PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF EXTRACTS OF *Xanthium strumarium*, *Chrysanthemum* AND THEIR MIXTURE

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ABSTRACT

Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the active constituents, which determine the medicinal properties of the plants. The present investigation was undertaken to screen the phytochemical analysis and antioxidant activity of *Xanthium strumarium*, *Chrysanthemum* and mixture of *Xanthium strumarium* and *Chrysanthemum* plant extracts.

Keywords: Asteraceae species, Phytochemical Screening, DPPH Assay, Antioxidant Activity

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INTRODUCTION

Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the active constituents, which determine the medicinal properties of the plants. It has now been a universally accepted fact that the plant drugs and remedies are far safer than of synthetic medicines.¹ *Xanthium strumarium* is an annual herb with a short, stout, hairy stem. Leaves are broadly triangular-ovate or suborbicular. It commonly grows in waste places, along with roadsides and river banks. The plant's component xanthinin shows antibacterial activity. It resembles sunflower oil and used in bladder infection, herpes, and erysipelas. The whole plant improves appetite, voice, complexion, and memory. It cures leucoderma, biliousness, poisonous bites of insects, epilepsy, salivation, teeth diseases and fever. Roots are used in cancer, strumous diseases. Fruits are useful in headache. Seeds fumes are inhaled for piles (burnt). *Chrysanthemum* is a bushy herb, densely branching, 40-80cm in height. Leaves are deeply cut, toothed, pale greyish green. Many flowered stems, often semi-double. It grows fairly well when the seeds are sown in the fall (September to November), and in the spring.² *Chrysanthemum* flowers used to make medicine. It is used to treat high blood pressure, diabetes, chest pain, cough, cold, fever and inflammation.³⁻⁶

EXPERIMENTAL

The plant materials *Xanthium strumarium* and *Chrysanthemum* were collected seasonally and authenticated by the taxonomists Dr. S.P. Rothe from the Department of Botany, Shri Shivaji College, Akola.

All the chemicals used in the study were obtained commercially and of analytical grade.

Phytochemical Screening⁷⁻¹¹

The chemical tests were performed for testing different chemical groups present in the ethanolic extract of leaves and flowers of *Chrysanthemum*, fruits and leaves of *Xanthium strumarium* and mixture of leaves of *X. strumarium* and *Chrysanthemum*

Table-1: Phytochemical Investigation of *X. Strumarium*, *Chrysanthemum* and Mixture of *X. Strumarium* and *Chrysanthemum* Plant Extracts

S. No.	Phyto-constituents	<i>X. strumarium</i>		<i>Chrysanthemum</i>		Mixture of <i>X. Strumarium</i> and <i>Chrysanthemum</i>
		Fruit	Leaves	Flower	Leaves	Leaves
1.	Carbohydrates	+	+	+	+	+
2.	Reducing Sugar	+	+	+	+	+
3.	Hexose Sugar					
	(i) Glucose	+	+	+	+	+
	(ii) Fructose	+	+	+	+	+
4.	Steroid / Triterpenoid	+	+	+	+	+
5.	Protein	+	+	+	+	+
6.	Alkaloids	+	+	+	+	+
7.	Amino acids	– (Ninhydrine)	–	–	–	–
8.	Flavonoids	–	+	–	+	+
9.	Tannins	+	+	+	+	+
10.	Coumarin Glycosides	+	+	+	+	+
11.	Anthraquinone Glycosides	+	–	–	–	–
12.	Cyanogenetic Glycosides	–	–	–	–	–
13.	Cardiac Glycosides	–	–	–	–	–
14.	Saponins	+	+	–	–	+
SPECIFIC TESTS						
15.	Barberine	+	+	+	+	+
16.	Morphine	–	+	–	+	+
17.	Aconitine	+	+	+	+	+
18.	Brucine	+	–	–	–	–
19.	Connine	–	+	–	+	+
20.	Ergometrine	+	+	+	–	+
21.	Hesperidin	–	–	+	–	–
22.	Papaverine	–	–	–	–	–
23.	Phygostigmine	–	+	–	+	+
24.	Quinine	+	+	+	–	+
25.	Quinidine	+	+	+	–	+
26.	Soalsodine	–	+	–	+	+
27.	Cinchonine	–	–	–	–	–
28.	Cinchonidine	+	+	+	+	+

The Leaves and fruits of *X. strumarium* and leaves and flowers of *Chrysanthemum* plants were shade dried at room temperature and ground in a manual mill to get a coarse powder. The coarse powdered materials of test plants were kept in the airtight polythene bag and stored in dry place. These powders were extracted with ethanol by using soxhlet apparatus. The extracts were concentrated at 40 °C using rotary evaporator. Finally it was dried, crushed and stored in airtight bottles at 4 °C for further study.

