



Isolation And Analysis Of Phytochemical Constituents Of *Wrightia Tinctoria*

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Abstract: *Wrightia tinctoria*, an important traditional medicinal plant is exploited for treating several diseases. The present study intends to reveal the presence of phytochemicals and test some biological activities of *Wrightia tinctoria* leaf extracts. The methodology includes identification and collection of *Wrightia tinctoria* leaves, preparation of leaf powder, isolation by Soxhlet extraction method, preliminary phytochemical screening and determination of antibacterial, anti-diabetic and anti-inflammatory activities on leaf extract. Results revealed the presence of more active metabolites in the ethanol plant extract of *Wrightia tinctoria* leaves which may be the reason for the promising antibacterial potential against the bacterial strains. Its constituents are of utmost interest to pharmaceutical industries owing to their many actions and biological activities.

Keywords: *Wrightia tinctoria*, Soxhlet extraction, Phytochemical screening, Antibacterial activity,

I. Introduction

Wrightia tinctoria is known by the names “pala indigo” and “indrajao,” and belongs to the Apocyanaceae family. It is extensively used in the treatment of skin diseases and liver disorders, and possesses broad spectrum biological activities¹. It is widely recognized medicinal plant of high significance in traditional healing². It is distributed throughout India up to 1200 m altitude³. In south India, it is very well recognized as the jaundice curing tree but it is also employed to treat other diseases. Tooth ache, as a result of decay, is relieved by filling the tooth cavity with pulverized fresh leaves. Fresh leaves of the plant may also be mixed with coconut oil to prepare “777 oil”, a medication which is used in the management of psoriasis⁴. The bark and seeds are effective against psoriasis and nonspecific dermatitis. It has antidandruff actions and is therefore employed in hair oil preparations⁵. *Wrightia tinctoria* is popularly anticipated as a therapeutic agent, owing to its varied pharmacological actions. It has been observed to have anti-inflammatory, antidiabetic, antinociceptive, hepatoprotective, antibacterial, antifungal, antiviral, antipsoriatic, anticancerous, anthelmintic, aphrodisiac, analgesic as well as antipyretic activities⁶.

Plant parts such as leaves, seeds, and fruits are consumed as vegetables. Indigenous people also utilize various parts of *Wrightia tinctoria* for dietary requirements. To coagulate milk, juice extracted from fresh unripe fruits is used. Its wood is utilized in furniture production due to its high quality and value. It also provides indigo-like blue dye which is a glycoside obtained from its leaves, flowers, fruits, and roots. Seeds of the plant exhibit aphrodisiac and anthelmintic properties. Ailments like psoriasis, stomach pain, toothache, and dysentery are managed by bark and leaf preparations. Leaves are used to ease tooth pain which occurs while chewing and can stop bleeding if used with salt and the milky juice of the plant⁷. In light of the above information, we were prompted to carry out extraction of *Wrightia tinctoria* leaves followed by screening its some of biological activities. The phytoconstituents found in the methanol extracts were analyzed by gas chromatography-mass spectrometry (GC-MS)

II. Experimental:

All required chemicals were procured from s d fine and all solvents used in the work were of analytical grade. Fluorescence measurements were performed by using Hitachi F-4500 spectrofluorimeter (JAPAN) equipped with a 150W Xenon lamp and 1 cm quartz cell. The widths of excitation and emission slits were set at 5.0 and 10 nm, respectively, and the scan rate was 1200 nm min⁻¹. The Thermo Scientific Nicolet Summit X FTIR Spectrometer with Thermo Scientific OMNIC Paradigm Software was used for taking the FTIR spectra of the the leaves extract.

GC-MS analysis of the extract was performed using a Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m x 0.25 mm ID x 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 2 ml/min. The injector temperature was set at 220°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 50°C (2 min); followed by 150°C at the rate of 15°C min⁻¹ and 150°C, where it was held for 2min and then followed by 250°C at the rate of 30°C min⁻¹, it was held for 8.00 min. The mass detector conditions were: Inlet line temperature 250°C, ion source temperature 230°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2014) library.

Anti-diabetic property of the given sample was determined spectrophotometrically at 540nm, α -amylase causative agent to release glucose by the hydrolysis of starch substrate blocking ability is tested for the samples. Anti-inflammatory property of the given sample was determined spectrophotometrically at 560nm. The antifungal activity was carried out by well diffusion method

Preparation of crude extract

Healthy, disease-free leaves of *Wrightia tinctoria* were collected from Maharani Cluster University campus, Bangalore, India. Freshly collected leaves were brought to the laboratory and washed with distilled water. Leaves were kept in Petri dishes and left at room temperature for drying. Then, the dried leaves were crushed in a mortar pestle. The dried powder (30g) was packed in a filter paper and loaded in a Soxhlet apparatus, with 200 cm³ of 90 % ethanol at 78°C. After exhaustive extraction, the solvent was filtered through Whatman 41 filter paper and concentrated and dried at low temperature. The extract was further purified by TLC and Column chromatographic methods. After purification the extract was used for various analyses.

Purification using thin layer chromatography method: A thin line was drawn on the activated TLC plate about 1.5 cm above the bottom. A spot of the extract was placed on the line and allowed to dry. This was followed by a repeated addition of the extract on the same spot. In a developing chamber, a mixture of petroleum ether and acetone in the ratio of 8.5:1.5 was taken and allowed the chamber for saturation. The TLC plate was placed inside the developing chamber and the TLC chamber was covered. The solvent mixture was allowed to rise on the plate till it reached 1.5 cm close to the top.

