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Research Article

Extraction and Isolation of Bioactive Compounds from a Therapeutic Medicinal Plant - *Wrightia tinctoria* (Roxb.) R. Br.

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ABSTRACT

Medicinal plants are a source of great economic value all over the world. Medicinal value of plants lies in some bioactive compounds that produce a definite physiological action on the human body. The most important bioactive (chemical) compounds of plants are alkaloids, flavonoids, tannins and phenol compounds. The present investigation was aimed to analysis the bioactive compounds of a therapeutically effective plant *Wrightia tinctoria* (Roxb.) R. Br. Leaf extracts (ethanol, petroleum ether, acetone and methanol) of *W. tinctoria* were used in various analyses of thin layer chromatography, column chromatography, gas chromatography-mass spectroscopy, UV-visible and FTIR spectroscopy. The findings of thin layer chromatographic study revealed visible spots at varying solvent system with different Rf values and the clear response showed in methanol extracts. Gas Chromatography-Mass Spectroscopy spectra reveal the presence of 26 bioactive compounds and 8 major compounds. UV-FTIR spectrum confirmed the presence of ethers, alcohols, alkanes, alkyls, carboxylic acids, alkynes in methanol extracts.

Keywords: *Wrightia tinctoria*, bioactive compound, chromatographic and spectroscopic analysis.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some bioactive compounds that produce a definite physiological action on the human body[1]. The bio active compounds that are present in the plants referred as phytochemicals. These phytochemicals derived from different parts of plants such as leaves, barks, seed, seed coat, flowers, roots and pulps and thereby used as some of direct medicinal agents[2]. According to the World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary health care needs[3].

Plants are being investigated extensively for their pharmacological purpose as the source of material of major modern drugs. Crude extracts of different parts of medicinal plants were being used to treat different type of infectious disease in Ayurvedic system of medicine[4]. Nowadays traditional medicinal practices form an integral part of complementary or alternative medicine[5]. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial response due to their active chemical constituents[6]. Hence the extraction of plant metabolites is indispensable to isolate biologically active compounds and in understanding their role in disease prevention and treatment and in knowing their toxic effects as well.

Wrightia tinctoria (Roxb.) R. Br. is belongs to the Apocynaceae family. It is a small to medium sized deciduous tree, which is widely distributed throughout India[7,8]. The plant is commonly known as Paalai and generally called "Sweet Indrajao". The plant is useful as stomachic, in the treatment of abdominal pain, anti-diarrheal and antihaemorrhagic[9]. Leaves indicated the presence of flavonoids, glycoflavones, iso-orientin and phenolic acids. The leaves of this tree yield a blue dye called pala indigo. Leaves of *W. tinctoria* were soaked in coconut oil for few hours and applied for eczema, psoriasis and other skin diseases[10, 11]. The bark and seeds are effective against psoriasis and non-specific dermatitis. It has anti-inflammatory and anti-dandruff properties and is used in hair oil preparations[12].

The preliminary studies on biochemical constituents of *W. tinctoria* have been reported, but no reports exist on the extraction of bioactive components from methanol leaf extracts. Thus, the present investigation aimed to extract, isolate and identify the bio active compounds from the leaves of *W. tinctoria* through various chromatographic and spectroscopic techniques.

MATERIALS AND METHODS

Collection and identifications of plant materials

The leaves of *Wrightia tinctoria* (Roxb.) R. Br. was collected from different regions of Tiruchirappalli District. The plant specimen was identified and confirmed by Rabinet Herbarium, St. Joseph's College, Tiruchirappalli.

Sample preparation and extraction procedure

The collected leaves were washed in running tap water in order to remove the surface adhered dust particles. The air dried leaves were pulverized to powder in a mechanical grinder. 15 gram of fine powder was mixed with 100 ml

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Table 1: TLC profile for *Wrightia tinctoria* (Roxb.) R. Br.

S.No	Plant Sample	Solvent Extracts	No.of Peaks	Rf Value
1.	*Wrightia tinctoria* (Roxb.) R. Br.	Ethanol	9	0.17,0.19,0.21,0.25, 0.29,0.33,0.35,0.44, 0.8.
		Methanol	8	0.07,0.11,0.1,0.19, 0.21,0.23,0.31,0.39
		Petroleum Ether	9	0.09,0.11,0.13,0.19 0.21,0.30,0.38,0.78 0.92.
		Acetone	8	0.91,0.14,0.16,0.18, 0.20,0.24,0.31,0.92.

