British Journal of Pharmaceutical Research
4(1): 1-22, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Phytochemical Screening, *In-vitro* Evaluation of Antioxidant and Free Radical Scavenging Activity of Leaves, Stems and Roots of *Xanthium strumarium* L., (Compositae)

Anjoo Kamboj[1*], Pooja Atri[1] and Ajay Kumar Saluja[2]

[1]Chandigarh College of Pharmacy, Landran, Mohali (Punjab), India.
[2]A. R. College of Pharmacy, V. V Nagar, Gujarat, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author AK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author PA managed the analyses of the study. Author AKS managed the overall checking of the manuscript. All authors read and approved the final manuscript.

Research Article

Received 11th March 2013
Accepted 12th May 2013
Published 5th October 2013

ABSTRACT

Introduction: Many diseases are associated with oxidative stress caused by free radicals. Current research is directed towards finding naturally occurring antioxidants of plant origin. *Xanthium strumarium* L. a cocklebur or bur weed is a reputed medicine in Europe, China, Indo-china, Malaysia and America. It is used in treatment of common disease such as hemicrania, leucoderma, biliousness, poisonous bites of insects, epilepsy, long standing malaria, relieving constipation, diarrhoea, vomiting etc. The present research deals with phytochemical screening and in-vitro evaluation of antioxidant activities of the various extracts of leaves, stems and roots of *X. strumarium* L.
Method: Successive extracts of leaves, stems and roots was subjected for phytochemical screening. Various extracts of leaves, stems and roots were screened in-vitro for total antioxidant potential. Inhibition of oxygen derived free radicals, viz., assay for free radical scavenging of nitric oxide, hydrogen peroxide, the antioxidant capacity by phosphomolybdenum, reducing power ability and determination of phenolic and flavonoids content in the extracts of leaves, stems and roots were performed. DPPH scavenging activity or the Hydrogen donating capacity was quantified in presence of

**Corresponding author: Email: anjookamboj@gmail.com;*

stable DPPH radical on the basis of Blois method. NO scavenging activity was performed in the presence of nitric oxide generated from sodium nitroprusside using ascorbic acid as standard in both methods. The phenolic content was determined by using Folin-Ciocalteu reagent and flavonoid content was determined by aluminium chloride.

Result: The preliminary phytochemical screening revealed the presence of saponins, sterols, flavonoids, alkaloids and phenolic compounds in the extracts. The scavenging activity was found to be dose dependent. The reducing capacity serves as significant indicator of antioxidant activity. The reducing power increased with the increasing concentration of sample. The 100mg powder of leaves yielded 0.069, 0.523, 1.620 mg/g phenolic content and 0.17, 0.45, 0.95 mg/g flavonoid content with solvents such as petroleum ether (60º-80ºc), chloroform, and ethanol respectively. Similarly, in case of stems and roots the phenolic content yielded 0.063, 0.324, 1.324 mg/g and 0.040, 0.159, 0.41 mg/g and flavonoids content 0.00, 0.11, 0.23 mg/g and 0.00, 0.05, 0.18 mg/g respectively, using quercetin as standard.

Conclusion: The present study provides evidence that *X. strumarium* L., is a potential source of antioxidants and the extracts have constituents which were capable of showing antioxidant activity and the said in-vitro antioxidant activity may also be due to the presence of antioxidant principles present in the extracts like flavonoid and phenolic compounds. So the folklore use of *X. strumarium* L. has been proved in present research work. These findings confirm the great interest of the herb whose phytochemistry and phytopharmacology should be investigated further in order to detect possible phytotherapeutic uses in the prevention of ageing related diseases, cardiovascular disorders and Alzheimer disease.

Keywords: Xanthium strumarium L.; DPPH scavenging activity; NO scavenging activity; phytochemical screening.

1. INTRODUCTION

Free radicals (FR) are molecules containing an unpaired electron; they are unstable and highly reactive. They are products of cellular metabolism. Our body cells replenish themselves and produce energy to run the processes of the body. During this biochemical processes certain molecules are left behind with missing electron called free radicals which seek stability through electron pairing with biological macromolecules such as protein and DNA along with lipid peroxidation. Free radicals are not only by-products of cellular metabolism but in recent era they introduced in to our bodies from other sources like cigarette or cigar smoke, radiation, alcohol, air and water pollution ingesting artificial products can lead to higher levels of free radical in the body. Certain gases and even sunlight can affect the FR level in our body. This is the main reason of ageing which appears like a disease; over degenerative diseases are now known to be linked to FR induced oxidative stress [1-2].

Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative disease and others. Reactive oxygen Species (ROS) or FR play an important role in oxidative stress related to the pathogenesis of various important diseases. Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions [3-9].

Antioxidants are the compounds that are inhibitor of oxidation process and are found to inhibit the chain reactions at small concentrations and thereby eliminate the threat of pathological process. The plants carotenoids, flavonoids, phenolic and polyphenols etc are the secondary metabolites present in most edible fruits, vegetables and are therefore are common in everyday diet of many people. Synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are commercially available but are quite unstable and toxic. Hence strong restrictions have been placed on their use and hence trend to substitute them with naturally occurring antioxidants. Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent developments due to their potent antioxidant activities, no side effects and economic viability [10-12].

Xanthium strumarium L. (Family: compositae) a medicinal plant commonly found as a weed, is widely distributed in North America, Brazil, China, Malaysia and hotter parts of India. Extract of the whole plant, especially leaves, stems, roots, fruits and seeds have been applied traditional medicine for treatment of leucoderma, poisonous bites of insects, epilepsy, salivation, long-standing cases of malaria, rheumatism, tuberculosis, allergic rhinitis, sinitis, urticaria, constipation, diarrhoea, leprosy, lumbago, bacterial and fungal infection. Most of the pharmacological effects can be explained by the constituents like sesquiterpene lactones, glycoside, phenols, polysterols present in all plant parts. The constituents which are responsible for antioxidant activity are phenol and the phenols isolated are caffeic acid, potassium3-O-caffeoylquinate, 1-O-caffeoylquinic acid, chlorogenic acid, 4-O-caffeoylquinicacid, 1,4-di-O-caffeoylquinic acid, 1,5-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinicacid, 4,5-di-O-caffeoylquinic acid, 1, 3, 5-tri-O-caffeoylquinic acid, 3, 4, 5-tri-O-caffeoylquinic acid and cynarin [13].

The present study was thought to carry out antioxidant and free radical scavenging activity of various extracts of leaves, stems and roots of *X. strumarium* L. by in-vitro studies by multi-mechanistic assays and determination of the total phenolic and flavonoids content.

2. MATERIALS AND METHODS

2.1 Chemicals and Instruments

1, 1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), potassium ferricyanide was purchased from sigma chemicals. Gallic acid, quercetin, ascorbic acid, folin-ciocalteu reagent and ferric chloride were purchased from Merck. All other reagents were of analytical grade. UV-spectra were recorded in Shimadzu 1800 UV-Visible spectrophotometer.

2.2 Plant Material

Fresh plant *X. strumarium* L. was collected from Landran, Mohali (Punjab) in month of October. The plant was originally authenticated by Dr. H. B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi. A herbarium sample of this plant is preserved in the department. The plant parts were separated and dried under shade until a constant weight was obtained and then coarsely powdered and passed through No. 40 sieve and was used for the extraction.

2.3 Experimental

2.3.1 Preparation of Extracts

The powdered plant materials (Leaves, stems & roots) were extracted successively in soxhlet apparatus with various solvents like petroleum ether, benzene, chloroform, acetone, ethanol and water. All the extracts were subjected to preliminary phytochemical screening for the identification of various phyto-constituents by using standard procedures.

