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Investigation on cytotoxic, antioxidant, antimicrobial and volatile profile of *Wrightia tinctoria* (Roxb.) R. Br. flower used in Indian medicine

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ABSTRACT

Objective: To investigate the total phenols, flavonoids, carotenoids, antioxidant activity, antimicrobial and cytotoxic activity of *Wrightia tinctoria* flower extract. **Methods:** Total phenols, flavonoids, carotenoids content, DPPH scavenging activity, the reducing power activity, phosphomolybednum activity, metal chelating activity, Hydrogen peroxide radical scavenging activity of crude extract, Cytotoxicity activity, GC-MS analysis and Antibacterial screening were evaluated. **Results:** Total phenols, flavonoids, carotenoids in the extract was found to be 55.29±0.45 mg GAE, 370.53±1.213 mg QE and 1.825±0.321 mg/g respectively, where the reducing power, phosphomolybednum activity and metal chelating activity were increasing with increasing concentration of the flower extract. The antioxidant activity (IC_{50}) of the flower extract was said to be 43.16 μg/mL by 2,2-Diphenyl-1-Picrylhydrazyl method and 124.07 mg AAE/100g of plant extract by phosphomolybednum method. The antibacterial studies of the ethanolic flower extract tested at different concentration of extracts, where 250mg/mL concentration of extract showed good inhibitory activity against all the test pathogens compared with standard antibiotics like streptomycin and penicillin. The cytotoxic activity of flower extract was evaluated by brine shrimp lethality bioassay method and the LC_{50} value found to be 3.544 μg/mL. **Conclusions:** The presence of major bioactive compound, hexadecanoic acid justifies the use of the whole plant for various ailments by traditional practitioners. Further studies are needed to explore the potential phenolics, flavonoid compounds from *W. tinctoria* for application in drug delivery, nutritional or pharmaceutical fields.

1. Introduction

In the recent years, the antimicrobial and antioxidant actions have received much attention. The antioxidant may be useful in retarding oxidative deterioration of food materials especially those with high lipid content. It is well known that reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species[1-7]. ROS, causing damage to DNA, proteins and lipids, have been associated with carcinogenesis, coronary heart disease, and many other health problems[8]. Minimizing oxidative damage may well be one of the most important approaches to the primary prevention of these oxidative stress-related diseases and health problems, since antioxidants terminate direct ROS attacks and radical-mediated oxidative reactions[9]. Plants are the primary sources of naturally occurring antioxidants for humans. The natural antimicrobial agents also protect living organisms from damages resulting in the prevention of various ailments. Although, much work has been done on the antimicrobial and antioxidant effects of different plants species but after that the current study is an attempt to determine the antioxidant and antibacterial activity of the flower extracts of *Wrightia tinctoria (W. tinctoria)* against the resistant clinical isolates of *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumonia)* and *Vibrio chlorea (V. chlorea).*

W. tinctoria (Roxb.) R. Br. (Apocynaceae) is a small

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deciduous tree, with a light grey, scaly smooth bark, growing up to 1.8 m tall and 60 cm in girth, is widely distributed throughout India[10]. The plant is commonly known as Paalai (Tamil) and generally called "Sweet Indrajao". It is considered to be very effective jaundice plant in Indian indigenous system of medicine. The leaves are applied as a poultice for mumps and herpes and sometimes, they are also munched to relieve toothache. In folk medicine, the dried and powdered roots of *Wrightia* along with *Phyllanthus amarus* (keezhanelli) and *Vitex negundo* (nochi) is mixed with milk and orally administered to women for improving fertility. The bark and seeds are effective against psoriasis and non-specific dermatitis. Selvam *et al*., studied the different extracts of leaf of *W. tinctoria* against replication of HIV-1, HCV and lymphocytes and results showed that chloroform extract exhibited significant cytotoxicity (10 μg/mL) against viral cell lines *in vitro*, showing its potential activity on viral pathogens[11]. The bark of this species is used as antidysenteric, antidiarrhoeal and antihaemorrhagic agent[12]. The woody stem of *W. tinctoria* registered significant activity against a wide range of bacteria and fungi[13]. Recently antioxidant potential of ethanolic extract of bark was studied by Lakshman Kumar *et al*.[14]. Past studies revealed that so far there is no study pertaining phytochemical constituents and pharmacological evaluation of the flowers of *W. tinctoria*. Hence it is imperative to evaluate the cytotoxic, antimicrobial and antioxidant property and also identifying the active compounds present in the *W. tinctoria* flower extract by GC-MS analysis.

