Research Article

# Phytochemical Profile of Crude Seed Oil of *Wrightia tinctoria* R.BR. and *Wrightia arborea* (DENNST.) MABB. by GC-MS

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**ABSTRACT**

Plants have been used from ancient times to attempt cures for diseases and to relieve physical suffering and found to be rich sources of important therapeutic aids for alleviating human ailments. *Wrightia tinctoria* and *Wrightia arborea* of the family Apocynaceae *are* known in the traditional medicine for anticancer activity along with other broad indications including in snake and scorpion bites, renal complications and menstrual disorders. The present study was carried out to analyse the seed oil of *W. tinctoria* and *W. arborea* by GC-MS technique. The phytochemical studies revealed the presence of medicinally important compounds like α amyrin, β amyrin, squalene, γ tocopherol, campesterol, lupeol, betulin, lupenone in *W. tinctoria* and squalene, γ tocopherol, campesterol, lupeol, phytol in *W. arborea.*

**Keywords:** Phytocomponents, Seed oil, *Wrightia tinctoria, Wrightia arborea.*

## INTRODUCTION

The genus *Wrightia* comprises of 23 flowering plants (Apocynaceae family), which are native to tropical Africa, Asia and Australia. Plants belonging to *Wrightia* are known in the traditional medicine for anticancer activity along with other broad indications including in snake and scorpion bites, renal complications, menstrual disorders etc.[1] Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents. So, the integration of scientific evaluation with traditional medicinal practices helps in drug development and synthesis and plays a vital role in complementary or alternative medical formulations.[2]

*Wrightia tinctoria* R.Br. commonly known as Indrajau is a small deciduous tree native to India, Burma. It is one of the 70 medicinal plants being exported from tropical forests of India.[3] In India, this plant grows in abundance in dry, hilly and rocky areas of Tamil Nadu, Andhra Pradesh, Madhya Pradesh and Rajasthan. The seeds of *Wrightia tinctoria* are astringent, acrid, thermogenic, carminative, digestive, stomachic, antidysenteric, constipating, depurative, anthelmintic, aphrodisiac, febrifuge and diuretic. Seeds are useful as a tonic, carminative, anthelmintic, astringent, aphrodisiac and febrifuge and for treatment of stomach disorder. In Unani medicine, the seeds of *W. tincoria* are differently known as "Lisanul-e-Asafir", Inderjao Shireen, and Meetha Inderjao and have been reported to have used for cure disorders of central nervous system and claimed to have analgesic, aphrodisiac, tonic and emmenagogue actions.[4] The seeds yield deep red, semi-drying oil, which has medicinal value.[5] The bark and seeds are effective against psoriasis and non-specific dermatitis. It has anti-inflammatory and anti-dandruff properties and hence is used in hair oil preparations.[6]

*Wrightia arborea* (Dennst.) Mabb. is an endangered species of Apocynaceae placed in the category I of Red Data.[7] It is used medicinally in Ayurveda, Siddha and other traditional systems of medicine for curing various ailments. The plant is reported to possess beneficial weakness in seeds.[8] The extracts of *W. arborea* were proved to have promising potential as a source of natural antioxidant and antimicrobial agents[9] and anti cancerous agent.[10] It is distributed throughout the warmer part of India at an altitude of 600 mt in the Himalayas and 1200 mt in the Nilgiris.[11]

Though numerous phytochemical works have been carried out using different parts of *W. tinctoria and W. arborea*, the seed oil has been left untouched. The objective of the present study is to determine the chemical composition of the oil from the seeds of *W. tinctoria* and *W. arborea* by GC-MS analysis and to chemically analyse the seed oil of these medicinally important plants.

## METERIALS AND METHODS

### Plant collection and preparation extraction of oil

*Wrightia tinctoria* and *W. arborea was* collected from Savanadurga hillocks, Karnataka, India in February 2013. The pods were shade dried and seeds were extracted. 100 gm of the air-dried seed powder of *W. tinctoria* as weighted, extracted with petroleum ether (60-80$^{0}$C) through soxhlet extraction apparatus and the extract was concentrated by recovering the solvent using rotary evaporator. The oil yield of was 2.12% and *W. arborea* was 1.76%.

In order to make fatty acid present in the oil volatile, derivatization was performed converting non volatile fatty acids into volatile fatty acids methyl esters (FAMEs). Derivatization was performed according to the AOAC standard reference method using Boron triflouride ($BF_3$) - Methanol as a derivitizing agent.[12] 2 g of oil was saponified by means of 25 ml 0.5 M methanolic NaOH. 300 mg of saponified oil sample was treated with 8ml of $BF_3$ – Methanol and boiled for 2-4 min. Thereafter, 2-3 ml of petroleum ether (40-60 $^0$C) was added to resultant solution to dissolve the esters. Saturated NaCl solution was added enough to float FAMEs on the top of the flask and collected by means of syringe and analysed.

