Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2012, 2 (6):688-691

ISSN : 2249-7412
CODEN (USA): AJPSKY

GC-MS analysis of leaf ethanolic extracts of *Wrightia tinctoria* - A high Medicinal value plant

T. Jayamathi*, N. Komalavalli* and V. Pandiyarajan**

** PG and Research Department of Botany, H. H. The Raja's College (Autonomous), Pudukkottai – 622 001, Tamil Nadu, India*
***Centre for Research and Post Graduate studies in Botany, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi-626 124, Tamil Nadu, India*

ABSTRACT

Wrightia tinctoria, a medicinally important plant belongs to the family Apocynaceae. Traditionally leaves are used in the treatment of abdominal pain and skin diseases. In the present study, the bioactive compounds of Wrightia tinctoria leaf have been evaluated using GC-MS. The chemical compositions of the ethanolic extract of W. tinctoria were investigated using Perkin-Elmer Gas Chromatography - Mass Spectroscopy. GC-MS analysis of W. Tinctoria ethanolic extract revealed the existence of the GC-MS chromatogram of the twenty two peaks presented. The major chemical constituents are 3-O-methyl-d-glucose (51.44%), Squalene (16.52%), n-hexadecanoic acid (6.17%), Phytol (4.47%) and 9,12-Octadecadienoyl chloride (Z,Z)- (4.31%).

Keywords : GC-MS analysis, Bioactive compounds, *Wrightia tinctoria*, Ethanol extract.

INTRODUCTION

Wrightia tinctoria Roxb.is a small to medium-size deciduous tree belongs to the family Apocynaceae which is distributed throughout India up to 1200m. The plant grows up to 18 m tall and to 20 cm DBH (Diameter at Breast Height) with green marks on the stem and producing milky-white resin. The bark is smooth, somewhat corky and pale greyamenable for carving. Traditionally *W. tinctoria*is commonly called as "Jaundice curative tree" in south India [1]. Different parts of this plant possesses very high medicinal value and used in Ayurveda, Siddha and other traditionalsystems of medicine for curing various ailments[2]. The plant has been assigned to have antidiarrhoeal[3], antihaemorrhagic[4], antipyretic [5], anthelmintic and diuretic [6,7], antinociceptive[8],stomachic[2], analgesic and antidiabetic[9,10], antiviral and cytotoxic [11], anti-inflammatory [12], hypolipidemic[13], antioxidant [14] and antiulcer [15]activities. It is also used in febrifuge and dog bite [1,3,16], toothache [4],skin diseases [4,17,18,19,20,21], psoriasis [7], seminal weakness and flatulence [1],leprosy, burns, enlargement of spleen, boils andpiles [21]. Moreover, a few drops of its sap in milk prevent curdling and enhance its shelf life, without the need to refrigerate owing to its preservative nature [10].The reported constituents in bark are alkaloids, terpenes, wrightial[22], Tryptanthrin[23], Indole and flavonoids [2].Active compounds present in the *W. tinctoria* flower extract by GC-MS analysis was reported [24]. Past studies revealed that so far there is no study pertaining phytochemical constituents of the leaves of *W. tinctoria*. Therefore the present study was carried out to determine the phytochemical constituents from *W. tinctoria* leaves by GC-MS using ethanolic extract.

MATERIALS AND METHODS

Plant material
Wrightia tinctoria was collected from Chadurakiri hills of Western Ghats, Virudhunagar district, Tamil Nadu, India. The plant specimen was identified and confirmed by Botanical Survey of India, Coimbatore, TamilNadu, India.

Preparation of leaf powder

The leaves were collected and washed in running tap water in order to remove the surface adhered dust particles. Then they were shade dried and pulverized to powder in a mechanical grinder. The powdered obtained were sieved in a cotton muslin cloth (hole size of 0.2mm) to get a fine powder. The fine powder of leaf was stored in a plastic container at 4^{o}C until further use.

Preparation of leaf extract

1gm of the leaf powder of *Wrightia tinctoria* was weighted, transferred to flask, treated with the absolute ethanol until the powder was fully immersed and incubated overnight. The extracts were then filtered through Whatmann filter paper No.41 along with 2gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted 95% ethanol. The filtrate is then concentrated to 1ml by bubbling nitrogen gas in to the solution. The extract contains both polar and non-polar components of the material.

GC –MS analysis

2µl of the ethanolic extract of *W. tinctoria* was employed for GC-MS for analysis of different compounds.

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: columnElite-1 fused silica capillary column (30 ×0.25 mm × ID x 1µm of capillary column, 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) inject or temperature 250°C; ion-source temperature280°C. The oven temperature was programmed from 110°C (isothermal for 2min), with an increase of 10°C/min, to200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C.Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450Da.