2. Materials and methods

2.1. Chemicals

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free radicals were obtained from Sigma Aldrich. Other chemicals, sodium phosphate, potassium ferricyanide, ammonium molybdate, quercetin, ascorbic acid, aluminium chloride, potassium hydroxide, potassium acetate, trichloroacetic acid, ferric chloride, ferrozine, EDTA, diethyl ether, and gallic acid, 2-thiobarbituric acid (TBA), butylated hydroxyl anisole (BHA), Follin-Ciocalteu, 2- deoxyribose and H_2O_2 (30 %, v/v) from Merck India Ltd and Himedia. All other chemicals and solvents used were of analytical grade.

2.2 Sample collection

The fresh flowers of *W. tinctoria* was collected from the campus area at Madurai Kamaraj University in Madurai, Tamilnadu, India and authenticated by the Director, Centre for Biodiversity and Forest Studies, Madurai Kamaraj University, and voucher specimens were deposited in the herbarium of Centre for Biodiversity and Forest Studies of our university (No. AM-06).

2.3 Plant preparation and extraction

The air-dried flowers of *W. tinctoria* were extracted with 95 % ethanol in a Soxhlet apparatus for 10 h at a temperature not exceeding the boiling point of the solvent. The extraction was repeated many times to obtain required quantity of extract. The extract was filtrated using Whatman filter paper (No.1) and then concentrated in a rotary evaporator at 40 ℃. The residues obtained were stored inside the refrigerator until further tests.

2.4 Determination of total phenolics

Folin-Ciocalteu (FC) assay described by Siddhuraju *et al* was used to determination of the total phenolics (TP) content of the flower extract of *W. tinctoria*[15]. Eight mL of water was added into 1 mL of extract in a 10 mL volumetric flask. 0.5 mL of FC reagent was added and mixed for 15 min followed by addition of 1.5 mL of 20 % sodium carbonate solution. After 2 h at ambient temperature the absorbance of the colored reaction product was measured at 765 nm, where different concentrations of standard gallic acid solutions were used for calibration curve and results were expressed as mg of Gallic acid Equivalent per gram (mg GAE/g) of dried extract.

2.5 Determination of total flavonoids

The total flavonoid content of *W. tinctoria* ethanol extracts was determined by using aluminium chloride colorimetric method[16]. Quercetin was used as a standard to make the calibration curve. The sample solution (0.5 mL) was mixed with 1.5 mL of 95 % ethanol, 0.1 mL of 10 % aluminium chloride hexahydrate, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. After incubation at room temperature for 40 min the absorbance of the reaction mixture was measured at 415 nm. The same amount (0.1 mL) of distilled water substituted for the amount of 10 % aluminum chloride as the blank and a seven point standard curve (0-500 μg/mL) was obtained.

2.6 Determination of carotenoids

Total carotenoids were determined by the method of Jensen[17]. One gram sample was extracted with 100 mL of 80 % methanol solution and centrifuged at 4000 rpm for 30 min. The supernatant was concentrated to dryness. The residue was dissolved in 15 mL of diethyl ether and after addition of 15 mL of 10 % methanolic KOH the mixture was washed with 5 % ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 h. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank.

2.7 Antioxidant activity

2.7.1 DPPH free radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Shen *et al*[18]. Plant extract (0.1 mL) was added to 3 mL of a 0.004 % methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0 - A1)/A0] × 100,

Where, A0 is the absorbance of the control, and A1 is the

absorbance of the extract/ standard.