Table 2: Column Chromatography profile of leaf extracts of *Wrightia tinctoria* (Roxb.) R. Br.

S.No	Solvent run ratio (Hexane: Acetone)	No. of Fraction	Colour of Fraction
1.	8.2	9	F1- Yellowish green F2-blakish green F3-dark green F4-pickle green F5-moss green F6-juniper green F7- soft green F8-olive green F9-colourless
2.	6.4	2	F1-pistachio green F2-colourless
3.	5. 5	4	F1-pale white F2-snow white F3-bluish white F4-colourless
4.	4.6	3	F1-whitish green F2-ritic blue F3-colourless
5.	2.8	3	F1-ink blue F2-light ink blue F3-colourless

of ethanol, methanol, acetone and petroleum ether in closed dark container and soaked for 3 days at room temperature (25-30^{0}C) and filtered using standard Whatmann No.1 filter paper. The extracts were used for further analysis.

Chromatographic analysis

Thin layer chromatography (TLC)

Solvent extracts of *W. tinctoria* (ethanol, methanol, acetone and petroleum ether) were subjected to thin layer chromatographic analysis to determine the presence of biochemical constituents in different extracts. About 0.1 mg of plant extract was separated on TLC plates using 15% acetone and 85%petroleum ether. The separated components (visible spots) were visualized under visible and ultra violet (UV) light. The qualitative evaluation of the plates was done by determining the migration behavior of the separated substances (visible spots) expressed as a retardation factor (Rf value). TLC is also used for the identification of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound.

Column chromatography

Column chromatography involves ion exchange, molecular sieves, and adsorption phenomenon. One ml of thick viscous methanol extracts were fractionated using Si-gel gas column with the dimension of 28cm×2cm. The n-hexane and methanol were used as eluents (Eluent ratio; n-hexane/methanol 100%, 80:20, 60:40, 40:60, and 20: 80). The compounds of the plant extract run down to the column forming bands based on their polarity and molecular nature. The eluted volumes were sequentially collected as fractions.

Spectroscopic analyses

GC-MS

GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900Model of Thermo fisher scientific make) employing the following conditions: HP-5fused silica gel used as capillary column.1µl of sample was injected into the column using PTV injector at 275ºC. The GC program was initiated by a column temperature at 60 ºC for 5 minutes, increased to 300 ºC at a rate of 8 ºC/min, held for 10 minutes. Helium was used as the carrier gas (1.5ml/min). The mass spectrometer was operated in E1 mode with mass source at 200 ºC. The chromatogram and spectrum of the peaks were visualized. The particular compounds presenting the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mss Spectral database (NIST-MS, 1998) library.

UV-VIS Spectroscopy

UV-VIS spectrophotometric analysis was conducted on the *W. tinctoria* methanol leaf extract using a UV-visible Spectrophotometer. The plant extracts was centrifuged for 15 minutes at 3000 rpm and sieved through Whatmann No. 1 filter paper. The diluted (1:10) sample was examined under visible and UV light in the wavelength ranging from 200-1100nm. The characteristic peak values were observed and noted.

Fourier-transform infrared (FTIR) spectroscopy

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract[13,14]. FTIR analysis of *W. tinctoria* was performed using Perkin Elmer spectrophotometer

Table 3: FTIR peak values and functional groups of methanol leaf extract of *Wrightia tinctoria* (Roxb.) R. Br.

S. No	Peak value	Functional Group	Functional Group Name
1.	3319.49	O-H Stretch	Alcohols
2.	2943.37	C-H Stretch	Alkanes and alkyls
3.	2831.50	O-H Stretch	Carboxylic acid
4.	2526.75	O-H Stretch	Carboxylic acid
5.	1450.75	C-H Bend	Alkanes and alkyls
6.	1415.75	S=O Stretch	Sulfate
7.	1114.86	C-O Stretch	Alcohol
8.	1020.34	C-O-C Symmetrical	Ethers
9.	653.87	C-H Bend	Alkynes
10.	605.65	C-Br Stretch	Alkyl halides
11.	489.92	C-I Stretch	Alkyl halides