### GC-MS analysis

GC - MS analysis was carried out at Shiva Analytical (India) PVT. LTD., Bangalore, India. GC clarus 500 Perkin – Elmer system comprising a AOC -20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) was used for analysis of seed oil. It was equipped with fused silica capillary column with30m x Id 0.25 mm x 1μM df (composed of 100 % dimethyl poly siloxone), operating in electron impact mode at 70 ev, carrier gas: Helium (99.99%) with constant flow rate of 1ml/min and an injection volume of 0.5μl was employed (split ratio of 0:1), Injector temperature 250 $^0$C, ion source temperature 280 $^0$C. The oven temperature was programmed from 50 $^0$C (isothermal for 1 min )to 300 $^0$C with an increase of 60 C /min; ending with 9 minutes isothermal at 300 $^0$C .Mass spectra are taken at 70ev; a scan interval of 0.5 seconds and fragments from 0 to 550 Da. The total GC running time for *W. tinctoria* and W. arborea was 47.17 min and 47.41 min respectively.

### Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Techniques (NIST) and the name and molecular weight of the component of the test material was ascertained.[13] The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. HPCHEM Software was adopted to handle mass spectra.

## RESULTS AND DISCUSSION

The GC-MS chromatogram of the seed oil of *W. tinctoria* shows 33 peaks indicating presence of compounds (Figure 1) and that of *W. arborea* shows 35 peaks indicating presence of compounds (Figure 2). The identification of the active principles present in the oil fraction was confirmed based on the peak area, retention time, molecular weight and peak area in percentage).The compounds of *W. tinctoria* were presented in Table 1 and the first compound identified with less retention time (6.95 min) was 2-Heptenal, whereas Lup-20 (29) -en-3-one was the last compound which took longest retention time (47.17 min). The major compounds present are lupenone (9.56%), Hexadecanoic acid, 2-hydroxy-1-(hydroxy methy) ethyl ester (7.64%), 4,4,6a,6a,8a,11,11,l4 b-octa methyl- 1, 4,4a,5,6,6a,6b, 7,8,8a,9,10, 11,12,12a, 14,14a, 14b octadecahydro-2H-picen-3-one (7.08%), 3,12 oleandione (5.98).The compounds of *W. arborea* were presented in Table 2 and the first compound identified with less retention time (3.55 min) was ethylene amine and 13,27 cycloursan 3-one was the last compound which took longest retention time (47.41 min). The major compounds are 13, 27 Cycloursan – one (11.17%), 4,4,6a,6a,8a,11,11,l4 b-octa methyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10, 11,12,12a,14, 14a, 14b octadecahydro-2H-picen-3-one (8.23%), 2,2H-1-Benzopyron-6-ol, 3,4 –dihydro -2.8-dimethyl-2-(4,8,trimethysilyl -[2R-[2R- (4R*,8R*)] ] (8.02%).The phyto components with many biological activities was presented in Table 3. The biological activities listed are based on Dr. Duke's phyto chemical and Ethno botanical database and past literature.