A blank is the absorbance of the control reaction (containing all reagents except the test compound). A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50 % inhibition was determined and represented as IC_{50} value for each of the test solutions.

2.7.2 Reducing power assay

Aliquots of each extracts were taken in test tubes and dissolved in 1 mL of 0.2 M phosphate buffer in a test tube to which was added 5 mL of 0.1 % solution of potassium ferric cyanide[19]. The mixture was incubated 50 ℃ for 20 min. Following this, 5 mL of trichloroacetic acid (10 %) (w/v) solution was added and the mixture was then centrifuged at 7000 rpm for 10 min. A 5 mL of aliquot of the upper layer was combined with 5 mL of distilled water and 1 mL of ferric chloride solution (0.1%) and absorbance was recorded at 700 nm against reagent blank. A higher absorbance of the reaction mixture indicates greater reducing power of the sample.

2.7.3 Metal chelating activity

The chelating of ferrous ions by *W. tinctoria* ethanolic flower extract was estimated by the method of Dinis *et al*[20]. Briefly the extract samples (250 μ) were added to a solution of 2 mmol/l $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mmol/l ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min, after which the absorbance was measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The metal chelating activity of the extract is expressed as mg EDTA equivalent/g extract.

2.7.4 Phosphomolybednum activity

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Kannan *et al*[21]. An aliquot of 100 μL of sample solution was combined with 1mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 mL vial. The vials were canned and incubated in a water bath at 95 ℃ for 90 min. After the samples have cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

2.7.5 Hydrogen peroxide radical scavenging activity

Hydrogen peroxide assay[22] was carried out for the determination of antioxidant activity of compounds for their ability to scavenge the oxidant hydrogen peroxide. The reaction mixture contained phosphate buffer (pH-7.4) and hydrogen peroxide solution prepared in phosphate buffer (40 mM). Plant extracts at the concentration of 10 mg/10 μL was added to hydrogen peroxide solution (0.6 mL, 40 mM). The total volume was made up to 3 mL. The absorbance of the reaction mixture was recorded at 230 nm. The blank solution contained phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the plant extract was calculated as follows:

Percentage of scavenged H_2O_2=A0-A1x100/A0

Where, A0-Absorbance of control

A1- Absorbance in the presence of plant extract.

2.8 Antibacterial activity

2.8.1 Test organisms

The ethanolic flower extract of *W. tinctoria* was screened against four bacterial strains. The test organisms *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonia*) and *Vibrio chlorea* (*V. chlorea*) were procured from the Microbial Type Culture and collection, Chandigarh, India.

2.8.2 Antibacterial screening

The different concentrations of the leaf extracts (50 mg/l, 100 mg/l and 250 mg/l) were tested for antibacterial activity using agar disc diffusion assay according to the method of Qaralleh *et al*[23]. The strains of microorganisms obtained were inoculated in conical flask containing 100 mL of nutrient broth. These conical flasks were incubated at 37 ℃for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 μL of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37 ℃ Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10 μg/disc) and penicillin (10 μg/disc) were used as standards.

2.9 Cytotoxicity bioassay

Brine shrimps (Artemia salina) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Twenty nauplii were drawn through a glass capillary and placed in each vial containing 4.5 mL of brine solution. In each experiment, 0.5 mL of the extract was added to 4.5 mL of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted with a hand lens. Experiments were conducted along with control (vehicle treated), different concentrations (10-1000 μg/mL) of the test substances in a set of three tubes per dose. Based on the percent mortality, the LD50 of the test compound was determined using probit scale[24].

2.10 GC-MS Analysis

2.10.1 Preparation of extract

2 μL of the ethanolic extract of *W. tinctoria* (Roxb.) R. Br. was

employed for GC/MS analysis.