The powdered plant materials (100g) (Leaves, stems & roots) extracted in soxhlet apparatus with petroleum ether (60º-80ºc), chloroform & ethanol solvent (95 %v/v). The extracts were concentrated to dryness in rotary evaporator under controlled temperature (40º-50ºC). The extracts were preserved in vacuum desiccators for subsequent use in the evaluation of total phenolic, total flavonoid content and in-vitro antioxidant evaluation.

23.2 Determination of total phenolic content

Total phenolic compounds were determined using Folin-Ciocalteu method and the results were expressed as gallic acid equivalents. In brief, 0.005 g of dried ethanol extract was dissolved in 10ml of methanol-water mixture (4:6 v/v ratios). 0.2 ml of such solution was pipette in to a glass tube and 1ml of Folin-ciocalteu reagent, 0.8 ml of sodium carbonate (7.5%) were added to it. The mixture was stored at room temperature for 30 min and then absorbance was read at 765 nm with Shimadzu UV-visible spectrophotometer. Total phenolic content was calculated using a standard curve prepared with dilutions of gallic acid. [14].

2.3.3 Determination of total flavonoid content

The total flavonoid content was determined using the Dowd method. 5 mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the ethanol extract solution (0.5 mg/mL). Absorption readings at 415 nm using Shimazdu UV-visible spectrophotometer were taken after 10 minutes against a blank sample solution consisting of a 5 mL extract solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (0-100 mg/L) as the standard [15-17].

2.4 Evaluation of Antioxidant and Free Radical Scavenging Activity

2.4.1 Scavenging of DPPH radical

DPPH scavenging activity or the hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of blois method [18]. Briefly, to a methanol solution of DPPH (100 mM, 2.95 ml), 0.05 ml of test extracts dissolved in methanol was added at different concentrations (20-100 µg/ml). Reaction mixture was shaken and absorbance was measured at 517 nm using ascorbic acid as standard. The degree of discoloration indicates the scavenging efficacy of the extracts. The experiment was performed in triplicate and percentage of scavenging activity was calculated using the following equation 1.

$$\%\ DPPH\ Scavenging\ Activity = \frac{Ac - At}{Ac} x100 \quad (1)$$

Where *Ac*= Absorbance of standard, *At* = Absorbance of test sample

2.4.2 Scavenging of nitric oxide

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction according to the method of Marcocci. The chemical source of NO was sodium nitroprusside (5 mM) in 0.5 M phosphate buffer, PH 7.4, spontaneously generates nitric oxide in aqueous solution. Nitric oxide interacts with oxygen to produce stable products, leading to the production of nitrites [19].

About 1 ml sodium nitroprusside (5 mM) in 0.5 M phosphate buffer was mixed with 3.0 ml of different concentrations (20-100µg/ml) of the extracts and incubated at 25 °C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 546 nm was measured against blank with spectrophotometer. Ascorbic acid was used as standard antioxidant. The experiment was performed in triplicate and the nitric oxide radicals scavenging activity was calculated according to the equation 2.

$$\%\ Nitric\ Oxide\ Scavenging\ Activity = \frac{Ac - At}{Ac} x100 \qquad (2)$$

Where *Ac* = Absorbance of the control, *At* =Absorbance in the presence of the extract.

2.4.3 Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (20-100µg/ml) in methanol were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide [20]. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds were calculated using equation 3.

$$\%\ Scavenging\ Activity = \frac{Ac - At}{Ac} x100 \qquad (3)$$

Where Ac = Absorbance of the control, *At* =Absorbance in the presence of the sample of extract and standard.

2.4.4 Reducing power determination

The Reducing power of extracts was determined according to the method of Yen and Chen (1995). The extracts (20-100 µg/ml) in methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6), potassium ferricyanide (2.5 ml, 1%). Then the mixture was incubated at 50ºC for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, to stop the reaction and then centrifuge at 1000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) & ferric chloride (0.5 ml, 0.1%) & then absorbance

was measured at 700 nm. Ascorbic acid was taken as standard, phosphate buffer used as blank solution [21].

2.4.5 Phosphomolybdenum method

The assay is based on the reduction of Mo (VI) - Mo (V) by the extract and subsequent formation of green phosphate/MO (V) complex at acidic pH. 0.3ml extract was mixed with 3ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate & 4 mM ammonium molybdate). Then the tubes containing the reaction solution was incubated at 95ºc for 90min. Then, the absorbance of solution measured at 695nm using a spectrophotometer against blank cooling to room temperature. Use methanol (0.3 ml) in the place of extract as the blank. The antioxidant activity is express as the number of equivalent of ascorbic acid [22-23].

2.4.6 Statistical analysis

All results are expressed as mean ± S.E.M. Linear regression analysis was used to calculate the IC_{50} values.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The following active constituents have been identified from the tests in the various successive extracts are alkaloids, flavonoids, carbohydrates, sterols, phenols and glycosides. The phytochemical constituents present in successive extracts of leaves, stems and roots are presented in Tables 1-3.

Table 1. Phytochemical Screening of various extracts of Leaves of *X. strumarium*

S. No.	Type of constituents	Pet. ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloid	-	-	-	+	+	+
2	Flavonoid	-	-	-	+	+	+
3	Saponin	-	-	-	-	+	+
4	Carbohydrate	-	+	+	+	+	+
5	Phytosterol	+	+	-	-	-	-
6	Tannin	-	-	-	+	+	-
7	Phenolic	-	-	+	+	+	-
8	Coumarin	-	-	-	-	+	-
9	Cardiac glycoside	-	-	-	+	+	+
10	Anthraquinone	-	-	-	-	-	-
11	Essential oil	+	-	-	-	-	-

+ *present,* **-** *lower than the limit of detection of the method.*

Table 2. Phytochemical Screening of various extracts of Stems of X*. strumarium*

S. No.	Type of constituents	Pet. ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloid	-	-	+	+	+	+
2	Flavonoid	-	-	-	+	+	-
3	Saponin	-	+	-	+	+	+
4	Carbohydrate	-	+	+	+	+	+
5	Phytosterol	+	+	-	-	-	-
6	Tannin	-	-	-	+	+	-
7	Phenolic	-	+	+	+	+	
8	Coumarin	-	-	-	-	-	-
9	Cardiac glycoside	-	-	+		+	-
10	Anthraquinone	-	-	-	+	-	-
11	Essential oil	-	-	-	-	-	-

+ *present,* **-** *lower than the limit of detection of the method*

Table 3. Phytochemical Screening of various extracts of Roots of *X. strumarium*

S. No.	Type of constituents	Pet. ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloid	-	-	-	+	+	+
2	Flavonoid	-	-	-	+	+	-
3	Saponin	-	-	-	+	+	+
4	Carbohydrate	-	+	+	+	+	+
5	Phytosterol	+	-	-	-	-	-
6	Tannin	-	-	-	+	+	-
7	Phenolic	-	-	+	+	+	-
8	Coumarin	-	-	-	-	-	-
9	Cardiac glycoside	-	-	-	-	-	-
10	Anthraquinone	-	-	-	-	-	-
11	Essential oil	-	-	-	-	-	-

+ *present,* **-** *lower than the limit of detection of the method*

3.2 Total Phenolic and Flavonoid Contents

Total phenolic compounds, as determined by Folin-ciocalteu method, are reported as gallic acid equivalents by reference to standard curve (y=0.008x-0.002, r^2=0.997). The total flavonoid contents are reported as mg quercetin equivalent/g of extract powder by reference (y=0.007x+0.028, r^2=0.995). It was noted that leaves of *X. strumarium* had significant higher total phenolic and flavonoid content than stems and roots. Phenols and polyphenolic compounds, such as flavonoid are widely found in plant products, and they have been shown to possess significant antioxidant activities. The high amount of phenols and flavonoids in extract may explain their high antioxidative activities.

