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Research Article

ANALYSIS OF THE ESSENTIAL OIL FROM THE LEAVES OF *WRIGHTIA TINCTORIA* R. BR. FROM SOUTH INDIA

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ABSTRACT

Wrightia tinctoria R.Br. (Apocyanaceae) is considered to be therapeutically very effective jaundice plant in Indian indigenous system of medicine. The juice of the tender leaves is used efficaciously in jaundice. The plant is known to be used for psoriasis and other skin diseases. In the present study, the chemical composition of the essential oil from the leaves of *Wrightia tinctoria* was analyzed by GC-MS (Gas Chromatography-Mass Spectrometry). GC-MS analytical technique provides conclusive confirmatory evidence for the identification and characterization of essential oil components. Thirty seven known compounds have been identified and quantified from the leaf essential oil of *Wrightia tinctoria* by GC-MS analysis. The major compounds present in the leaf essential oil are urs-12-en-24-oic acid-3-oxo-methyl ester (34.28%), hydroquinone (13.24%), 1, 6-cyclodecadiene,1-methyl-5-methylene- 8- (1-methylethyl) (9.70%), 3-methyl-2-(2-pentenyl)- 2-cyclopentene-1-one (6.76%) and 9, 12, 15-octadecatrienoic acid (4.52%). This is the first report of extraction of essential oil from the leaves of *Wrightia tinctoria*.

KEYWORDS: *Wrightia tinctoria*, Apocyanaceae, Essential oil, Gas Chromatography-Mass Spectrometry (GC-MS).

INTRODUCTION

The genus of *Wrightia* is named after a Scottish physician and botanist William Wright (1740 - 1827). The leaves of this tree yield a blue dye called pala indigo [1]. *W. tinctoria* belongs to family Apocynaceae. It is commonly known as "indrajav" (fig.2). It has got very important place in traditional healing and also is widely recognized medicinal plant [2]. *Wrightia tinctoria* is also known as the jaundice curative tree which is useful for various diseases especially in south India. The crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. *Wrightia tinctoria* R. Br has shown the presence of lipid, saponin, tannin, alkaloid, steroid and some other chemical constituents [3, 4]. Oil 777 prepared out of the fresh leaves of the plant has been assigned to analgesic, anti-inflammatory and antipyretic activities and to be effective in the treatment of psoriasis [5, 6]. Leaves of this plant showed the presence of flavonoids, glycoflavones-iso-orientin and phenolic acids [7, 8]. The leaves are applied as a poultice for mumps and herpes. 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 [9].The bark and seeds are effective against psoriasis and non-specific dermatitis. It has anti-dandruff

properties and hence is used in hair oil preparations [10].

Gas chromatography (GC) is undoubtedly one of the key techniques used for screening / identification / quantification of many groups of non-polar and/or semi-polar components present in plant extracts. The high attainable separation power in combination with a wide range of the detectors employing various detection principles to which it can be coupled makes GC an important, often irreplaceable tool in the analysis of (ultra)trace levels of components [11].

Most of the previous chemical investigations on *Wrightia tinctoria* have focused mainly on the isolation of compounds from the leaves, bark and flowers of the plant. So far no data about the volatiles from *W. tinctoria* leaves has been published. In this work, the *W. tinctoria* leaf oil was extracted by steam distillation and chemical composition of the volatile compounds of the oil was determined by GC-MS.

MATERIALS AND METHODS

Plant material

Wrightia tinctoria leaves were collected from Thrissur district of Kerala, South India during September 2013 and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur.

Essential oil extraction

The fresh leaf (250g) of *Wrightia tinctoria* was cut into small pieces and ground to a paste using an electric mixer grinder and subjected to steam distillation for three hours. About 2 liters of the distillate were collected and extracted with diethyl ether (3X100ml) and dried using anhydrous sodium sulphate. The dry ether extract on evaporation yielded 0.39g (0.15% of fresh weight of the sample) of pale yellow oil. Chemical composition of the leaf essential oil was analyzed by GC-MS.

Steam Distillation

It is a special type of distillation (fig.3) for temperature sensitive materials. Many organic compounds tend to decompose at high sustained temperatures. Separation by normal distillation would then not be an option, so steam is introduced into the distillation apparatus. By introducing the steam, the boiling point of the compounds were depressed, allowing them to evapourate at lower temperature, preferably below the temperature at which deterioration of the material becomes appreciable.

When a mixture of two practically immiscible liquids is heated while being agitated to expose the surfaces of both the liquids to the vapour phase, each constituent independently exerts its own vapour pressure as a function of temperature as if the other constituent were not present. Consequently, the vapour pressure of whole system increases. Boiling begins when the sum of the partial pressures of the two immiscible liquids just exceeds the atmospheric pressure (approximately 101 k Pa at sea level). In this way, many organic compounds insoluble in water can be purified at a temperature well below the point at which decomposition occurs.

