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Research Article

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Phytoconstituents profiling and antioxidant potential of *Wrightia tinctoria* R. Br.

S. Rajkumar[1,2]*, G. Sathyaprabha[1], Maghimaa Mathanmohun[3]*

[1]PG and Research Department of Microbiology, Maruthupandiyar College (Affiliated to Bharathidasan University, Trichy-24), Vallam, Thanjavur-613403, Tamil Nadu, India, [2]Department of Biotechnology, Don Bosco College (Autonomous), Maram-795015, Manipur, India, [3]Department of Microbiology, Muthayammal College of Arts and Science, Rasipuram, Namakkal-637408, Tamil Nadu, India

ABSTRACT

In the Indian traditional system of medicine, *Wrightia tinctoria* R.Br. (Apocyanaceae) is recognized as a biologically effective therapeutic plant for the treatment of jaundice. It is a natural medicinal tree possessing antidiabetic, anti-inflammatory, hepatoprotective, antinociceptive, antifungal, antibacterial, antipsoriatic, antiviral, anthelmintic, anticancerous, analgesic, antipyretic, and aphrodisiac activities. Methanolic extract of *W. tinctoria* plant was investigated with Gas Chromatography–Mass Spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR) and the antioxidant activity of *W. tinctoria* was analysed by most probable hydrogen peroxide scavenging (H_2O_2) activity, DPPH activity and Thiobarbutaric acid. The phytochemical in *W. tinctoria* leaf and bark have been assessed by GC-MS analysis. GC-MS analysis of W. tinctoria methanolic extract exposed the existence of the GC-MS chromatograms of 10 peaks in the leaf and 20 peaks in the bark. The FTIR spectroscopy analyses were identified by various functional compounds in the extracts with distinctive peak values. The FTIR analysis of methanolic leaf extracts of *W. tinctoria* confirmed the presence of alcohol, amine, alkane, carboxylic acid, sulfur compounds, halogen compounds and alkyne which showed major peaks. Also the methanolic bark extracts of *W. tinctoria* confirmed the presence of amines, phenol, alcohols, alkane, aldehydes, carboxylic acid, nitrogen compounds, sulfur compounds, and halogen compounds which also exhibited major peaks. Significant antioxidant activity is displayed by the plant part of leaf and bark sample. The results obtained in the determination of antioxidant activity of MEWT displayed considerable free radical scavenging capacity against DPPH which generated free radicals. This study includes the identification of phytochemicals and antioxidant potential of methanolic and aqueous extract of *W. tinctoria* which assist in therapeutic claims about this species in the traditional medicinal plant system.

KEYWORDS: *Wrightia tinctoria*, Plant extracts, GC-MS, FTIR, Antioxidant activity

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***Corresponding Authors:**
S. Rajkumar
E-mail: rajkumar@dbcmaram.ac.in
Maghimaa Mathanmohun
E-mail: maghimaam@gmail.com

INTRODUCTION

Owing to their traditional uses and therapeutic actions medicinal plants are popular worldwide. They are measured as an excellent source to obtain a variety of bioactive compounds which can further be developed into novel drugs. *W. tinctoria* is referred to by the names "pala indigo" and "indrajao," which belongs to the Apocyanaceae family. *W. tinctoria* is a small to medium-sized deciduous tree found throughout India with up to 1200 feet of plant height. The bark is suitable for carving since it is a smooth, slightly corky, and pale grey. In south India, traditionally *W. tinctoria* is referred to as "the tree that cures jaundice" (Joshi, 2000). *W. tinctoria* is popularly predicted as a therapeutic agent, owing to its varied pharmacological actions. It possesses broad spectrum of biological activities and which is extensively used in the treatment of skin diseases and liver disorders. It is extensively recognized as a therapeutic medicinal plant of high significance in traditional healing. The present study is determined to screen the phytochemical compounds in the methanolic leaf and bark extracts of W. tinctoria by GC-MS and FTIR analysis (Khan *et al.*, 2021).

According to Gas Chromatography and Mass-Spectrometry (GC-MS), the *W. tinctoria* R.Br leaf and bark extract has a variety of phytochemical components. The results of the characterization tests substantiated the properties of *W. tinctoria* (Rajagopal *et al.*, 2021). Spectra from gas chromatography-mass spectrometry showed the existence of 26 bioactive chemicals and 8 significant compounds. Ethers, alcohols, alkanes, alkyls, carboxylic acids, and alkynes were all present in the methanol extracts based on the UV-FTIR spectra (Hemavathy *et al.*, 2019). For the detection and comparison of biomolecules between two

species of medicinal plants, FTIR Spectroscopy was used as a sensitive and effective assay. From that, it is demonstrated that the plants *Tephrosia tinctoria* and *Atylosia albicans* contain high phenolic content (Kumar & Prasad, 2011). From the plant extracts the FTIR spectroscopic studies revealed different characteristic peak values with various functional compounds. In the methanol and chloroform leaf extracts of *Wedelia biflora*, the FTIR analysis confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amines compounds, which exhibited major peaks. On the spectrophotometer system, the FTIR method was performed, which was used to identify their functional groups and characteristic peak values (Sahayaraj *et al.*, 2015).