2.10.2 Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100 % Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999 %) was used as carrier gas at a constant flow of 1mL/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250 ℃; ion-source temperature 280 ℃. The oven temperature was programmed from 110 ℃(isothermal for 2 min), with an increase of 10℃/min, to 200 ℃/min, then 5 ℃/min to 280 ℃/min, ending with a 9 min isothermal at 280℃. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

2.10.3 Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.11 Statistical analysis

Results were expressed as the means of three replicates ± the standard deviation of triplicate analysis.

3.Results

3.1 Determination of total phenols, flavonoids

Total phenolic content of the ethanolic extract of *W. tinctoria* flower extract is (55.29±0.445) mg gallic acid equivalent per gram of plant extract (Table 1). The total phenolics of essential oil of Geranium sanguineum L. flowers ranged from 54.43 to 88.25 μg/mL[25]. The total phenolics of oleander (Nerium oleander) flower was (136.54±3.32) mg as gallic acid/g essential oil[26]. The phenolic compounds present in natural products have higher antioxidant activity than synthetic antioxidants, also by acting as free radical terminators[27]. Gowri and Vasantha, 2010 reported that Sesbania grandiflora flowers have less phenolic content than leaf extract[28].

The flavonoid contents of the *W. tinctoria* ethanolic flower extract is (370.53±1.2130 mg Quercetin equivalent per gram of plant extract (Table 1). The flavonoids possess antioxidant activity acting through scavenging or chelating process thereby having considerable effect on human health and nutrition[29]. Since the flower extract of *W. tinctoria* shows higher amount of phenolic compounds, suggesting their usage as a good source of natural antioxidant, preventing free radical-mediated oxidative damage.

The carotenoid contents of the *W. tinctoria* ethanolic flower extract were found to be (1.825±0.321) mg/g (Table 1). Carotenoids have a positive role on the epithelisation process and influence the cell cycle progression of the fibroblasts[30]. Carotenoids act as photoprotective agents, reducing skin cancer, skin related diseases, photo-allergy and sun burns[31].

3.2 Antioxidant activity (DPPH free radical scavenging activity) determination

The radical scavenging activity of the *W. tinctoria* ethanolic flower extract was tested using stable free radical DPPH (deep purple colour), as DPPH has the advantage of being uneffected by certain side reactions. Figure 2 shows the DPPH radical scavenging activity of *W. tinctoria* with ascorbic acid as reference, where the IC_{50} values for the *W. tinctoria* ethanolic flower extract (43.16 μg/mL) which was said to be little less than the standard ascorbic acid (IC_{50}=30.31 μg/mL). Thus the antioxidants present in the extract quenches the DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless product (2, 2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine) resulting in a decreasing absorbance at the 517 nm[32]. The results of the antioxidant activity by DPPH of the extracts of Pyrostegia venusta (Ker Gawl) Miers flowers was said to be 95 % comparable with that of ascorbic acid (98.9 %) and BHT (97.6 %)[33]. The antioxidant activity of oleander (Nerium oleander) flower studied by DPPH method showed significant activity (2.11±0.12-EC_{50}) compared to standard ones, trolox (6.75±0.22 μg/mL) and BHT (4.61±1.61) μg/mL)[26].

Figure 1. Flowers of *W. tinctoria* (Roxb.) R. Br.

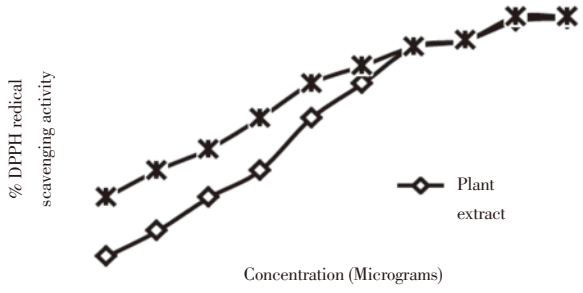

Figure 2. DPPH radical scavenging activity of *W. tinctoria* ethanolic flower extract.

