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## Isolation and structure elucidation of a new glyceride from seeds of *Wrightia tinctoria* found in Jharkhand region of India

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### Abstract

A glyceride compound was isolated from the seeds of *Wrightia tinctoria* (Apocynaceae). Their structure was established, on the basis of spectroscopic data. The ethanolic extract of *Wrightia tinctoria* seeds packed in silica column and column was eluted with 10%, 20%, 30%, 40%, 50% petroleum ether and chloroform mixture solvents. The F<sub>6</sub> fraction of 20% elutes assigned as Glyceryl-1-linoleryl-2-achidyl-3-docos-9'', 12''-dienoate. It was found to be pale yellow color liquid; its structure was assigned on the basis of IR, <sup>1</sup>H NMR, <sup>13</sup>CNMR and MS analysis.

**Keywords:** *Wrightia tinctoria*, Column chromatography, ethanolic extract

### Introduction

Natural products, like plants extract, either as purified compounds or in the form of standardized extracts, provide immense opportunities for new drug discoveries or researches, the reason behind is, because of the unbeaten availability of chemical diversity [1]. In Asia a large number of Plants used as traditional medicine which contains a wide spectrum of natural substances that can be used for treatment of chronic as well as infectious diseases [2]. *Wrightia tinctoria* R.Br. belongs to family Apocynaceae, commonly known as Indra jau and dudhi, the common vernacular names known are as: sweet indrajao, ivory wood, pala indigo plant in English, duhi, mitaindarjau kapar in Hindi, dandappala, ayyappala, vettupala in Malayalam, kala kuda in Marathi, Paalai in Tamil. It is distributed throughout the world and found in north and central part of India [3]. In continuation on our previous report on the GC-MS analysis of pet ether fraction obtained from elution of ethanolic extract of *W. Tinctoria* packed in silica column revealed 22 components. The prevailing significant compounds from fraction F<sub>6</sub> to F<sub>9</sub> were 6, 9, 12, 15-Docosatetraenoic acid, methyl ester (3.39%, Anticholesterol compound) and Trilinolein (7.74%, anti-ischemic, antiarrhythmic, and antioxidant) [4]. The plant is reported to contain the presence of flavanoid, glycoflavones-iso-orientin, and phenolic acids and so many other pharmaceutically significant natural compounds [5]. The numbers of phytoconstituents reported from various plant parts of the plant till date as follows: lupeol, stigmasterol, and campesterol, 3,4-Seco-lup-20 (29)-en-3-oic acid, triterpene, tryptanthrin, isatin, anthranillate, and rutin, Triacontanol and tryptanthrin Wrightial, cycloartenone, cycloeucaenol, beta-amyrin, and beta-sitosterol, Cycloartenone, cycloeucaenol and four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol, and 24-dehydropollinastanol, were isolated and identified in addition to several more common phytosterols [3].

### Methodology

#### Plant Material

The *Wrightia tinctoria* seed were collected from its natural habitat in Jharkhand, identified by botanist at (NBRI) National Botanical Research Institute; Lucknow, India, voucher specimens (NBRI-SOP-202) were preserved at the Herbarium of the same institute for future reference.

#### Preparation of *Wrightia tinctoria* seed Extracts

The seeds of *Wrightia tinctoria* thoroughly washed dried and powered, extracted with petroleum ether, chloroform, ethanol in successive session in soxhlet percolator. Extractable value calculated by evaporating the solvent in vacuum evaporator. Pet. Ether, Chloroform, Alcohol, W soluble extractives were reported 20%, 6%, 10%, 4% w/w. In pet ether no crystalline component obtained, while chloroform and ethanolic extracts were semisolid in nature. Ethanolic extract of the plant was selected to carry out the isolation of natural compounds.