When compared to the standard ascorbic acid (IC_{50} = 17.45 g/mL), the aqueous fraction of the bark and the chloroform fraction of the fruits had the highest antioxidant activity (IC_{50} = 7.22 and 4.5 g/mL, respectively) (Jannat *et al.*, 2022). Natural antioxidants have garnered a lot of attention because they may be crucial in preventing the formation of free radicals and oxidative chain reactions in tissues and membranes (Carini *et al.*, 1990). Using unique in-vitro methods, we discover the antioxidant activity of methanolic W. *tinctoria* Roxb root extract/fraction. It contains the 1,1-Diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity, the ABTS radical scavenging activity, the total antioxidant capacity, and the o-phenanthroline assay/Iron chelating activity (Charanjeet *et al.*, 2019). In light of the above information, we were provoked to carry out extraction of W. *tinctoria* leaf and bark followed by screening its antioxidant potential. The phytoconstituents found in the methanol extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) and also by FTIR analysis.

MATERIALS AND METHODS

Collection of Plant

The fresh and healthy *Wrightia tinctoria* R. Br. were collected in Thanjavur, Tamil Nadu, India and the plant was identifed by botanist Prof. C. Rajasekaran, FSEDI, FRSB (UK), FLS (Lon), FISPP Professor, School of BioSciences and Technology, VIT University, Vellore-632 014, India. Prof. C. Rajasekaran has following credentials IUCN-Species Survival Commission-Member, IUCN-Commission on Environmental, Economic and Social Policy-Member, Zonal Secretary-Indian Society for Plant Physiology. The leaves and barks of the plant properly washed under running water followed by washing with distilled water and then shade dried at room temperature to completely remove the moisture content. After being homogenized into a fine powder using a mixer grinder, the dried leaves were then preserved in airtight containers and used for the crude extract preparation.

Sample preparation

10 g of W. *tinctoria* dried leaf and bark powder were weighed out individually and placed in marked airtight bottles with 50 mL of each solvent namely aqueous and methanol separately.

Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

Using GC-MS (Model: GC-MS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μm film thickness the plant extract was analysed. An electron ionization system with an ionization energy of 70eV was used for the GC-MS detection. At a constant flow running rate of 1.51 mL/min Helium (99.99 percent) is utilized as the carrier gas. The temperature of the Injector and mass transfer line was set at 200 and 240 °C, correspondingly. The temperature of the oven was set from 70 to 220°C at 10°C/min which is held isothermal for 1min and then finally raised upto 300°C at 10°C/min. A split mode infusion of 2 μL of the sample was performed using a scan range of 40-1000 m/z. The GC-MS ran for 35 minutes in total. With peak area normalization, the relative percentage of extract constituents was denoted in percentage. The name, structure, and molecular weight of the test samples were checked twice using the NIST database and evaluated with mass-spectrum GC-MS (Vakayil *et al.*, 2021).

Fourier Transform Infra-red Spectroscopy (FTIR) Analysis

An infrared spectrum of an objects absorption or emission can be obtained using the Fourier-transform infrared spectroscopy (FTIR) method. High-spectral-resolution data are concurrently gathered over a broad spectral range using an FTIR spectrometer. By recognizing different kinds of chemical bonds (functional groups), Fourier transform infrared spectroscopy (FT-IR) can be used to identify some of the constituents of an unknown mixture. Translucent pellets were produced by grinding one milligram of partially purified bio-surfactant with 100 mg of K Br and pressing it with 7500 kg for 30 seconds. On an AVATAR-NICOLAT FTIR system with a spectral resolution and wave number precision of 4 and 0.01 cm^{-1}, respectively, infrared absorption spectra were captured.

Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity Assay

As per the Indian Pharmacopoeia 1996 standards the standard solution of 0.2 M potassium dihydrogen phosphate (KH_2PO_4) and 0.2 M sodium hydroxide (NaOH) solutions were prepared. In a 200 mL volumetric flask, 50 mL potassium dihydrogen phosphate solution was placed and 39.1 mL of 0.2 M sodium hydroxide solution was added into it and finally with distilled water the volume was made up to 200 mL to prepare phosphate buffer (pH 7.4). Consequently, 50 mL of phosphate buffer solution was added to an equal amount of hydrogen peroxide solution to generate free radicals, for that the solution was kept aside at room temperature for 5min to complete the reaction. To 0.6 mL hydrogen peroxide solution 1 mL plant extract was added then the absorbance was measured at 230 nm in a spectrophotometer and phosphate buffer solution without hydrogen peroxide was kept as blank. In the extract the percentage of scavenging of H_2O_2 was measured (Singh & Singh,

2008). The ability to scavenge the H_2O_2 radical was calculated using the following equation:

$$H_2O_2 \text{ scavenging activity } (\%) = (A0 - A1)/A0 \times 100$$

Where,
A0 is referred as absorbance of the control and A1 is referred as absorbance in the presence of the extract sample. Like same concentration of the extract a standard solution of ascorbic acid was prepared and ran. The antioxidant activity of the sample was denoted as concentration (mg/mL) of the sample which inhibited 50% of the formation of H_2O_2 radicals.