The standard curve of gallic acid and quercetin has shown in Fig. 1 and Table 4 indicates the total phenolic and flavonoid contents in the ethanol extracts of leaves, stems and roots of *X. Strumarium* L.

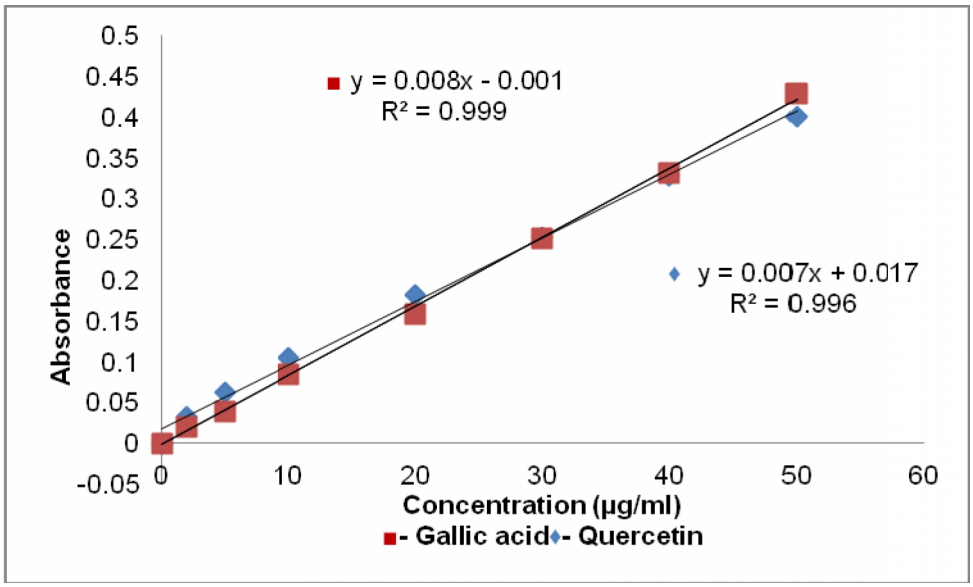

Fig. 1. Standard curve of gallic acid and quercetin

Table 4. Total phenolic and flavonoids contents of leaves, stems and roots of *Xanthium strumarium*

Extract	Total phenolic content (%w/w)			Total Flavonoid content (%w/w)		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Petroleum ether	0.069±0.001	0.063±0.001	0.040±0.002	0.17±0.18	–	–
Chloroform	0.523±0.23	0.324±0.19	0.159±0.29	0.45±0.43	0.11±0.22	0.05±0.001
Ethanol	1.620±0.59	1.324±0.52	0.41±0.49	0.95±0.59	0.23±0.31	0.18±0.12

All values are expressed as mean ± SEM (n=3)

3.3 DPPH Scavenging Activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen abstraction or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. It was found that the radical scavenging activities of all the extracts increased with increasing concentration. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity [15-16]. Leaves with high level of phenolic contents and highest amount of flavonoids showed the best activity. The antioxidant activity of ethanol extracts of various parts of *X. strumarium* increases with concentration from 13.5% to 62.1% in leaves and from 8% to 44.06% in stem and 8.38 to 37.8% in roots. The antioxidant activity of petroleum ether and chloroform extracts also increase with increase in concentration. (Table 5, Fig. 2a-c).

3.4 Nitric Oxide-Scavenging Activity

The extracts showed good nitric oxide-scavenging activity and percentage inhibition was found to increases with increasing concentration of the extract. The ethanol extracts of leaves, stems and roots were found to have significant free radical scavenging activity against nitric oxide (NO) induced release of free radicals. The ethanol extracts of leaves and stems had shown better reducing power than other. (Table 6, Fig. 3a-c).

3.5 Hydrogen Peroxide Method

Scavenging of H_2O_2 by extracts may be attributed to their phenolic, which can donate electrons to H_2O_2, thus neutralizing it to water. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch, where they are compared with that of ascorbic acid as standard [20]. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Scavenging capacity is increase with increase in concentration and leaves shows maximum scavenging effects then stems and roots. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems. (Table 7, Fig. 4a-c).

3.6 Reducing Power determination

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [20]. In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance indicates an increase in reductive power. Figure shows dose-response curves for the reducing powers of the extract. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. All extracts had shown good reducing power that was comparable with ascorbic acid but ethanol extracts showed more reducing power ability than the other extracts. (Table 8, Fig. 5a-c).

3.7 Phosphomolybdenum Reducing Capacity

Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH [18]. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. The reducing capacity increases with increase in concentration of extracts (Table 9, Fig. 6a-c).

Table 5. In-Vitro Free radical scavenging activity of petroleum ether, chloroform & ethanolic extracts of leaves, stems & roots of *X. strumarium*, by DPPH method

Conc. (µg/ml)	% Scavenging activity of Different Extracts								
	Leaves			Stem			Root		
	PE	CE	EE	PE	CE	EE	PE	CE	EE
20	-	6±0.12	13.5±0.14	-	9.7±0.16	8±0.19	-	5.5±0.18	8.38±0.21
40	-	15.2±0.23	25.4±0.21	-	18±0.25	18.6±0.28	-	9.7±0.23	11.8±0.23
60	11±0.19	26.3±0.31	35.4±0.18	-	33.3±0.31	32±0.31	-	13.4±0.25	22±0.16
80	15.2±0.29	37.5±0.29	45.7±0.26	-	37.5±0.16	35±0.23	-	24.8±0.31	23.7±0.18
100	20.8±0.34	48.6±0.19	62.1±0.17	18±0.23	43.5±0.34	44.0±0.26	-	27.4±0.28	37.8±0.26
IC_{50}	162	102	82	172	122	110	-	220	117

PE- Petroleum ether extract, CE- Chloroform extract, EE- Ethanol extract; All values are expressed as mean ± SEM (n=3).

Table 6. In- Vitro Free radical scavenging activity of petroleum ether, chloroform & ethanol extracts of leaves, stems & roots of *X. strumarium* by Nitric oxide scavenging method

Conc. (µg/ml)	% Scavenging activity of different Extracts								
	Leaves			Stem			Root		
	PE	CE	EE	PE	CE	EE	PE	CE	EE
20	-	8± 0.19	12.6± 0.23	-	5± 0.18	9.7± 0.21	-	8± 0.16	7.4±0.18
40	-	14.3± 0.23	26.1± 0.29	-	19.8± 0.26	19± 0.29	-	11.4±0.23	18.2±0.23
60	-	27.2± 0.26	35.1± 0.31	-	28.6± 0.29	28.3± 0.31	-	27.9±0.29	26.1±0.25
80	12±0.23	36± 0.29	57.8± 0.34	-	36.7± 0.36	45.8± 0.39	-	30.5±0.32	33.5±0.32
100	26± 0.29	45.9± 0.24	70.2± 0.21	23± 0.29	46.6± 0.42	57.8± 0.26	-	32.7±0.28	45.1±0.30
IC_{50}	148	112	72	144	112	88	-	191	112

PE- Petroleum ether extract, CE- Chloroform extract, EE- Ethanol extract; All values are expressed as mean ± SEM (n=3).

