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Isolation and Characterization of Constituents from the Leaves of *Xanthium strumarium* and their Evaluation for Antioxidant and Antimicrobial Potential

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Abstract

Xanthium strumarium L., belonging to the family compositae, is a medicinal plant commonly found as a weed, is widely distributed in North America, Brazil, China, Malasia and hotter part of India. The herb is traditionally used mostly in treating several ailments. The present research includes isolation and characterization of chemical constituents and *in vitro* evaluation of antioxidant potentials by three different assay methods of isolated constituents from leaves of *Xanthium strumarium* as the plant is an ingredient of various traditional preparations used in the treatment of various diseases. Three constituents (hexadecanoic acid, α-amyrin, 14-methyl-12,13-dehydro-sitosterol-heptadeconate) were isolated and the structures of these compounds have been established by spectroscopic methods (UV,^{1}H-NMR, IR, MS). All these constituents were isolated from this plant for the first time. These chemical constituents showed significant antioxidant and antimicrobial activity in dose dependent manner by different assay methods. The result obtained in the present study indicates that leaves of *Xanthium strumarium* could be a potential source of natural antioxidant and antimicrobial. This also justified the traditional use of herb in preventing disease induced by oxidative stress and microorganism.

Keywords: DPPH scavenging activity; *Xanthium strumarium*; Gram positive; Gram negative

Introduction

Medicinal plants are used as herbs or traditional medicines for various types of diseases since ancient times. Medicinal plants are used for the preparation of various modern drugs or used as the principal sources of raw materials. The medicinal value of these plants lies in bioactive phytochemical constituents that produce specific physiological action on the human body [1]. Some of the most important bioactive constituents are alkaloids, saponins, flavonoids, tannins, terpenoids, phenolic compounds, essential oils, steroids, glycosides [2]. These naturally occurring compounds form the backbone of the modern medicines or drugs [3]. Phytochemical are natural compounds that are found in medicinal plants, vegetables, fruits, flowers, leaves, roots, stems and work together with nutrients and fibres to act as a defence system or to protect humans against diseases [4].

Xanthium strumarium is a cocklebur or burweed belonging to family Asteraceae and commonly found as a weed in roadsides, rice fields, hedges throughout the tropical parts of India [5,6]. The word "xanthium" is derived from an ancient Greek word "xanthos" meaning yellow and "strumarium" means "cushion like swelling," with reference to the seedpods which turn from green to yellow as they ripen (later they become deep yellow to brown) [7]. It is commonly called chotagokhru due to the shape of its fruit which look likes the cow's toe. The herb as such is suspected to be poisonous but the toxic substances are removed by washing and cooking [8].

The whole plant is used as medicine. According to Ayurveda, the plant has cooling, laxative, fattening, anthelmintic, tonic, digestive, antipyretic activities and improves appetite, voice, complexion and memory. It cures leucoderma, biliousness, and poisonous bites of insects, epilepsy, salivation and fever. The plant has been reported as fatal to cattle and pigs [9]. It is used by various Native American tribes to relieve constipation, diarrhoea and vomiting. Indigenous Chinese applications are as a headache remedy and to assist with cramping and numbness of the limbs, ulcers and sinus problems. The plant is considered to be useful in treating long-standing cases of malaria [8] and is used as an adulterant for *Datura stramonium*.

In continuation of investigation of bioactive metabolites from *Xanthium strumarium*, the present work deals with the isolation, structure elucidation and identification of chemical constituents from the petroleum ether extract of leaf part of the plant and further the isolated constituents were evaluated for their antioxidant and antibacterial potential.

Materials and Methods

General

IR spectra was recorded (KBr disc) on FT-IR spectrometer; Bruker ADVANCE II 400 NMR spectrophotometer was used to record ^{1}H-NMR chemical shifts (δ) are reported as downfield displacements from TMS used as internal standard and coupling constants are reported in Hz; WATERS Q-T OF MICROMASS (LC-MS) was used to record mass spectrum of compounds from SAIF, Punjab University, Chandigarh. SHIMAZDU UV -1800 spectrophotometer was used for UV analysis. Electronic balance (CY 200) of Denver instruments was used for weighing. TLC was performed with precoated aluminium silica gel GF_{254}. All solvents were analytical grade.

