, rheumatism, wounds, ulcers, and inflammatory disorders. The widespread traditional use of *X. strumarium* suggests the presence of biologically active constituents responsible for its therapeutic properties (Fan *et al.*, 2019) [3].

Phytochemical investigations of *X. strumarium* have revealed the presence of numerous primary and secondary metabolites including carbohydrates, proteins, phenolic compounds, flavonoids, alkaloids, tannins, phytosterols, saponins, and terpenoids. These compounds play important physiological roles in plants and are known to contribute significantly to their pharmacological activities. Phenolic compounds and flavonoids, in particular, have attracted considerable attention because of their ability to scavenge reactive oxygen species (ROS), thereby protecting biological systems against oxidative stress. The distribution and concentration of these metabolites often vary among different plant organs, making comparative phytochemical studies essential for identifying the most bioactive plant parts (Sharifi Rad *et al.*, 2015) [6].

Oxidative stress caused by the excessive generation of free radicals has been implicated in the pathogenesis of numerous chronic diseases, including cancer, diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, and premature aging. Antioxidants act by neutralizing these reactive species and preventing cellular damage. Although synthetic antioxidants are widely used, concerns regarding their safety and potential adverse effects have encouraged the search for effective natural antioxidants from plant sources. Therefore, the evaluation of antioxidant activity has become an important criterion for assessing the medicinal potential of plant extracts (Arora *et al.*, 2002) [2].

In view of the medicinal importance of *Xanthium strumarium* and the need for natural antioxidant agents, the present study was undertaken to evaluate the phytochemical composition and antioxidant potential of different plant parts using aqueous, methanolic, and petroleum ether extracts. The study aims to compare the distribution of primary and secondary metabolites among various organs and to assess their free radical scavenging activity. The findings are expected to contribute to the scientific validation of the traditional uses of *X. strumarium* and provide a basis for its future utilization in pharmaceutical, nutraceutical, and herbal product development.

Materials and Methods

Plant Material Collection and Preparation

Healthy specimens of *Xanthium strumarium* were collected from the study area and authenticated by standard floristic literatures. The plant material collected was washed thoroughly with distilled water to remove the adhering dust and debris. Roots, stems, leaves and flowers were separated, shade dried at room temperature and ground to fine powder for further analysis.

Extraction of Plant Material

Powdered samples of different plant parts were extracted with solvents of different polarity such as distilled water, methanol and petroleum ether. The plant material was incubated with the respective solvents at room temperature with constant shaking. After extraction the samples were centrifuged and the supernatants were collected. The solvents were evaporated to yield crude extracts and the yield of extractives was calculated on a dry weight basis. The extracts obtained were kept for further phytochemical and antioxidant studies.

Qualitative Phytochemical Analysis

Preliminary Phytochemical Screening: Major primary and secondary metabolites in the extracts were identified by preliminary phytochemical screening. Detection of carbohydrates, proteins, tannins, alkaloids, flavonoids, glycosides, phenols, saponins, phytosterols, terpenoids and other bioactive compounds was carried out using standard qualitative tests. Characteristic color changes or precipitates were observed and considered as an indication of the presence of specific phytochemicals.

Quantitative Estimation of Phytochemicals

Concentrations of selected primary and secondary metabolites were measured by standard biochemical methods. Spectrophotometrically, total soluble sugars, starch, proteins, lipids, ascorbic acid, chlorophyll, carotenoids, phenols, flavonoids, alkaloids, phytosterols, tannins and saponins were quantified. Calibration was performed with suitable standards and results are expressed as mg per gram dry weight of plant material.

Evaluation of Antioxidant Activity

The antioxidant activity of different extract was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging free radical assay. Different concentrations of each extract were mixed with DPPH solution and incubated under dark circumstances. The decrease in absorbance was measured spectrophotometrically and the percentage radical scavenging activity was calculated with respect to control. The antioxidant capacity of different parts of the plant and solvent extracts were compared by their ability to inhibit DPPH radicals.

Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation. Comparisons were made to assess differences in phytochemical profile and antioxidant activity between plant parts and extraction solvents.

Results

Extractive Yield of Different Plant Parts

The extractive values of different parts of *Xanthium strumarium* varied widely with solvent used. Methanol solvent had highest extraction yield in all parts of plant and stem showed highest extractive value (384 mg/g dry weight) followed by leaves (352 mg/g dry weight). Petroleum ether extracts produced generally lower extractive values while aqueous extracts gave moderate yields. The lowest yield was obtained from petroleum ether extract of roots (22 mg/g dry wt). Differences among solvents and plant parts were observed for color and consistency of extracts, indicative of differences in the composition of extracted phytochemicals.

Table 1: Extractive values, extract color and appearance in different parts of *Xanthium strumarium*.

Plant	Solvent	Weight of extract (mg/g.dw)	Drying time (days)	Color	Consistency
Root	Water	123	9	Light brown	powder
	Methanol	246	7	brown	powder
	Pet ether	22	2	Yellowish-brown	sticky
Stem	Water	267	11	Brown	Powder
	Methanol	384	6	Brown	Powder
	Pet ether	46	1	Yellow	Sticky
Leaves	Water	136	7	Dark green	sticky
	Methanol	352	6	Green	sticky
	Pet ether	94	1	Yellow	sticky
Flowers	Water	124	8	Dark brown	powder
	Methanol	176	6	Cream	sticky
	Pet ether	152	1	white	sticky

Qualitative Phytochemical Screening

Preliminary phytochemical screening showed the presence of different primary and secondary metabolites in *Xanthium strumarium*. Carbohydrates and flavonoids were identified in all plant parts and solvent extracts. Proteins, tannins and alkaloids were mainly present in aqueous and methanolic extracts but generally absent in petroleum ether extracts. Majority of the extracts were found to contain phytosterols and saponins and terpenoids were mostly found in methanolic and petroleum ether extracts. In general, methanol was the best solvent for extraction of most variety of phytochemical constituents.

Table 2: Preliminary Phytochemical Screening of different extracts of *different parts of Xanthium strumarium* showing presence and absence of various phytochemicals

Phytochemical test	Solvent	Root		Stem		Leaves		Flowers	
		Observation	Result	Observation	Result	Observation	Result	Observation	Result
Total carbohydrates (Fehling method)	Water	Red	+	Red	+	Red	+	Red	+
	Methanol	Red	+	Red	+	Red	+	Red	+
	Pet ether	Red	+	Red	+	Light blue	+	Red	+
Protein (Ninhydrin)	Water	blue	+	blue	+	Dark brown	+	blue	+
	Methanol	blue	+	blue	+	blue	+	blue	+
	Pet ether	brown	-	brown	-	Lighy yellow	-	brown	-
Tannins (FeCl3)	Water	Green	+	Green	+	Green	+	Green	+
	Methanol	Green	+	Green	+	Light green	+	Green	+
	Pet ether	yellow	-	yellow	-	transparent	-	yellow	-
Alkaloids (Dragendorff's method)	Water	Red	+	Red	+	Light yellow	+	Red	+
	Methanol	Red	+	Red	+	Yellow	+	Red	+
	Pet ether	yellow	-	yellow	-	Transparent	-	yellow	-
Flavonoids (alkaline solution test)	Water	Yellow	+	Yellow	+	Yellow	+	Yellow	+
	Methanol	Yellow	+	Yellow	+	Dark brown	+	Yellow	+
	Pet ether	Yellow	+	Yellow	+	brown	+	Yellow	+
Phytosterols (Salkowski's test)	Water	Brown	+	Brown	+	Foams +nt	+	Brown	+
	Methanol	Brown	+	Brown	+	Foam+nt	+	Brown	+
	Pet ether	brown	+	brown	+	No foam	-	brown	+
Saponins (Sofowora)	Water	No frothing	-	No frothing	-	No ppt	-	No frothing	-
	Methanol	frothing	+	frothing	+	Ppt +nt	+	frothing	+
	Pet ether	frothing	+	frothing	+	Ppt +nt	+	frothing	+
Terpenoids (Sofowora)	Water	pink	-	pink	-	Yellow	+	pink	-
	Methanol	purple	+	purple	+	Green	+	purple	+
	Pet ether	purple	+	purple	+	Transparent	-	purple	+

