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Phytochemical Study and Antioxidant Activity of Methanolic Extract of *Xanthium strumarium* L.

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Abstract— *Antioxidants serve a critical function in preventing cell injury caused by ROS by scavenging free radicals. Plant-derived antioxidants protect cells by scavenging free oxygen radicals and balancing reactive oxygen species (ROS). This is due to the presence of bioactive components in plants, such as phenolic compounds, flavonoids, and essential oils, which provide antioxidant action.The main objective of this study is to evaluate the antioxidant activity of methanol extract of aerial part of Xanthium strumarium L. The methanol extract of the aerial part of plant was subjected to preliminary phytochemical analysis. Free radical scavenging activity of the methanolic extract at different concentration was determined with 1, 1-diphenyl-2 picrylhydrazyl (DPPH) assay. The goal of current study is to uncover naturally occurring antioxidants from plants. The current study focuses on phytochemical screening and in-vitro antioxidant activity evaluation of methanol extract of areal parts of Xanthium strumarium.*

Keywords— *Antioxidant; DPPH Assay; Oxidative stress; Phytochemical screening, Xanthium strumarium*.

I. INTRODUCTION

Oxidants and antioxidants are crucial elements of the signal transductory system in cells. Oxidative stress takes place when the pro-oxidant/antioxidant equilibrium is altered in a manner that results in an excess of prooxidant agents, which negatively affects particular organs/organ systems.[1] Antioxidants are compounds that eliminate oxidative stress in biological systems. Basically, there are two mechanisms that lead to oxidative stress: either the concentration of antioxidants is decreased (for example, because of mutated antioxidant enzymes, toxins, or a decrease in the intake of natural antioxidants), or the number of oxygen/nitrogen/carbon-based reactive species produced by activated phagocytes is increased, as in the case of chronic inflammation (disease).[1,2] In the etiology of many chronic diseases, oxidative stress plays a significant risk factor. It is now established that free radicals and other reactive oxygen species have a role in the pathophysiology of diseases like atherosclerosis, Parkinson's disease, diabetes, inflammatory arthropathies, and asthma.[3,4] Antioxidants serve a critical function in preventing cell injury caused by ROS by scavenging free radicals. Plant-derived antioxidants protect cells by scavenging free oxygen radicals and balancing reactive oxygen species (ROS). This is due to the presence of bioactive components in plants, such as phenolic compounds, flavonoids, and essential oils, which provide antioxidant action.[4] *Xanthium strumarium* belongs to family asteraceae, commonly known as cocklebur, is annual herb commonly found in India, North America, Brazil, China and Malaysia.[3] Various parts of this plant species were discovered to have beneficial medicinal properties. Phenylpropenoids, Sesquiterpenoids, flavonoids, glycosides, coumarins, steroids, lignans, thiazides, naphthoquinones, anthraquinones, and other chemicals have all been isolated and identified from *X. strumarium* to date.[5,6] According to recent studies, *X. strumarium* extracts and compounds have a wide range of pharmacological effects, including antitrypanosomal[6], hypoglycemic[8], anthelmintic, antifungal[9], antileishmanial[9], antiulcerogenic[10], and anti-inflammatory[11] activities. The goal of current study is to uncover naturally occurring antioxidants from plants. The main aim of the present study was to explore Phytochemical screening of methanolic extract of aerial parts of *Xanthium strumarium* L. and to carry out antioxidant activity of methanolic extract of aerial parts of *Xanthium strumarium L.* from India.

II. MATERIALS AND METHODS

A. Collection of Plant material

Meticulously examined healthy plant parts (stem, leaves and fruits) of *Xanthium strumarium* L. were collected from Haldia, West Bengal in the month of April-May 2022. The selected plants were sent for botanically identification and authentication. The plant parts were washed. After being washed, the plant parts were dried in the shade for 36 hours with natural air flow and a temperature of 25 °C.

Plant materials were completely dried then processed into a powder using a electric grinder. The powder was then packed in brown bottles to be used in the experimental methods.

B. Preparation of extracts

100 gm of Dry powder was added 300ml Methanol (70%) and then Mixture was poured into Volumetric flask and keep it for 4 hours with continuous shaking. After that it was kept aside for 3 days. Then extracts were filtered by using Whatmann's filter paper No. 1 and then used for further experiments.

C. Phytochemical Screening

To validate the presence of phytochemicals, tests for alkaloids, carbohydrates, glycosides, saponins, phenols, tannin, flavanoids, protein and amino acids, steroids, and terpinoids were conducted.

