

GC-MS Analysis of Phytocomponents in, Pet Ether Fraction of *Wrightia tinctoria* Seed

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ABSTRACT

Introduction: *Wrightia tinctoria* R.Br. (Family: Apocynaceae) commonly called “Indrajau” is well known in Indian traditional system for its traditional uses. **Materials and Methods:** The present investigation was carried out to determine the possible bioactive components of plant seed ethanolic extract, pet ether fraction using GC-MS analysis. 22 components were identified from pet ether fraction obtained from elution of ethanolic extract packed in silica column. **Results:** The prevailing compounds from fraction F6 to F9 were [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (21.39%) , Trilinolein (7.74%), 2-Myristinoyl pantetheine (18.07%), 9-Octadecan-12-ynoic acid, methyl ester (4.46%), 1Hexadecanol,2-methyl (3.77%), Cyclopropane tetradecanoic acid, 2-octyl-, methyl ester (2.36%), 1b, 4a-Epoxy-2H-cyclopenta [3,4] cyclopropano [8,9]cycloundec [1,2-b]oxiren-5 (6H)-one, 7-(acetyloxy) decahydro-2,9,10-trihydroxy-3,6,8,8,10a-pentamethyl (38.91%), Geranyl isovalerate (23.58%), cis-13-Octadecenoic acid (5.91%), Quassin (3.82%), cis-10-Heptadecenoic acid (3.08%), 9,12,15-Octadecatrienoic acid 2-phenyl-1, 3-dioxan-5-yl ester (31.50%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-2,3-dihydroxypropyl ester (14.35%), Cyclopropanebutanoic acid, 2-[2-[2- [(2-pentylcyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl] methyl]-, methyl ester (10.13%), 6,9,12,15-Docosatetraenoic acid, methyl ester (3.39%), 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-(2.73%), 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-(4.34%), Ursodeoxycholic acid (7.14%), Bufa-20,22-dienolide, 3-(acetyloxy)-14,15-epoxy-16-hydroxy-, (3á,5á,15á,16á)-(4.75%), 5H-Cyclopropano [3,4] benz [1,2-e]azulen-5-one, 9a (acetyloxy)-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, [1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-(6.59%), Docosahexaenoic acid, 1,2,3-propanetriyl ester (10.86%), Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)-(4.40%) found as the major components. **Conclusion:** It could be concluded that, *Wrightia tinctoria* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance.

Key words: Bioactive components, Ethanolic extract, GC-MS, Indrajau, *Wrightia tinctoria*.

INTRODUCTION

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant

extracts with potentially useful properties for further chemical and pharmacological investigations.¹ GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc.² *Wrightia tinctoria* R.Br. (Family: Apocynaceae) commonly called “Indrajau” is distributed throughout the world and occurs abundantly in India. It is a deciduous tree with white fragrant flowers. The seeds and bark of this plant are used in Indian traditional medicine as anti-diarrheal and anti-dysenteric.³

Medicinal uses: Ethno medically, the bark of this plant is used as a galactagogue, to treat abdominal pain, skin diseases and wounds, as an antipyretic, antidysenteric, antidiarrheal and antihemorrhagic agents and as an antidote for snake poison. Seeds of this plant are also used as an aphrodisiac.