**Isolation of compound through Column Chromatography**

Column chromatography used for the separation of natural compounds. The solid - liquid technique of isolation was used, in which the stationary phase was a solid silica gel of column grade & mobile phase was in a liquid state. The principle behind column chromatography based on differential adsorption of substance by the adsorbent. The adsorbent of silica gel was made into slurry with petroleum ether and placed in a cylindrical chromatographic column that is plugged at the bottom by a piece of glass wool or porous disc. The ethanolic extract was selected for isolation of natural compounds. Attached the column to a ring stand and fastened in a vertical position. Continued to transfer the slurry to the column until all the silica added, after finished packing, drained the excess solvent and closed the pinch clamp. The properly packed column used for separation of compounds. A small amount of sand is added to the top of the column to prevent it from being disturbed when fresh solvent is added.

**Loading and elution of gravity chromatography column**

The sample to be analyzed was ethanolic extract of *Wrightia tinctoria* dissolved in a very small amount of solvent ethanol (90%) and made slurry with silica, allowed to dry at room temp, finally sieved through sieve to form uniform powder, added to the top of the column. The pinch clamp was opened and the solvent was allowed to drain just to the top of the column. A small amount of the eluting solvent (pet ether) was added and allowed to drain in until the mixture is a little way into the adsorbent, then the column was filled to the top with eluting solvent (pet ether), continued adding solvent at the top and collecting fractions at the bottom until the compounds elute at the bottom. The purified ethanolic extract subjected to isolation process by column chromatography. The ethanolic extract of *Wrightia tinctoria* packed in silica column and column was eluted with 10%, 20%, 30%, 40%, 50% pet ether and chloroform mixture solvents. The F<sub>6</sub> fraction of 20% elutes were selected for FTIR, GC-MS and NMR analysis.

FT-NMR Cryo-magnet Spectrometer 400 MHz (Bruker) was used for NMR study and F.T. Infra-Red Spectrophotometer Model RZX (Perkin Elmer) was used for IR analysis from Panjab University.

**Isolation and characterization of natural compounds of *Wrightia tinctoria* alcoholic extract.**

The ethanolic extract of *Wrightia tinctoria* packed in silica column and column was eluted with 10%, 20%, 30%, 40%, 50% pet ether and chloroform mixture solvents. The F<sub>6</sub> fraction of 20% elutes assigned as compound 1 were selected for FTIR, GC-MS and NMR analysis.

**Results and discussions**

The column chromatographic separation of ethanolic extract of *Wrightia tinctoria* seed yielded one new glyceride structure of this was confirmed through spectral analysis.

**Identification of isolated component from *Wrightia tinctoria* alcoholic extract**

The compound-1 identified and assigned as: Glyceryl-1-linoleyl-2-achidyl-3-docos-9'', 12''-dienoate. Compound-1 from *Wrightia tinctoria* alcoholic extract was found to be pale yellow color liquid its structure (Figure-A) was assigned as Glyceryl-1-linoleyl-2-achidyl-3-docos-9'', 12''-dienoate on the basis of IR, <sup>1</sup>H NMR, <sup>13</sup>CNMR and MS analysis.

**IR(KBr)**

2927,2855,1737,1721,1645,1463,1377,1272,1244,1176,1115,

1059,985,724 cm<sup>-1</sup>

**<sup>1</sup>H NMR(CDCl<sub>3</sub>)**

δ 5.50(2H,m,H-10',H-12''),5.37(1H,m,H-10''),5.36(1H,m,H12''),5.35(1H,m,H9''),5.34(2H,m,H-9''),H13''),5.33(1H,m,H13),4.20(1H,m,H2),4.16(2H,m,H2),4.13(2H,m,H23),2.80(2H,m,H211),2.76(2H,m,H211''),2.36(2H,t,J=7.2Hz,H-2''),2.33(2H,t,J=7.5Hz,H-2''),2.31(2H,t,J=6.9Hz,H-2''),2.07(2H,m,H28),2.05(2H,m,H214''),2.03(2H,m,H28''),2.01(2H,m,H214''),1.62(4H,m,2CH<sub>2</sub>),1.60(4H,m,2CH<sub>2</sub>),1.36(6H,m,3CH<sub>2</sub>),1.28(14H,7CH<sub>2</sub>),1.25(16H,8CH<sub>2</sub>),1.22(30H,18CH<sub>2</sub>),0.86(3H,t,J=6.3Hz,Me18'),0.84(3H,t,J=6.5Hz,Me20''),0.82(3H,t,J=6.6Hz,Me-22'')