DPPH Assay

The antioxidant activity of the *W. tinctoria* were performed on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical method with slender alterations. The subsequent concentrations of aqueous and methanol extracts were prepared at 100, 200, 300, 400 and 500 µg/mL. The concentrated 5ml of prepared extract was mixed with 0.5 mL of 1mM DPPH solution. All the experiments were done in triplicate. At room temperature the test tubes were incubated for 30 min and finally the absorbance was measured at 517 nm. When the absorbance of the reaction mixture is low the free radical scavenging activity is higher. Furthermore, 0.1 mg/mL of Vitamin C was kept as standard and as the test solution a similar concentrations were prepared (Brand-Williams *et al.*, 1995). The variation in absorbance among the test and the control (DPPH in methanol) was calculated and expressed in % scavenging of DPPH radical.

$$\% \text{ Scavenged [DPPH]} = [(AC - AS)/AC] \times 100.$$

Thiobarbituric Acid (TBA) Assay

For the Preparation of TBA Reagent the standard solution of 4.0 mM of TBA with glacial acetic acid was prepared. Consequently, in 100 mL of glacial acetic acid, 57.66 mg of TBA was dissolved. The *W. tinctoria* leaf and bark extract sample were added with 100% glacial acetic acid (AA) and 50% glacial acetic acid with water (AW). With 1 mL of TBA reagent 1ml of leaf extract was mixed and the above procedure was repeated for five times ($nn = 5$) (Zeb & Ullah, 2016). The TBARS was evaluated using the formula as $\mu\mu$M/g of the sample:

$$\text{TBARS } (\mu\mu\text{M/g}) = (\text{Ac} \times VV)/WW$$

Where
Ac is referred as the amount determined from the calibration curve and WW is weight of the sample taken while VV is the volume in mL or dilution factor of the total leaf extract prepared.

Statistical Analysis

Experimental data found from the antioxidant activity were reported as mean ± standard error of mean (SEM), whenever possible. Data were evaluated by Student's *t*-test using GraphPad Software, USA. *p* values less than 0.05 were considered statistically significant.

RESULTS

The crude extract recovered was 0.5 g. The distribution of several phytochemical components in methanolic extracts of *W. tinctoria* leaf and bark was analyzed qualitatively and shown in Figure 1 and 2 and summarized in Table 1 and 2. In the present investigation, the GC-MS analysis of *W. tinctoria* leaf extracts revealed 10 distinct phytochemical components (Figure 1). The results showed that the percentage of significant bioactive compounds i.e. Neophytadiene, 2-Hexadecen-1-Ol,3,7,11,15 Tetramethyl-,3,7,11,15-Tetramethylhexadec-2-En-1-ol (2E)-3,7,1, erythro-7,8-Bromochlorodisparlure, Strychane, 1-acetyl-20.alpha.-hydroxy-16-methylene, Stigmasterol, Silicone Grease, Siliconfett, 5.beta.-Pregnane-3.alpha.,17,20.alpha.-triol, cyclic 17,20-methaneboronate, TMS derivative, cis-9-Tetradecenoic acid, isobutyl ester, Silicone Grease, Siliconfett and Silikonfett were discovered as the primary substances in the methanolic extract of *W. tinctoria* leaf (Table 1).

W. tinctoria bark extracts GC-MS chromatogram displayed 20 peaks, signifying the presence of 20 different phytochemical constituents found to be recorded respectively. The percentages of significant bioactive chemicals were shown in Figure 2 and Table 2. The phytochemicals such as Cyclononasiloxane, octadecamethyl, Cyclononasiloxane, octadecamethyl, Hexadecanoic acid, methyl ester, Cyclooctasiloxane, hexadecamethyl, Methyl stearate, Cyclononasiloxane, octadecamethyl, Cyclononasiloxane, octadecamethyl, Cyclononasiloxane, octadecamethyl, Cyclononasiloxane, octadecamethyl-, (5S,6aR,10aS)-5-Propyldecahydrodipyrrolo[1,2-a:1',2'-c] pyrimidine, Benzene, 1-(1,1-dimethylethyl)-4-(2-ethoxyethoxy)-, Cyclononasiloxane, octadecamethyl-, 3,3,5,5,7,7,9,9,11,11,13,13-Dodecamethyl-1,15-bis(1,3,3,5,5-pentamethyl-2,4,6-trioxa-1,3,5-trisilacyclohexyl 0-4,6,8,10, 12-tetraoxa-3,5,7,9,11-, Nonanoic acid, 2,2,2-trichloroethyl ester, Silicone Oil $$ Silikonfett Se30 (Grevels), Cyclononasiloxane, octadecamethyl-, benzoic acid, 4-[(trimethylsilyl)oxy]methyl]-, trimethylsilyleste, Barbituric acid, 5-allyl-5-(cyclohex-2-en-1-yl)-, Cyclohexanone, 2,6-bis(2-methylpropylidene)-, beta.-Sitosterol and TMS derivatives were found as the major compound in the methanolic bark extract of *W. tinctoria* plant.