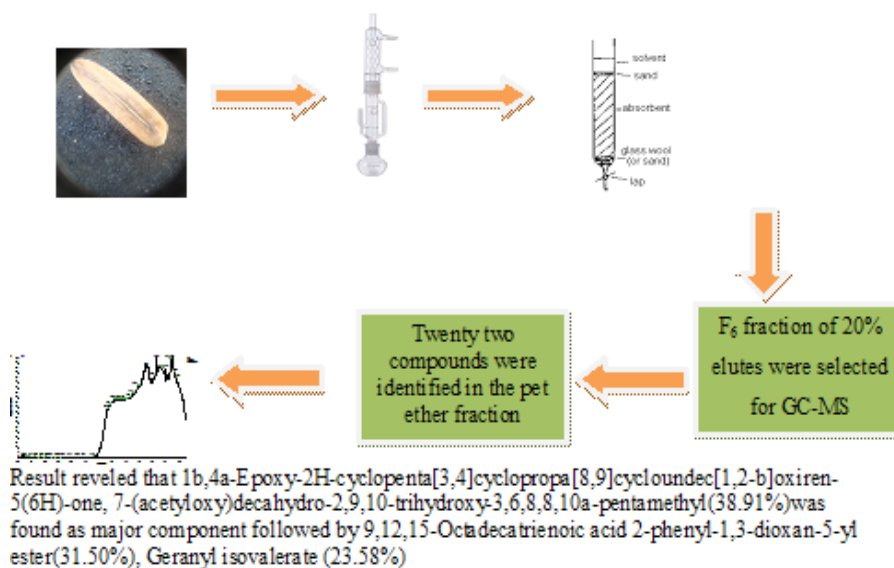
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Graphical Abstract

In view of the reported severe health hazards of estrogen, such as increased risk of endometrial hyperplasia and carcinoma breast cancer and thromboembolic diseases. A large number of natural products showing promising antifertility activity in preliminary studies could not be pursued due to their associated estrogen-agonistic activity.⁴

MATERIALS AND METHODS

Collection of plant material

The *Wrightia tinctoria* seed were collected from its natural habitat in Jharkhand and identified by Botanist at (NBRI) national botanical research institute, Lucknow, Voucher specimens were preserved at the Herbarium of the institute.

Preparation of extract and fraction

The seeds of *Wrightia tinctoria* washed dried and powered, extracted with per ether, chloroform, ethanol and water in successive session in soxhlet percolator. Extractable value calculated by evaporating the solvent in vacuum evaporator. In pet ether no crystalline component obtained instead get rubbery material, while chloroform and ethanolic extracts are semisolid in nature. Ethanolic extract packed in silica column and column is eluted with 10%, 20%, 30%, 40%, 50% pet ether and chloroform mixture solvents. The F₆ fraction of 20% elutes were selected for GC-MS analysis.

GC-MS analysis

MS sample has been analyzed on THERMO Scientific TSQ 8000_(triple quadrupole Gas Chromatograph-Mass

Spectrometer) This mass spectrometer comes paired with the TRACE 1300 GC along with Auto-sampler for automated sample. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min. Ion Source Type: EI source programmable to 350°C. Scans: 731. Condition is: Initial temperature is kept at 60°C, then temperature is increased to 280°C with a max ramp of 90°C/min. Then temperature is allowed to cool down. MASS range was kept between 50-500 m/z. injection volume 10 (µl). Run Time: 2.48 min, This GC-MS is equipped with NIST Library.

Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and molecular formula and probability of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC-MS analysis

The components present in the ethanolic extract of seed pet ether fraction was identified by GC-MS (Figures 1-6). The prevailing compounds from fraction F₆ to F₉ were [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (21.39%) , Trilinolein (7.74%), 2-Myristynoyl pantetheine (18.07%), 9-Octadecen-12-ynoic acid, methyl ester (4.46%), 1Hexadecanol, 2-methyl