Results and Discussion

Thin layer chromatographic method

After complete elution, the TLC plate was then taken out from the chamber, the distance moved by the solvent is marked and the plate was air dried. The distance moved by different spots as well as the distance moved by the solvent also measured and the R_f values of different spots were calculated (Table 1).

Table 1: R_f values of different spots of leaf extract of *Wrightia tinctoria*

Spots	Distance travelled by solvent in cm	Distance travelled by solute in cm	Retention factor (R _f)
1	7.3	4.2	0.57
2	7.3	4.6	0.63
3	7.3	5.1	0.69
4	7.3	5.5	0.75
5	7.3	5.8	0.79

Preliminary phytochemical screening: The extracts were used for qualitative identification of various phytochemical constituents as per the standard procedures^{8,9}.

In the present study, occurrence of wide range of active phytochemical compounds such as flavonoids, glycosides, phenols, tannins, saponins, anthracyanin, coumarin, terpenoids, sugar, and cholesterol were seen in ethanol extracts indicated that these compounds were not disturbed during the preparation and storage.

Ethanol is one of the good solvents in plant extractions which include low toxicity, inability to cause the extract to complex or dissociate easy evaporation at low heat, preservative action and stable. Hence the ethanol extract of leaf of *Wrightia tinctoria* was used for further investigation which includes identification of pharmacologically active phytochemical compounds which were evaluated qualitatively and summarized in Table 2

Table 2: Qualitative phytochemical screening of leaf extract of *Wrightia tinctoria*

TEST	PROCEDURE	INFERENCE	RESULT
Flavanoids	Extract + 2% NaOH + few drops of dil.HCl	An intense yellow colour becomes colourless on addition dil.HCl	+ve
Phenolic	Aqueous extraction + 5% FeCl ₃	Dark green colour	+ve
Reducing Sugar	Extract + 1ml water + few drops of aq.NaOH Solution	Yellow colour	+ve
Alkaloids (Dragendroff's test)	Extract + dragendroff's reagent	Reddish brown precipitate	-ve
Picric Acid	Extract + iodine solution	A blue colour which disappears on boiling re appear on cooling	-ve
Cardiac glycosides	Extract + Pyridine + sodium nitroprusside + 20% NaOH	A red colour fades to brownish yellow	+ve
Phlobatannins	Extract + 1% HCl	Red colour precipitate	-ve
Tannins (Braymer's test)	Extract + distilled water + 10% FeCl ₃	Blue green	+ve
Carbohydrates (Molish test)	Extract + alc.α -Naphthol + 1ml of con.H ₂ SO ₄ (along the sides of the test tube)	Violet ring	+ve
Cholesterol	Extract + chloroform + acetic anhydride + 2-3 drops of con.H ₂ SO ₄	Red rose colour	+ve
Quinone	Extract + Conc.HCl	Green colour	+ve
Anthracyanin	Extract + 2N HCl + few ml Ammonia	Pink – red Solution which turns blue violet after addition of ammonia	-ve
Coumarin	Extract + 10% NaoH + chloroform	Yellow colour	+ve
Terpenoids	Choloform + Extract (Evaporated on water bath) + 3ml Con.H ₂ SO ₄ (boiled on water bath)	A grey coloured solution	+ve

From the Table 2, it was evident that a wide range of active compounds like flavonoids, glycosides, phenols, tannins, saponins, anthracyanin, coumarin, terpenoids, sugar, and cholesterol were present in the ethanol extract of leaf of *Wrightia tinctoria*.

Fluorescence analysis

Fluorescence analysis is effectively sensitive which can be used to characterize the crude drugs¹⁰. The fluorescent colour is specific for each compound. The crude drug when viewed under UV light showed different fluorescence at different wavelengths. This is due to the presence of different phytochemical constituents in the drug^{11,12}. In the present study, the fluorescent analysis clearly showed that the powdered leaf of *Wrightia tinctoria* exhibited clear fluorescence behaviours at 660 nm after excitation at 450 nm (Figure 1). different radiations. This fluorescence behaviour may be due to the presence of bioactive compounds like Tannins or Quinones¹³.