**<sup>13</sup>CNMR (CDCl<sub>3</sub>)**

δ 172.58(C-1'), 169.231(C-1''),168.71(C-1'''),131.96(C-9''),130.23(C-13'),130.01(C-9''), 128.30(C-13''), 128.08(C-10'),127.90(C-12'), 127.76(C-11'') 127.11(C-12''),70.28(C-2), 66.16(C-1), 62.35(C-3), 41.35(CH<sub>2</sub>), 36.07(CH<sub>2</sub>) 34.66(CH<sub>2</sub>), 34.14(CH<sub>2</sub>)33.89(CH<sub>2</sub>)32.71(CH<sub>2</sub>),31.91(CH<sub>2</sub>), 31.51(CH<sub>2</sub>), 29.83(CH<sub>2</sub>), 29.69(6 CH<sub>2</sub>),29.65(CH<sub>2</sub>) 29.59(CH<sub>2</sub>), 29.42(CH<sub>2</sub>), 29.34(CH<sub>2</sub>), 29.08(CH<sub>2</sub>), 29.05(5 CH<sub>2</sub>), 28.99(CH<sub>2</sub>), 28.20(CH<sub>2</sub>) 27.66(CH<sub>2</sub>), 27.19(2CH<sub>3</sub>), 26.91(2 CH<sub>2</sub>),25.83(CH<sub>2</sub>),25.62(CH<sub>2</sub>), 25.53(CH<sub>2</sub>), 25.28(CH<sub>2</sub>), 24.89(CH<sub>2</sub>), 24.72(CH<sub>2</sub>), 22.80(CH<sub>2</sub>), 22.68(CH<sub>2</sub>),22.60(CH<sub>2</sub>),22.56(CH<sub>2</sub>),20.68(CH<sub>2</sub>), 20.54(CH<sub>2</sub>), 19.42(CH<sub>2</sub>), 18.75(CH<sub>2</sub>), 14.25(Me-18'), 14.09(Me-20''), 11.40(Me-22'')

**ESIMS m/z**

968[M]<sup>+</sup> (C<sub>63</sub>H<sub>116</sub>O<sub>6</sub>)(1-8),335(27.6) 311(70.1), 295(8.3), 279(18.2)

Glyceryl-1-linoleyl-2-achidyl-3-docos-9'',12''-dienoate (Compound-1) from *Wrightia tinctoria* alcoholic extract was found to be pale yellow color liquid its structure (Figure-A) was assigned as Glyceryl-1-linoleyl-2-achidyl-3-docos-9'',12''-dienoate on the basis of IR, <sup>1</sup>H NMR, <sup>13</sup>CNMR and MS analysis.

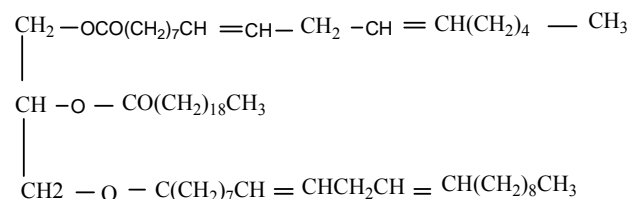


Figure A. compound 1: Glyceryl-1-linoleyl-2-achidyl-3-docos-9'',12''-dienoate

**Conclusion**

Plant-derived and natural product have provided many novel bioactive molecules, some of which have led to important drugs that are available on the market today. The present investigation regarding isolation of natural compounds from *Wrightia tinctoria* seed based on traditional claims in Jharkhand region of India. But study needs attention to explore its possible biological activities for development of plant derived safe pharmaceuticals.

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