In the present study numerous functional groups including phenols, amines, alcohols, alkenes, carboxylic acids, aliphatic compounds, carbonyl compounds, and esters, were revealed using FT-IR spectroscopy. Figure 3 displays representative FT-IR spectra of the extracts from *W. tinctoria* leaf methanol extract. Bands have been seen at 3656.76 cm^{-1}, which were related to the vibration of the Acye Halide Stretching Vibrations, Miscellaneous chromophoric groups, Alcohols and phenols, O – H stretching hydrogen bonded (change on dilution), Amines, Secondary, free; one band Imines (= N –H), one band at peak of 3396.17cm^{-1}; Hydrocarbon chromophore, C–H Stretching, alkane were absorbed at the peak of 2946.82 17 cm^{-1}; Hydrocarbon chromophore, C–H Stretching, alkane

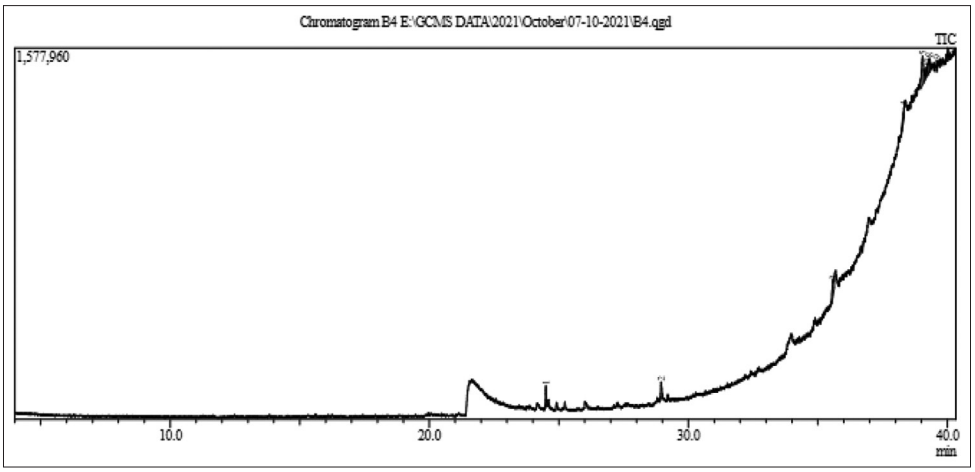

Figure 1: GC-MS chromatogram of *W. tinctoria* leaf extract

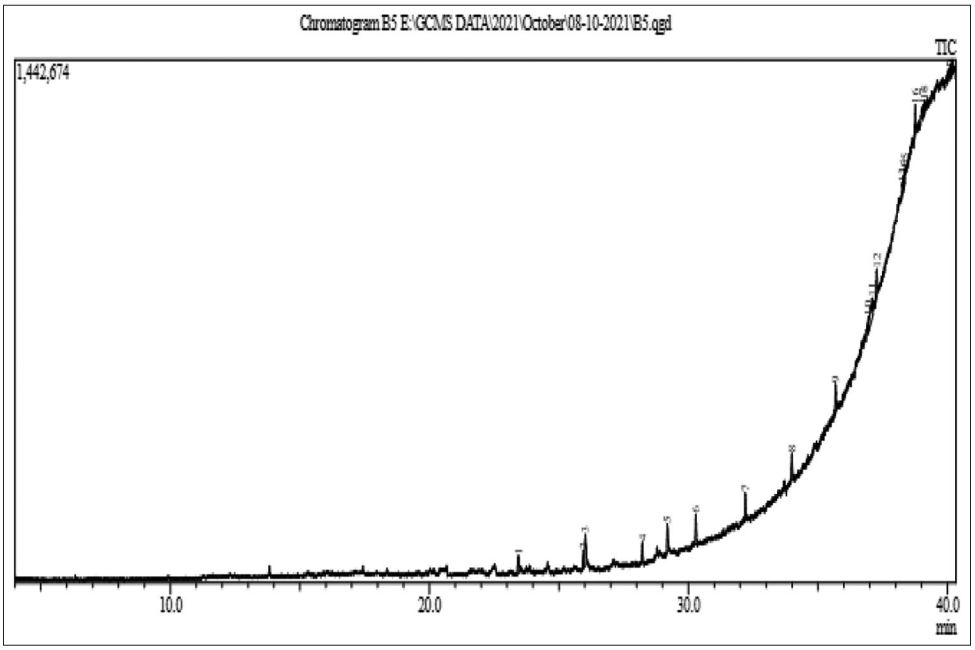

Figure 2: GC-MS chromatogram of *W. tinctoria* bark extract

Table 1: Phytochemical constituents in leaf extract of *W. tinctoria* by GCMS

S. No	Retention time	Name of the compounds	Molecular name	Molecular weight
1	24.500	Neophytadiene	$C_{20}H_{38}$	278.5
2	28.955	2-Hexadecen-1-Ol, 3,7,11,15 Tetramethyl-,3,7,11,15-Tetramethylhexadec-2-En-1-ol (2E)-3,7,1	$C_{20}H_{40}O$	296.5
3	35.580	1-bromo-19-chlorononadecane	$C_{19}H_{38}BrCl$	381.9
4	38.320	Strychane, 1-acetyl-20.alpha.-hydroxy-16-methylene	$C_{21}H_{26}N_2O_2$	338.4
5	39.040	Stigmasterol	$C_{29}H_{48}O$	412.7
6	39.145	Stigmast-5-en-3-ol	$C_{47}H_{82}O_2$	679.2
7	39.235	5.beta.-Pregnane-3.alpha.,17,20.alpha.-triol, cyclic 17,20-methaneboronate, Trimethylsilyl derivative	$C_{25}H_{45}BO_3Si$	432.5
8	39.310	cis-9-Tetradecenoic acid, isobutyl ester	$C_{18}H_{34}O_2$	282.5
9	39.585	Stigmasterol	$C_{47}H_{82}O_2$	679.2
10	40.020	Stigmasterol	$C_{47}H_{82}O_2$	679.2

were found at the peak of 2865.83 cm^{-1}, Aldehydes, C–H Stretching vibration at the peak of 2841.71 cm^{-1}; at the peak of 2525.39 cm^{-1} Carboxylic acids, hydroxyl stretching (bounded) several bands were found; Carboxylic acids, several bands

Table 2: Phytochemical constituents in bark extract of *W. tinctoria* by GC-MS

S. No.	Retention time	Name of the compounds	Molecular name	Molecular weight
1	23.440	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666
2	25.945	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666