Table 7. In-Vitro Free radical scavenging activity of petroleum ether, chloroform & ethanol extracts of leaves, stems & roots of *X. strumarium* by Hydrogen peroxide method

Conc. (µg/ml)	% Scavenging activity of different Extracts								
	Leaves			Stem			Root		
	PE	CE	EE	PE	CE	EE	PE	CE	EE
20	-	9.2±0.21	13±0.18	-	5±0.15	10.4±0.22	-	6.5±0.15	9.8± 0.12
40	-	18.4±0.29	29.6±0.23	6±0.16	13.3±0.21	22.2±0.28	-	13.3±0.17	18.4±0.28
60	-	30.8±0.13	46.5±0.29	11±0.29	22.6±0.26	30.8±0.19	-	22.6±0.24	27.7±0.33
80	9±0.19	39±0.49	54.1±0.31	17±0.23	32.2±0.33	36.4±0.24	-	28.1±0.23	34.6±0.29
100	14±0.23	47.6±0.39	66.4±0.29	19±0.16	35.5±0.31	47.6±0.24	12± 0.01	32.3±0.29	42.4±0.33
IC_{50}	220	110	62	214	167	110	222	146	128

PE- Petroleum ether extract, CE- Chloroform extract, EE- Ethanol extract; All values are expressed as mean ± SEM (n=3).

Table 8. In-Vitro Reducing Capacity of petroleum ether, chloroform & ethanol extracts of leaves, stems & roots of *X. strumarium*

Concentration (µg/ml)	Absorbance of different Extracts								
	Leaves			Stem			Root		
	PE	CE	EE	PE	CE	EE	PE	CE	EE
20	0.011	0.043	0.032	0.012	0.018	0.022	0.009	0.017	0.020
40	0.017	0.064	0.052	0.017	0.032	0.047	0.012	0.029	0.041
60	0.018	0.107	0.094	0.018	0.048	0.077	0.017	0.042	0.068
80	0.023	0.133	0.151	0.021	0.098	0.107	0.021	0.084	0.096
100	0.058	0.147	0.168	0.033	0.123	0.143	0.029	0.099	0.126

PE- Petroleum ether extract, CE- Chloroform extract, EE- Ethanol extract; All values are expressed as mean ± SEM (n=3).

Table 9. In-vitro Antioxidant Capacity of petroleum ether, chloroform & ethanol extracts of leaves, stems & roots of *X. strumarium* by Phosphomolybdenum method

Concentration (µg/ml)	Absorbance of different Extracts (n=3)								
	Leaves			Stem			Root		
	PE	CE	EE	PE	CE	EE	PE	CE	EE
20	0.022	0.047	0.064	0.007	0.041	0.052	0.00	0.027	0.048
40	0.029	0.059	0.087	0.023	0.054	0.072	0.013	0.034	0.068
60	0.043	0.078	0.123	0.29	0.067	0.103	0.018	0.047	0.107
80	0.067	0.104	0.154	0.041	0.085	0.132	0.034	0.072	0.127
100	0.071	0.136	0.168	0.057	0.106	0.148	0.039	0.093	0.131

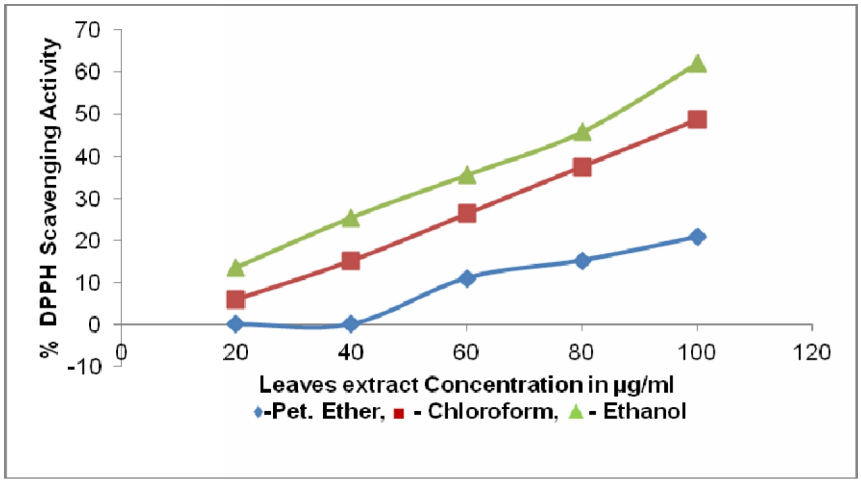

Fig. 2a. DPPH radical scavenging activity of petroleum ether, chloroform & ethanol extracts of leaves of *X. strumarium*

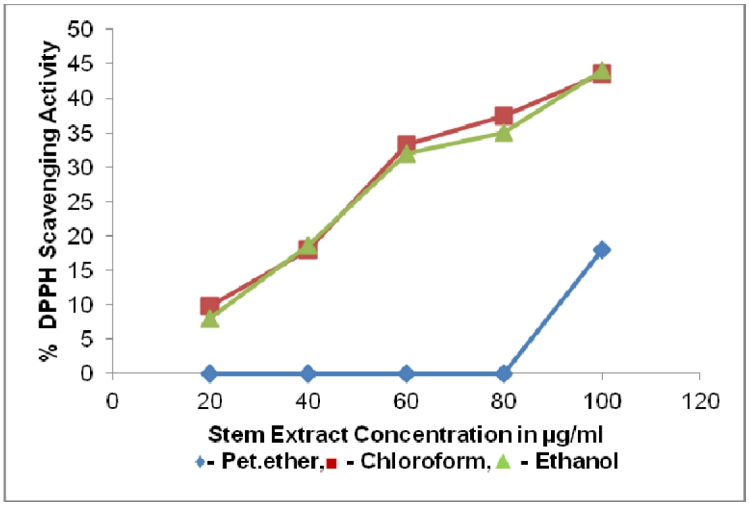

Fig. 2b. DPPH radical scavenging activity of petroleum ether, chloroform & ethanol extracts of stems of *X. strumarium*

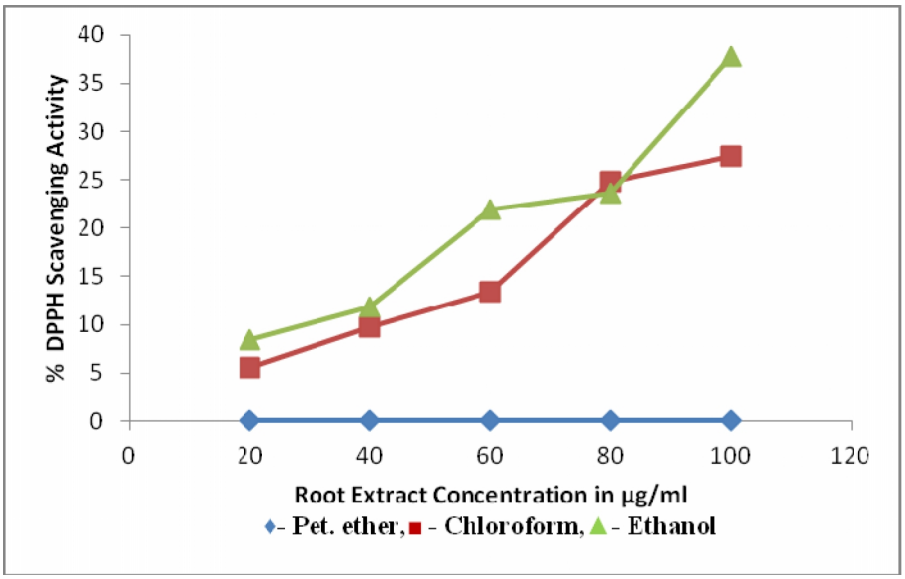

Fig. 2c. DPPH radical scavenging activity of petroleum ether, chloroform & ethanol extracts of roots of *X. strumarium*