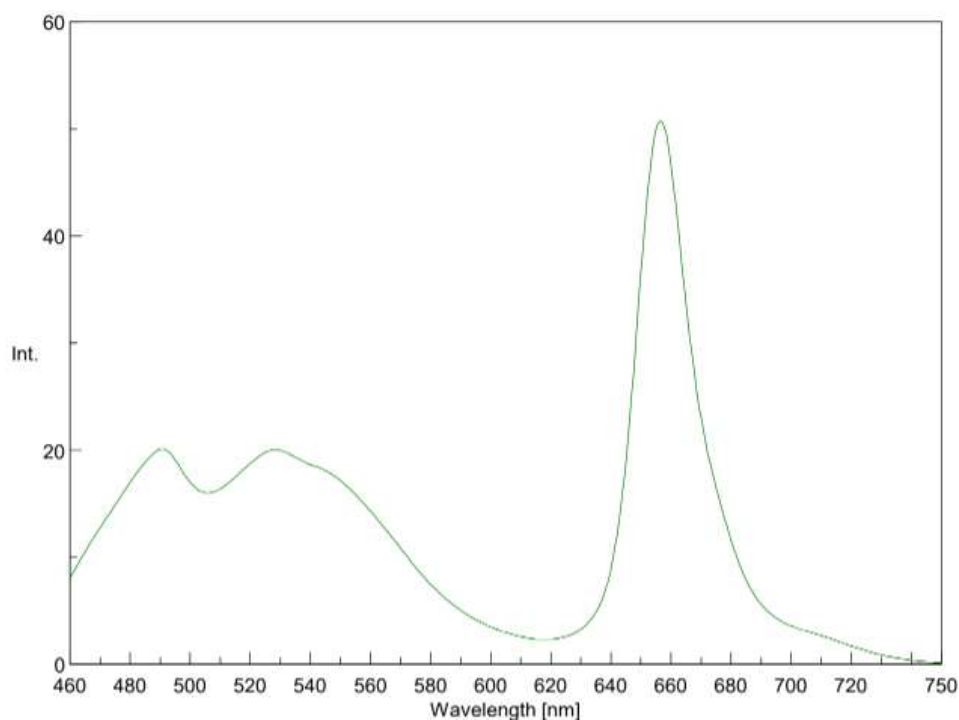


Figure 1: Fluorescence spectra of ethanolic extract of *Wrightia tinctoria* leaf

FT-IR spectroscopy study

FT-IR spectroscopy was used to detect the characteristic peaks and their functional groups of plant extracts. The crude extract of leaf of *Wrightia tinctoria* was subjected to FT-IR analysis. The functional groups of the components were separated based on its peak ratio. The FT-IR spectrum chromatogram profile of ethanolic extract of leaf of *Wrightia tinctoria* was illustrated in Figure 2. The characteristic FT-IR peak values and functional groups were depicted in Table 3.

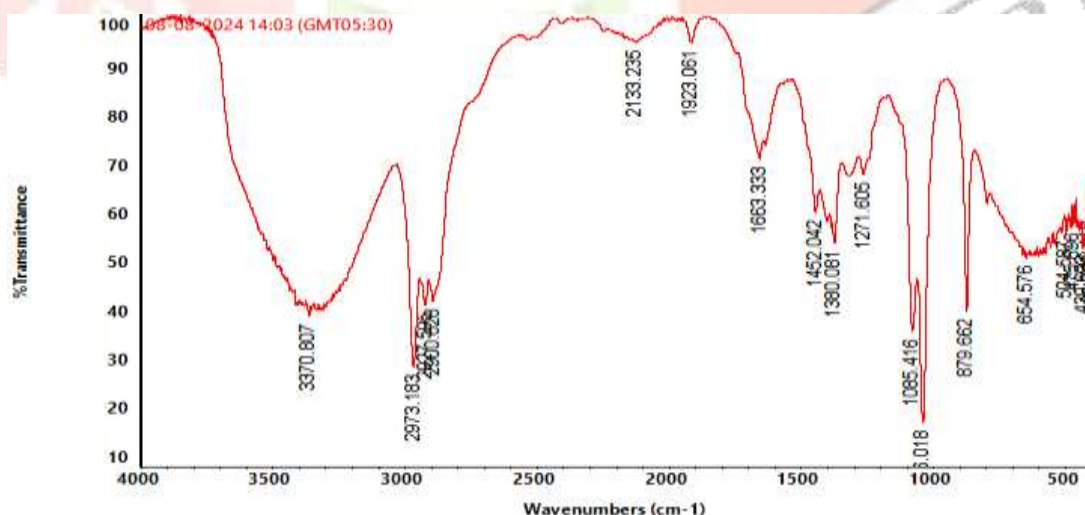


Figure 2: FT-IR chromatogram of ethanolic extract of *Wrightia tinctoria* leaf

FT-IR is proved to be a reliable and sensitive method used for identification of different bonds. Secondary metabolites functional groups were separated based on its peak values in the region of infrared radiation¹⁴. The spectra showed substantial overlap of each absorption spectrum of various components, each band represents an overall overlap of some characteristic absorption peaks of functional groups in the test sample. In the current study, the FT-IR analysis of ethanolic extract of leaf of *Wrightia tinctoria* showed different peaks. It confirmed the presence of different functional groups such as alcohols, phenolics, primary alcohols, alkanes, alkynes and ketones which might be a perfect product for any type of pharmaceutical applications. The above (Table: 3) infrared functional group characteristics were cited in literature^{15,16}.

Table 3: FT-IR peak values and functional groups of ethanolic extract of leaf of *Wrightia tinctoria*

Wavelength in cm^{-1}	Functional groups
3370 cm^{-1}	O-H Stretching frequency, indicating phenolic group
2973 cm^{-1}	C-H stretching frequency, indicating alkyl chains
2900 cm^{-1}	C-H stretching frequency, indicating of methyl groups
1923 cm^{-1}	C=O stretching frequency, indicating carbonyl group
1663 cm^{-1}	C=C stretching frequency, indicating of unsaturated compounds
1271 cm^{-1}	C-O stretching frequency, indicating ether linkages
879 cm^{-1}	C-H bending, indicating of the aromatic rings

GC-MS analysis

GC-MS analysis is used to determine whether the plant species contains any individual compound or group of compounds and also used to understanding the nature of active principles in medicinal plants. The heights of the peak indicate the relative concentrations of the components present in the extract. Different phytochemical compounds of the ethanolic extract of leaf of *Wrightia tinctoria* was examined by using GC-MS. The chromatogram of the extract was revealed in Figure 3 and summarized in Table 4.