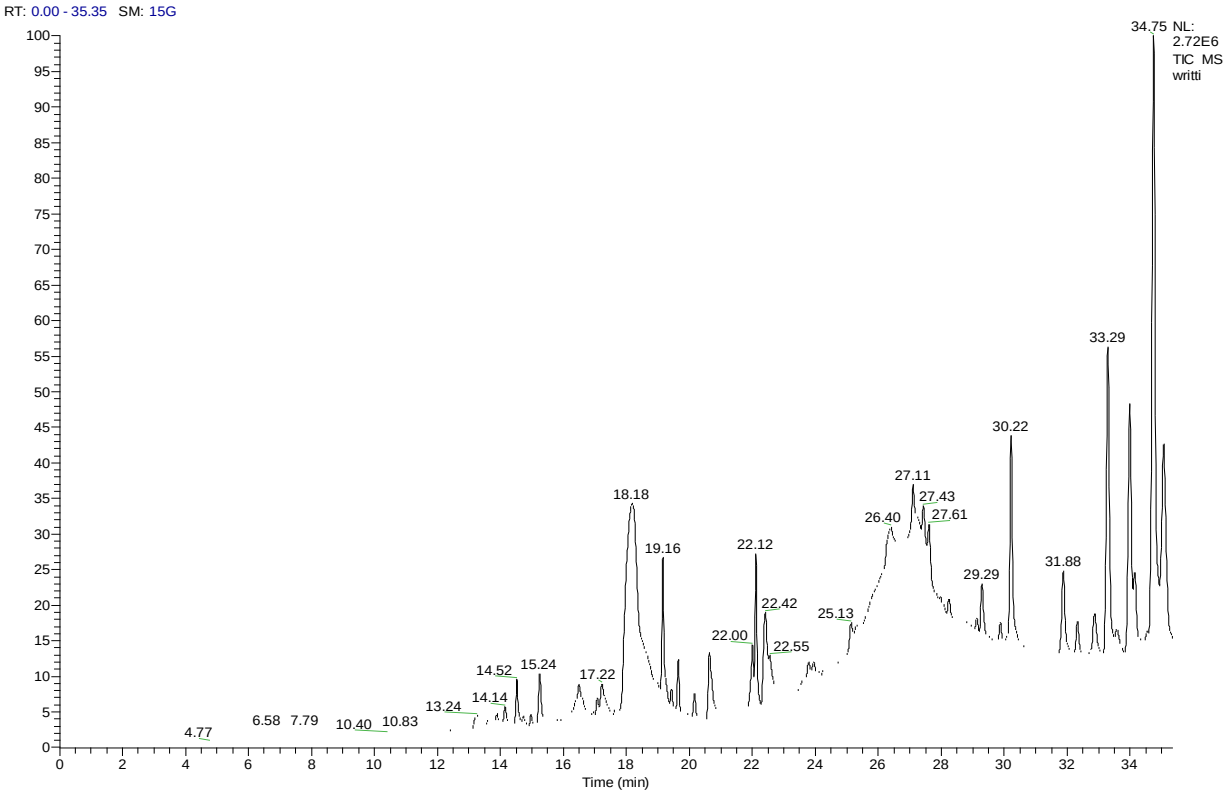

Plate 1: Gas Chromatography and mass spectroscopy (GC-MS) analysis of methanol leaf extract of *Wrightia tinctoria* (Roxb.) R. Br.

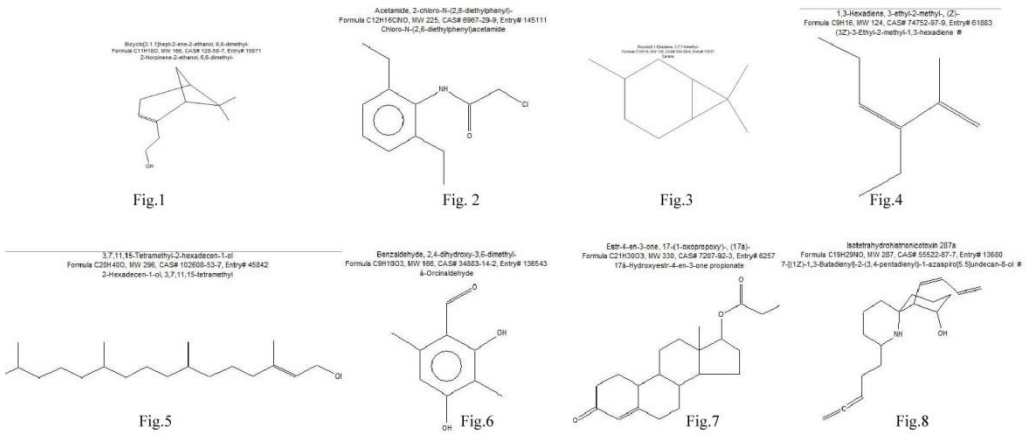

Plate 2: Major bioactive compounds of methanol leaf extract of *Wrightia tinctoria* (Roxb.) R. Br.

