Phytochemical Profiling of Ethanolic Bark Extract of *Wrightia tinctoria* using GC-MS

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Abstract
This study investigates the phytochemical composition and biological activities of the ethanolic bark extract of *Wrightia tinctoria*. GC-MS analysis revealed 14 bioactive compounds, including triterpenoids, phthalates, fatty acids, and steroids.

Keywords: *Wrightia tinctoria*, GC-MS, triterpenoids, fatty acids, phytochemical profiling

1. Introduction

Wrightia tinctoria (Roxb.) R. Br. is a small to medium-sized deciduous tree belonging to the family Apocynaceae (Dogbane family). It is an economically and medicinally significant plant native to the Indian subcontinent and Southeast Asia. The species is widely known by several vernacular names, with its most common English names being the Pala indigo plant and Dyer's oleander, referencing its historical use as a source of blue dye. In India, it is often called *Sweet Indrajao*, *Dudhi* (Hindi), and *Veppalai* or *Vetpalai* (Tamil). It is naturally distributed across countries including India, Myanmar, Nepal, Timor, and Vietnam, with a significant presence in peninsular and central India [1]. It thrives in a variety of habitats, primarily found in dry and moist deciduous forests on plains and hillsides [2]. Revered in traditional systems of medicine such as Ayurveda and Siddha, various parts of the plant are used to treat a range of ailments [3]. It is most renowned for its traditional application in treating skin disorders like psoriasis and eczema, often in the form of an oil prepared from the leaves [4]. Traditional uses also include treating fever, dysentery, gastrointestinal issues, and toothache. Modern phytochemical studies have identified bioactive components like flavonoids, alkaloids, and triterpenoids, supporting its traditional claims of having anti-inflammatory, antioxidant, and antimicrobial properties [5].

Gas Chromatography-Mass Spectrometry (GC-MS) has emerged as a powerful and highly sensitive tool for the separation, identification, and quantitative analysis of volatile and semi-volatile secondary metabolites in complex plant extracts [6]. GC-MS analysis allows for the detection of compounds like fatty acids, esters, terpenoids, steroids, and other organic molecules, providing a detailed phytochemical information of the plant material.

Medicinal plants are a rich source of therapeutic agents, with bark extracts often used in traditional medicine. *Wrightia tinctoria* (Apocynaceae) is widely employed in Indian ethnomedicine for treating skin disorders, inflammation, and microbial infections. Despite its traditional relevance, comprehensive studies on its phytochemical constituents and biological activities are limited. This study aims to characterize the phytochemical profile of *Wrightia tinctoria* bark using GC-MS.

2. Materials and Methods

2.1 Plant Material and Extraction

Bark of *Wrightia tinctoria* was collected from Tanjore district, Tamil Nadu, shade-dried, powdered, and extracted with ethanol using a Soxhlet apparatus for 48 hours.

2.2 GC-MS Analysis

The ethanolic extract was analyzed using an Agilent 8890 GC system coupled with a 7000 Triple Quadrupole Mass Spectrometer (GC/TQ), designated CH-GCMSMS02. Chromatographic separation was achieved using an HP-5MS capillary column (30 m × 250 µm × 0.25 µm). Helium was used as the carrier gas at 1.0 mL/min. The oven temperature program ranged from 50 °C to 280 °C over 38 minutes. Mass spectra were recorded in scan mode (m/z 30–900) and compounds were identified.

3. Results

3.1 Phytochemical Profiling

The GC-MS analysis revealed the presence of 14 bioactive compounds. The most abundant compound detected was Olean-12-en-3-ol, acetate, (3.beta.)-, a triterpenoid with a retention time (RT) of 37.7569 minutes, contributing 29.27% to the total composition. Phthalates were prominently represented, with Diethyl Phthalate appearing twice (RT: 20.3635 and 20.2423) and Phthalic acid, di(2-propylpentyl) ester (RT: 30.5272), collectively accounting for over 39% of the total area. Dimethyl ether (RT: 3.3963) was the most significant ether compound, contributing 9.03%, while Methylal (RT: 3.1908) was present in trace amounts (0.22%). Triterpenoids also included Ursa-9(11),12-dien-3-one at two retention times (36.3988 and 37.0951), contributing 4.68% and 4.48% respectively. Fatty acids such as n-Hexadecanoic acid (RT: 24.7473), 9-Octadecenoic acid (RT: 26.6160), and Octadecanoic acid (RT: 26.8562) were detected, with relative percentages ranging from 0.89% to 4.14%. Steroidal content was represented by 17-(1,5-Dimethylhexyl)-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-7-ol (RT: 33.5097), contributing 2.74%. Other compounds included hydrocarbons like Pentacosane (RT: 30.0398), Tetradecane (RT: 16.4587), and Dodecane (RT: 11.1960), each contributing around 1%. Methyl salicylate (RT: 11.2351) was also present at 0.76%. The GC-MS chromatogram of the ethanolic bark extract of *Wrightia tinctoria* showed several peaks (Figure 1). Table 1 summarizes the compounds identified, their retention times, molecular formulas, peak area percentages and classification.