GC-MS study of ethanolic leaf extract of *Wrightia tinctoria* revealed the presence of ten largest peaks in chloral and n-Hexadecanoic acid. The major chemical constituents include Tetra decanoic acid (10.2%), Tridecanoic acid (7.41%), Dodecanoic acid (6.83%), Pentadecanoic acid (5.23%) and Chloral hydrate (24.7%), Trichloromethane (15.5%), Trichloroacetic acid (3.64%), Dichloromethanesulphonyl chloride (0.49%), Trichloroacetamidoxime (0.32%). GC-MS analysis of leaf extract shows 10 peaks at 82, 84, 47, 29, 111, 83, 113, 48, 85, and 35 in chloral and 60, 73, 57, 43, 55, 41, 129, 71, 69, and 83 in n-Hexadecanoic acid.

Table 4: Bioactive compounds present in ethanolic extract of *Wrightia tinctoria* leaf extract.

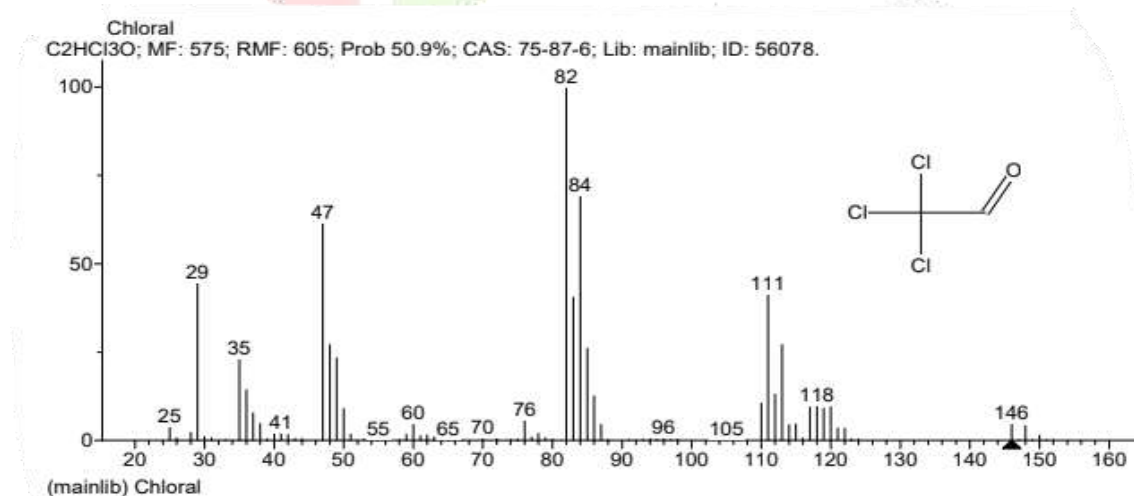
Name	Molecular formula	Molecular weight	% Probability	Compound nature	Biological importance
Chloral	$\text{C}_2\text{HCl}_3\text{O}$	145.90	50.9	Trichloroacetaldehyde	Sedative effect Anesthesia, Psychoactive effect, pesticide.
Chloral hydrate	$\text{C}_2\text{H}_3\text{Cl}_3\text{O}_2$	163.91	24.7	Trichloralacetaldehyde	Xenobiotic
Trichloromethane	CHCl_3	117.91	15.5	-	Antibiotic, anesthetic
Trichloroacetic acid	$\text{C}_2\text{HCl}_3\text{O}_2$	162	3.64	Monocarboxylic acid	Herbicide
Methane oxybis[dichloro]	$\text{C}_2\text{H}_2\text{Cl}_4\text{O}$	182	1.99	Sulfonyl chloride	Natural gas
Dichloromethanesulphonyl chloride	$\text{CHCl}_3\text{O}_2\text{S}$	181.87	0.49	Sulphonyl	Synthesis of sulphonamides, pharmaceutical
Trichloroacetamidoxime	$\text{C}_2\text{H}_3\text{Cl}_3\text{N}_2\text{O}$	176	0.32	Acetamide group	Herbicide, potential in drug