Isolated phytoconstituents were tested by performing chemical test such as Libermann- buchard's reagent, Salkowski test, Spot test, Litmus paper test etc and determining the physical parameters like appearance, solubility and melting point by comparing with literature.

Plant material

Fresh and fully grown plants were collected near the roadside and fields of village Jhande Majra Mohali (Pb.) in the month of

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September and October. The sample was authenticated by comparing the morphological characters as described in the literature. The authentication was further confirmed by Dr. H.B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi.

Collection and preparation of plant materials

Xanthium strumarium were collected in bulk quantity after confirmed authenticity from village Jhande Majra, Mohali (Punjab) in months of September and October, 2012. The plant parts such as leaves, stems, and fruits were manually separated. The plant material was washed thoroughly 2-3 times with water to remove soil, mud, debris and other adhering materials and dried in air under shade at room temperature. Coarse powder of each drug was prepared and stored in air tight container in a dry place at room temperature.

Extraction

About 400 g of powered leaves of *Xanthium strumarium* were extracted in soxhlet apparatus with petroleum ether as solvent. The extract was concentrated in rotary evaporator under control temperature 40°-50°C and dried in desiccator yields 13.9 g extract. Petroleum ether extract was saponified using 1M alcoholic potassium hydroxide and then subsequently picked up in anhydrous ether. This fraction contained a lesser number of components than the unsaponified extract. The ether fraction was reduced to minimum volume.

Isolation

6.58 g of the extract after saponification was subjected to chromatography on silica gel (60-120 mesh, Merck) and was eluted with solvents in increasing order of polarity using hexane, hexane: chloroform, pure chloroform, chloroform: ethyl acetate and methanol.

462 fractions each of volume 50 mL were collected. The solvent of each of the fraction was completely removed by evaporation at 50°C under a steam. The residue was reconstituted with 0.5mL chloroform. After reconstitution, TLC of these fractions was carried out to find out in which fractions similar compounds were present in.

Fractions with components having same R_f value were combined together and labelled. Fractions 25-93 were combined and label as compound A (Hexane: Chloroform 70:30), fractions 117-198 were combined and label as compound B, C (Hexane: Chloroform 20:80), fractions 199-216 were combined and label as compound C (Chloroform: Ethyl acetate 90:10).

Fractions 333-396 were combined and label as compound D (Ethyl acetate: Methanol 90:10). But none of fractions obtained were exclusively pure by this method. So further purification was carried by re-column chromatography and Preparative Thin Layer Chromatography (PTLC) over silica gel G.

Pharmacological evaluation

Antioxidant activity

DPPH radical scavenging capacity: DPPH scavenging activity or the hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method [10]. DPPH (0.002%) was dissolved in pure methanol. The radical stock solution was prepared freshly. The DPPH solution (1 mL) was added to 1 mL of sample extracts of different concentration. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 517 nm using ascorbic acid as standard [11]. The degree of discoloration indicates the scavenging efficacy of the extracts. Methanol (1mL) with DPPH solution (0.002%, 1 mL) was used as blank.

The equation used to measure free radical scavenging activity is:

$$\%\ DPPH\ \ Scavenging\ Activity = \frac{Control - Test}{Control} \times 100$$

'Control' was the absorbance of DPPH; 'Test' was the absorbance in the presence of the sample of compound and standard.

The experiment was performed in triplicate and average absorbance was noted for each concentration. Results are expressed as mean inhibitory concentration (IC_{50}). A lower value of IC_{50} indicates a higher free radical scavenging activity.

Hydroxyl radical scavenging capacity: The hydroxyl radical scavenging activity was determined according to the method of Beara et al. 2 mL of sample solution (5-100µg/mL), 1.0 mL of *ortho*-phenanthroline (7.5 mmol L^{-1}), 5.0 mL of phosphate buffer (0.2 M, pH 6.6), 1.0 mL of ferrous sulphate (7.5 mmol L^{-1}) and 1.0 mL of H_2O_2 (0.1%) were mixed and diluted to 25 mL with distilled water. After incubation at room temperature for 30 min, the absorbance was measured at 510 nm using ascorbic acid as standard.