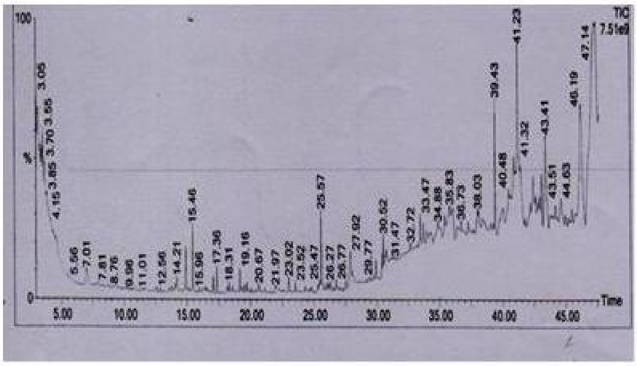

**Figure 1:** GC-MS Chromatogram of petroleum ether seed oil extract of *Wrightia tinctoria*

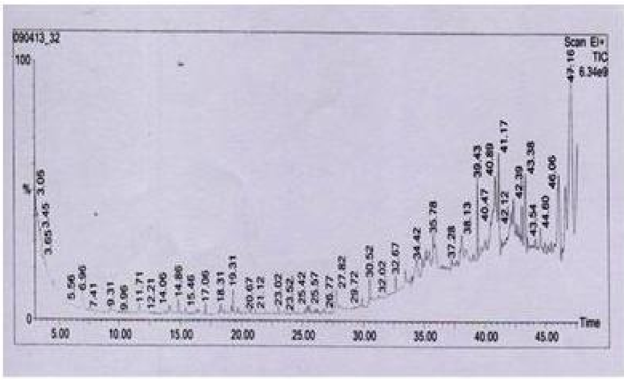

**Figure 2:** GC-MS Chromatogram of petroleum ether seed oil extract of *Wrightia arborea*

Fatty acids always occur in plants. Fatty acids in plants react with alcohols in an esterification reaction to form esters. The composition of saturated fatty acids and fatty acid esters in the seeds of *W. tinctoria* and *W. arborea* was found to be 14.05% and 11.45% respectively. The constituent with the highest quantity in the seed oil of *W. tinctoria* was n-hexadecanoic acid having a composition of 8.46%. Fatty acids present in the oil were suggested to be responsible for its anti-inflammatory activity and have been reported to exhibit antibacterial and antifungal activity. Thus the combination of hexadecanoic acid, 9,12,15-octadecanoic acid-(Z,Z,Z), present in the oil may be useful for the management of inflammatory conditions arising from bacterial and fungal infections as well as bruises and erupted skins. A long chain fatty acid alcohol, heptatriacontanol, may inhibit platelet aggregation.[14]

**Table 1**: Composition of seed oil of *W. tinctoria* R.Br.

| RT | Name of compound | Peak area % | MW |
|---|---|---|---|
| 6.956 | 2-Heptenal, (Z)- | 0.38 | 114.18 |
| 27.820 | n-Hexadecanoic acid | 0.82 | 256.24 |
| 30.522 | Octadecanoic acid,3-hydroxy-, methyl ester | 0.76 | 312.48 |
| 31 .223 | Urs-12-en-24-oic acid,3-oxo-, methyl ester | 2.85 | 468.71 |
| 32.674 | 9-Octadecenoic acid, [2-phenyl-1,3-dioxolan-4-yl)methyl ester,cis | 1.85 | 444.64 |
| 33.124 | Lupeol | 2.36 | 426.73 |
| 33.474 | 2-[4-methyl-6-[2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl)cyclohex-1-en-1-carboxaldehyde | 1.86 | 324.49 |
| 34.425 | 13,27 -cycloursan-3-one | 3.49 | 253.23 |
| 34.875 | 4,4,6a,6a,8a,11,11,l4 b-octa methyl-1, 4, a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a, 14b octadecahydro-2H-picen-3-one | 7.08 | 423.25 |
| 35.426 | cholest-22-ene-21-o1,3-5-dehydro-6-methoxy- Pivalate | 2.24 | 498.78 |
| 35.776 | Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl)ethyl ester | 7.64 | 330.5 |
| 37.277 | E,E,Z-1,3,12-Nonadecatriene-5, 14-diol | 1.66 | 225.23 |
| 37.677 | 9, 10-secocholesta-5,7, 10( 19 )-triene -3,24,25- triol,(3β,5Z,7E) | 1.26 | 365.24 |
| 38.127 | 9-Octadecenoic acid (Z), 2-hydroxy-1-(hydroxy methyl)ethyl ester | 2.98 | 326.51 |
| 38.428 | α amyrin | 3.14 | 426.72 |
| 39.428 | Squalene | 2.04 | 410.71 |
| 40.054 | 9,l0-Secocholesta-5,7,10(19)triene-1,3-diol, 25- [ (tri methylsilyl) oxy] -, (3β,5Z,7 E) | 4.01 | 254.58 |
| 40.889 | 4,8,13-Cyclotetradecatriene-l,3-diol, 1,5,9 trimethyl- 12-(1 -methylethyl)- | 9.75 | 289.14 |
| 41.169 | 2H-1-Benzopyran-6-o1,3-4 dihydro-2,8-dimethyl-2 (4,8,12-trimethyl tridecyl ) -, [ 2R- [ 2R * ( 4 R *, 8 R *)) ] | 2.49 | 345.25 |
| 41.419 | l-Heptatriacontanol | 2.46 | 536.99 |
| 42.195 | 3,12-oleandione | 5.98 | 352.23 |
| 42.39 | γ-Tocopherol | 2.33 | 416.68 |
| 42.615 | 9, 19-cycloergost-24(28)-en-3-ol,4, 14-di methyl acetate, (3β,4α,5α) | 1.34 | 321.23 |
| 42.800 | Stigmastan-3,5-diene | 1.24 | 396.69 |
| 42.925 | 1-Hexatriacontane | 0.99 | 506.97 |
| 43.080 | 9,19-cyclolanostan-3-ol, acetate,(3β | 1.36 | 452.12 |
| 43.38 | vitamin E | 2.88 | 430.70 |
| 43.930 | Lup-20(29)-en-3β-ol, acetate(3β) | 1.54 | 468.75 |
| 44.595 | Campesterol | 1.89 | 400.68 |
| 44.976 | Cholesto-22,24-dien-5-o1,4,4-dimethyl- | 0.72 | 352.25 |
| 45.236 | Betulin | 0.76 | 456.70 |
| 45.491 | Urso-9(11),12-dien-3-one | 0.57 | 253.21 |
| 46.656 | β -Amyrin | 2.34 | 426.72 |
| 47.172 | Lup-20(29)-en-3-one | 9.56 | 424.70 |