3.3 Reducing power assay

Figure 3 shows the reducing power of the *W. tinctoria* ethanolic flower extract using potassium ferricyanide reduction method. The absorbance value of the extract shows higher increase with increase in concentration, when compared to standard ascorbic acid. The yellow colour of test solution changes to various shades of green and blue due to the reduction of Fe^{3+}/ Ferric cyanide complex to ferrous form by the antioxidants present in the extract. Thus the reducing power of medicinal plants and vegetables are said to be well associated with the antioxidant activity and its phenolic constituents[15].

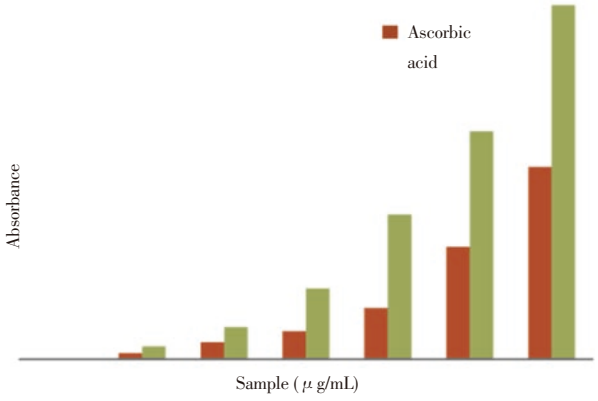

Figure 3. Reducing power of the *W. tinctoria* ethanolic flower extract

3.4 Metal chelating activity

Figure 4 shows the metal chelating activity of the *W. tinctoria* flower extract, where IC_{50} of plant extract was said to be 30.12 μ g/mL compared to the standard 1.89 μ g/mL. In this metal chelating activity, the presence of chelating agents in the extract of *W. tinctoria* disrupts the ferrozine-Fe^{2+} complex formation, thus decreasing the red colour. It is reported that chelating agents are effective as secondary antioxidants as they stabilise the oxidised form of the metal ion by reducing the redox potential [34].

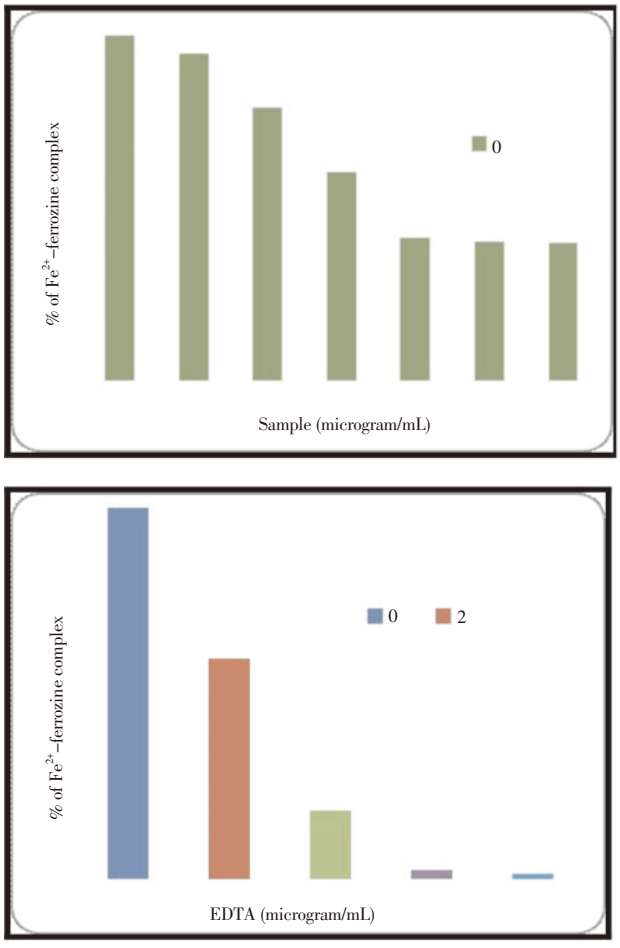

Figure 4. Metal chelating activity of *W. tinctoria* ethanolic flower extract

Table 2.
Antibacterial activity of ethanolic flower extract of *W. tinctoria*.