Lakshmi Kanta Kanthal, Suman Pattanayak, Susmita Roy, Krishnendu Manna, Moulik Roymahapatra, Sk Ayneuddin Ahamed, and Partha Biswas, "Phytochemical Study and Antioxidant Activity of Methanol Extract of Xanthium strumarium L.," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 5, Issue 5, pp. 82-84, 2022.

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D. Antioxidant activity

On the basis of the radical scavenging ability of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) molecule, the antioxidant activity of the methanol extracts of *X. strumarium* aerial parts was evaluated .[12] To prepare DPPH standard solution, 4mg of DPPH was mixed with 100ml of ethanol and sealed the volumetric flax with aluminium foil, then kept for 30 minutes in incubation. After that, to prepare control solution 4ml of DPPH standard solution was mixed with 6 ml of ethanol and after that sealed with aluminium foil, then kept in incubation for 30 minutes. Next, in order to prepare Ascorbic acid standard solution, 100 mg of ascorbic acid was mixed with 100 ml distilled water (1000µg/ml concentration was prepared as standard stock solution), from which five different concentration 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml of standard solution were prepared. After that, all the above 5 different concentration of standard solution were kept in a dark place for 30 minutes. Then, 100 mg of plant (*Xanthium strumarium*) extract and 100 ml of ethanol (prepared at a concentration of 1000 g/ml as a sample stock solution) were mixed to create a test sample solution from which solutions at various concentrations (20 g/ml, 40 g/ml, 60 g/ml, 80 g/ml, and 100 g/ml) were created. After that, all the above 5 different concentration of sample solution were kept in a dark place for 30 minutes. After 30 minutes the absorbance of the mixtures were measured at 518 nm. Further, % radical scavenging activity of plant extract and ascorbic acid solution was calculated using the following formula:

% Inhibition for scavenging activity = (Absorbance of control-Absorbance of sample)/Absorbance of control × 100

III. RESULT

A. *Phytochemical Test*

Table no I shows the phytochemical constituent present in the methanol extract of aerial parts of *Xanthium strumarium.*

TABLE I. Phytochemical constituent present in the methanol extract of aerial parts of *Xanthium strumarium*

Type of phytoconstituent	Name of the tests	Methanol extract of X strumarium
Alkaloid	Mayer"s test	Present
	Wagner test	Present
Carbohydrate	Molish"s test	Present
	Fehling"s test	Present
Glycoside	Brontragers test	Present
	Killer-killani test	Absent
Saponins	Foam test	Present
Phenols	Ferric chloride test	Present
Tannin	Lead Acetate test	Present
Flavonoids	Alkaline reagent test	Present
Proteins and Amino acid	Ninhydrin test	Present
Terpenoids	Salkowski test	Present

B. *DPPH radical scavenging activity*

The results of antioxidant activity of the tested extract is summarized in Table no II,III and Figure no I,II as comparable with known antioxidant Vitamin C. Absorbance of different concentration extract of *Xanthium strumarium L* with standard Ascorbic acid at 518nm by UV visible spectrophotomer are given in Table no II. By calculating % inhibition, the antioxidant activity was determined BY gradually increasing concentration. Percentage inhibition of different concentration of extract with Ascorbic acid is given in Table no III. The effective concentration at which the antioxidant activity was 50% inhibited is known as the IC50 (g/ml), which is calculated by interpolating the results of a linear regression analysis. Inverse correlation exists between IC50 values and antioxidant and DPPH radical scavenging capacity levels.

TABLE II. Absorbance of different concentration extract of *Xanthium strumarium* L with standard Ascorbic acid at 518nm by UV visible spectrophotomer

Sr No	Concentration ((µg/ml)	Absorbance at 518nm	
		Test	Standard
1	20	0.0932	0.0310
2	40	0.0608	0.0026
3	60	0.0328	0.0058
4	80	0.0325	0.0054
5	100	0.0348	0.0056

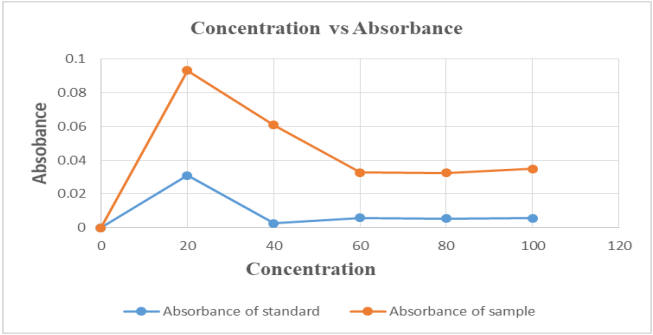

Fig. 1. Absorbance of different concentration of extract in comparison with standard solution