Quantitative Determination of Primary and Secondary Metabolites

There were considerable variations in the levels of metabolites among the different plant parts. The leaves had the highest concentration of starch (21.82 ± 1.18 mg/g dw), total soluble sugars (16.88 ± 0.38 mg/g dw), ascorbic acid (1.23 ± 0.16 mg/g dw), chlorophyll (3.11 ± 0.08 mg/g dw), carotenoids (1.92 ± 0.11 mg/g dw), Phenols (1.11 ± 0.11 mg GAE/g dw), phytosterols (2.27 ± 0.11 mg/g dw) and saponins (0.93 ± 0.15 mg/g dw). Flowers had highest protein content (20.91 ± 0.72 mg/g dw), whereas roots had highest concentration of flavonoids (0.55 ± 0.03 mg QE/g dw) and tannins (1.37 ± 0.18 mg CE/g dw). The alkaloid content was quite uniform in all parts of the plant with the highest value in the stems (1.26 ± 0.12 mg AE/g dw).

Table 3: Quantitative determination of primary and secondary metabolites in different parts of *Xanthium strumarium*

Phytochemical name	Root	Stem	Leaves	Flowers
Starch (mg/g.dw)	9.02±0.11	7.18±1.24	21.82±1.18	3.12±0.16
Total soluble sugar (mg/g.dw)	8.14±0.41	5.56±0.14	16.88±0.38	8.81±0.32
Ascorbic acid (mg/g.dw)	0.93±0.14	0.56±0.33	1.23±0.16	0.33±0.81
Lipids (mg/g.dw)	8.17±0.41	9.28±1.03	11.31±1.13	9.38±0.23
Proteins (mg/g.dw)	12.13±0.16	9.16±0.37	10.87±2.57	20.91±0.72
Chl.-a+b (mg/g.dw)	0.02±0.002	0.08±0.004	3.11±0.08	0.04±0.010
Carotenoid (mg/g.dw)	0.31±0.10	0.56±0.06	1.92±0.11	1.41±0.36
Phenols (mg/g.dwGAE)	0.81±0.11	0.70±0.31	1.11±0.11	0.31±0.05
Flavonoids (mg/g.dwQE)	0.55±0.03	0.26±0.01	0.32±0.06	0.23±0.14
Alkaloids (mg/g.dwAE)	1.22±0.21	1.26±0.12	0.75±0.04	1.23±0.21
Phytosterols (mg/g.dw)	2.15±0.51	1.58±0.12	2.27±0.11	0.17±0.08
Tannins (mg/g.dwCE)	1.37±0.18	0.18±0.11	0.25±0.11	1.12±0.18
Saponins (mg/g.dw)	0.13±0.06	0.58±08	0.93±0.15	0.25±0.08

Antioxidant Activity

All extracts showed antioxidant activity in DPPH free radical scavenging assay in a concentration dependent manner. The antioxidant potential increased progressively with increasing concentration of the extract (50–250 µg/mL). The leaf and flower extracts showed higher

antioxidant activity among the plant parts than roots and stems. Maximum scavenging activity was observed in aqueous leaf extract (28.74 ± 0.46%) at 250 µg/mL followed by methanolic flower extract (28.25 ± 2.13%). Root and stem extracts showed relatively lower antioxidant activities at all the concentrations studied. Overall, the results indicated that the antioxidant capacity of the leaves and flowers is probably related to higher contents of phenolic compounds, flavonoids and other bioactive metabolites.