Acetic acid trichloro-ethyl ester	$C_4H_5Cl_3O_2$	190	0.24	Acetic acid ester	Anesthetic, Neurotransmitter
Tetraborane(10)	B_4H_{10}	54	0.14	Boron hydride	Antimicrobial Fungicide
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	48.8	Fatty acid	Cardiovascular, Neurodegenerative diseases
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	10.2	Fatty acid	Metabolic effect, Antimicrobial properties
Tridecanoic acid	$C_{13}H_{26}O_2$	214	7.41	Fatty acid	Gut health, Immunomodulatory agent
Decanoic acid	$C_{12}H_{24}O_2$	200.17	6.83	Fatty acid	Antimicrobial, Gut health
Pentadecanoic acid	$C_{15}H_{30}O_2$	242	5.23	Fatty acid	Anti-inflammatory, Antimicrobial Activity
L-(+)-Ascorbic acid 2, 6 dihexadecanoate	$C_{38}H_{68}O_8$	652	3.17	Sugar acid	Antioxidant, tuberculosis, skin infection
Eicosanoic acid	$C_{20}H_{40}O_2$	312	3.05	Fatty acid	Skin and hair Health, Anti-inflammatory effects
Undecanoic acid	$C_{11}H_{22}O_2$	186	2.69	Fatty acid	Antimicrobial Activity,
Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.74	Fatty acid	Cardiovascular disease prevention
Isopropyl palmitate	$C_{19}H_{38}O_2$	298	1.26	Ester of isopropyl alcohol	Antimicrobial
3-O-Methyl-D-glucose	$C_7H_{14}O_6$	194	30.4	Carbohydrate	Glucose uptake Regulation, Antidiabetic
3,4-Di-O-methyl-L-arabinopyranose	$C_7H_{14}O_5$	178	8.12	Carbohydrate	Antioxidant Activity
Methyl-4,6-di-O-methylmannoside	$C_9H_{18}O_6$	222	7.49	Carbohydrate	Glycosylation
2-O-Methyl-D-mannopyranosa	$C_7H_{14}O_6$	194	6.04	Carbohydrate	Glycosylation
2-O-Methyl-D-xylose	$C_6H_{12}O_5$	164	6.04	Carbohydrate	Glycosylation
2-[2-(2-Butoxyethoxy) ethyl acetate	$C_{12}H_{24}O_5$	248	4.62	Ethoxide	Antimicrobial Activity, Anaesthetic
α -D-Xylofuranoside,	$C_7H_{14}O_5$	178	3.26	Hydrocarbon	Glycosylation

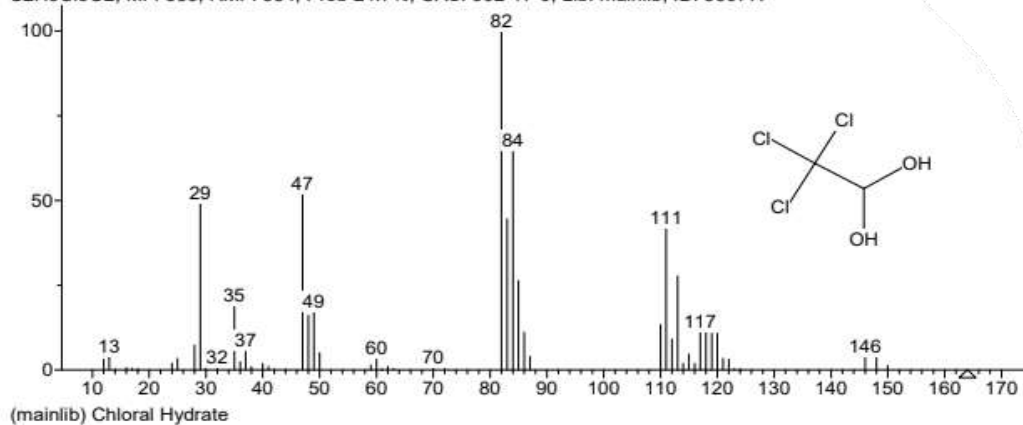
methyl-3-O-methyl							
Ethanol,2-(2-butoxyethoxy)-acetate	C ₁₀ H ₂₀ O ₄	204	3.01	Hydrocarbon	Antimicrobial		
4-O-Methylmannose	C ₇ H ₁₄ O ₆	194	2.89	Carbohydrate	Glycosylation		
α – D-Xylofuranoside, methyl 2-O-methyl	C ₇ H ₁₄ O ₅	178	2.33	Hydrocarbon	Glycosylation		
Oleic acid	C ₁₈ H ₃₄ O ₂	282	13.3	Fatty acid	Hormone Regulation		
9-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	12.7	Fatty acid	Hormone Regulation		
Trans-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	9.50	Fatty acid	Energy Production, Lipid metabolism		
Cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282.25	9.50	Fatty acid	Lipid metabolism		
Cis-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282.25	8.77	Fatty acid	Neurological Function	MS	GC-mass spectra
6-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	2.67	Fatty acid	Anti-inflammatory therapies		detected some bioactive compounds
E-9-Tetradecenoic acid	C ₁₄ H ₂₆ O ₂	226	2.25	Fatty acid	Immuno-modulator		from ethanolic extract of
9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	2.17	Fatty acid	Wound healing treatment		<i>Wrightia tinctoria</i> leaf.
Cyclopentadecanone, 2-hydroxy	C ₁₅ H ₂₈ O ₂	240	1.83	Ketone	Anti-inflammatory		Mass matching of the
Cis-10-Nonadecenoic acid	C ₁₉ H ₃₆ O ₂	296	1.83	Fatty acid	Skin care		spectrums of

prominent compounds present in the experimental sample was done with the spectrum of standard compounds of NIST library as shown in Figure 3.