TABLE III. Percentage inhibition of different concentration of extract in comparison with Ascorbic acid

Sr No	Concentration ((µg/ml)	Percentage of Inhibition	
		Extract	Standard
1	20	77.091	92.52
2	40	85.59	99.38
3	60	92.22	98.62
4	80	92.30	98.72
5	100	91.74	98.67
IC50 value		18.29 µg/ml	7.11 µg/ml

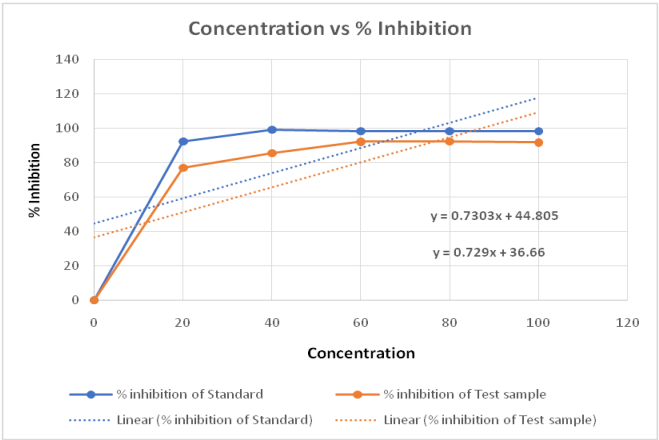

Fig. 2. % of inhibition in different concentration of extract in comparison with the % of inhibition in different concentration of standard solution

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IV. DISCUSSION

The preliminary phytochemical tests of methanolic extract (aerial part) of *Xanthium strumarium* indicates the presence of alkaloids, carbohydrates, glycosides, saponins, phenol, tannins, flavonoids, steroids, terpenoids, protein and amino acids.

Antioxidants are extremely significant compounds that have the potential to shield the body from damage caused by oxidative stress led by free radicals. In the search for new bioactive chemicals from natural resources, the methanol extracts of *Xanthium strumarium* were looked into for their antioxidant potential. More than 170 chemical components, including phenylpropenoids, sesquiterpenoids, flavonoids, glycosides, coumarins, steroids, lignans, thiazides, naphthoquinones, anthraquinones, and other substances, have so far been isolated and identified from *X. strumarium*.[12]

Antioxidant activity of methanol extract (aerial part) of *Xanthium strumarium* L was measured at five different concentration levels (/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml). The antioxidant activity was measured by calculating the percentage inhibition. The absorbance of gradually increasing concentration of methanol extract of *Xanthium strumarium* aerial part at 518nm by UV visible spectrophotometer were found 0.0932, 0.0608, 0.0328, 0.0325, 0.0348 respectively. The absorbance of gradually increasing concentration of Ascorbic acid at 518nm by UV visible spectrophotometer were found 0.0031, 0.0026,0.0058, 0.0054, 0.0056 respectively.

In line with the absorbance data for test sample methanol extract of *Xanthium strumaium* aerial part and standard solution ascorbic acid, the percentage inhibition for scavenging activity is determined by the formula % Inhibition for scavenging activity = (Absorbance of control- Absorbance of sample)⁄Absorbance of control × 100 .

These findings show that extract (aerial part) of *Xanthium strumarium* possesses antioxidant activity. Percentage inhibition for scavenging activity of different concentration 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml of Aerial part of *Xanthium strumarium* extract were 77.091, 85.59, 92.22, 92.30, and 91.74 percent respectively. While Percentage inhibition for scavenging activity of different concentration 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml of Ascorbic acid were 92.52, 99.38, 98.62, 98.72, 98.67 percent.

Further, IC50 value is calculated from the graph (Figure no 2) by interpolation from linear regression analysis.

DPPH assay revealed that IC 50 value of Aerial part of *Xanthium strumarium* is 17.88 µg/mL and Ascorbic acid is 7.12 µg/mL. As the lower the IC50 value, the stronger the antioxidant activity, DPPH assay revealed that the extract (aerial part) of *Xanthium strumarium* possesses significant antioxidant activity. Howevere, in comparison to standard the extract (aerial part) of *Xanthium strumarium* possesses less antioxidant activity.

V. CONCLUSION

It becames evident that *Xanthium strumarium*'s aerial portion has significant antioxidant activity when compared to the standard antioxidant Ascorbic acid for DPPH scavenging activity. The antioxidant properties displayed by the methanolic extract may confirm the plant's widespread historical use as a folk treatment. Further, an isolation and characterisation of phytochemical constituents are necessary to reflect the current understanding.

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