The equation used to measure free radical scavenging activity is:

$$\%\ Scavenging\ Activity = \frac{Control - Test}{Control} \times 100$$

'Control' was the absorbance value of the system with all solution including H_2O_2, 'Test' was the absorbance in the presence of the sample of compound and standard [12]. The experiment was performed in triplicate and average absorbance was noted for each concentration.

Phosphomolybdenum method: The total antioxidant capacities of samples were evaluated by phosphomolybdenum complex formation method. 0.3 mL sample (5-100 µg/mL) of each sample was mixed with 3mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in test tubes. The blank solution contained 3 mL of reagent solution. The test tubes were capped and incubated in water bath at 95°C for 90 minutes. After the samples had been cooled to room temperature, the absorbance of these was measured at 695 nm using a spectrophotometer against blank. Ascorbic acid was used as standard [13].

Antimicrobial activity

Microorganisms, media and standard drugs: Three bacterial strains (one Gram positive i.e. *Staphylococcus aureus* MTCC Code – 737 and two Gram negative i.e. *Escherichia coli* MTCC Code – 68 & *Pseudomonas aeruginosa* MTCC Code – 1688) and two fungal strains (*Candida albicans* MTCC Code – 3017 & *Kluyveromyces marxianus* MTCC Code – 9769) were used for evaluating antimicrobial activity. These strains were obtained from Microbiology Department, Institute of Microbial Technology (IMTECH) Chandigarh Sec-39A. The microorganisms, which were maintained by sub-culturing, were used at regular intervals in nutrient agar medium and Sabouraud media. Ciprofloxacin was used as standard drug for antibacterial activity and Fluconazole for antifungal activity.

Experimental procedure (agar diffusion method)

Nutrient agar was prepared, sterilized and cooled to 45°C with gentle shaking to bring about uniform cooling. It was then poured into the sterilized petri dishes. The poured material was allowed to set and plates were incubated for 24 hours to check for sterility. Plates which

showed any kind of contamination were discarded. Thereafter, 100 µl culture of microorganism was spread uniformly over the solidified agar with the help of spreader. Cups were made by punching into the agar surface with sterile cork borer and scooping out the punched part of the agar. 0.1 mL of test compounds was added into the cups with the help of micropipette. Two-fold diluted solutions of the compounds and reference drugs were used (2000, 1600, 800, 400, 100, 6.5 µg/mL). The drug solution was allowed to diffuse for some time into the medium for 30 minutes. The plates were incubated at 30-35°C for 24-48 hours. The incubation chamber was kept sufficiently humid. The minimal inhibitory concentration (MIC) values were determined at the end of the incubation period. DMF was used as control [14-16].

Statistical analysis

All data were expressed as the mean ± SD and where applicable, the data were analysed statically by student's t-test using and the level of significance was from $P<0.05$.

Results and Discussion

Although large number of isolates were separated from successive column chromatography but quantitatively only three compounds (A, B and C) were isolated, purified and characterized from petroleum ether extract of leaves part of *Xanthium strumarium* by column chromatography. Isolated components were further purified by PTLC and recrystallization method. Compounds were further identified and confirmed by melting point, TLC and UV, ^{1}H-NMR, IR, MS spectra of the compounds. The compounds were characterized on the basis of spectroscopic analysis and compared with reported data in literature (Supplementary file).

Hexadecanoic acid (Compound A)

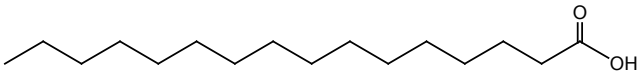
The compound A isolated from petroleum ether extract of leaves part of *Xanthium strumarium* appeared as colourless crystals with R_f 0.38 in hexane: ethyl acetate (9.8:0.2). It gave positive spot test and Litmus paper test. From the positive test it was assumed to be a fatty acid. The melting point of compound A 62°-65°C was in good agreement with melting point of hexadecanoic acid (63°-64°C). The λ_{max} of the compound A was observed at 290 nm (DMF).

IR spectrum showed absorption band at 2917.14 cm^{-1}, 2849.18 cm^{-1}, 2655.52 cm^{-1} was due to merged O-H stretching with C-H stretching, at 1703.20 cm^{-1} indicated the presence of C=O stretching, at 1464.40 cm^{-1} showed the presence C-H bending due to -CH_2 group, at 1310.41 cm^{-1} peak revealed as C-H bending due to CH_3 group, at 1296.39 cm^{-1} is characteristic of C-O stretching and absorption at 720.49 cm^{-1} peak due to $(CH_2)_n$ group i.e. long aliphatic chain band.