Study of Antioxidant Activity By DPPH¹²⁻¹³

The antioxidant activity of the ethanol extracts of leaves and fruits of *X. strumarium*, and leaves and flowers of *Chrysanthemum* plants and mixture of leaves of *X. strumarium* and *Chrysanthemum* plants were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the test plant extracts were prepared in ethanol. 0.004% of DPPH was prepared in ethyl alcohol and 3 ml of this solution was mixed with 3 ml of sample solutions. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. Alcohol (3 ml) with DPPH solution (0.004%, 3 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

$$\text{Percentage (\%) Inhibition of DPPH (\% AA)} = \frac{A - B}{A} \times 100$$

Where A=Optical density of the blank and B=Optical density of the sample.

RESULTS AND DISCUSSION

The stock solution 1 mg/ml of ethanol was prepared. The required dilutions 0.1 mg/ml to 0.9 mg/ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity were calculated.

Table-2: Optical Density And Percent Antioxidant Activity For Ethanolic Extract Of *X. strumarium* Fruits: (O.D. of Blank DPPH = 0.595)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
O.D. of <i>X. strumarium</i>	0.435	0.346	0.285	0.243	0.214	0.198	0.126	0.116	0.103	0.035
%AA <i>X. strumarium</i>	26.89	41.84	52.10	59.15	64.03	66.72	78.82	80.50	82.68	94.11

IC₅₀ = 0.044 mg/ml.

Fig.-1: Decrease in Optical Density of Sample With Increase In Concentration of Ethanolic Extracts of *X. strumarium* Fruits.

Table-3: Optical Density and Percent Antioxidant Activity For Ethanolic Extract of *X. strumarium* Leaves: (O.D. of Blank DPPH = 0.595)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
O.D. of <i>X. strumarium</i>	0.388	0.342	0.329	0.309	0.278	0.265	0.240	0.202	0.172	0.143
%AA <i>X. strumarium</i>	34.78	42.52	44.70	48.06	53.27	55.46	59.66	66.05	71.09	75.96

IC₅₀ = 0.061mg/ml.

Fig.-2: Increase in Percent Antioxidant Activity With Increase In Concentration of Ethanolic Extracts of X. *Strumarium* Fruits

Fig.-3: Decrease in Optical Density of Sample with Increase in Concentration of Ethanolic Extract of X. *Strumarium* Leaves

Fig.-4: Increase in Percent Antioxidant Activity with Increase in Concentration of Ethanolic Extract of X. *Strumarium* Leaves

Calculation of IC₅₀ Value for *X. strumarium* leaves extract = max - ½ (max-min)
 = 75.96 - ½ (75.96 - 34.78)
 = 75.96 - 20.59
 = 55.37

IC₅₀ value from graph corresponding to *X. strumarium* leaves extract is 0.061 mg/ml.

Calculation of IC₅₀ Value for *X. strumarium* fruits extract = max - ½ (max-min)
 = 94.11 - ½ (94.11 - 26.89)
 = 94.11 - 33.61
 = 60.5

IC₅₀ value from graph corresponding to *X. strumarium* fruits extract is 0.044 mg/ml.

Table-4: Optical Density And Percent Antioxidant Activity For Ethanolic Extract Of *Chrysanthemum* Flowers: (O.D. of Black DPPH = 0.595)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
O.D. of <i>Chrysanthemum</i>	0.345	0.311	0.283	0.262	0.243	0.211	0.183	0.165	0.143	0.127
%AA <i>Chrysanthemum</i>	42.01	47.31	52.43	55.96	59.15	64.53	69.24	72.26	75.96	78.65

IC₅₀ = 0.053 mg/ml

Fig.-5: Decrease in Optical Density of Sample with Increase in Concentration Of Ethanolic Extract of *Chrysanthemum* Flowers

Fig.-6: Increase in Percent Antioxidant Activity with Increase in Concentration of Ethanolic Extract of *Chrysanthemum* Flowers