The oils of *W. tinctoria* contain many bioactive compounds like γ tocopherol[15,16,17], campesterol[17,18], squalene,[17,19] α amyrin, β amyrin,[17,20] betulin[17, 21] and n-hexadecanoic acid [17] and lupeol.[22] In *W. arborea*, though α amyrin and β amyrin are absent, bioactive compounds γ tocopherol[15,16,17], campesterol[17,18], squalene[17,19] phytol[17] are present. Tocopherols, phytosterols are the most important natural antioxidants in crude oils [23]. In recent past, plant derived antioxidant is intensively studied as they are found to be better in their activity than that of synthetic antioxidants and they are extensively used in pharmacology. Natural antioxidants may function (a) as reducing agents, (b) as free radical scavengers, (c) as complexers of pro-oxidant metals, and (d) as quenchers of the formation of singlet oxygen. They can be used in the food industry and there is evidence that they may exert their antioxidant effects within the human body. Crude oils are consumed in their natural states, thus conserving a number of minor substances, which are usually removed from at various stages of refining. These minor constituents can have either pro-oxidative (free fatty acids) or antioxidative (tocopherols, phenols) effects. The nutritionally important antioxidants such as tocopherols improve the stability of the oils. Phytosterols contained in oils are hypocholesterolemic and their antioxidant activity has been attributed to the formation of an allylic free radical and its isomerization to other relatively stable free radicals.[24] Volatile terpenoid compounds like α amyrin, β amyrin, lupenone, lupeol are proved to have multifunctional anticancer activities. The presence of lupenone (9.56%), a melanogenetic agent[25], along with squalene, a major component of skin surface lipid and a quencher of singlet oxygen[19] indicated the application of oil for skin related disorders.