Microorganisms	Inhibition zone in diameter (mm)				
	Streptomycin	Pencillin	50mg/L	100mg/L	250mg/L
V. chlorea	16.80±0.87	19.34±0.38	12.65±0.37	14.56±0.78	16.76±0.29
E. coli	14.70±0.60	10.10±0.25	8.65±0.45	10.85±0.46	14.02±0.39
K. pneumonia	12.10±0.25	17.60±0.71	8.21±0.43	9.90±0.21	10.20±0.35
S. aureus	22.80±0.35	23.10±0.45	10.8±0.42	14.26±0.38	15.89±0.41

Values are inhibition zone (mm), and an average of triplicate.

Table 3.
Phytocomponents identified in the ethanolic flower extract of *W. tinctoria* by GC-MS.

RT	Name of the compound	Peak Area(%)
14.09	Propanoic acid, 2-mercapto, 1-methyl ester	17.79
16.52	3-methyl-3-butanoic acid	12.74
19.58	Styrene	1.50
26.17	Hexadecanoic acid, 15-methyl, methyl ester	58.31
27.80	3-Pyrimidinecarboxylic acid, 2,4-bis	1.83
29.30	Phenol, 4-ethyl-2-methoxy	1.83
30.00	2, 5, 7-Nonatrien-4-one, 9-(3-furanyl)	1.07
32.40	Pentadecanoic acid, ethyl ester	4.66
37.28	1-[4, 4-Dimethyl-6-(2-oxopropyl)-1-oxo	1.29
39.67	3, 6-Dioxa-2, 7-Disilanone-4,5-dicarboxylic acid, 2, 2, 7,7-tetra methyl- dimethyl ester	1.27
44.37	Diethyl phthalate	1.02

3.5 Phosphomolybednum activity

The total antioxidant activity of *W. tinctoria* ethanolic flower extract was found to be 124.07 mg equivalent of ascorbic acid/100 g of plant extract as determined by phosphomolybdenum method. This method is based on the formation of green phosphomolybdenum complex at 95℃ measured at an intensity of absorbance at 695 nm. In this method, reduction of Mo (VI) to Mo (V) by the antioxidant compounds present in the plant extract, forming green phosphate/Mo (V) complex takes place[35].

3.6 Hydrogen peroxide radical scavenging activity

The figure 5 shows the hydrogen peroxide radical scavenging activity of *W. tinctoria* ethanolic flower extract, showing an IC50 value of 242.76 μg compared with standard ascorbic acid (IC_{50}=51.23 μg). Hydrogen peroxide, though not reactive, is said to be highly important because of its ability to penetrate biological membranes, releasing toxic hydroxyl radicals in the cells[36]. Thus the flower extract showed significant scavenging activity of H_2O_2. (Figure 5)

3.7 Antibacterial Screening

The *W. tinctoria* ethanolic flower extract showed good inhibition against both gram positive and gram negative organisms at higher concentration of 250 mg/L (Table.2). The highest inhibition was noted in order of *V. chlorea* (16.76±0.29 mm), *E. coli* (14.02±0.39 mm), *S. aureus* (15.89±0.41 mm) and *K. pneumonia* (10.2±0.35 mm). The hexane extract of flowers of Hypericum scabrum, possessed significant antioxidant activity, antimicrobial activity due to the presence of omega-3 fatty acid[37]. The essential oil of *Geranium sanguineum* L. flowers showed significant antioxidant activity (IC_{50}=85 μg/mL) and remarkable antibacterial activity against all test pathogens due to presence of various phenolic compounds[25].