In ^{1}H-NMR spectrum of compound A, H-2 proton appeared as triplet at δ 2.35 (J=7.5Hz) and H-16 protons showed a triplet at δ 0.88 (J=7.0Hz), twelve CH_2 protons revealed as multiplet at δ 1.27 (H-3 to H-14). A multiplet appeared at δ 1.63 indicates H-15 protons.

Mass spectra revealed that it's molecular mass [M^{+1}] peak was observed at m/z 257 calculated mass for $C_{16}H_{32}O_2$, 256.42) and having characteristic fragments observed at m/z 239, 200, 127, 97, 83.

Hence the structure was identified and confirmed by comparison with the reported melting point and spectral data such as UV, IR, ^{1}H-NMR and MS of isolated compound A from petroleum ether extract of *Xanthium strumarium* that the compound A was found to be in good agreement with hexadecanoic acid in the literature [17,18].



Structure of hexadecanoic acid ($C_{16}H_{32}O_2$; Mol. Wt. 256.42)

α- amyrin (Compound B)

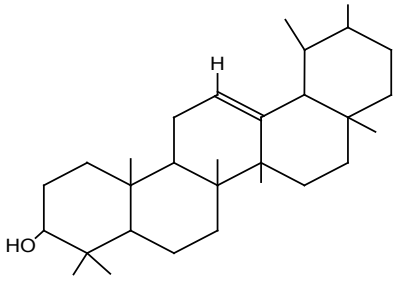
The compound B isolated from petroleum ether extract of leaves part of *Xanthium strumarium* appeared as colourless amorphous solid with R_f 0.45 in hexane: ethyl acetate (8:2). It gave positive Salkowski test. From the positive test it was assumed to be a triterpenoid. The melting point of compound B 184-186°C was in good agreement with melting point of α-amyrin (185°C). The λ_{max} of the compound B was observed at 292nm (DMF).

The IR spectrum revealed the presence of absorption band at 3368.68cm^{-1} that is characteristic of O-H stretching. Absorption at 2941.48 cm^{-1} and 2851.59 cm^{-1} indicated the presence of C-H stretching, 1640.76 cm^{-1} showed the presence of C=C stretching, 1464.67 cm^{-1} is due to C-H bending of -CH_2 group indicated the presence of unsymmetric ethylenic double bond, 1380.68 cm^{-1} indicated the presence of C-H bending of -CH_3 group and absorption at 1043.68 cm^{-1} is characteristic of C-O stretching.

In ^{1}H-NMR spectra data of compound B, H-12 proton appeared as multiplet at δ 5.11, H-3 proton showed a multiplet at δ 3.15 and eight methyl protons appeared as multiplet in region δ 1.0-0.66 (H-23, H-24, H-25, H-26, H-27, H-28, H-29, H-30).

Mass spectra revealed that molecular mass [M$^+$] peak observed at m/z 426.0 (calculated mass for $C_{30}H_{50}O$, 426.27) and characteristic fragments observed at m/z 411, 409, 393, 218 (100), 207, 191. The fragmentation pattern was in line with that of α-amyrin.

Hence the structure was confirmed by comparison with the reported melting point and spectral data such as UV, IR, ^{1}H-NMR and MS of isolated compound B from petroleum ether extract of *Xanthium strumarium* that the compound B was found to be in good agreement with α-amyrin in the literature [19,20].



Structure of α- amyrin ($C_{30}H_{50}O$; Mol. Wt. 426)

14-methyl-12, 13-dehydro-sitosterol-heptadeconate (Compound C)

The compound C isolated from petroleum ether extract of leaves part of *Xanthium strumarium* appeared as colourless crystals with R_f 0.74 in hexane: ethyl acetate (8:2). It gave positive test for Salkowski test. From the positive test it was assumed to be a steroid molecule. The $_{max}$ of this compound was observed at 265nm (DMF).

