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Sucheta Gaikwad

A) Dr. T. R. Ingle Research Laboratory, Dept. of Chemistry, S. P. College, Pune-411030, India.

B) Dept. of Chemistry, S. P. College, Pune-411030, India

Rasika Torane

A) Dr. T. R. Ingle Research Laboratory, Dept. of Chemistry, S. P. College, Pune-411030, India.

B) Dept. of Chemistry, S. P. College, Pune-411030, India

Kavita Mundhe

Anantrao Thopte College, Bhor, Pune, India.

Correspondence

Sucheta Gaikwad

A) Dr. T. R. Ingle Research Laboratory, Dept. of Chemistry, S. P. College, Pune-411030, India.

B) Dept. of Chemistry, S. P. College, Pune-411030, India

Preliminary screening and comparative evaluation of antioxidant potential of medicinally important plant *Xanthium strumarium* L.

Sucheta Gaikwad, Rasika Torane, Kavita Mundhe

Abstract

Xanthium strumarium L. a medicinal plant commonly found as a weed, is widely distributed in North America, Brazil, China, Malaysia and hotter parts of India. The genus *Xanthium* includes 25 species; two species of *Xanthium*, *X. indicum* and *X. strumarium* have been reported in India. The herb is traditionally used mostly in treating several ailments. Various parts of *Xanthium strumarium* L. are found to possess useful medicinal properties such as antibacterial, antitumor, anticancer, antifungal, anti-inflammatory, antinociceptive, antitussive, hypoglycaemic, antimutagenic, antitrypanosomal, antimalarial, diuretic, antioxidant, analgesic, repellent and insecticidal activities. The present work deals with the detail study of extractive values, Phytochemical test and fluorescence analysis. Fluorescence test of stem of the plant followed by the spectrophotometric study of phenolic and flavonoid content in semi polar to polar stem extracts. It was found that the polar methanol extract was found to be potent compared to acetone and ethanol extracts. This preliminary study undeniably useful to do further biological study.

Keywords: *Xanthium strumarium* L, Phytochemical test, fluorescence analysis Phenolic, Flavonoid, Spectroscopic study.

Introduction

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs [1], antimicrobial drugs [2], antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [3]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4, 5]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [6]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro [7]. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [8]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [9, 10, 11]. In the present work, qualitative and quantitative phytochemical analysis were carried out in seven plants, Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia, and *Xanthium strumarium*, of North eastern region of India.

Materials and Methods

UV-Vis S1700 Pharma spectrophotometer (Schimadzu) was used for the measurement of absorbance. All solvents used were of AR-grade and were obtained from Merck, Mumbai (India).

Collection of Plant Materials

Fresh stem were collected from Indapur, Districts of Pune. The plant materials were taxonomically identified and authenticated by The Botanical survey of India, Pune.

Extraction

Air-dried and powdered plant material (10 g) was extracted with methanol (50 ml) by keeping for 24 hours at room temperature. Solvent was recovered under reduced pressure to obtain crude methanol extract 11.6%.

Phytochemical Test

1. Detection of alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hagers reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.
2. Detection of glycosides: Extracts were hydrolysed with dil. HCL and then subjected to test for glycosides. Legals Test : Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide formation of pink to blood red colour indicates the presence of cardiac glycosides
3. Detection of Saponins: Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for 10 min it indicates the presences of saponins.
4. Detection of Phytosterols: Salkowskis Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour Indicates the presence of triterpenes.
5. Detection of phenols: Ferric chloride Test: Extract were treated with 3-4 drops of ferric chloride solution, formation of bluish black colour indicates the presence of phenols.
6. Detection of Tannins: To the extract, 1% gelatin solution containing sodium chloride was added, formation of white precipitate indicates the presence of tannins.
7. Detection of flavonoids : Extract were treated with 4-5 drops of sodium hydroxide solution, formation of intense yellow colour which becomes colourless on addition of dilute acid, indicates the presences of flavonoids.
8. Detection of proteins and amino acids: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes, formation of blue colour indicates the presences of aminoacids.