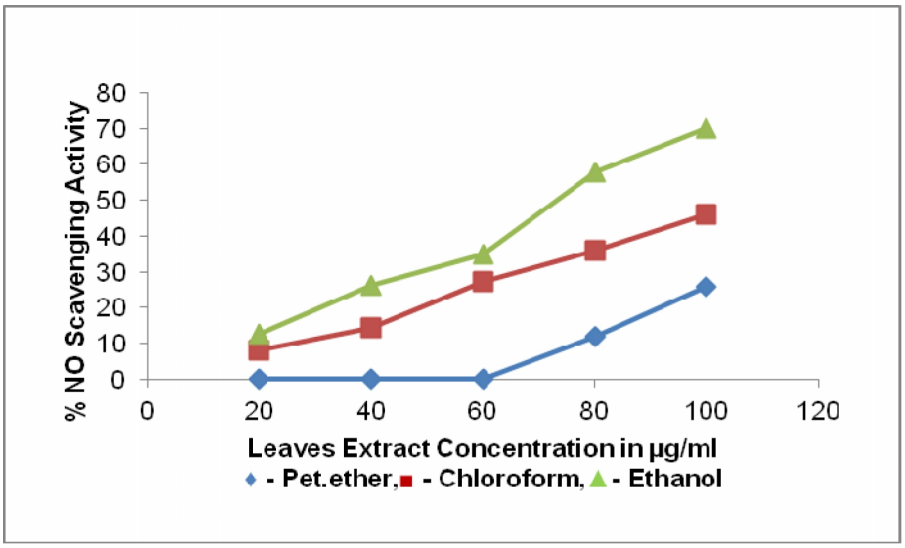

Fig. 3a. Nitric oxide scavenging activity of petroleum ether, chloroform & ethanol extracts of leaves of *X. strumarium*

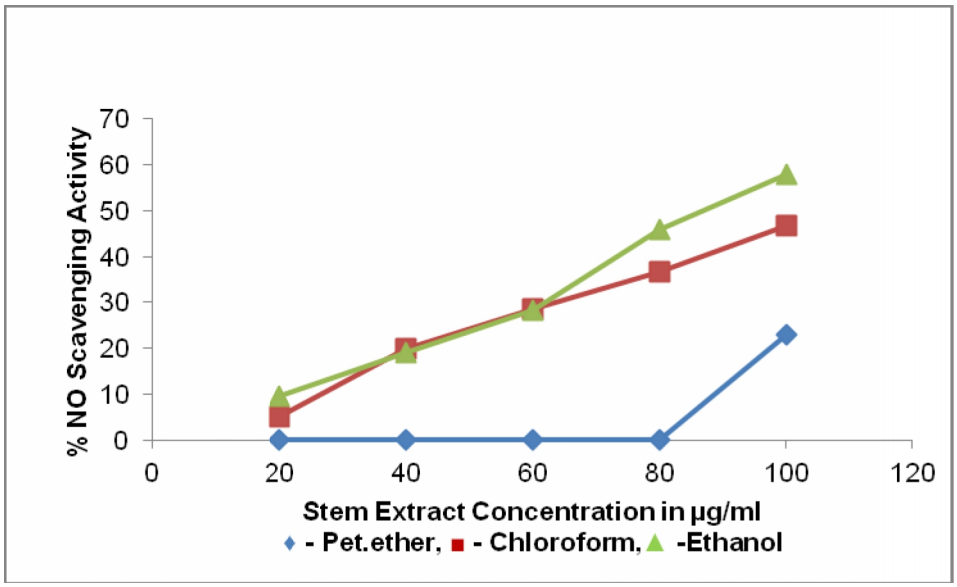

Fig. 3b. Nitric oxide scavenging activity of petroleum ether, chloroform & ethanol extracts of stems of *X. strumarium*

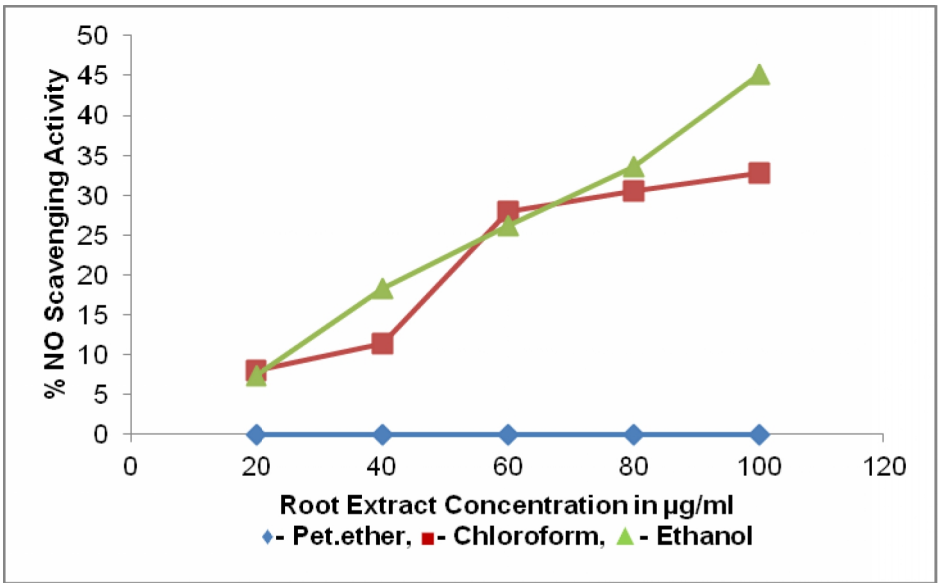

Fig. 3c. Nitric oxide scavenging activity of petroleum ether, chloroform & ethanol extracts of roots of *X. strumarium*

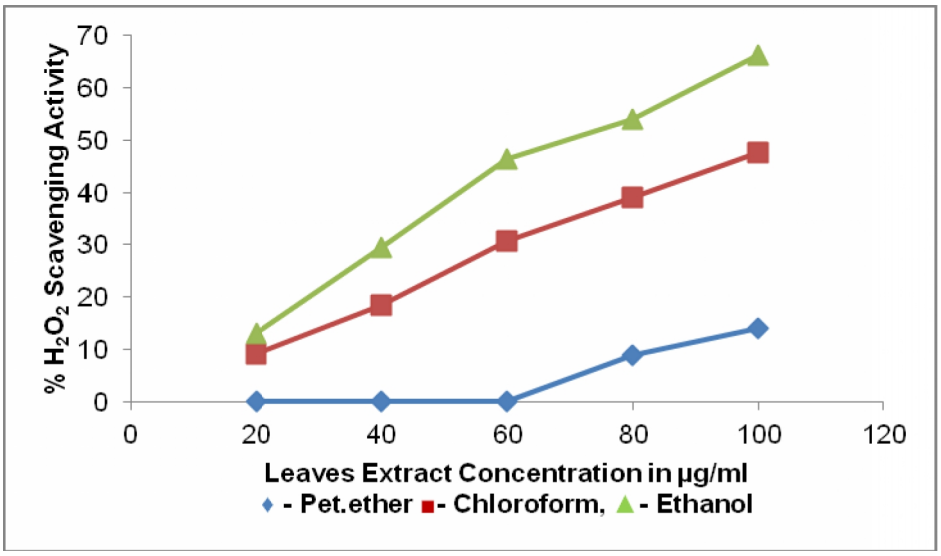

Fig. 4a. Hydrogen peroxide scavenging activity of petroleum ether, chloroform & ethanol extracts of leaves of *X. strumarium.*