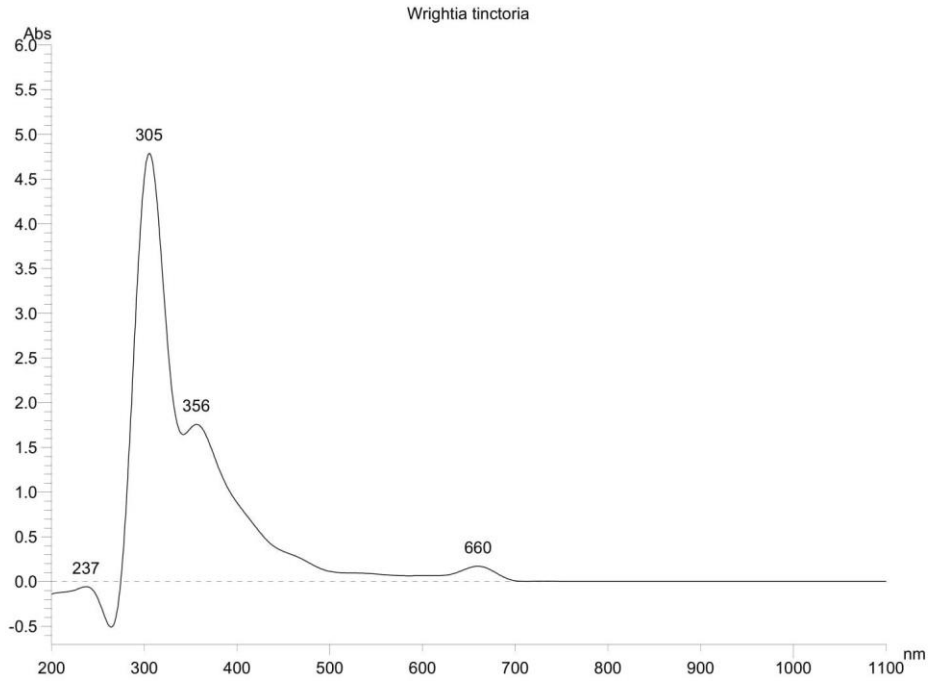

Plate 3: UV analysis of methanol leaf extract of *Wrightia tinctoria* (Roxb.) R. Br.

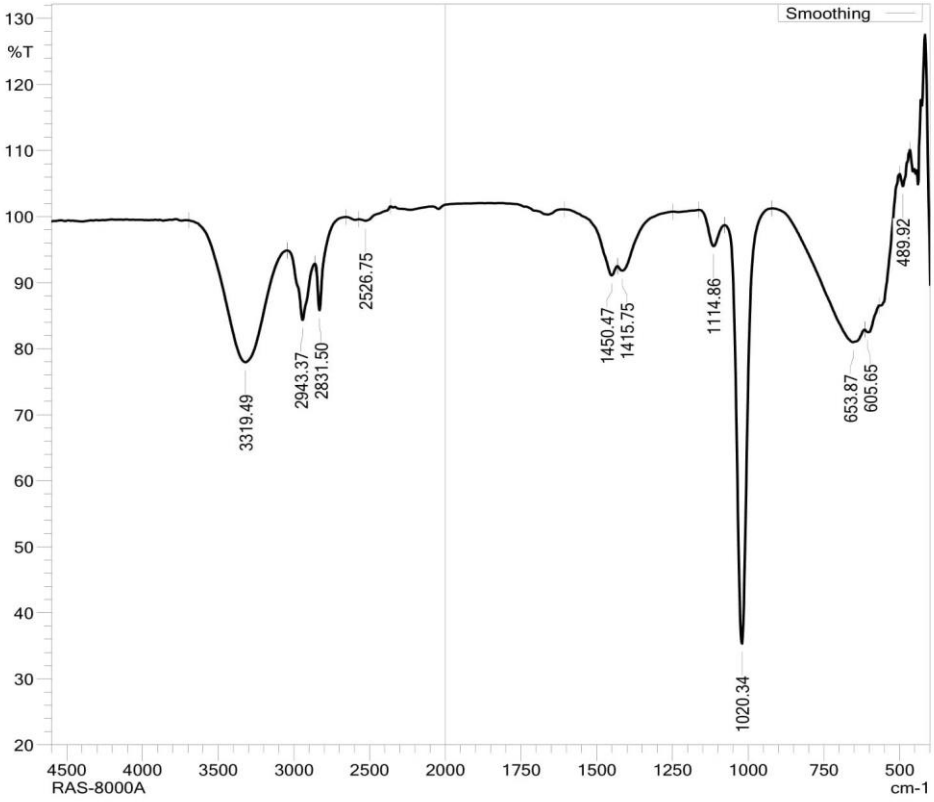

Plate 4: FTIR analysis of methanol leaf extract of *Wrightia tinctoria* (Roxb.) R. Br.