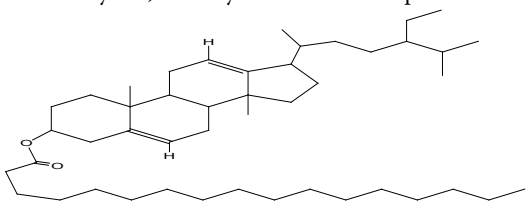
The IR spectrum revealed the presence of absorption band at 3307.60cm^{-1} due to O-H stretching. Absorption at 2917.29 cm^{-1} showed the presence of C-H stretching, 1726.64 cm^{-1} is due to C=O stretching, 1473.56 cm^{-1} revealed the presence of C=C stretch, 1462.54 cm^{-1} is characteristic of C-H bending of –CH_2 bend, 1379.61 cm^{-1} indicated the presence of C-H bending of –CH_3 bend, 1072.47 cm^{-1} revealed the C-O stretch and absorption at 719 cm^{-1} is due to $(CH_2)_n$ group indicated the presence of long aliphatic side chain.

In ^{1}H-NMR spectra data of compound C, H-6; H-12 protons

appeared as triplet at δ 5.02; H-3 Proton showed a multiplet at δ 3.55; H-4, H-2′, H-17, H-7, H-11, H-27, H-20,H-3′ were reported as multiplet at δ 2.2-1.8; region between δ 1.75-1.29 revealed the presence of protons at H-2, H-8, H-9, H-16, H-16′, H-1, H-15, H-24, H-25, H-22, H-23, H-4′-H-15′ as multiplet and seven CH_3 protons appeared as a multiplet at δ 1.15-0.7 (H-19, H-18, H-28, H-29, H-21, H-26, H-17′).

Mass spectra of compound C revealed that molecular mass [M^{-1}] peak at m/z 663.6 (calculated mass for $C_{46}H_{80}O_2$, 664.63) having characteristic fragments observed at m/z 663, 411, 315, 301, 270, 255, 187, 108, 95.

Hence the structure was confirmed by comparison with the reported melting point and spectral data such as UV, IR, ^{1}H-NMR and MS of isolated compound C from petroleum ether extract of *Xanthium strumarium* that the compound C was found to be good agreement with 14- methyl-12, 13-dehydro-sitosterol- heptadeconate.



Structure of 14-methyl-12, 13-dehydro-sitosterol-heptadeconate ($C_{46}H_{80}O_2$; Mol. Wt. 664.62)

In vitro evaluation of antioxidant and free radical scavenging activity

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 the stable nitrogen centred free radical the colour of which changes from violet to yellow upon reduction either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [21-24].

The radical scavenging activities of all isolated compounds were found to increase with increase in concentration. The IC_{50} of compound A, compound B, compound C and Ascorbic acid was found to be 106.41, 64.16, 76.18 and 11.41 µg/mL respectively. Antioxidant potential by DPPH method was found to maximum with compound B followed by compound C and compound A (Table 1, Figure 1).

Hydroxyl scavenging activity: The compounds were capable of scavenging hydrogen peroxide in a concentration dependent manner. Scavenging capacity increases with increase in concentration. 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 [25].

The radical scavenging activities of all isolated compounds were found to increase with increase in concentration. The IC_{50} of compound A, compound B, compound C and Ascorbic acid (standard) was found to be 127.43, 83.96, 84.48, 44.15 µg/mL respectively. Antioxidant potential by Hydroxyl method was found to maximum with compound B followed by compound C and compound A (Table 2, Figure 2).

By comparing the antioxidant results, it revealed that the total antioxidant capacity increases in a concentration dependent manner and further revealed that the total antioxidant capacity was higher with the compound B which is followed by compound C and compound A by all the methods (Table 3, Figure 3).

Phosphomonybdenum method: Total antioxidant capacity by phosphomolybdenum method assay based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since total antioxidant activity is expressed as the number of equivalents of ascorbic acid [26] (Table 4, Figure 4).

In vitro evaluation of antimicrobial activity

Antimicrobial activity of isolated compounds was carried out by agar diffusion method against *Staphylococcus aureus* (Gram positive bacteria), *Escherichia coli, Pseudomonas aeruginosa* (Gram negative bacteria), *Candida albicans* (Fungal strain), *Kluyveromyces marxianus* (Yeast). Zone of inhibition (mm), Minimum inhibitory concentration (MIC µg/mL) were recorded. MIC values of the test compounds and standard are presented in (Table 5, Figures 5 and 6).