3	26.020	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
4	28.225	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592
5	29.185	Methyl stearate	$C_{19}H_{38}O_2$	298
6	30.285	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666
7	32.195	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666
8	33.995	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666
9	35.680	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666
10	36.940	(5S,6aR,10aS)-5-Propyldecahydrodipyrrolo[1,2-a: 1′,2′-c] pyrimidine	$C_{13}H_{24}N_2$	208
11	37.095	Benzene, 1-(1,1-dimethylethyl)-4-(2-ethoxyethoxy)-	$C_{14}H_{22}O_2$	222
12	37.275	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666
13	38.245	:3,3,5,5,7,7,9,9,11,11,13,13-Dodecamethyl-1,15-bis (1,3,3,5,5-pentamethyl-2,4,6-trioxa-1,3,5-trisilacyclohexyl0-4,6,8,10,12-tetraoxa-3,5,7,9,11-	$C_{26}H_{74}O_{11}Si1_2$	898
14	38.300	Nonanoic acid, 2,2,2-trichloroethyl ester	C11H19Cl3O2	288
15	38.350	2,3-bis (trimethylsilyloxy) propyl (9Z,12Z)-octadeca-9,12-dienoate	$C_{27}H_540_4Si_2$	498.9
16	38.760	Cyclononasiloxane, octadecamethyl-	C18H54O9Si9	666
17	39.050	benzoic acid, 4-[(trimethylsilyl) oxy] methyl]-, trimethylsilyleste	C14H24O3Si2	296
18	39.110	Barbituric acid, 5-allyl-5-(cyclohex-2-en-1-yl)-	C13H16N2O3	248
19	40.120	Cyclohexanone, 2,6-bis (2-methylpropylidene)-	C14H22O	206
20	40.185	beta.-Sitosterol, TMS derivative	C32H58OSi	486

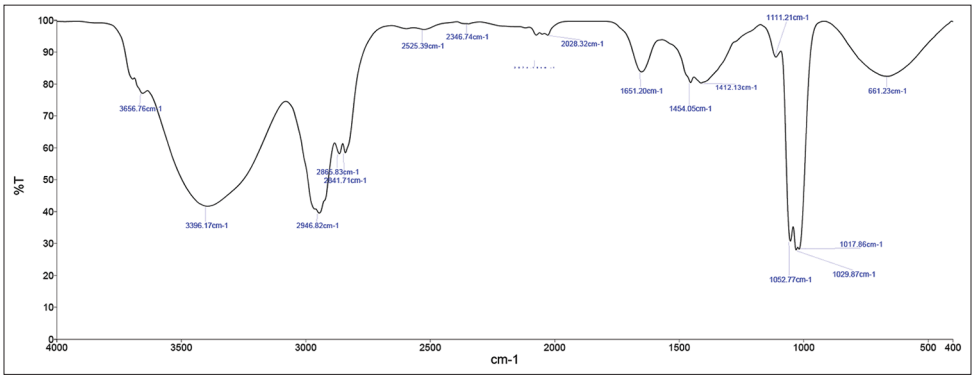

Figure 3: FT-IR chromatogram of *W. tinctoria* leaf extract

at were found at peak value of 2346.74 cm^{-1}; Miscellaneous chromophoric groups Alcholosand phenols, chelate compounds were found at 2028.32 cm^{-1}; at peak of 1651.20 cm^{-1} C–C Multiple bond stretching, Alkene, disubstituted, gem were absorbed; Hydrocarnon chromophore, C–H Bending, alkane, CH2– were found at the peak of 1454.05 cm^{-1}; at peak of 1412.13 cm^{-1}Alkene, disubstituted, gem were found; Sulfur compounds, C=S Stretching vibrations were absorbed at 1111.12 cm^{-1}; Sulfur compounds, S=O Stretching vibrations, sulfoxides were found at 1052.77 cm^{-1}; at the peak of 1029.87 cm^{-1} Amines, C–N Vibration, Aliphatic groups were absorbed, Halogen compounds, C–X, Stretching vibration were absorbed at the peak of 1017.86 cm^{-1}; at the peak of 661.23 cm^{-1} C–F and Hydrocarbon chromophore, C–H bending, Alkyne were absorbed (Figure 3 and Table 3).