Fluorescence Analysis of Powder of *Xanthium Strumarium L*

The powdered plant material was treated with different reagents and observed under the UV light at 254 nm and 365 nm.

Determination of Total Phenolics

The total phenolic contents of aerial parts of plant material were determined using Folin-Ciocalteau reagent and Na_2CO_3 according to the method described by Malik and Singh^[12]. The concentration of phenol was determined as equivalent of phenol /g of extract by measuring absorption at 650 nm using pre-calibrated standard curve employing pyrocatechol. Experiment was carried out in triplicate and

results were recorded as mean \pm SEM

Determination of total flavonoids

the aluminum chloride method was used for the determination of the total flavonoid content of the sample extracts^[13]. After addition of AlCl_3 , sodium-potassium tartarate and incubation absorbance was measured at 415 nm. The concentration of flavonoid in the test extracts was calculated from the calibration plot and expressed as mg quercetin equivalent /g of extract. Experiment was carried out in triplicate and results were recorded as mean \pm SEM

Results and Discussion

The observed percent extractive values are less in non-polar extract and more in polar in polar extract which indicates existence of polar compounds more in test material.

Table 1: Percent Extractive Values

Sr. No	Solvent Used	Color & Consistency	Average Value of Extractive (%)	
			Cold	Hot
1.	Hexane	Green	0.44	0.66
2.	Ethyl acetate	Dark Green	2.69	3.10
3.	Acetone	Dark Green	1.16	3.08
4.	Chloroform	Light Green	1.11	3.42
5.	Ethyl alcohol	Green	7.12	8.22
6.	Methyl alcohol	Green	8.11	9.91

Phytochemical evaluation is preliminary step towards the content of various phyto constituents. In case of hot solvent extracts alkaloid content is prominent. Saponin content in stem is exhaustive. Protein content is more in all extracts of stem.

Table 2: A) Phytochemical Test for –Cold solvent extracts.

Sr. No.	Test /Reagent used	1	2	3	4	5	6	7
1.	Detection of alkaloid-Hangers Test	-	+	+	+	-	-	-
2.	Detection of glycosides- Legals Test	-	-	-	-	-	-	+
3.	Detection of saponins - foam Test	+	+	+	+	+	+	+
4.	Detection of phytosterols- Salkowaski's test	-	+	+	+	+	+	+
5.	Detection of phenols - Ferric Chloride Test	-	-	+	+	+	+	+
6.	Detection of flavonoids- a. Alkaline reagent Test	+	-	-	-	+	+	+
	b. Lead acetate Test	-	-	-	+	+	+	+
7.	Detection of amino acids - Ninhydrin Test	-	+	-	+	-	-	+
8.	Detection of proteins - Xanthoprotic Test proteins (HNO_3)	-	+	-	+	+	+	+

*1- Hexane, 2- Chloroform, 3- Acetone, 4- Ethylacetate, 5- Ethylalcohol, 6- Methylalcohol 7-Aqueous

Table 3: B) Phytochemical Test -Hot solvent extracts.