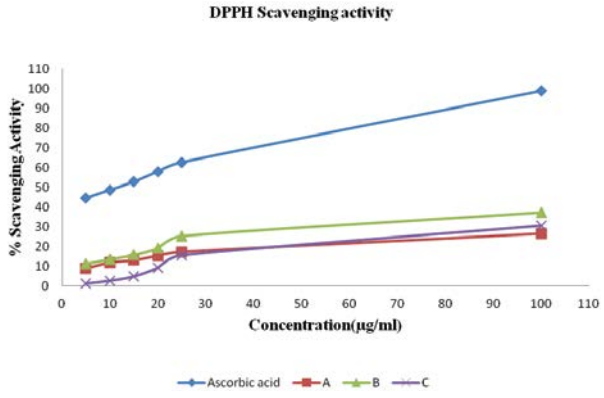
From the results it was found that compound B exhibit higher antibacterial potential followed by compound C and compound A respectively. Similarly antifungal potential were found highest with the compound B followed by compound A and compound C.

Conclusion

The result of the present study suggested that three compounds (A, B, C) were isolated from petroleum ether extract of *Xanthium strumarium*. Compounds were identified as fatty acid, triterpenoid and sterol respectively. By spectroscopic data compounds A, compound B and compound C were characterized as hexadecanoic acid, α-amyrin, 14-methyl-12,13-dehydro-sitosterol-heptadeconate. From the result, it was also concluded that isolated compounds may have some role in exhibiting the antioxidant and antimicrobial activities. This also

Conc. µg/mL	% Scavenging activity [Mean ± SD (n=3)]			
	A	B	C	Ascorbic Acid
5	8.9 ± 0.85	11.49 ± 0.86	1.2 ± 0.96	44.5 ± 0.85
10	11.86 ± 0.76	13.47 ± 0.56	2.6 ± 0.57	48.6 ± 1.69
15	13.00 ± 0.94	15.71 ± 0.74	4.8 ± 0.85	52.74 ± 0.87
20	15.26 ± 1.28	19.10 ± 0.68	9.2 ± 1.32	57.92 ± 0.75
25	17.26 ± 0.58	25.29 ± 1.85	15.6 ± 0.96	62.63 ± 0.68
100	26.39 ± 0.79	37.25 ± 0.68	30.59 ± 0.92	98.79 ± 0.74
IC_{50}	106.4 ±0.86	64.16 ± 0.89	76.18 ± 0.93	11.41 ± 0.93

Table 1: DPPH Scavenging activity of compounds isolated from leaves of *Xanthium strumarium*



Ascorbic acid = Standard

A= Compound A (hexadecanoic acid)

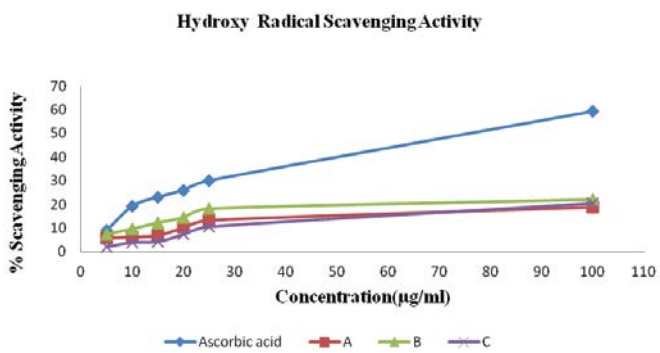
B= Compund B (α- amyrin)

Figure 1: DPPH Scavenging Activity of Compounds Isolated from Leaves of *Xanthium strumarium*

C= Compound C (14-methyl-12,13-dehydro-sitosterol-heptadeconate)

Conc. µg/mL	% Scavenging activity [Mean ± SD (n=3)]			
	A	B	C	Ascorbic Acid
5	5.84 ± 0.52	7.25 ± 0.56	1.96 ± 1.02	44.5 ± 1.46
10	6.2 ± 0.13	9.56 ± 0.79	3.89 ± 0.68	48.6 ± 0.58
15	10.11 ± 0.68	14.46 ± 0.83	7.25 ± 0.96	52.74 ± 0.68
20	13.25 ± 1.76	18.25 ± 0.48	10.54 ± 1.29	57.92 ± 0.84
25	18.87 ± 0.59	22.16 ± 1.84	20.54 ± 0.46	62.63 ± 0.69
100	26.39 ± 0.95	36.25 ± 0.65	30.59 ± 0.89	98.79 ± 0.89
IC_{50}	127.4 ±0.05	83.96 ±0.85	84.4 ± 0.88	44.15 ± 0.85