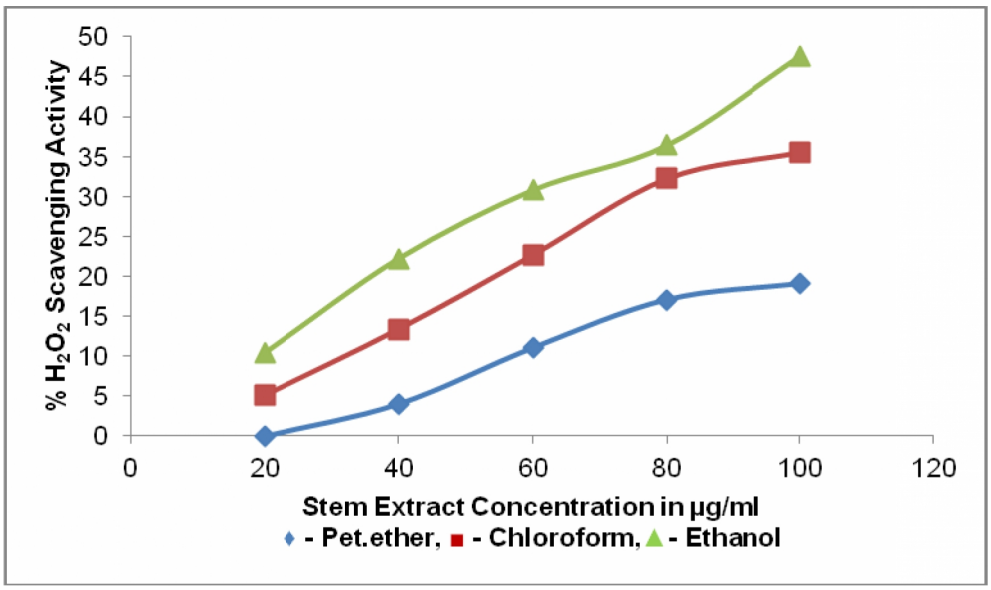

Fig. 4b. Hydrogen peroxide scavenging activity of petroleum ether, chloroform & ethanol extracts of stems of *X. strumarium.*

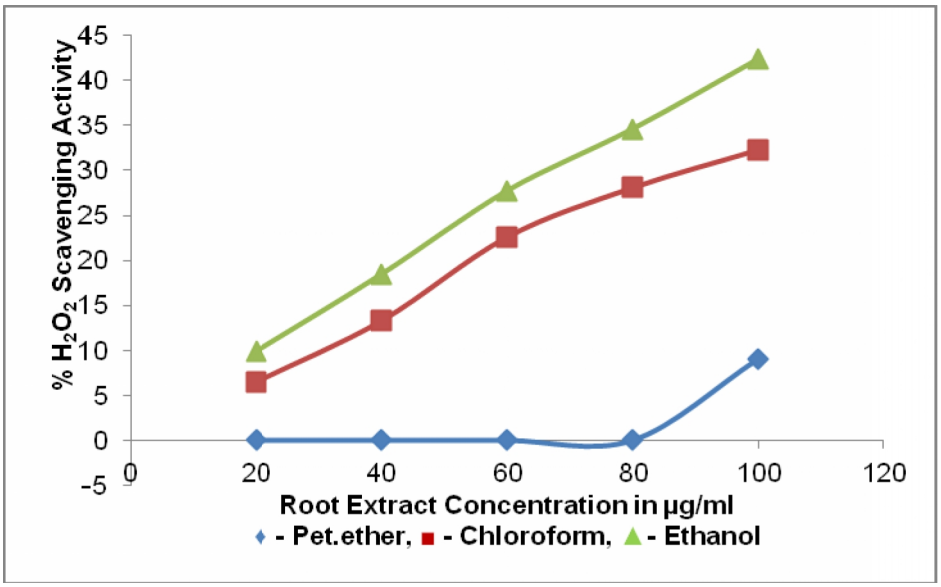

Fig. 4c. Hydrogen peroxide scavenging activity of petroleum ether, chloroform & ethanol extracts of roots of *X. strumarium*

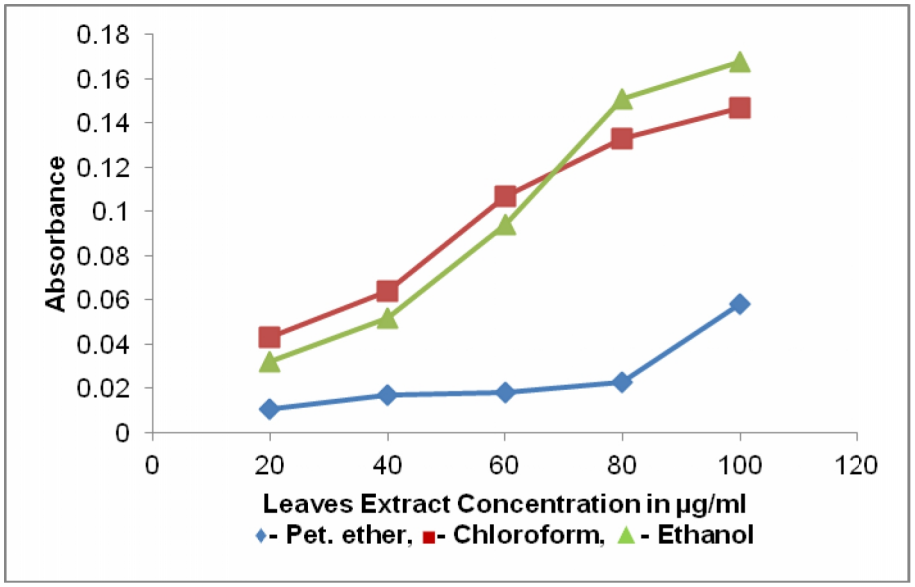

Fig. 5a. Reducing Capacity Determination of petroleum ether, chloroform & ethanol extracts of leaves of *X. strumarium*

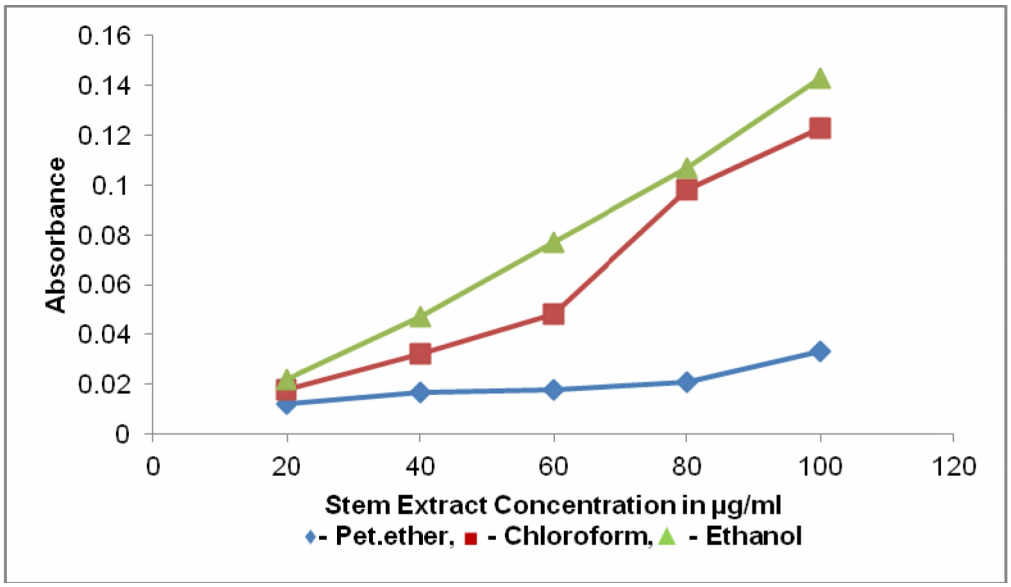

Fig. 5b. Reducing Capacity Determination of petroleum ether, chloroform & ethanol extracts of stems of *X. strumarium*