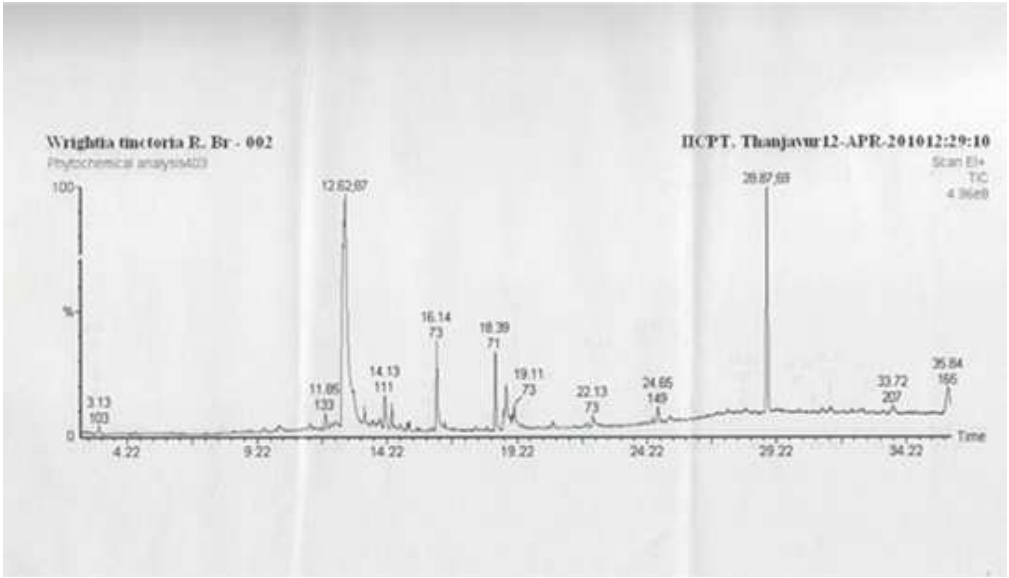
Identification of phytocompounds

Identification of phytocompounds and interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components using computer searches on a NIST Ver.2.1 MS data library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The studies to determine the possible chemical components from the leaf of *W. tinctoria*was carried out by GC-MS.The ethanol extract analysis clearlyrevealed twenty two peaks indicating the presence of twenty two phytochemical compounds. The GC-MS chromatogram of the twenty two peak of the compounds detected was shown in Figure-1.

Fig 1 GC - MS analysis of *Wrightia tinctoria*



The twenty two phytoconstituents were characterized and identified on comparison of the mass spectra of the constituents with the NIST library. The active principles with their retention time (RT), molecular formula, molecular weight (MW),and concentration (peak area%)are presented in Table-1.

Table 1 Phytocomponents identified in the *Wrightia tinctoria*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area (%)
1	3.13	Butane,1,1-diethoxy-3-methyl-	$C_9H_{20}O_2$	160	0.69
2	4.49	Propane,1,1,3-triethoxy-	$C_9H_{20}O_3$	176	0.16
3	5.95	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	$C_{10}H_{18}O_2$	170	0.19
4	8.89	α-Cubebene	$C_{15}H_{24}$	204	0.21
5	9.49	Bicyclo[7.2.0]Undec-4-Ene,4,11,11-Trimethyl-8-Methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	204	0.50
6	10.05	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	$C_{13}H_{18}O$	190	0.76
7	10.97	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.34
8	11.85	Megastigmatrienone	$C_{13}H_{18}O$	190	1.16
9	12.62	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	51.44
10	13.36	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.88
11	13.95	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol	$C_{14}H_{22}O_2$	222	0.85
12	14.13	10-Methyl-8-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	2.80
13	14.40	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.38
14	16.14	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.17
15	16.44	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.64
16	18.39	Phytol	$C_{20}H_{40}O$	296	4.47
17	18.71	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.87
18	18.80	9,12-Octadecadienoyl chloride (Z,Z)-	$C_{18}H_{31}ClO$	298	4.31
19	19.11	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.15
20	22.13	Eicosanoic acid	$C_{20}H_{40}O_2$	312	1.39
21	28.87	Squalene	$C_{30}H_{50}$	410	16.52
22	35.84	Vitamin E acetate	$C_{31}H_{52}O_3$	472	3.13

The results showed out of twenty two compounds ten and twelve were major and minor constituents respectively. The ten major compounds include 3-O-Methyl-d-glucose (51.44%), squalene (16.52%), n-Hexadecanoic acid (16.17%), Phytol (4.47%), 9,12-Octadecadienoyl chloride (Z,Z)- (4.31%), Vitamin E acetate (3.13%), 10-Methyl-8-tetradecen-1-ol acetate (2.80%), Eicosanoic acid (1.39%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.38%) and Megastigmatrienone (1.16%).