Table 2: Hydroxyl radical scavenging activity of compounds isolated from leaves of *Xanthium strumarium*



Ascorbic acid = Standard

A= Compound A (hexadecanoic acid)

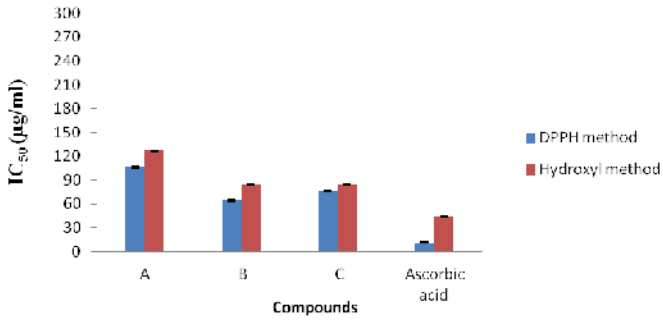
B= Compund B (α- amyrin)

C= Compound C (14-methyl-12,13-dehydro-sitosterol-heptadeconate)

Figure 2: Hydroxyl Radical Scavenging Activity of Compounds Isolated from Leaves of *Xanthium strumarium*

Compound	DPPH scavenging activity (IC_{50} µg/mL) [Mean ± SD (n=3)]	Hydroxyl ion scavenging activity (IC_{50} µg/mL) [Mean ± SD (n=3)]
A	106.41 ± 0.86	127.43 ± 0.50
B	64.16 ± 0.89	83.96 ± 0.85
C	76.18 ± 0.93	84.48 ± 0.88
Ascorbic acid	11.41 ± 0.93	44.15 ± 0.85

Table 3: Comparison of (IC_{50}) values of antioxidant activity of isolated compounds from petroleum ether extract of *Xanthium strumarium*



Ascorbic acid = Standard

A= Compound A (hexadecanoic acid)

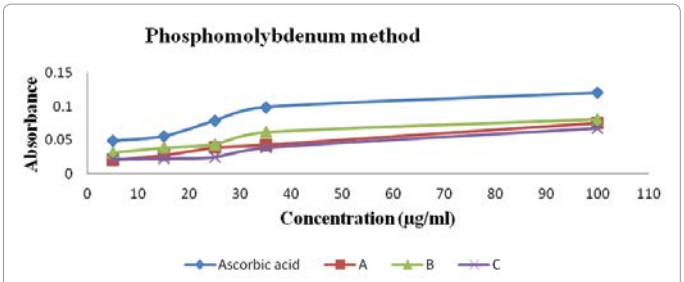
B= Compund B (α- amyrin)

C= Compound C (14-methyl-12,13-dehydro-sitosterol-heptadeconate)

Figure 3: Comparison of (IC_{50}) Values of Antioxidant Activity of Isolated compounds from Petroleum Ether Extract of Xanthium strumarium by DPPH and Hydroxyl ion Scavenging Method.

Conc. µg/mL	Absorbance [Mean ± SD (n=3)]			
	A	B	C	Ascorbic Acid
5	0.021 ± 0.04	0.032 ± 0.01	0.022 ± 0.02	0.049 ± 0.04
15	0.028 ± 0.03	0.039 ± 0.10	0.023 ± 0.03	0.056 ± 0.05
25	0.039 ± 0.02	0.044 ± 0.05	0.025 ± 0.01	0.079 ± 0.02
35	0.043 ± 0.06	0.062 ± 0.02	0.035 ± 0.03	0.099 ± 0.03
100	0.075 ± 0.01	0.081 ± 0.04	0.068 ± 0.04	0.12 ± 0.01

Table 4: Antioxidant activity by phosphomonybdenum method of isolated compounds from leaves of *Xanthium strumarium*



Ascorbic acid = Standard

A= Compound A (hexadecanoic acid)

B= Compund B (α- amyrin)