Table-5: Optical Density and Percent Antioxidant Activity for Ethanolic Extract Of
Chrysanthemum Leaves: (O.D. of Black DPPH = 0.595)

Conc.mg/ml	0.01	0.02	0.03	0.04		0.05	0.06	0.07	0.08	0.09	0.1
O.D. of <i>Chrysanthemum</i>	0.385	0.356	0.303	0.252		0.216	0.192	0.165	0.141	0.126	0.101
%AA <i>Chrysanthemum</i>	35.29	40.16	49.07	57.64		63.69	67.73	72.26	76.30	78.82	83.02

$$IC_{50} = 0.049 \text{ mg/ml}$$

Fig.-7: Decrease in Optical Density of Sample with Increase in Concentration of Ethanolic Extract of *Chrysanthemum* Leaves

Fig.-8: Increase in Percent Antioxidant Activity with Increase in Concentration Of Ethanolic Extract of *Chrysanthemum* Leaves

$$\begin{aligned}
 \text{Calculation of } IC_{50} \text{ value for } Chrysanthemum \text{ leaves extract} &= \max - \frac{1}{2} (\max - \min) \\
 &= 83.02 - \frac{1}{2} (83.02 - 35.29) \\
 &= 83.02 - 23.86 \\
 &= 59.16
 \end{aligned}$$

IC_{50} value from graph corresponding to *Chrysanthemum* leaves extract is 0.049 mg/ml.

$$\begin{aligned}
 \text{Calculation of } IC_{50} \text{ Value } Chrysanthemum \text{ flowers extract} &= \max - \frac{1}{2} (\max - \min) \\
 &= 78.65 - \frac{1}{2} (78.65 - 42.01) \\
 &= 78.65 - 18.32 \\
 &= 60.33
 \end{aligned}$$

IC_{50} value from graph corresponding to *Chrysanthemum* flowers extract is 0.053 mg/ml.

Table-6: Optical Density and Percent Antioxidant Activity For Mixture Of Ethanolic Extract of *X. Strumarium* and *Chrysanthemum* (Leaves): (O.D. of Black DPPH = 0.595)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
O.D. of Mixture	0.442	0.403	0.381	0.356	0.325	0.301	0.284	0.251	0.224	0.193
%AA of Mixture	25.71	32.26	35.96	40.16	45.37	49.41	52.26	57.81	62.35	67.56

IC₅₀ = 0.052 mg/ml

Fig.-9: Decrease in Optical Density of Sample with Increase in Concentration For Mixture of Ethanolic Extract of *X. Strumarium* and *Chrysanthemum* (Leaves)

Fig.-10: Increase in Percent Antioxidant Activity with Increase in Concentration For Mixture of Ethanolic Extract of *X. Strumarium* And *Chrysanthemum* (Leaves)

Calculation of IC₅₀ Value for a mixture of ethanolic extract of *X. Strumarium* and *Chrysanthemum* leaves:
 $= \max - \frac{1}{2} (\max - \min)$

$$\begin{aligned}
 &= 67.56 - \frac{1}{2} (67.56 - 25.71) \\
 &= 67.56 - 20.92 \\
 &= 46.64
 \end{aligned}$$

IC₅₀ value from graph corresponding to mixture of ethanolic extract of *X. strumarium* and *Chrysanthemum* leaves is 0.052 mg/ml.

CONCLUSION

On the basis of chemical tests performed for testing different chemical groups present in the ethanolic extract of leaves and flowers of *Chrysanthemum*, fruits and leaves of *Xanthium strumarium* and mixture of leaves of *X. strumarium* and *Chrysanthemum* were presented in Table-1.

The results obtained for the antioxidant assay by DPPH performed for various extracts of *Xanthium strumarium* and *Chrysanthemum* plants and Mixture of *Xanthium strumarium* and *Chrysanthemum* plants

were presented (Table no 2 to 6). A remarkable decrease in O. D. values of test plant extracts was observed from graph, showed good to moderate antioxidant activity. The IC₅₀ values of the various extracts were calculated from the figures which are given below:

Table-7

S. No.	Test plant extracts	IC ₅₀
1.	<i>X. strumarium</i> leaves	0.061
2.	<i>X. strumarium</i> fruits	0.044
3.	<i>Chrysanthemum</i> leaves	0.049
4.	<i>Chrysanthemum</i> flowers	0.053
5.	Mixture of <i>X. strumarium</i> and <i>Chrysanthemum</i> leaves	0.052

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