GC-MS study of the ethanolic flower extract of *W. tinctoria* revealed the presence of elevenpeaks[24]. The major chemical constituents include hexadecanoic acid, 15-methyl (58.31 %), 2-mercapto-propanoic acid (17.79 %), pentadecanoic acid (4.66 %) and 3-methyl-3-butanoic acid (12.74 %). The flower extract also shows presence of many methyl and ethyl esters such as propanoic acid, 2-mercapto; 3-methyl-3-butanoic acid; pentadecanoic acid, 4-ethyl-2-methoxy, and disilanone, dicarboxylic acid at retention time of 14.09, 16.52, 29.30, 32.40 and 39.67 respectively.

GC-MS analysis flower shows eleven peaks whereas leaf shows twenty two peaks. The presence of several constituents in the ethanolic leaf extract of *W. tinctoria* justifies the use of the leaf for various ailments by traditional practitioners.

CONCLUSION

It was concluded that ethanolic extract of leaves of *Wrightia tinctoria* possess variouspotentbioactive compounds and is recommended as a plant of phytopharmaceutical importance. Further studies are needed to explore the potential compounds responsible for the biological activity from *W. tinctoria* for application in drug delivery, nutritional or pharmaceutical fields.

Acknowledgement
The authors express their gratitude to Principal, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, for providing laboratory facility and encouragement.

REFERENCES

[1]JoshiSG,*Medicinal plants*, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, **2000**.
[2]MurugandandamAV, BhattacharyaSK, GhosaS, *Indian J Chem*., **2000**, 39,125.
[3]ChopraRN, NayarSI,ChopraIC,*Glossary of Indian Medicinal Plants*, CSIR, New Delhi, India, **1956**.
[4]NadkarniKM, *Indian MateriaMedica*, Popular Prakashan, Bombay,**1976**.

[5]GhoshD, ThejmoorthP, VeluchamyG, *Bull. Med. Ethnobot. Res*.,**1985**, 6, 141.
[6]MitraSK, SeshadriSJ, VenkatarangannaMV,GopumadhvanS,*Ind. J.Dermat*.,**1998**,43,102.
[7]SathianarayananS, AshaJ, RajasekaranA, GeorgeRM,AmruthaBC,*Int. J. Phytopharmac*.,**2011**,2, 7.
[8]KrishnamoorthyJR, RanganathanS, *Ind. J. Dermat*., 45, **2000**, 125.
[9]MadhavaChettyK,*Wrightia tinctoria Linn. - Chittor medicinal plants*, Himalaya Book Publications, Tirupathi, **2008**.
[10]Ashok RajR, Saravana KumarA, GandhimathiR, *Int. J. Phytopharm*., **2010**,1, 47.
[11]selvamP, MurugeshN, VitvrouwM, KeyaertsE,NeytsJ, *Ind. J. Pharm. Sci*., **2009**, 71, 670.
[12]TharkarPR, TatiyaAU, SuranaSJ, BhajipaleNS, DeoreSR, *Int. J. Pharm. Tech. Res*., **2010**,2, 2434.
[13]ShruthiA, LathaKP, VagdeviHM, VaidyaVP, PushpaB, ShwethaC, *Int. J. ChemTech.Res*., **2010**, 2, 2043.
[14]Lakshman KumarD, RaoKN, BinduMadhaviV, Sathis KumarD, BanjiD,*J. Pharm. Res*.,**2011**,4,396.
[15]MadhuCD,Lakshmi DeviS, *Der Pharm.Sinica*.,**2011**, 2, 355.
[16]AgarwalCK,*Economic Plants of India*,KailashPrakashan, Culcutta, **1986**, pp406.
[17]VarierPS,*Indian Medicinal plants*, Orient Longman Ltd., Madras,**1997**.
[18]KrishnamurthiJR, KalaimaniS, VeluchamyG, *J. Res. Ayur. Sidd*.,**1981**, 2, 58.
[19]KothariMJ, LondheAN, In:MaheshwariJK(Ed.),*Ethnobotany and Medicinal plants of Indian Subcontinent*, Scientific Publisher,Jodhpur, India,**2000**,pp324.
[20]KothariMJ, RaoKM, In:MaheshwariJK(Ed.),*Ethnobotany and Medicinal plants of Indian Subcontinent*, Scientific Publisher,Jodhpur, India,**2000,**pp 120.
[21]KhareCP, Indian medicinal plants, Springer-Verlag Berlin, Heidelberg, Newyork, **2007**.
[22]RamachandraP, BasheermiyaM, KrupadanamGLD, MannarayanaG, *J. Nat. Prod*.,
1993, 56, 1811.
[23]GeorgeV, KoshyAS, PushpangandanP, *Fitoterapia*.,**1996**, 67, 553.
[24] Ramalakshmi S, EdayadullaN, RameshP, MuthuchelianK, *Asian Pac. J. Trop. Biomed.*, **2012**, 154.