W. tinctoria with methanol extract displayed recognisable absorption bands at peak of 3693.42 cm^{-1} are Amines, N – H Stretching vibrations primary, free, two bands; Amines Imines (=N–H); one bands at peak of 3399.73 cm^{-1}, Miscellaneous chromophoric groups Alcholosans phenols, chelate compounds at peak of 2979.95 cm^{-1}, Miscellaneous chromophoric groups Alcholos and phenols, chelate compounds at peak of 2965.09 cm^{-1}; Hydrocarbon chromophore, C–H Stretching, alkane were absorbed at the peak of 2948.84 cm^{-1}; Hydrocarbon chromophore, C–H Stretching, alkane showed their presence at the peak of 2922.26 cm^{-1}; at the peak of 2864.00 cm^{-1} Aldehydes, C–H Stretching vibration were found; Aldehydes, C–H Stretching vibration were present at the peak of 2842.72 cm^{-1}; Carboxylic acids, hydroxyl stretching (bounded) several bands were found at 2524.64 cm^{-1}, at peak of 2075.22 cm^{-1} Unsaturated Nitrogen Compounds, C–N Stretching vibrations, isocyonides are found; Alkyne, monosubstituted groups are found at peak of 2052.47 cm^{-1}, at peak of 2026.01 cm^{-1} Miscellaneous chromophoric groups Alcoholsand phenols, chelate compounds are present; C–C Multiple bond stretching, Alkene, disubstituted, gem are found in 1650.78 cm^{-1}; at 1453.66 cm^{-1} Hydrocarnon chromophore, C–H Bending, alkane, CH_2 are identified; at peak range of 1412.61 cm^{-1} Alkene, disubstituted, gem were found; Sulfur compounds, C=S

Stretching vibrations are identified at peak of 1113.15 cm^{-1}; Sulfur compounds, S=O Stretching vibrations, sulfoxides are present at the peak range of 1052.10 cm^{-1}; also at peak of 1032.22 cm^{-1} Sulfur compounds, S=O Stretching vibrations, sulfonic acids are found; Halogen compounds, C–X, Stretching vibration, C–F are found at peak of 1017.80 cm^{-1} and at peak of 691.936 cm^{-1}Alkene, disubstituted, *cis* are identified (Figure 4 and Table 4).

In this study, the concentration of the analysed plant extracts capable of Hydrogen peroxide scavenging (H_2O_2) assay (%) activity was also determined. The results showed that highest values of (21.1±3.68) 400 µg/mL in aqueous extract and (19.3±1.21) 500 µg/mL in methanol extract of *W. tinctoria* leaf. The *W. tinctoria* bark results were expressed in (18.3±1.21) 400 µg/mL in aqueous extract and (17.2±5.69) 400 µg/mL in methanol extract (Table 5). The DPPH method was used to measure the antioxidant activity of the plant extracts against the aqueous extract and methanolic extract of *W. tinctoria* plant. Whereas the aqueous extract of *W. tinctoria* leaf showed maximum values at range of (25.5±9.12) 400 µg/mL and (15.1±8.19) 500 µg/mL in methanolic extract. The *W. tinctoria* bark results showed that the maximum values for aqueous extract (15.0±1.37) at 300 µg/mL and methanol extract of (16.4±2.40) in 500 µg/mL. The Thiobarbutaric acid test concentration showed that the aqueous extract of *W. tinctoria* leaf produced significantly higher absorbance value of (18.4±4.64) 500 µg/mL and methanolic extract showed value of (19.5±4.29) 500 µg/mL respectively. *W. tinctoria* bark aqueous extract exhibited (19.5 ±7.43) 500 µg/mL and methanol extract exhibited the result of (14.9±8.15) 300 µg/mL which is found to be the higher absorbance values.

DISCUSSION

The *Withania somnifera* whole plant's GC fraction of the methanolic extract chromatogram displayed the peaks of a variety of substances which is shown by Debendranath and Sunita (2020). The existence of numerous components with differing retention duration was confirmed by the GC-MS spectra. To determine the type and structure of a compound and the mass spectrometer examines the compounds that have been eluted at various periods. Numerous phytochemicals were found in the ethanolic extract of *Phyllodium pulchellum* leaf