Gas chromatography – Mass spectrometry

The GC-MS analyses (fig. 4) were carried out on Agilent 6890 GC system equipped with a 5973 inert mass selective detector (Agilent Technologies, USA). A CO Sil 8 CB (Varian, Middleburg, Netherlands) column of 30m length, 0.25mm i.d, and 0.25µm film thickness was used. The oven was programmed from an initial temperature 50^0C (hold for 2 min) to the final temperature 280^0C at the rate of 10^0C/min. The final temperature hold up time was 5min. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode. The inlet and interface temperatures were kept at 280^0C. The EI source was operated at 230^0C and the quadrupole temperature was 150^0C. The MS was scanned from 30 to 500 mass units. One micro litre of the sample was injected in split mode at a split ratio of 10:1. For compound

identifications, Wiley 275 library spectra were used (online).

Thirty seven known compounds have been identified and quantified from the leaf essential oil of *Wrightia tinctoria* by GC-MS analysis (fig.1).

RESULS AND DISCUSSION

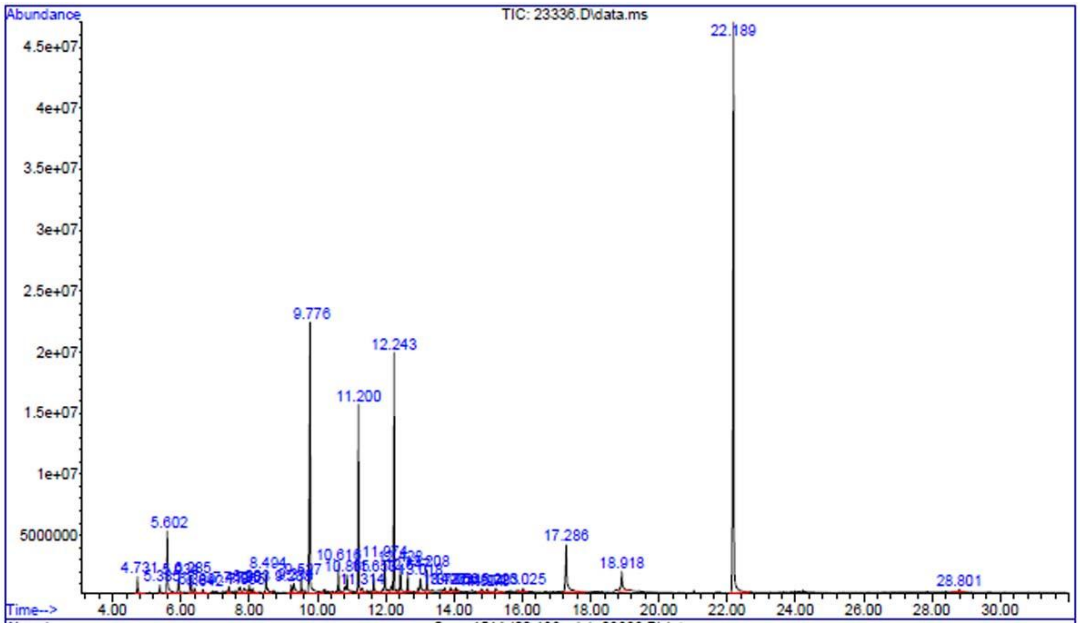

Figure 1: Gas Chromatogram of *Wrightia tinctoria* leaf essential oil

The major compounds present in the leaf essential oil are urs-12-en-24-oic acid-3-oxo-methyl ester (34.28%), hydroquinone (13.24%), 1, 6-cyclodecadiene,1-methyl-5-methylene- 8- (1-methylethyl) (9.70%), 3-methyl-2-(2-pentenyl)- 2-cyclopentene-1-one (6.76%) and 9, 12, 15-octadecatrienoic acid (4.52%). The compounds identified and the corresponding concentrations (%) are given in the following table 1.

Table 1: Composition of the leaf essential oil of *Wrightia tinctoria* obtained by steam distillation

Identified compounds	Retention time (min)	Molecular weight	Percentage (%)
Phenol	4.731	94	0.83
1-methyl-4-(1-methylethyl)-Benzene	5.385	134	0.30
Benzyl alcohol	5.602	108	3.49
N-methyl-N-2-pyridinyl formamide	5.934	136	0.96
4-methyl phenol	6.285	108	1.04
2-methoxy phenol	6.399	124	0.43
1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	6.642	136	0.22
2,4-dimethyl phenol	7.410	122	0.37
4-ethyl phenol	7.735	122	0.65
3,5-dichloro phenol	7.855	162	0.74
2,3-dimethyl phenol	8.003	122	0.96
2-methoxy-4-methyl- phenol	8.494	138	1.65
Benzoic acid,2-hydroxy,methyl ester	9.238	152	0.42
2,3-dihydro Benzofuran	9.311	120	1.12
4-methyl -2-methoxy phenol	9.527	152	0.78