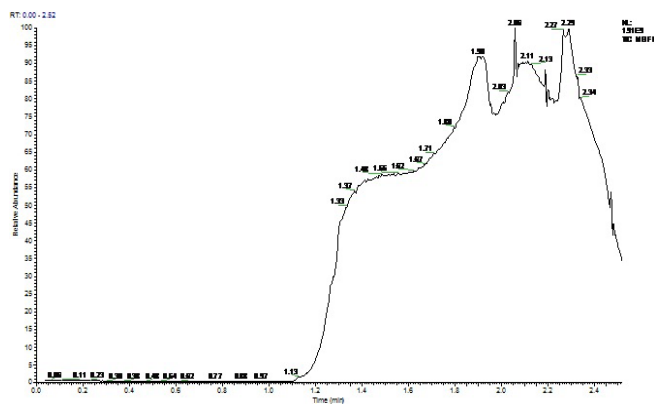


Figure 1: GC-MS total ion current chromatogram of pet ether fraction

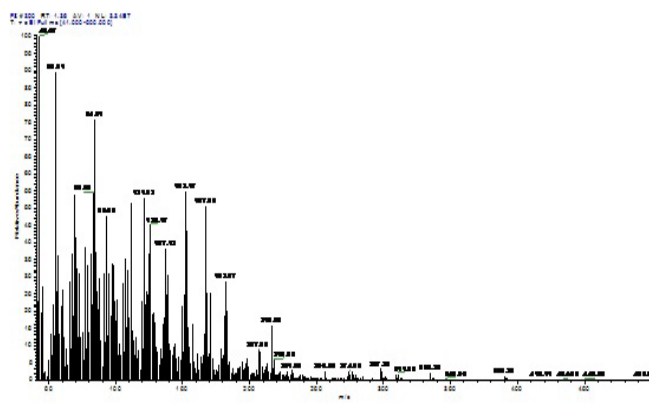


Figure 2: GC-MS Spectra 1

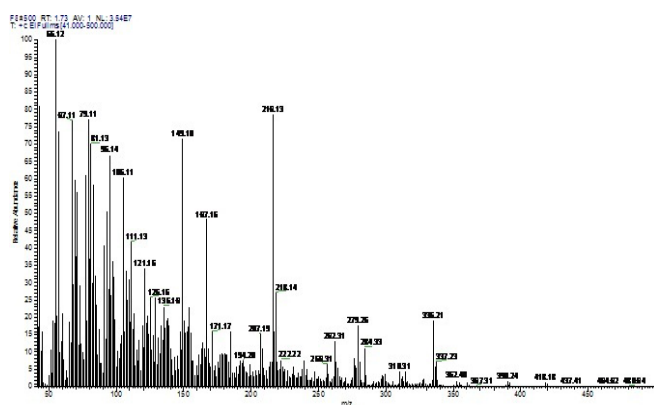


Figure 3: GC-MS Spectra 2

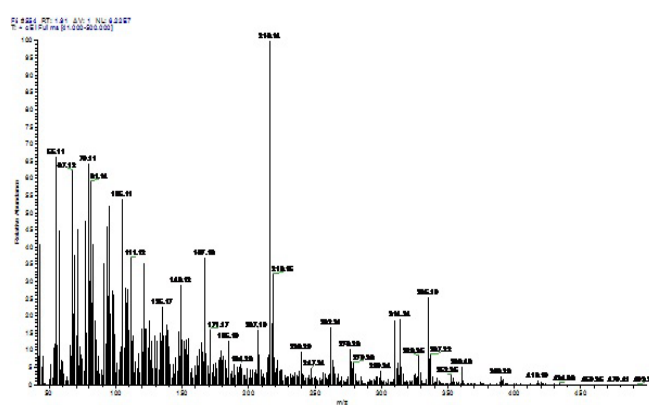


Figure 4: GC-MS Spectra 3

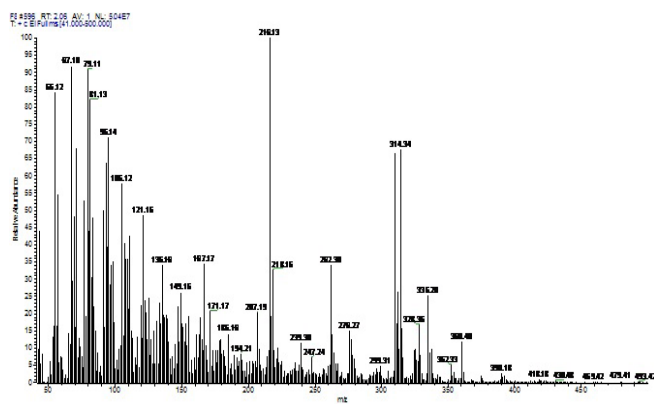


Figure 5: GC-MS Spectra 4

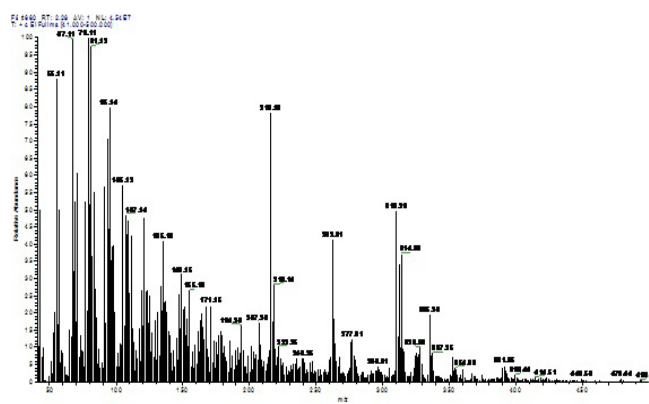


Figure 6: GC-MS Spectra 5

(3.77%), Cyclopropane tetradecanoic acid, 2-octyl-methyl ester (2.36%), 1b, 4a-Epoxy-2H-cyclopenta[3,4] cyclopropa[8,9]cycloundec[1,2-b]oxiren-5(6H)-one, 7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,10-pentamethyl (38.91%), Geranyl isovalerate (23.58%), cis-13-Octadecenoic acid (5.91%), Quassin (3.82%), cis-10-Heptadecenoic acid (3.08%), 9,12,15-Octadecatrienoic acid 2-phenyl-1,3-dioxan-5-yl ester (31.50%), 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)-2,3-dihydroxypropyl

ester (14.35%), Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-,methyl ester (10.13%), 6,9,12,15-Docosatetraenoic acid, methyl ester (3.39%), 9, 12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-(2.73%), 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-(4.34%), Ursodeoxycholic acid (7.14%), Bufa-20,22-dienolide, 3-(acetyloxy)-14,15-epoxy-16-hydroxy-,

Compound name (con%)	Pharmacological activity
Trilinolein (7.74%)	Anti-ischemic, Antiarrhythmic, and Antioxidant
2-Myristylnoyl pantetheine(18.07%)	---
9-Octadecen-12-ynoic acid, methyl ester (4.46%)	Immunotoxicity effects, and Antioxidant activity