Table 3: FT–IR analysis of *W. tinctoria le*af extract

S. No.	Group frequency cm^{-1} of the sample	Functional group assignment
1	3656.76	Acye Halide Stretching Vibrations, Miscellaneous chromophoric groups, Alcohols and phenols, O – H stretching hydrogen bonded (change on dilution)
2	3396.17	Amines, Secondary, free; one band Imines (= N – H) ; one band
3	2946.82	Hydrocarbon chromophore, C–H Stretching, alkane
4	2865.83	Hydrocarbon chromophore, C–H Stretching, alkane
5	2841.71	Aldehydes, C–H Stretching vibration
6	2525.39	Carboxylic acids, hydroxyl stretching (bounded) several bands
7	2346.74	Carboxylic acids, several bands
8	2028.32	Miscellaneous chromophoric groups Alcholosans phenols, chelate compounds
9	1651.20	C–C Multiple bond stretching, Alkene, disubstituted, gem
10	1454.05	Hydrocarnon chromophore, C–H Bending, alkane, CH2–
11	1412.13	Alkene, disubstituted, gem
12	1111.12	Sulfur compounds, C=S Stretching vibrations
13	1052.77	Sulfur compounds, S=O Stretching vibrations, sulfoxides
14	1029.87	Amines, C–N Vibration, Allphatic
15	1017.86	Halogen compounds, C–X, Stretching vibration, C–F
16	661.23	Hydrocarbon chromophore, C–H bending, Alkyne.

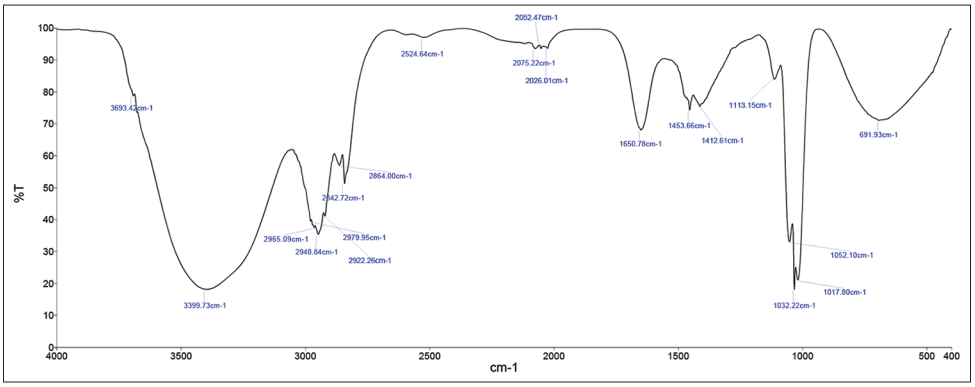

Figure 4: FT-IR chromatogram of *W. tinctoria* bark extract

Table 4: FT-IR analysis *W. tinctoria* bark extract

S. No.	Group frequency cm^{-1} of the sample	Functional group assignment
1	3693.42	Amines, N – H Stretching vibrations primary, free; two bands
2	3399.73	Amines Imines (=N–H); one bands
3	2979.95	Miscellaneous chromophoric groups Alcholosans phenols, chelate compounds
4	2965.09	Miscellaneous chromophoric groups Alcholosans phenols, chelate compounds
5	2948.84	Hydrocarbon chromophore, C–H Stretching, alkane
6	2922.26	Hydrocarbon chromophore, C–H Stretching, alkane
7	2864.00	Aldehydes, C–H Stretching vibration
8	2842.72	Aldehydes, C–H Stretching vibration
9	2524.64	Carboxylic acids, hydroxyl stretching (bounded) several bands
10	2075.22	Unsaturated Nitrogen Compounds, C–N Stretching vibrations, isocyonides
11	2052.47	Alkyne, monosubstituted
12	2026.01	Miscellaneous chromophoric groups Alcholosans phenols, chelate compounds
13	1650.78	C–C Multiple bond stretching, Alkene, disubstituted, gem
14	1453.66	Hydrocarnon chromophore, C–H Bending, alkane, CH2–
15	1412.61	Alkene, disubstituted, gem
16	1113.15	Sulfur compounds, C=S Stretching vibrations
17	1052.10	Sulfur compounds, S=O Stretching vibrations, sulfoxides
18	1032.22	Sulfur compounds, S=O Stretching vibrations, sulfonic acids
19	1017.80	Halogen compounds, C–X, Stretching vibration, C–F
20	691.936	Alkene, disubstituted, *cis*

Table 5: Antioxidant activity of *W. tinctoria* leaf and bark extracts

Name of the Extracts		Antioxidant activity (% of inhibition)		
W. tinctoria	Concentrations (µg/mL)	H_2O_2	DPPH	TBA
Leaf aqueous extract				
	100	14.2±3.75	14.1±1.15	13.0±6.00
	200	15.9±4.20	21.0±7.74	14.0±0.30
	300	16.1±2.06	23.4±5.16	14.3±0.17
	400	21.1±3.68	25.5±9.12	17.4±4.20
	500	19.5±9.46	25.2±5.59	18.4±4.64
Leaf methanolic extract				
	100	14.6±0.28	12.7±4.44	13.9±3.98
	200	15.3±9.00	13.0±6.69	16.6±0.43
	300	15.9±8.26	13.3±2.31	17.6±1.31
	400	19.3±1.21	15.1±8.19	19.4±6.84
	500	18.1±5.96	14.5±7.97	19.5±4.29
Bark aqueous extract				
	100	12.6±9.53	11.8±6.56	12.2±7.32
	200	14.8±2.19	12.1±2.67	13.6±7.67
	300	17.8±6.57	15.0±1.37	16.8±3.31
	400	18.3±1.21	14.1±1.15	19.5±7.43
	500	17.8±8.90	13.5±0.37	17.7±1.03
Bark methanolic extract				
	100	10.4±2.26	12.3±2.61	12.0±6.48
	200	11.3±5.60	13.0±9.53	12.2±0.59
	300	12.2±8.94	14.0±0.70	14.9±8.15
	400	17.2±5.69	14.7±1.93	14.1±7.01
	500	13.6±4.49	16.4±2.40	12.4±0.48