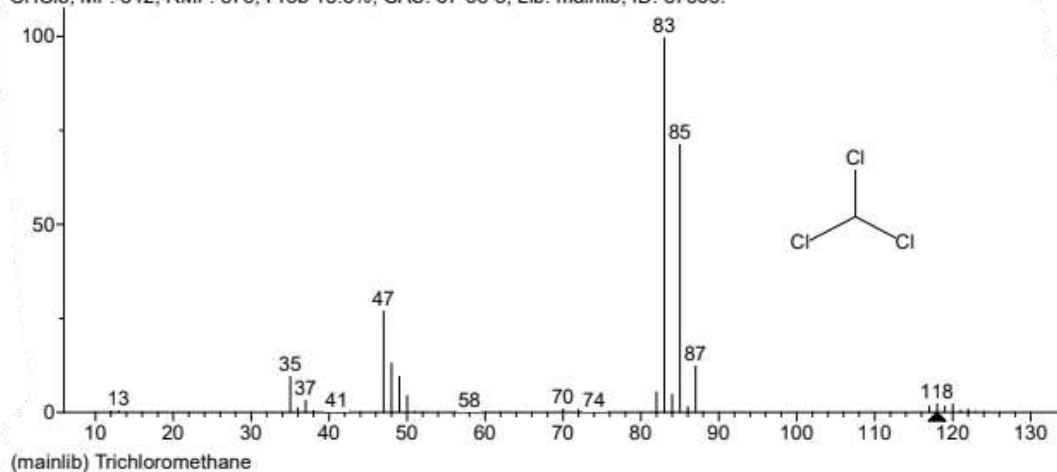
Figure 3: GC-MS mass spectra of a few compounds detected from ethanolic extract of *Wrightia tinctoria* leaf extract.



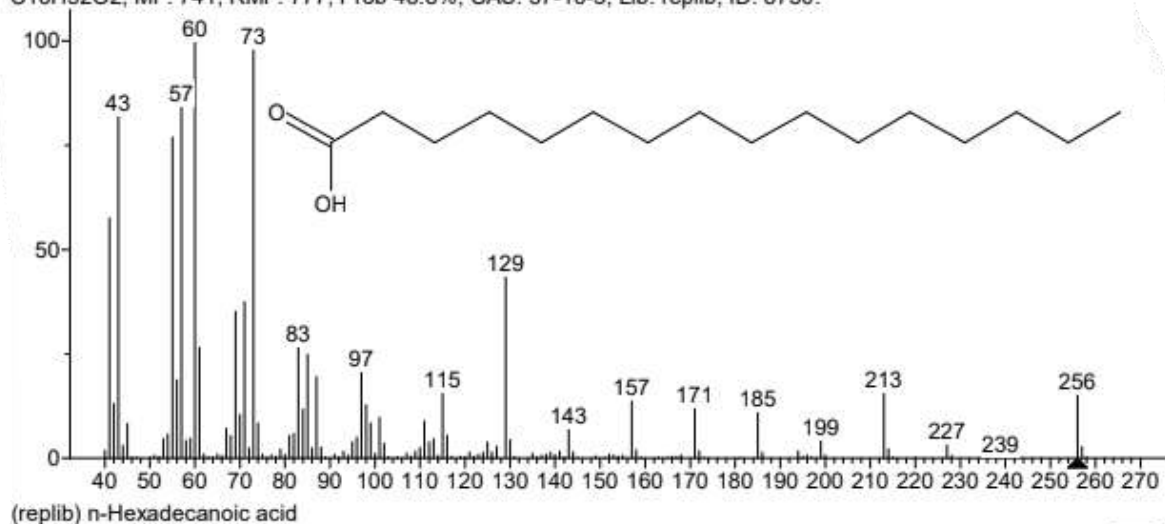
Chloral Hydrate
C₂H₃Cl₃O₂; MF: 556; RMF: 584; Prob 24.7%; CAS: 302-17-0; Lib: mainlib; ID: 56077.