1-Hexadecanol, 2-methyl (3.77%)	Antimicrobial
Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester (2.36%)	Antimicrobial
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (21.39%)	Antibacterial
1b,4a-Epoxy-2H-cyclopenta[3,4] cyclopropa[8,9] cycloundec[1,2-b]oxiren-5(6H)-one, 7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,8,10a-pentamethyl(38.91%)	---
Geranyl isovalerate (23.58%)	Antimicrobial
cis-13-Octadecenoic acid (5.91%)	dopaminergic stimulatory activity
Quassin(3.82%)	Antiplasmodial and antiviralactivity
cis-10-Heptadecenoic acid (3.08%)	Anticancer
9,12,15-Octadecatrienoic acid 2-phenyl-1,3-dioxan-5-yl ester(31.50%)	---
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-2,3-dihydroxypropyl ester (14.35%)	Analgesic, Antipyretic, Anticonvulsant, Antiseptic
Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester(10.13%)	---
6, 9, 12, 15-Docosatetraenoic acid, methyl ester (3.39%)	Anticholesterol compound
9, 12-Octadecadienoic acid, (2-phenyl-1, 3-dioxolan-4-yl) methyl ester, trans-(2.73%)	---
9, 12-Octadecadienoic acid, (2-phenyl-1, 3-dioxolan-4-yl) methyl ester, cis-(4.34%)	---
Ursodeoxycholic acid (7.14%)	---
Bufa-20, 22-dienolide, 3-(acetyloxy)-14, 15-epoxy-16-hydroxy-, (3á,5á,15á,16á)-(4.75%)	---
5H-Cyclopropa[3,4] benz [1,2-e]azulen-5-one, 9a-(acetyloxy)-1,1a,1b,4,4a,7a,7b,8,9,9a-de cahydro-4a,7b,9-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-,[1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-(6.59%)	---
Docosahexaenoic acid, 1, 2, 3-propanetriyl ester(10.86%)	---
Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15á,16á,21á,22à)-(4.40%)	---