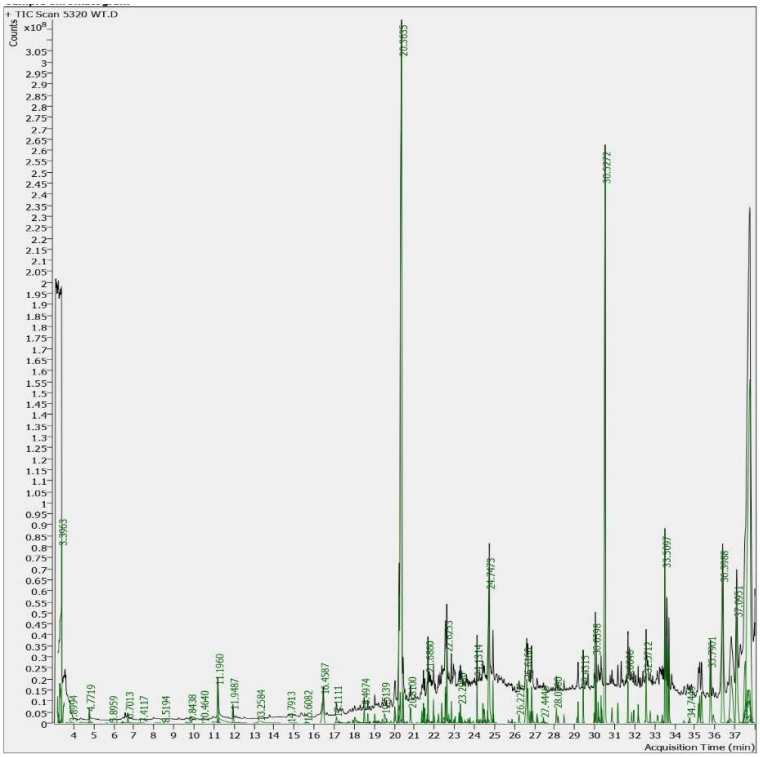

Fig 1. **GC-MS Chromotogram of Ethanolic extract of *Wrightia tinctoria* bark**

Table 1: GC-MS Profile of Bioactive Compounds: Retention Time, Molecular Data, and Classification

S.No.	RT	Compound	Molecular formula	Area	Match	Relative %	Class
1	37.7569	Olean-12-en-3-ol, acetate, (3.beta.)-	C32H52O2	1659839129.6	75.9	29.27	Triterpenoids
2	20.3635	Diethyl Phthalate	C12H14O4	1375300380.1	97.3	24.25	Phthalates
3	30.5272	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	670498811.4	95.7	11.82	Phthalates
4	3.3963	Dimethyl ether	C2H6O	511932194.5	85.4	9.03	Ethers
5	36.3988	Ursa-9(11),12-dien-3-one	C22H23NO7	265656378.0	93.6	4.68	Triterpenoids
6	24.7473	n-Hexadecanoic acid	C16H32O2	234568460.6	92.8	4.14	Fatty Acids
7	33.5097	17-(1,5-Dimethylhexyl)-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-7-ol	C27H48O	155302467.2	70.5	2.74	Steroids
8	26.6160	9-Octadecenoic acid, (E)-	C18H34O2	81333799.3	84.5	1.43	Fatty Acids
9	30.0398	Pentacosane	C25H52	61459423.7	90.8	1.08	Alkane
10	16.4587	Tetradecane	C14H30	60996873.8	91.6	1.08	Alkane
11	26.8562	Octadecanoic acid	C18H36O2	50709531.7	86.3	0.89	Fatty Acids
12	11.1960	Dodecane	C12H26	43484753.2	91.7	0.77	Alkane
13	11.2351	Methyl salicylate	C8H8O3	43292340.0	91.1	0.76	Non Steroids
14	3.1908	Methylal	C3H8O2	12699714.8	73.4	0.22	Aliphatic ethers

4. Discussion

The most prominent bioactive compound was Olean-12-en-3-ol, acetate, (3. beta.)- (RT 37.7569), a triterpenoid with well-documented anti-inflammatory, hepatoprotective, and anticancer properties [7]. Its high component area and retention time suggest significant abundance, reinforcing the therapeutic potential of the extract. Three fatty acids were identified: n-hexadecanoic acid (palmitic acid, RT 24.7473), 9-octadecenoic acid (E) (oleic acid, RT 26.6160), and octadecanoic acid (stearic acid, RT 26.8562). These are ubiquitous in plant lipid metabolism and have been implicated in modulating inflammation, apoptosis, and membrane dynamics [8]. Methyl salicylate (RT 11.2351), a well-characterized plant volatile, functions as a signaling molecule in systemic acquired resistance and exhibits anti-inflammatory and analgesic properties [9]. Its presence supports the extract's potential therapeutic relevance.

17-(1,5-dimethylhexyl)-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-7-ol (RT 33.5097), was detected with moderate match factor. Although its precise biological role remains unclear, its structural similarity to phytosterols suggests possible hormonal or membrane-modulating activity [Piironen et al., 2000].

Continuing GC-MS investigation of *W. tinctoria* bark extract with different solvents is crucial for isolating and structurally elucidating novel or major bioactive compounds, paving the way for the development of new plant-based drugs and standardized herbal formulations.

5. Conclusion

The ethanolic bark extract of *Wrightia tinctoria* exhibits a rich phytochemical profile with pharmacologically relevant compounds. The findings validate its traditional medicinal use and suggest potential for further drug development.

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