system which was used to detect the characteristic peaks and their functional groups.

RESULTS AND DISCUSSION

Thin layer chromatography

In *W. tinctoria*, thin layer chromatographical analysis using varying solvent system was carried out. A maximum of 9 visible spots were noticed in ethanol and petroleum ether extracts. In methanol and acetone extracts, 8 visible spots were observed with different Rf values which were depicted in Table 1. Of the four solvent extracts used, the maximum visible spots were observed in ethanol and petroleum ether extracts. However, the bands were very clear and more visible in methanol extract. Hence for

further studies, the methanol extracts was used. Similar findings were reported in methanol extracts of *Enhydra fluctuans*, *Leucas aspera* and *Dillinia indica*, showed the presence of various phytoconstituents with different Rf values[15].

Column chromatograhy

The column chromtographical analysis of *W. tinctoria* showed a total of 5 fractions with different solvent run ratio of hexane: methanol (8:2, 6:4, 5:5, 4:6, 2:8) which were collected sequentially and the results shown in Table 2. A maximum of 5fractions were obtained in 8:2 solvent run ratio with different colours.

GC-MS spectral analysis

The GC-MS analysis revealed 26 peaks (Plate 1). Each peak area was directly proportional possible bioactive components from the methanol leaf extracts of *W. tinctoria.* Of these, eight major bioactive compounds were identified (Plate 2). They are Bicyclo (3.1.1) hept-2-ene-2 ethanol, ,6-dimethyl(fig1), Acetamide, 2-chloroN-(2,6-diethylphenyl)(fig2), Bicyclo (4.1.0) heptanes, 3,7,7-trimethyl(fig3), 1,3-Hexdine,3-ethyl2-methyl-(Z)(fig4), 3,7,11,1-Tetramethyl-2-hexadecen-1-01(fig 5),Benzaldehyde,2,4-dihydroxyl-3-6-dimethyl(fig6), Estr-4-en-3-one, 17-(1-oxoproxy)-(1a)(fig7) and isotetrahydrohistrionicotoxin287a(fig8). Similarly, the ethanolic flower extract of *W. tinctoria* were reported eleven peaks[16] and the ethanol leaf extracts of *W. tinctoria* were reported twenty two compounds. Of these, ten were major and twelve were minor compounds[17]. The essential oils of *W .tinctoria* leaves showed 37 compounds by GC-MS technique[18].

UV-FTIR- Functional Groups Identification

The UV spectrum profile of *W. tinctoria* methanol leaf extract showed four peaks at 237, 305,356 and 660 nm with the absorption of - 0.0055, 4.790, 1.756 and 0.173 respectively; the results were showed in Plate 3.

The peak values and functional groups of *W. tinctoria* obtained from FTIR spectroscopy were represented in Table 3. The various peak values of FTIR indicates the presence of O-H stretching-H stretching, C-H bending, S=O stretching, C-O stretching, C-O-C symmetrical, C-Br stretching and C-I stretching confirmed the presence of ethers, alcohols, alkanes and alkyls, carboxylic acids, sulfate, alkynes and alkyl halides (Plate 4). Hence, the extracts subjected to UV and FTIR spectroscopy is analyzed for the identification of bioactive compounds present in *W. tinctoria*. In addition, UV and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of molecular composition of medicinal plants which contains therapeutic properties.

CONCLUSION

From the above investigation, it was concluded that, the *W. tinctoria* is an important therapeutic medicinal plant with varied pharmacological spectrum. The results revealed that among the organic solvents used, methanol was more suitable to fractionate the bioactive compounds from the leaf extracts of *W. tinctoria*. The plant shows the presence of many bioactive constituents which are responsible for varied pharmacological and therapeutic property. The evaluation needs to be carried out on *W. tinctoria* in order to use the plant in various practical and clinical applications, which can be used further for the welfare of the mankind.

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