Hydroquinone	9.776	110	13.24
Indole	10.616	117	1.61
α-cubebene	10.865	204	1.68
3-methyl-2-(2-Pentenyl)-2-cyclopenten-1-one	11.200	164	6.76
5-chloro-2-thiophenecarbaldehyde oxime	11.314	161	1.01
α-caryophyllene	11.653	204	2.41
1-methyl-5-methylene- 8-(1-methylethyl) -1,6-cyclodecadiene	11.974	204	9.7
Butylated hydroxy toluene	12.243	220	1.62
Dodecanoic acid	12.428	200	1.62
Caryophyllene	12.647	204	0.51
1,2,3,5,6,8 a-hexahydro-4, 7-dimethyl-1-(1-methylethyl) Naphthalene	13.018	204	0.90
Caryophyllene oxide	13.208	220	1.27
1-ethyl heptyl-Benzene	13.737	204	0.40
1,2,3,4,4a,5,6,8a-(octahydro-7-methyl-4-methylene-1- (1methylethyl)-naphthalene	13.903	204	0.37
1-pentylheptyl-Benzene	14.056	246	0.32
τ-cardinol	14.804	204	0.34
1-ethyldecyl Benzene	14.978	246	0.30
6,10,14-trimethyl 2-penta decanone	15.223	250	0.54
n-Hexadecanoic acid	16.025	256	0.52
9,12,15-Octadecatrienoic acid	17.286	278	4.52
1,2-Benzenedicarboxylic acid, diisooctyl ester	18.918	280	1.84
Urs-12-en-24-oic acid 3-oxo-methyl ester	22.189	468	34.28

CONCLUSION

The present study is the first report of the extraction of essential oil from the leaves of *Wrightia tinctoria* by steam distillation. The essential oils are known to possess antimicrobial activity and further investigation be carried out to find out the activity of the oil and to incorporate it in the drug formulations.

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PHOTOGRAPHS

Figure 2: Indrajav (*Wrightia tinctoria* R.Br.)

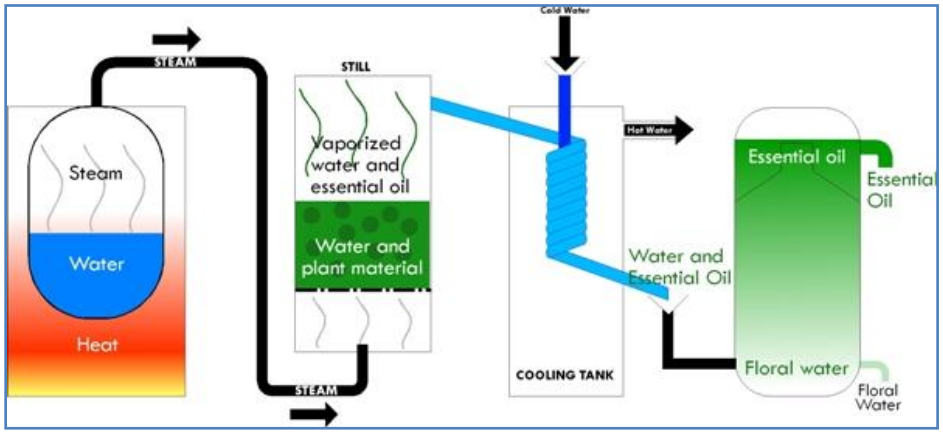

Figure 3: Extraction of essential oil by steam distillation

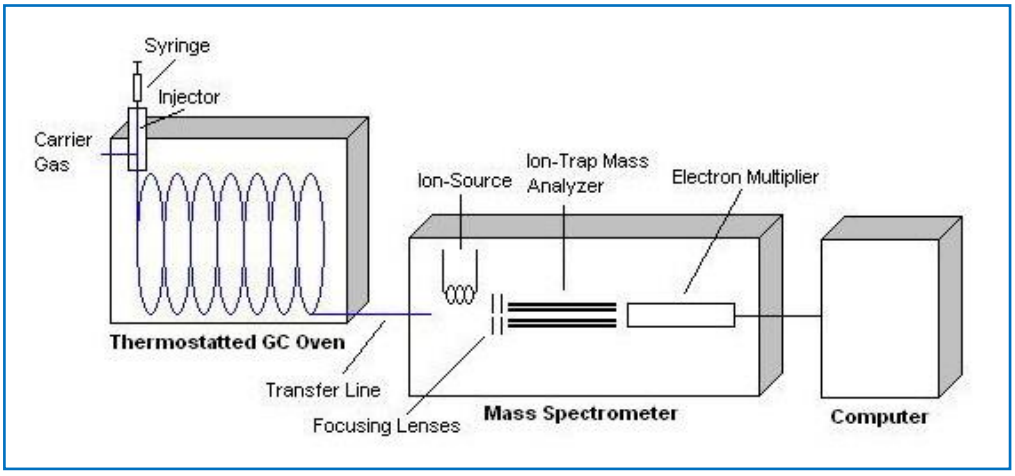

Figure 4: Schematic diagram of Gas Chromatography-Mass Spectrometry (GC/MS)