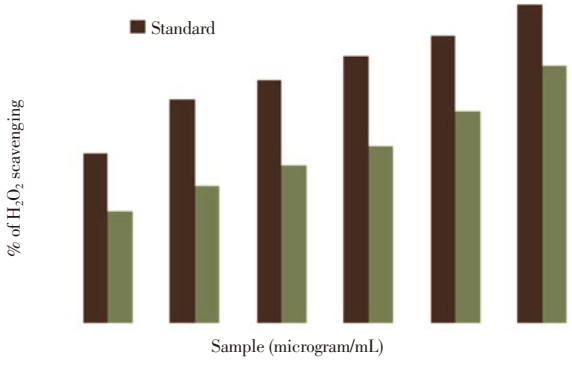

Figure 5. Hydrogen peroxide radical scavenging activity of *W. tinctoria* ethanolic flower extract

3.8 Cytotoxicity activity

The ethanolic flower extract of *W. tinctoria* showed cytotoxic activity against brine shrimp and the LC_{50} value was found to be 3.544 μg/mL compared to taxol 0.85 μg/mL (figure 6). The cytotoxicity bioassay against Artemia salina is a simple and inexpensive method to test cytotoxicity, to biodirect fractionation of natural products and as a predictor of antitumor and pesticidal activity[38]. The inhibitory effect of the *W. tinctoria* flower extract might be due to the prsesnce of toxic compounds such as hexadecanoic acid present in the extract that possess hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, haemolytic and 5- Alpha reductase inhibitor activity. So the cytotoxic effects of the flower extracts enunciate that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts[39]. The results on brine shrimps assay indicate that the extract has LC50 value greater than 20 μg/mL; the recommended cutoff point for detecting cytotoxic activity[40]. (Figure 6)

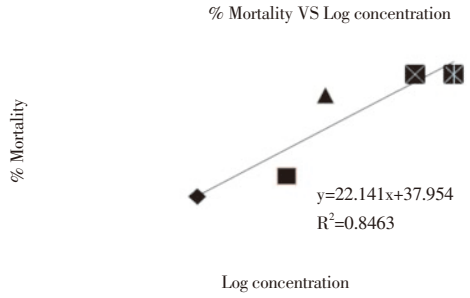

Figure 6. The toxicity effects of the *W. tinctoria* flower extract using brine shrimp lethality assay after 24 hour

3.9 GC-MS analysis

(Figure 7)

On comparison of the mass spectra of the constituents with the NIST library, eleven peaks were obtained; all the phytoconstituents were characterized and identified (Table 1). GC-MS chromatogram of the ethanolic flower extract of *W. tinctoria* (Roxb.) R. Br. is given in Figure7. The retention times (RT) are in minutes. The major chemical constituents in ethanolic flower extract studied through GC-MS are hexadecanoic acid, 15-methyl (58.31 %), 2-mercapto-propanoic acid (17.79 %), pentadecanoic acid (4.66 %) and 3-methyl-3-butanoic acid (12.74 %) (Table 3). The major constituent, hexadecanoic acid, ethyl ester at retention time of 26.17 min was said to possess various activities such as antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, haemolytic and 5-Alpha reductase inhibitor. The extract also shows the presence of phenolic substance, 4-ethyl-2-methoxy, phenol at a retention time of 29.30 min.

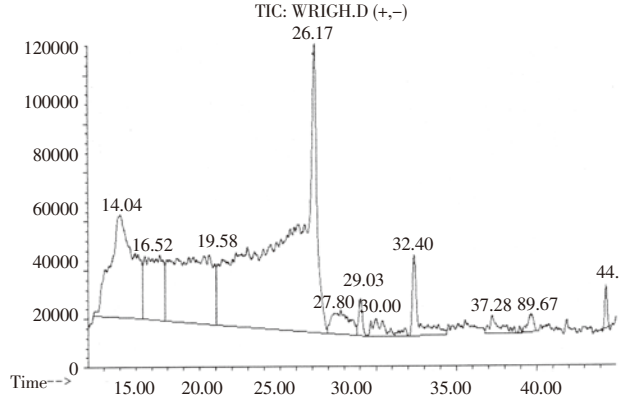

Figure 7. GC-MS Chromatogram of ethanolic flower extract of *W. tinctoria*

Table 1.
Total phenols, flavonoids and carotenoids contents of *W. tinctoria* flower extract