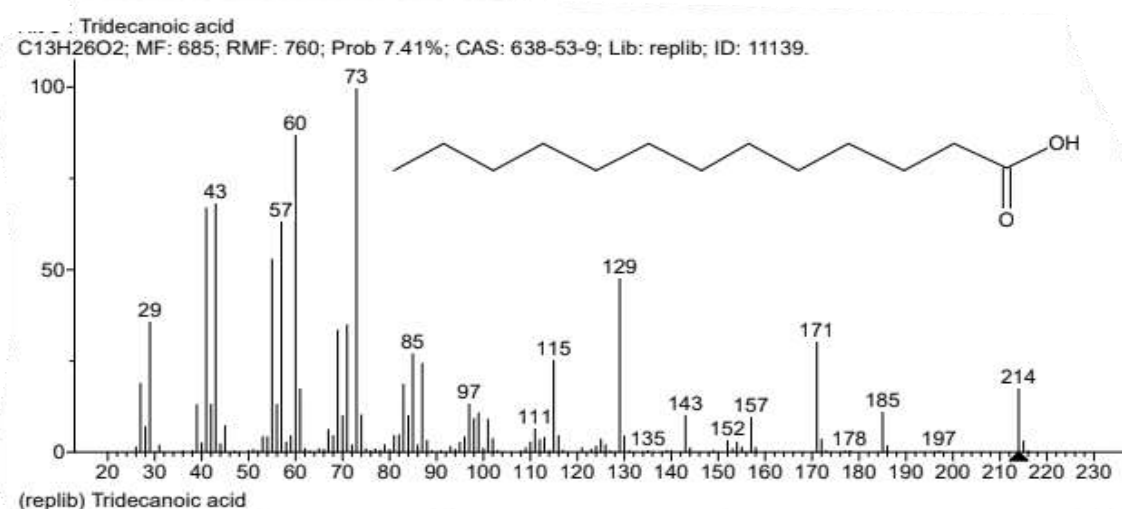
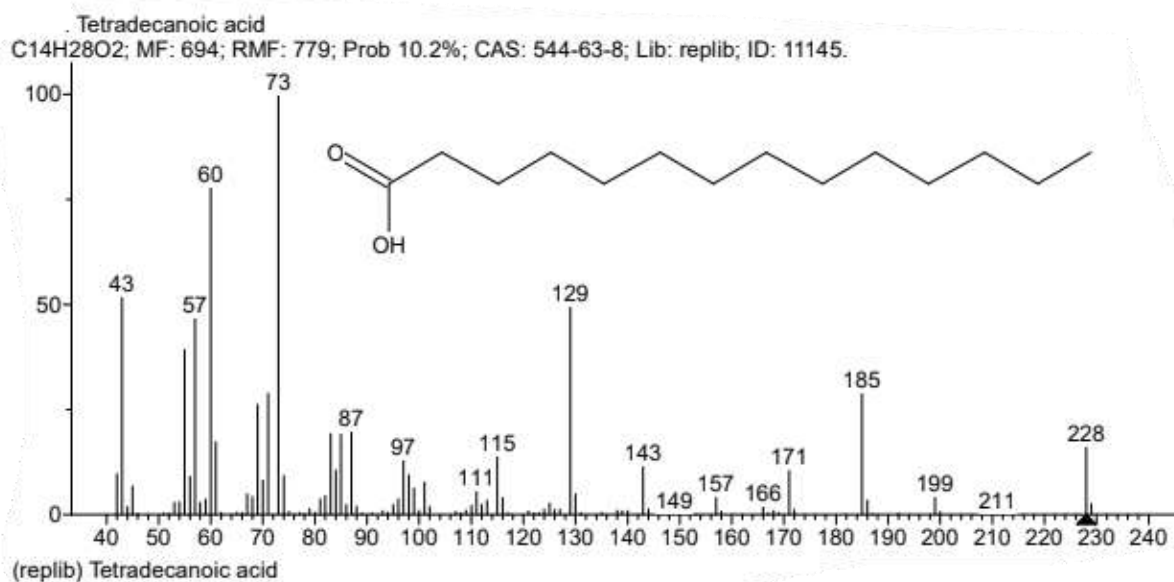


Trichloromethane
CHCl₃; MF: 542; RMF: 573; Prob 15.5%; CAS: 67-66-3; Lib: mainlib; ID: 57506.



n-Hexadecanoic acid
C₁₆H₃₂O₂; MF: 741; RMF: 777; Prob 48.8%; CAS: 57-10-3; Lib: replib; ID: 8750.





Biological activity of ethanolic extract of leaves of *Wrightia tinctoria*

Detection of Anti-diabetic Property

Test Sample	Anti-diabetic Property	Methods ¹⁷
Wrightia tinctoria	22.2%	O. James, O. P. Nnacheta, H. S. Wara, and U. R. Aliyu, "International Journal of PharmTech Research, vol. 1, no. 3, pp. 474-482, 2009.

Anti-diabetic property of the given sample was determined spectrophotometrically at 540nm, α - amylase causative agent to release glucose by the hydrolysis of starch substrate blocking ability is tested for the samples. Test samples are treated with α -amylase for a period of time later the reaction mixture is treated with starch to check the efficiency of α -amylase to release glucose, the concentration of glucose liberated is determined at 540nm using Di-nitro salicylic acid and the percentage of anti-diabetic property is determined by the below formula¹⁶.

$$\text{Anti-diabetic Property \%} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

$$\text{Anti-diabetic property \%} = 22.2\%$$

Detection of Anti-Inflammatory Property

Anti-inflammatory property of the given sample was determined spectrophotometrically at 560nm, HRBC (Human red blood cells) suspension was prepared from the blood of healthy individual, the blood

sample collected was mixed with equal volume of aqueous alsever's solution containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride and isosaline, the mixture was centrifuged at 5000rpm for 10 minutes, the supernatant obtained after centrifugation is used as HRB suspension for the test. The compound to be tested is treated with equal volume of HRBC suspension and incubated at 35°C for 30 minutes, later the test sample mixture is centrifuged at 3000rpm for 5 minutes; absorbance of the obtained supernatant is measured at 560nm. The absorbance of HRBC suspension is taken as control. The percentage of haemolysis and the percentage of protection is calculated using below formula to know the anti – inflammatory property¹⁷.

$$\text{Percentage of Haemolysis} = \frac{\text{Test Absorbance}}{\text{Control Absorbance}} \times 100$$

$$\text{Percentage of Haemolysis} = 45.64\%$$

$$\begin{aligned} \text{Percentage of Protection} &= 100 - \text{Percentage of Haemolysis} \\ &= 100 - 45.64 = 54.36\% \end{aligned}$$