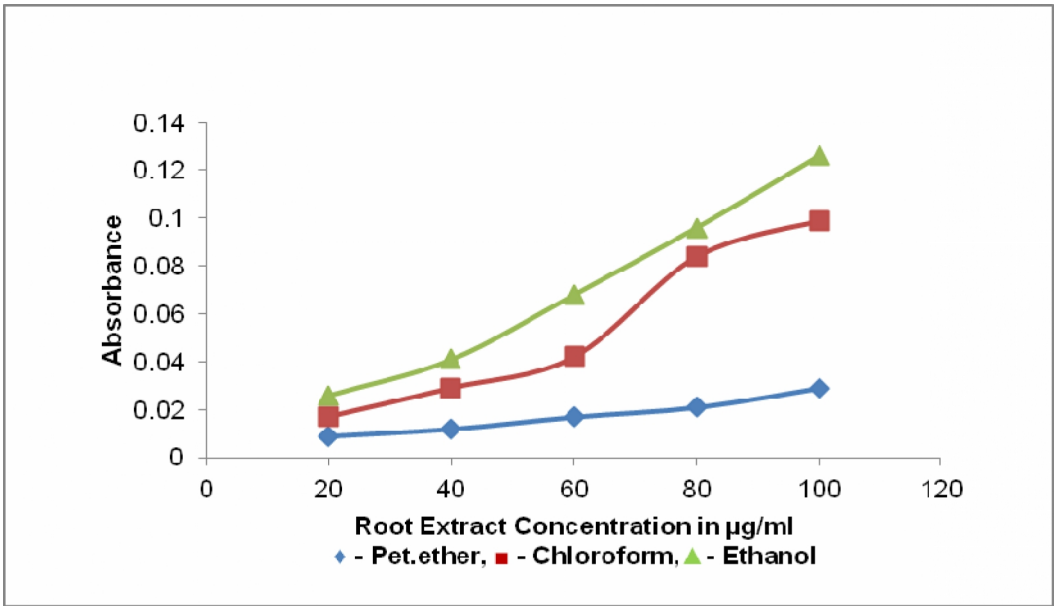

Fig. 5c. Reducing Capacity Determination of petroleum ether, chloroform & ethanol extracts of roots of *X. strumarium*

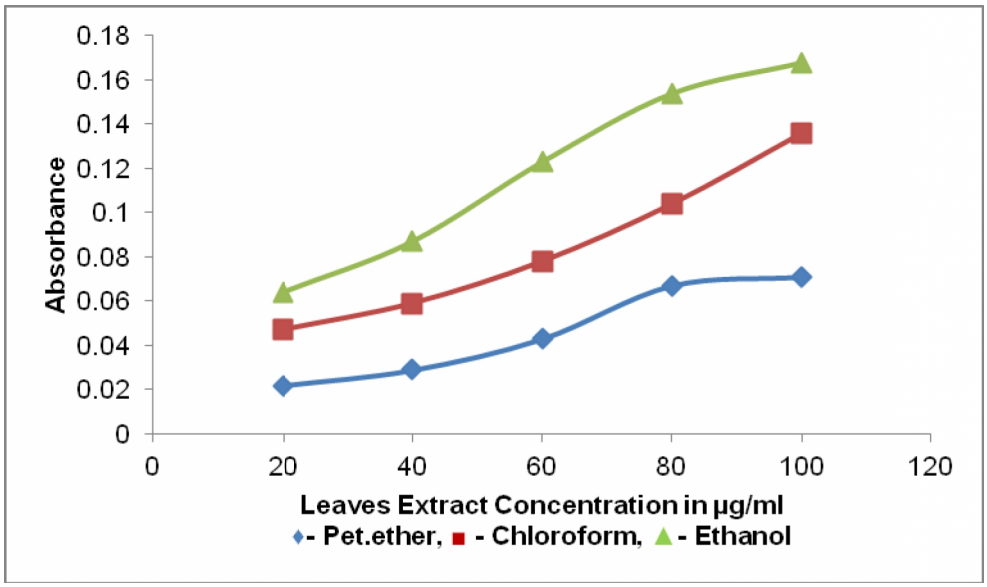

Fig. 6a. Antioxidant Capacity by Phosphomolybdenum method of petroleum ether, chloroform & ethanol extracts of leaves of *X. strumarium*

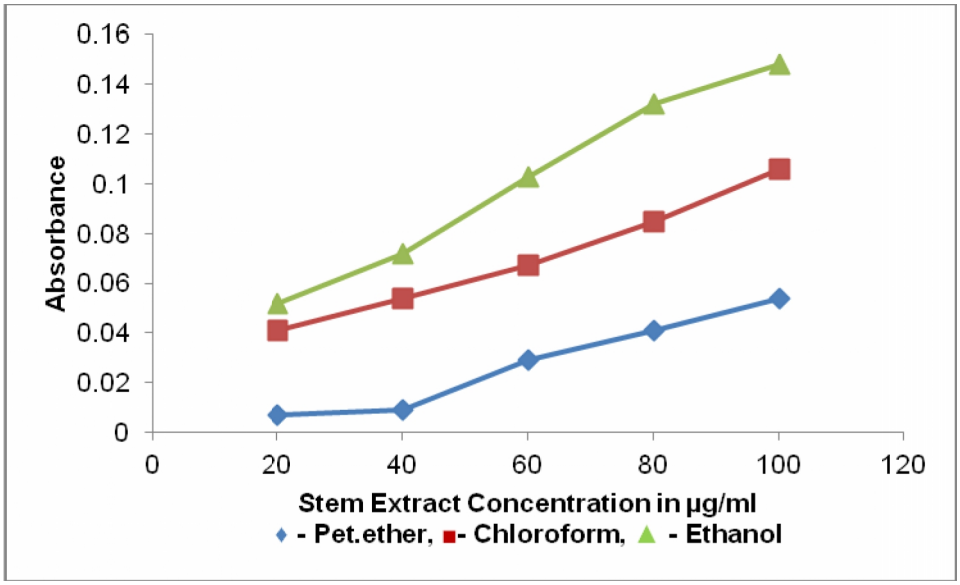

Fig. 6b. Antioxidant Capacity by Phosphomolybdenum method of petroleum ether, chloroform & ethanol extracts of stems of *X. strumarium*

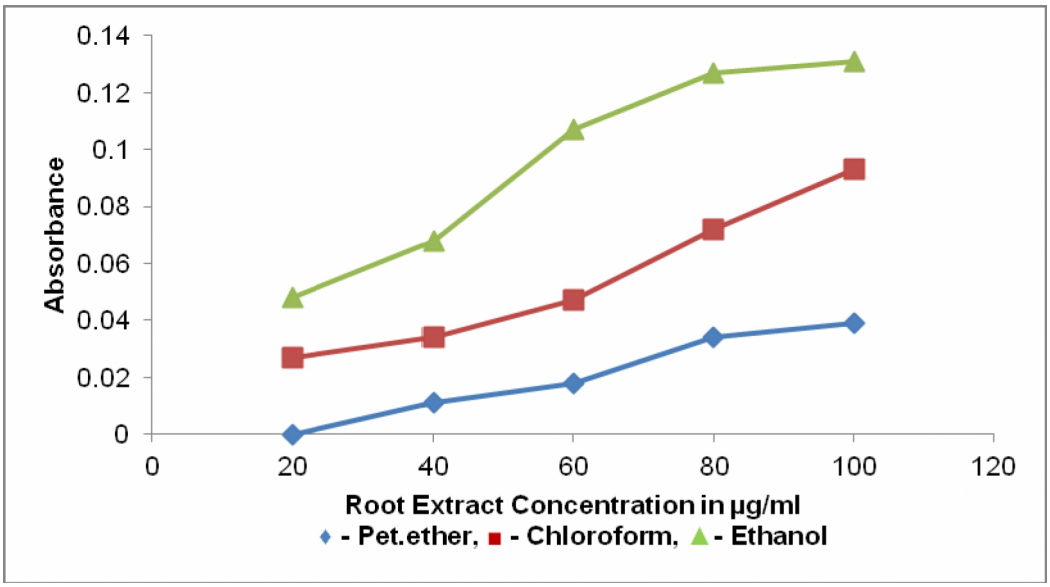

Fig. 6c. Antioxidant Capacity by Phosphomolybdenum method of petroleum ether, chloroform & ethanol extracts of roots of *X. strumarium*

4. DISCUSSION

In Indian system of medicine, certain herbs are claimed to cure various pathological conditions. Reactive oxygen species (ROS) generated endogenously or exogenously are associated with pathogenesis of various diseases. Thus antioxidants which can scavenge ROS are expected to improve these disorders.