Determination of Phytoconstituent	*W. tinctoria* flower extract
Total phenols	55.29±0.445 mg GAE/g
Flavonoids	370.53±1.213 mg QE/g
Carotenoids	1.825±0.321 mg/g

The extract shows presence of many methyl and ethyl esters such as propanoic acid, 2-mercapto; 3-methyl-3-butanoic acid; pentadecanoic acid and disilanone, dicarboxylic acid at retention time of 14.09, 16.52, 32.40 and 39.67 respectively. GC-MS study of Pyrostegia venusta (Ker Gawl) Miers flowers revealed the presence of myoinositol, hexadecanoic acid, linoleic acid, palmitic acid and oleic acid in the flower extracts[33]. The major components of Rosa damascena petals essential oil were linalool (3.8 %), nerol (3.05 %), geraniol (15.05 %), 1-nonadecene (18.56 %), n-tricosane (16.68 %), hexatriacontane (24.6 %) and n-pentacosane (3.37 %)[41]. The GC-MS of Chimonanthus praecox flower extract revealed the presence of elemene, muurolene, caryophyllene, cadinol and spathulenol[42] suggesting the use of flowers of Chimonanthus praecox for pharmaceutical benefits, and as potential source of natural antioxidants and biocides.

The major phytochemical constituent, hexadecanoic acid present in ethanolic flower extract of *W. tinctoria* is presented as mass spectra and compound structures in figure 8.

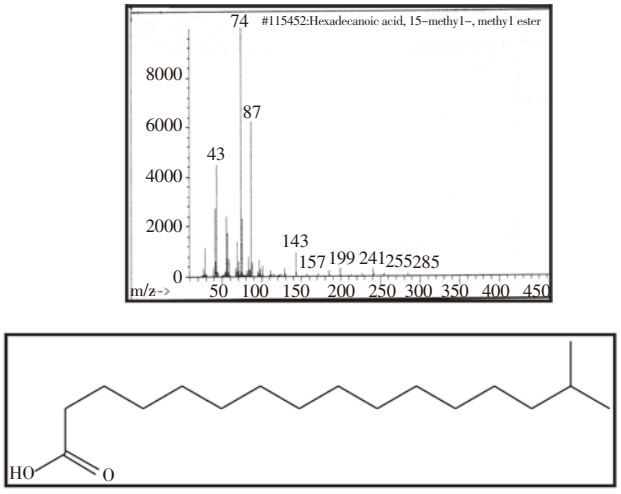

Figure 8. The mass spectrum analysis and structure of Hexadecanoic acid, 15-methyl

4. Discussion

The present study indicates that the ethanolic flower of *W. tinctoria* are high in phenolic and flavonoid content. Flavonoids are potent antioxidants having characteristics of scavenging free radical, chelating metal and inhibiting lipid peroxidation. The extract possessed strong antioxidant, reducing activity. Also the results of scavenging activities observed against DPPH, reducing power, phosphomolybednum activities, show that *W. tinctoria* as promising natural sources of antioxidants suitable for preventing free radical-mediated diseases. The extract also shows high antibacterial against important bacterial strains. The cytotoxic activity of flower extract on Brine shrimp, Artemia salina was said to possess significant toxicity. GC-MS analysis have been found useful in the identification of several constituents such as hexadecanoic acid, pentadecanoic acid, butanoic acid and propanoic acid present in the ethanolic flower extract of *W. tinctoria* (Roxb.) R. Br. The presence of major bioactive compound, hexadecanoic acid justifies the use of the whole plant for various ailments by traditional practitioners. Further studies are needed to explore the potential phenolics, flavonoid compounds from *W. tinctoria* for application in drug delivery, nutritional or pharmaceutical fields.

Conflict of interest statement

We declare that we have no conflict of interest.

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