Detection of Anti-bacterial activity

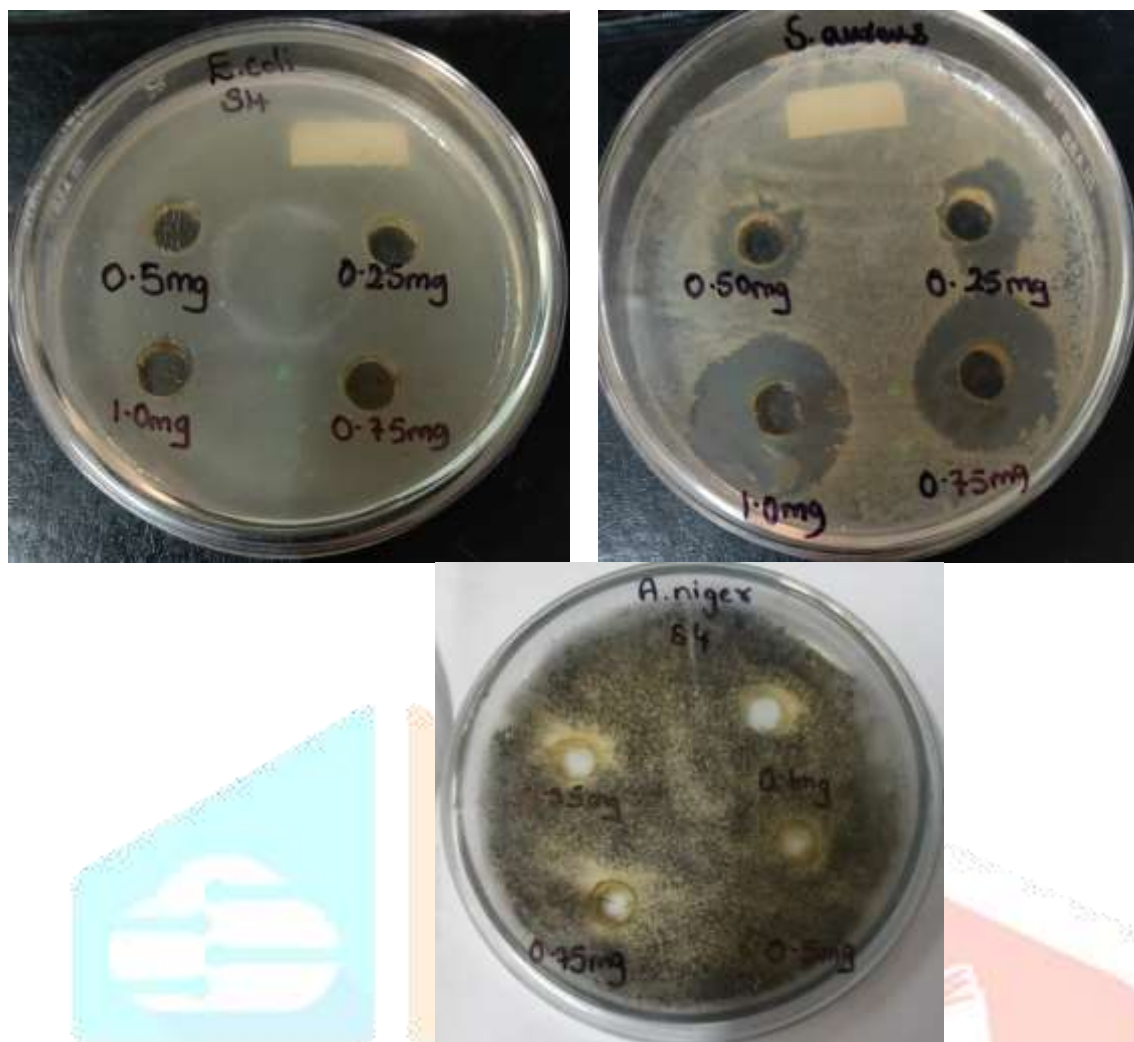
The antibacterial activities were performed on nutrient agar medium. The nutrient agar was prepared as per the composition mentioned below (Table-1), autoclaved for 121°C for 15 lbs and cooled and poured on sterilized petri plates and allowed for solidification. After solidification, the two samples were used. Antibacterial activity was carried out by well diffusion method. Incubate the plates for 37°C for 24 hours for bacteria and results were recorded (zone of inhibition).

Table-1: Nutrient agar

Sl. No	Composition	Concentration (%)
1	Peptone	0.5%
2	Yeast extract	0.3%
3	Sodium chloride	0.5%
4	Agar	1.5%
5	pH	5.6±0.2
	Distilled water-1000ml (1Lit)	

Detection of Anti-fungal activity

The antifungal activities were performed on Czapek's agar (CZA) medium. The CZA was prepared as per the composition, autoclaved for 121°C for 15 lbs and cooled and poured on sterilized petri plates and allowed for solidification. After solidification, All the two samples were used. The antifungal activity was carried out by well diffusion method. Incubate the plates for 28°C for 48 hours to 72hrs for fungi and results were recorded (zone of inhibition).



Report: For both anti-bacterial and anti-fungal

Inhibition to E.coli & A.niger - Nil at all concentration

Test organism	Zone of inhibition in test sample			
	Sample dyer's oleander			
	Concentration in mL			
	0.25	0.50	0.75	1.0
Staphylococcus aureus	Nil	3mm	7mm	8mm

The ethanolic extract of leaves of *Wrightia tinctoria* has Anti-diabetic, Anti-Inflammatory and Anti-bacterial properties.

Conclusion

Medicinal plants are used for screening of the secondary metabolites in research institutes which proves to be helpful in pharmaceutical companies for manufacturing of new drugs for the treatment of various diseases and disorders. From this study, it can be concluded that the leaf of *Wrightia tinctoria* contains different phytochemicals, which might be a perfect product for Anti-diabetic, Anti-Inflammatory and Anti-bacterial activities.

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CONFLICT OF INTEREST Conflict of interest declared none.

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