The present study demonstrates the free radical scavenging activity of various extract of XS which confirms that extracts from plant possesses antioxidants. The phytochemistry of plant revealed the presence of steroids, alkaloids, flavonoids and phenolic compounds. These constituents have been found to possess antioxidant properties. Thus the potent antioxidant properties of XS may be related to presence of these constituents.

Polyphenols (electron rich compounds) have the ability to go into electron donation reactions with oxidizing agents to form stable species and thus inhibit or delay the oxidation of different biomolecules. Hence various plant phenols such as vitamin E exhibit antioxidant properties. Phenolic antioxidants are potent free radical terminators and this is thought to be due to ability to donate hydrogen to free radicals and their presence is a good marker of potential antioxidant activity. The high potential of phenolic compounds to scavenge free radicals may be explained by their phenolic hydroxyl groups. Detection of phenols and flavonoids in XS extracts was a preliminary evidence of its possible antioxidant activity. Also a high correlation between antioxidant capacity and the total phenol content suggesting that the phenols detected may be responsible for antioxidant effect found in XS extracts [24-26].

The DPPH is used as a reagent to evaluate free radical scavenging activity of antioxidants. It is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH is determined by the decrease in the absorbance at 517 nm induced by antioxidants. The extracts ability to concentration

dependently reduce DPPH forming yellowish-colored diphenylpicrylhydrazine suggest that XS extracts are free radical scavenger and acts so by donating electron or hydrogen radical [27-28].

The extracts showed a concentration dependent increase in its reducing power. This measurement describes the Fe^{3+} to Fe^{2+} and Mo^{6+} to Mo^{5+} transformation in the presence of extract. A direct correlation between antioxidant activity and reducing power of plant extract may be due to presence of reductones. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Increase in the absorbance of the extracts indicated the antioxidant activity. However activity of antioxidants are attributed by various mechanisms, among which some of them are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging [29-30].

It can be concluded that the extracts have constituents which were capable of showing antioxidant activity and the said in-vitro antioxidant activity may also be due to the presence of antioxidant principles present in the extracts like flavonoids and phenolic compounds. Suggests XS is an antioxidant that can be classified as preventive, scavenging or chain breaking.

5. CONCLUSION

The result of the present study clearly demonstrates significant antioxidant effects of *X. strumarium* L extract which may play a role of traditional uses of the plant. Extracts contains the significant amount of phenolic and flavonoids and exhibits the significant antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing. Further research to isolate individual compounds, their in-vivo antioxidant activities with different mechanism is needed.

CONSENT

Not Applicable.

ETHICAL APPROVAL

Not Applicable.

ACKNOWLEDGEMENT

Authors thankfully acknowledge Professor Dr. H. B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi for authentication of plant material, also thankful to Chandigarh College of pharmacy, Landran, Mohali (Pb) for providing the facilities for work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Devasagayamn TP, Tilak JC, Boloor KK, Sanek S, Ghaskadbi SS, Lele RD. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. J Assoc Physicians India. 2004;52:794-804.
2. Anonymous, Understanding Antioxidants and Free Radicals A Guide to Understanding – Special reports, 12/11/2011. http://www.natural-holistic health.com/.
3. Diaz MN, Frei B, Keaney JF. Antioxidants and atherosclerotic heart disease. N. Engl. J. Med. 1997;339:408-416.
4. Metodiewa D, Koska C. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders, An overview. Neurotox. Res. 2000;1:197-233.
5. Young IS, Woodside JV. Antioxidants in health and disease. J. Clin. Pathol. 2001;54:176-186.
6. Halliwell B, Gutteridge JMC. Free radicals in Biology and Medicine. 3rd edition, Oxford University Press, Oxford, U.K.; 1999.
7. Conner EM, Grisham MB. Inflammation, free radicals, and antioxidants. Nutrition. 1996;12(4):274-277.
8. Korish AA. Oxidative stress and nitric acid deficiency in inflammatIon of chronic renal failure. Possible preventive role of L-arginine and multiple antioxidants. Saudi Med J. 2009;30(9):1150-1157.
9. Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. Clin Chim Acta. 2001;303(1-2):19-24.
10. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408:239-247.
11. Wijaya S, Nee TK, Jin KT, Hon LK, San LH, Wiart C. Antibacterial and antioxidant Activities of *Synedrella nodiflora* (L.)Gaertn. (Asteraceae). Journal of complementary and Integrative Medicine. 2011;8(1):1-13.
12. Amarowicz R, Troszynska A, Shahidi F. antioxidant activity of Almond Seed Extract and its Fractions. J Food Lipids. 2005;12:344-358.
13. Chu YH, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food and Agric. 2000;80:561-566.
14. Kamboj A, Saluja AK. Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). International journal of green pharmacy. 2010;4(3):129-139.
15. Singleton VL, Rossi JA. Colorimetry of total phenolic with Phosphomolybdic acid phosphotungistic acid reagents. Am Jenol viticult. 1965;1:144-158.
16. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol. 2008a;32:43-49.
17. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant activities of Iranian corn silk. Turk. J.Biol. 2008 32:43-49.
18. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline ontents in *burkina fasan* honey, as well as their radical scavenging activity. Food Chemistry. 2005;91:571–577.
19. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, Songklanakarin. J. Sci. Technol. 2004;26(2):211-219.
20. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide- Scavenging properties of Ginkgo biloba extract EGb761. Biochem. Biophys. Res. Commun. 1994;201:748-755.

21. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Be khradnia AR. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. Pharmacologyonline. 1994;2:560-567.
22. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agri. Food Chem. 1995;43(1):27-32.
23. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex; Specific application to the determination of vitamin E. Anal Biochem. 2001;269:337-341.
24. Utkina NK. Antioxidant activity of aromatic alkaloids from marine sponges *Aaptos aaptos* and *Hyrtios* SP. Chemistry of Natural Products. 2009;45:849-853.
25. Seidel V, Verholle M, Malard Y, Tillequin F, Fruchart JC, Dureiz P, et al. Phenylpropanoids from *Ballota nigra* L. inhibit in vitro LDL peroxidation. Phytother Res. 2000;26(5):1429-1434.
26. Ozgova S, Hermanek J, Gut I. Different antioxidant effects of polyphenols on lipid peroxidation and hydroxyl radicals in the NADPH-, Fe-ascorbate and Fe-microsomal systems. Biochem Pharmcol. 2003;66(7):1127-1137.
27. Soares JR, Dinis TC, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygis. Free Radic Res. 1997;26(5):469-478.
28. Krings U, Berger RG. Antioxidant activity of some roasted foods. Food Chemistry. 2001;72(2):223-229.
29. Duh PD. Antioxidant activity of burdock (*Arctium lappa* linne): its scavenging effect on free radical and active oxygen. Journal of American Oil Chemist's Society. 1998;75:455-465.
30. Diplock AT. Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease? Free Radic Res. 1997;27(5):522-532.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=280&id=14&aid=2162