Table 4: Antioxidant potential of extracts of various parts of *Xanthium strumarium.*

		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
Root	Water	6.49±0.38	9.88±0.87	12.37±0.78	16.63±1.29	19.46±0.66
	Methanol	8.32±2.47	10.24±2.60	15.31±1.67	19.29±2.12	24.39±2.88
	Pet ether	9.23±1.71	12.28±3.32	17.92±2.25	22.52±3.33	25.73±5.66
Stem	Water	5.10±2.46	7.16±2.64	12.01±3.16	15.43±3.55	18.88±4.94
	Methanol	6.05±1.03	9.01±0.65	13.93±0.93	16.84±2.09	19.49±2.10
	Pet ether	11.20±3.65	15.36±5.34	19.89±4.66	23.23±4.74	24.89±4.28
Leaf	Water	13.96±5.18	16.27±3.22	20.21±3.10	24.85±1.99	28.74±0.46
	Methanol	12.26±3.76	16.03±2.41	17.59±2.49	22.54±4.53	24.07±4.52
	Pet ether	11.19±1.34	14.01±0.59	18.64±2.19	24.02±3.61	27.94±5.32
Flower	Water	11.01±6.41	14.51±8.37	18.14±9.26	21.39±10.24	23.54±11.99
	Methanol	11.37±0.52	15.79±0.75	19.13±0.93	24.27±0.49	28.25±2.13
	Pet ether	12.28±3.38	16.55±2.57	20.38±2.79	24.32±3.03	27.05±2.21

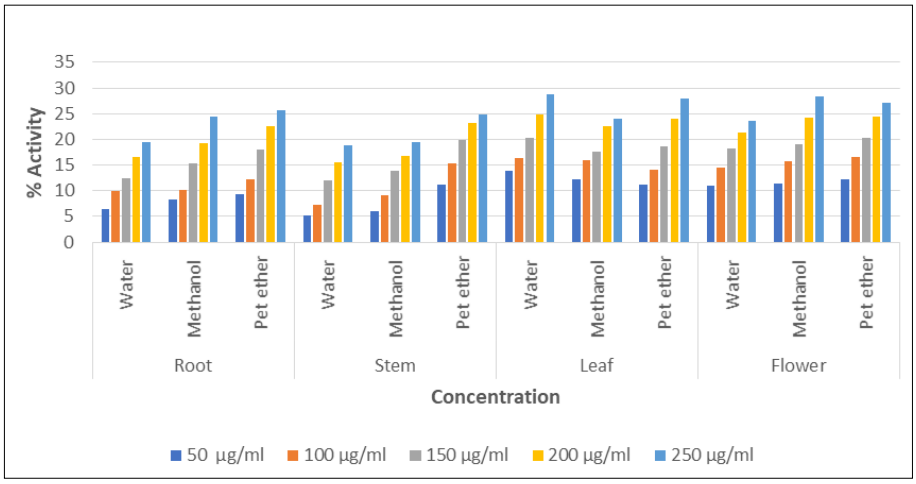

Fig 1: Antioxidant potentiaon of extracts of various parts of Xanthium strumarium

Discussion

The present study revealed that *Xanthium strumarium* has a rich phytochemical profile and considerable antioxidant potential and supports its traditional medicinal use. The solvents differed significantly in their extraction efficiency with methanol being the most effective solvent for all plant parts, particularly for stems and leaves. The better performance of methanol could be due to its intermediate polarity that allows to extract a wide variety of phytochemicals including phenolics, flavonoids, alkaloids, tannins and glycosides. This agrees with the observations of Harborne (1998) [4] and Sofowora (2008) [7] who reported that methanol is one of the best solvent for extraction of biologically active plant metabolites. Higher extractive values in leaves and stems suggest higher accumulation of secondary metabolites in these tissues, indicating their active involvement in plant defense and metabolism.

The preliminary extracts were subjected to phytochemical screening and presence of various important bioactive compounds such as carbohydrates, proteins, tannins, alkaloids, flavonoids, phytosterols, saponins and terpenoids were indicated. The frequent distribution of these compounds in different plant parts accentuates the chemical diversity of *X. strumarium*. The presence of flavonoids and phenolic compounds in the current study is of special importance, because these metabolites are known to possess strong antioxidant, anti-inflammatory, antimicrobial and cytoprotective properties. Similar phytochemical profiles have been reported for *Xanthium species* by Fan *et al.*, (2019) [3] and Kumar *et al.*, (2021), who identified phenolic acids, flavonoids, sesquiterpene lactones, and sterols as major constituents responsible for the pharmacological activities of the plant.