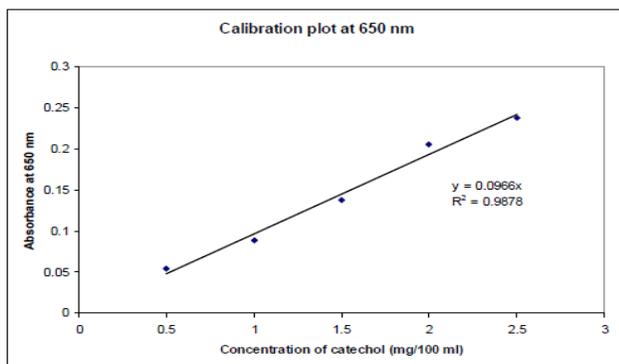
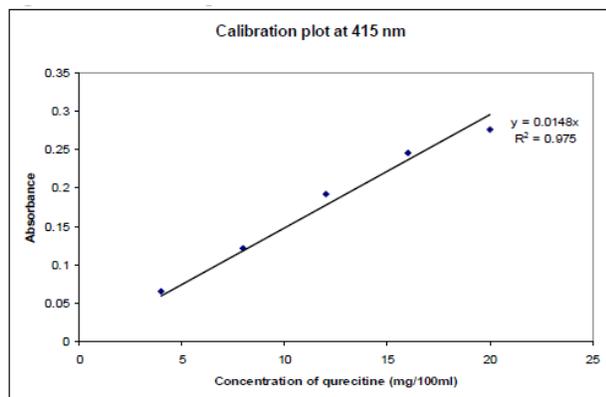
Sr. No.	Test /Reagent used	1	2	3	4	5	6	7
1.	Detection of alkaloid-Hangers Test	+	+	+	-	-	-	-
2.	Detection of glycosides-Legals Test	-	-	-	-	-	-	+
3.	Detection of saponins - foam Test	+	+	+	+	+	+	+
4.	Detection of phytosterols-Salkowski's test	+	+	-	-	+	+	+
5.	Detection of phenols - Ferric Chloride Test	-	+	+	+	+	+	+
6.	Detection of flavonoids- a. Alkaline reagent Test	-	-	+	+	+	+	+
	b. Lead acetate Test	+	-	-	-	+	+	+
7.	Detection of amino acids - Ninhydrin Test	+	-	-	+	+	+	+
8.	Detection of proteins - Xanthoprotic Test proteins (HNO ₃)	+	+	+	+	-	-	+

Flourescence study of the whole plant powder showed the color with different chemical reagents. These colours changes in different wavelengths used for observations indicates,

Table 4: Fluorescence Analysis of powder of *Xanthium strumarium* L.

Sr. No.	Treatment	Visible light	UV(254 nm)	UV (365 nm)
1	Powder as such	Green	Yellow	Green
2	Powder + NaoH in Methanol	Green	Brown	Dark Brown
3	Powder + 1N Hydrochloric acid	Green	Yellow	Greenish brown
4	Powder + Nitric acid	Green	Dark Yellow	Yellow
5	Powder +Sulphuric acid	Green	Green	Brown
6	Powder +Acetic acid	Green	Dark Green	Brown
7	Powder +Picric acid	Yellowish Brown	Brown	Yellowish Green

Total phenolic content for *Xanthium strumarium* L is obtained from the regression equation of calibration curve of pyrocatechol ($y=0.0900x$, $R^2=0.9678$) and expressed as pyrocatechol equivalent. Total flavonoid content for *Xanthium strumarium* L is obtained from the regression equation of calibration curve of quercetin ($y=0.0148x$, $R^2=0.975$) and expressed as quercetin equivalent. The phenolic and flavonoid content of *Xanthium strumarium* L recorded (Table 5, 6)

**Fig 1:** Calibration plot for phenolic determination**Fig 2:** Calibration plot for flavonoid determination**Table 5:** Phenolic content

Stem Extracts	mg of Catechol equivalent/g of material
Acetone	0.00546
Ethanol	0.00634
Methanol	0.00947

Table 6: Flavonoid content

Stem Extracts	mg of quercetin equivalent/g of material
Acetone	0.00846
Ethanol	0.01064
Methanol	0.0142

Conclusion

The results of present spectrophotometric study conclusively demonstrated that *Xanthium strumarium* L possess high antioxidant potential. The plant is found to be rich in phenolic compounds as well as flavonoid content of semi polar to polar stem extracts. It was found that the polar methanol extract was found to be potent compared to acetone and ethanol extracts. This would be responsible for its high antioxidant potential. This preliminary study undeniably useful to identify the molecule/s having strong antioxidant potential.

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