which strongly contributed to the plant's therapeutic bioactive compounds according to a GC-MS analysis (Velmurugan & Anand, 2017). A total of 15 peaks representing the bioactive compounds were identified in the GC-MS chromatogram of the methanol and ethyl acetate leaf extracts of *A. nilgiricum* by comparing their peak retention time, peak area (%), height (%), and mass spectral fragmentation patterns to those of the recognized compounds listed in the National Institute of Standards and Technology (NIST) library. Results showed that methanol and ethyl acetate extracts of A. *nilgiricum* leaf contained 9 and 6 chemicals respectively (Konappa *et al.*, 2020). From ethanolic extracts of *T. dioica* leaf and fruit the GC-MS mass spectra detected some of the compounds. Mass matching of the spectrums of prominent compounds was done with the spectrum of standard compounds of NIST library present in the experimental samples (Kavitha, 2022). The GC-MS study of the methanol fraction of *Hibiscus asper* leaves revealed a total of 23 substances with different phytochemical activities and a chromatogram was displayed by (Olivia *et al.*, 2021).

FTIR analysis of *Rhynchosia minima* leaves showed lipid, protein, phosphate ions, carboxylic acid, hydroxyl compound and aliphatic bromo compound (Patil & Jadhav, 2020). The characteristic absorption band was displayed at 2865-2845 cm^{-1} (for C-H stretching), for C-H bending at 1492 cm^{-1} and at 1700-1725 cm^{-1} for carbonyl groups (C=O) which was showed by CF extract. The ME extract of *Wedelia biflora* displayed characteristic absorption bands at 1410-1310 cm^{-1} for a hydroxyl (-OH) group and at 2929 cm^{-1}, 2850-2815 cm^{-1} for C-H stretching. The typical absorption bands were exposed at 2865-2845 cm^{-1} for C-H stretching also for the C-H group and at 1740-1725 cm^{-1} for the carbonyl group (C=O) which was exhibited by CF extract. The ME extract of *Wedelia biflora* showed characteristic absorption bands at 3400-3200 cm^{-1} and 1055 cm^{-1} for a hydroxyl (-OH) group, and 2970-2950 cm^{-1} for C-H stretching and at 800-700 cm^{-1} for C-Cl stretch (Sahayaraj *et al.*, 2015). The distinctive FTIR PAS and DRIFT spectra were performed for five different herbals including chamomile, silver birch, Hibiscus, peppermint and corn flower. At two spectral areas 850–1850 cm^{-1} and 2700–3200 cm^{-1}, the organic matter and bonds in the sample are visible in the FTIR spectra of the examined HMs (Brangule *et al.*, 2020). Multiple functional groups including amines, phenols, alkenes, alcohols, aliphatic compounds, carboxylic acids, esters and carbonyl compounds were identified using FT-IR spectroscopy. The extracts from

ethanol, methanol, and ethyl acetate are represented by indicative FT-IR spectra. The bands at 3465, 3458 and 3460 cm^{-1} were connected to the vibration of the stretched hydroxy (-OH) groups (Ayalew, 2020).

Generally, in absorbance values at a wavelength of 700 nm, the methanolic extracts of three studied medicinal plants exhibited remarkable concentration-dependent increases. DPPH radical scavenging activities of methanolic extracts of *C. volkensii*, *V. lasiopus* and *A. hockii* has demonstrated remarkable in vitro DPPH radical scavenging activity in a dose-dependent manner (Guchu *et al.*, 2020). At times of 2, 4, and 6 h for all extracting solvents and the extraction methods the data referring to the mean antioxidant activity of the tests using t-test application for the comparison of means value is undergone (Oleinik *et al.*, 2022). 3 mL of reagent solution containing 50, 100, and 200 g/mL of MECM in methanol was added with 0.6 mL H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added to test solutions containing (Rajasree *et al.*, 2021). Most of the plants extracts at dissimilar concentrations exhibited more than 70 % scavenging activity (Mistry *et al.*, 2021).

CONCLUSION

The current research demonstrates a considerable similarity between GC-MS of *W. tinctoria* leaf and bark extracts, which include a variety of powerful bioactive chemicals recommended as a plant of phytopharmaceutical importance. FTIR analysis of *W. tinctoria* leaf and bark extract possesses different functional groups including phenols, amines, alcohols, alkenes, carboxylic acids, aliphatic compounds, carbonyl compounds, and esters which were revealed using FT-IR spectroscopy. Our study demonstrates that the antioxidant hydrogen peroxide scavenging assay, DPPH assay, and Thiobarbutaric acid assay showed maximum values in the methanolic extract of *W. tinctoria* leaf, and also highest value is found in the aqueous extract of *W. tinctoria* bark.

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