The quantitative analysis showed significant differences in the distribution of metabolites among plant parts. The highest levels of starch, soluble sugars, ascorbic acid, chlorophyll, carotenoids, phenols, phytosterols and saponins were recorded in leaves. Such high concentrations are expected as leaves are the major sites of photosynthesis and biosynthesis of secondary metabolites. The high phenolic and flavonoids contents in the leaves agree with the reports that the foliar tissues usually accumulate antioxidant compounds as defense strategies against UV-radiation, oxidative stress and herbivory. On the other hand, flowers had the highest protein content, likely due to their metabolic activity during reproductive development. The accumulation of tannins and flavonoids in roots may be related to their defensive role against soil-borne pathogens and environmental stress factors.

The antioxidant activity of *X. strumarium* extracts was increased gradually with the increase in concentration indicating the clear dose dependent effect of free radical scavenging. The extracts of leaf and flower showed highest antioxidant activity among all the parts of the plant, while comparatively lower activity was observed in roots & stems. The better antioxidant activity of leaves was strongly correlated with the higher levels of phenolic compounds, flavonoids, carotenoids and ascorbic acid, all of which are known to be good free radical scavengers. Phenolic compounds neutralize reactive oxygen species through the donation of hydrogen atoms or electrons and thereby prevent oxidative damage to cellular components. Several medicinal plants such as *Xanthium strumarium* and other related Asteraceae species also showed similar positive correlations between phenolic content and antioxidant activity.

Interestingly, methanolic and petroleum ether extracts showed considerable antioxidant activity suggesting the involvement of both polar and non-polar antioxidant constituents. Phenolics and flavonoids are major contributors to the activity of polar extracts. Non-polar fractions may have terpenoids, sterols and lipid-soluble antioxidants that can scavenge free radicals. Moreover, the high antioxidant activity of flower extracts indicates that reproductive tissues can act as reservoirs of protective phytochemicals that protect pollen and developing seeds against oxidative damage.

In conclusion, the results of the present study suggest that *Xanthium strumarium* is a rich source of biologically active compounds possessing significant antioxidant potential. The high phenolics, flavonoids, tannins and other secondary metabolites especially in leaves and flowers appear to be playing a major role in the free radical scavenging activity observed. The results of this investigation support previous reports concerning the medicinal importance of *X. strumarium* and imply that its extracts may be used as natural antioxidants in pharmaceutical, nutraceutical and functional food industries. More studies on isolation, characterization and *in vivo* biological evaluation of compounds are needed to determine which constituents are responsible for the observed activities and to investigate their therapeutic potential.

Conclusion

The present study showed that *Xanthium strumarium* is a good source of primary and secondary metabolites with significant antioxidant potential. The phytochemical screening revealed the presence of high bioactive compounds such as phenolics, flavonoids, alkaloids, tannins, phytosterols, saponins and terpenoids in different plant parts. The quantitative analysis showed that leaves contain the highest concentrations of some metabolites, especially phenols, ascorbic acid, pigments and carbohydrates, whereas flowers and roots are rich in proteins, tannins and flavonoids. In addition, the antioxidant assay showed concentration-dependent free radical scavenging activity in all extracts, with leaf and flower extracts exhibiting the highest antioxidant potential. The results indicated that the antioxidant activity of *X. strumarium* might be related to its phytochemical composition, especially the phenolic and flavonoid contents. The study scientifically validates the medicinal importance of *Xanthium strumarium* and its potential as a natural source of antioxidant compounds for the development of pharmaceutical, nutraceutical and herbal products. Further studies such as isolation of the active constituents and *in vivo* studies are recommended to explore its full therapeutic potential.

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