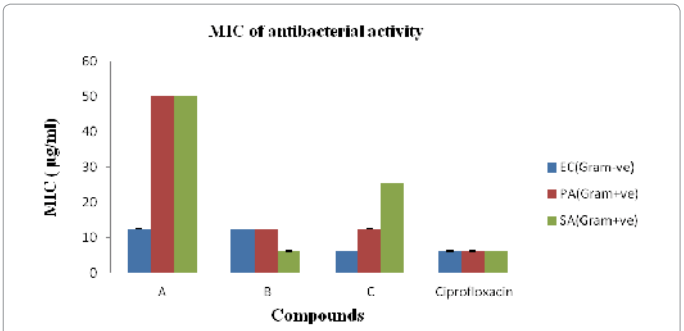
C= Compound C (14-methyl-12,13-dehydro-sitosterol-heptadeconate)

Figure 4: Antioxidant Activity by Phosphomolybdenum Method of Isolated Compounds from Leaves of *Xanthium strumarium*

Compound	Minimum inhibitory concentration (MIC) in µg/mL [Mean ± SD (n=3)]				
	EC	SA	PA	CA	KM
A	12.5 ± 0.06	50 ± 0.03	50 ± 0.05	12.5 ± 0.03	12.5 ± 0.06
B	12.5 ± 0.03	12.5 ± 0.04	6.25 ± 0.07	6.25 ± 0.04	12.5 ± 0.04
C	6.25 ± 0.02	12.5 ± 0.05	25.5 ± 0.05	6.25 ± 0.04	50±0.07
Std_1	6.25 ± 0.05	6.25 ± 0.03	6.25 ± 0.05	-	-
Std_2	-	-	-	3.25 ± 0.03	3.25 ± 0.06
Control	-	-	-	-	-

Where, EC- *Escherichia coli.*, PA- *Pseudomonas aeruginosa*, SA- *Staphylococcus aureus*, CA- *Candida albicans*, KM-*Kluyveromyces marxianus*, Std_1- Ciprofloxacin and Std_2-Fluconazole, Control-DMF

Table 5: Minimum Inhibitory Concentration (MIC) in µg/mL of isolated compounds from petroleum ether extract of leaves of *Xanthium strumarium*



Ciprofloxacin = Standard

A= Compound A (hexadecanoic acid)

B= Compund B (α- amyrin)

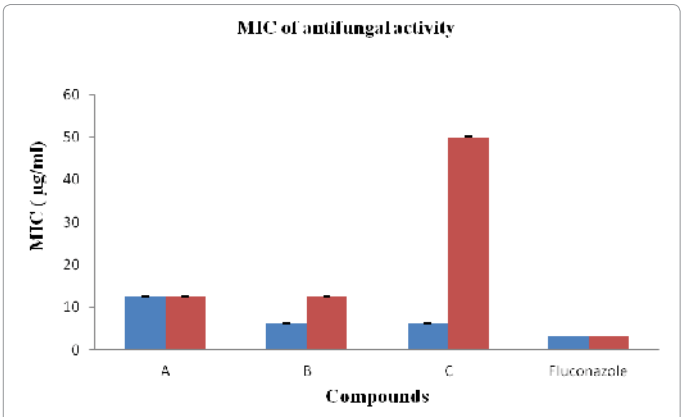
C= Compound C (14-methyl-12,13-dehydro-sitosterol-heptadeconate)

EC= *Escherichia coli* (gram negative)

PA- *Pseudomonas aeruginosa* (gram negative)

SA- *Staphylococcus aureus* (gram positive)

Figure 5: Comparison of Minimum Inhibitory Concentration (MIC) of Compounds Isolated from Leaves of Petroleum Ether Extract of *Xanthium strumarium* against Bacterial Strains (EC, PA and SA)



Fluconazole = Standard

A= Compound A (hexadecanoic acid)

B= Compund B (α- amyrin)

C= Compound C (14-methyl-12,13-dehydro-sitosterol-heptadeconate)

CA= *Candida albicans* (fungal Strain)

KM= *Kluyveromyces marxianus* (yeast)

Figure 6: Comparison of Minimum Inhibitory Concentration (MIC) of Compounds Isolated from Leaves of Petroleum Ether Extract of *Xanthium strumarium* against Fungal Strains (CA and KM)

justified the traditional use of herb in preventing disease induced by oxidative stress and microorganism.

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