**Table 2:** Composition of seed oil of *Wrightia arborea* (Dennst) Mabb.

| RT | Name of compound | Peak area % | MW |
|---|---|---|---|
| 3.554 | Ethylene amine | 0.507 | 133.02 |
| 7.006 | 2- Heptanal,(Z) | 0.723 | 114.18 |
| 14.211 | Tetradecane | 0.449 | 198.39 |
| 15.462 | 2,4 - Decadienal | 1.472 | 152.23 |
| 25.569 | 2-Pentadecanone,6, I0, 14-trimethyl | 1.487 | 268.47 |
| 27.921 | n-Hexadecanoic acid | 1.727 | 256.24 |
| 29.922 | Phytol | 0.426 | 296.53 |
| 30.522 | Octaethylene glycol monododecyl ether | 1.194 | 538.75 |
| 30.773 | 9,12-Octadecadienoyl chloride, (Z,Z) | 1.264 | 298.89 |
| 31.473 | E-10, 13,13-Trimethyl-11-tetradecen-1-ol acetate | 2.192 | 296.48 |
| 32.724 | Oxiraneoctanoic acid, 3-octyl-, cis- | 4.338 | 298.46 |
| 33.474 | 4,8,12,16-tetra methyl hepta decan-4-oxide | 3.672 | 275.56 |
| 33.725 | 9,12-Octadecadienoic acid,methyl ester | 1.366 | 294.47 |
| 33.975 | Hexanedioic acid, bis(2-ethylhexyl) ester | 3.375 | 370.56 |
| 34.875 | 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyllcyclohex-1-en-1-carboxaldehyde | 3.862 | 324.49 |
| 35.426 | Ethanol,2-(octadecyloxy) | 2.001 | 314.55 |
| 35.826 | Lup-20(29)-en-3-ol, acetate, (3β) | 4.981 | 468.75 |
| 36.477 | 9,12 Octadecadienoic acid (Z,Z)-/Linoleic acid | 4.981 | 280.44 |
| 36.727 | Heptadecane2,6,10,15 tetramethyl | 1.433 | 296.57 |
| 37.277 | Safranin | 1.442 | 350.84 |
| 36.677 | Lupeol | 3.306 | 426.73 |
| 38.02 | Octadecane,3-ethyl-5-(2-ethylbutyl) | 2.03 | 366.70 |
| 39.078 | 4,4,6a,6b,8a,11,11,14b-octa methyl-1, 4,4a,5, 6, 6a, 6b,7,8,8a,9, L0,L1,,12,12a,14, 14 a, 14b octadecahydro-2 H-picen-3-one | 8.235 | 423.25 |
| 39.429 | Squalene | 2.083 | 410.71 |
| 40.044 | Tricyclo [20.8.0.0(7,16)triacontane , t(22),7 (3.6)diepoxy | 1.78 | 342.12 |
| 40.484 | 3,12-Oleandione | 2.483 | 440.70 |
| 40.899 | Oxirane, 2,2,-dimethy -3-(3,7,12,16,20 - pentamethyl - 3,7,11,15,19-heneicosapentaenyl )- (all E) | 3.124 | 260.13 |
| 41.229 | 2H-1-Benzopyron-6-ol, 3,4-dihydro-2.8- dimethyl-2-(4,8, 1 2-trimethylsilyl)-, [2R-[2R- (4R*,8R*)] ] - | 8.02 | 450.25 |
| 42.205 | Cedrone. B-propoxy- | 0.642 | 362.12 |
| 42.44 | r-Tocopherol | 4.89 | 416.68 |
| 42.92 | Octacosane | 0.613 | 394.76 |
| 43.095 | Podocarp-7-en-3-one,13β-methy-`13-vinyl- | 0.764 | 286.45 |
| 43.94 | 1-Heptatriacontanol | 0.825 | 536.99 |
| 44.646 | Campesterol | 1.222 | 400.68 |
| 47.417 | 13,27 -Cyclo ursan-3-one | 11.173 | 247.23 |

**Table 3:** Biological activity of chemical compounds present in the *Wrightia tinctoria* and *W. arborea*

| Name of the compound | Compound nature | Medical/ biological activity |
|---|---|---|
| Campesterol | Sterol | Antioxidant; Hypocholesterolemic; |
| Beta amyrin | pentacyclic triterpene | Analgesic; Antiedemic; Anti inflammatory; Antinociceptive; Antiulcer; Gastroprotective; Hepatoprotective; Larvicide; Mosquitocide; |
| Gamma tocopherol | Methylated phenol | AntiCRP; Antiatherosclerotic; Anticancer; Antiinflammatory; Antioxidant; Antiprostaglandin; Cardioprotective; Cyclooxygenase- Inhibitor; Hypocholesterolemic; NO-Inhibitor; Natriuretic; PKC-Inhibitor; |
| Squalene | Triterpene | Antibacterial; Antioxidant; Antitumor; Cancer Preventive; Chemo preventive; Immunostimulant; Lipoxygenase-Inhibitor; Perfumery; Pesticide; |
| Alpha amyrin | Pentacyclic triterpene | Analgesic; Anti inflammatory; Anti nociceptive; Gastroprotective; Hepatoprotective; Insectifuge |
| n-Hexadecanoic acid | Palmitic acid | 5-Alpha-Reductase-Inhibitor; Antiandrogenic; Antifibrinolytic antioxidant, Flavor; hemolytic, hypercholesterolemic, lubricant, nematicide, pesticide, propecic, soap |
| Betulin | Triterpene | Anti HIV; Anti carcinomic; Anti feedant; Antiflu; Anti inflammatory, Antitumor; Antiviral; Aphidifuge, Cytotoxic; Hypolipemic; Prostaglandin-Synthesis-Inhibitor, Topoisomerase-II-Inhibitor |
| Lupeol | Triterpene | Anti protozoal, anti inflammatory, anti tumor, neutraceutical , chemo preventive agent, antimicrobial |
| Phytol | Diterpene (acyclic) | Cancer preventive |

## CONCLUSION

Screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule and drug-like properties present the identification of multi- target compound. The present study to investigate the phytochemical identification of seed oil of *W. tinctoria* and *W. arborea* by GC-MS analysis indicated a concentrated dependent antioxidant, anti inflammation and anticancerous ability which indicates the great potential as an interesting source for natural health products.

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