(3á,5á,15á,16á)-(4.75%), 5H-Cyclopropa [3,4] benz [1,2-e] azulen-5-one, 9a (acetyloxy)-1,1a,1b,4,4a,7a,7b,8,9,9a-de cahydro-4a,7b,9-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-,[1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-(6.59%), Docosahexaenoic acid, 1,2,3-propanetriyl ester(10.86%), Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15á,16á,21á,22à)-(4.40%) found as the major components.

Twenty two compounds were identified in the pet ether fraction; principal components with their % concentration and pharmacological activity were presented in (Table 1).

Result revealed that 1b, 4a-Epoxy-2H-cyclopenta [3,4] cyclopropa [8,9] cycloundec [1,2-b]oxiren-5 (6H)-one, 7-(acetyloxy) decahydro-2, 9, 10-trihydroxy-3,6,8,8,10a-pentamethyl (38.91%) was found as major component followed by 9,12,15-Octadecatrienoic acid 2-phenyl-1,3-dioxan-5-yl ester (31.50%), Geranyl isovalerate (23.58%) .

CONCLUSION

In the present study, 22 components were identified from seed alcoholic extract, pet ether fraction by

Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. However, isolation of individual constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *Wrightia tinctoria* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

CONFLICTS OF INTEREST

Authors declared no conflict of interest.

Highlights of Paper

- *Wrightia tinctoria* R.Br. (Family: Apocynaceae) commonly called “Indrajau” is well known in Indian traditional system for its traditional uses.
- The components present in the ethanol extracts of *Wrightia tinctoria* seed pet ether fraction was identified by GC–MS.
- Twenty two compounds were identified in the pet ether fraction.
- Result revealed that 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxiren-5(6H)-one, 7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,8,10a-pentamethyl(38.91%) was found as major component followed by 9,12,15-Octadecatrienoic acid 2-phenyl-1,3-dioxan-5-yl ester(31.50%), Geranyl isovalerate (23.58%).
- *Wrightia tinctoria* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance

Author Profile

- **Dr. Alok Mukerjee:** Presently working as Principal in UNITED INSTITUTE OF PHARMACY, Industrial Area, Naini, Allahabad. (U.P.), he has 19 years of teaching experience, more than 30 paper presented, organised and attended number of conferences and workshops, delivered a number of scientific talks, published 13 research articles in various reputed journals. Member of Board of Study Assam University, Silchar, Assam V.B. Singh University, Jaunpur (U.P.) SHIATS, AAI Deemed University, Allahabad (U.P.).
- **Dr. Amita Verma:** Presently Working as Associate professor and Head in Deptt. of Pharmaceutical sciences, SHIATS, Allahabad. She has published more than 79 research article in various reputed journals. She awarded with prestigious two months summer research fellowship by Indian Science Academy and also attended 26 conferences/ seminars and organized various events.
- **Rajani Srivastava:** Presently working as Asstt. Prof. in dept of pharmaceutical sciences, faculty of health sci. SHIATS, Allahabad. She has teaching experience of 8 years. She has 03 publication in journals, she has attended and research paper presented/ participated in 02 international and 25 national seminar/ conferences and various workshop attended. She has life membership of APTI (Association of Pharmaceutical Teachers of India) and pharmaceutical society.

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ABBREVIATION

GC–MS: Gas Chromatography–Mass Spectrometry.

NIST: National Institute of standard and Technology.