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Phytochemical & GC-MS Analysis of Hexane Extract from the Leaves of *Artabotrys odoratissimus* (R. Br.)

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Abstract

Artabotrys Odoratissimus (R. Br.), a member of the Annonaceae family, is a medium-sized shrub known for its fragrant flowers and medicinal properties. This study investigates the phytochemical composition of hexane extract derived from the leaves of *Artabotrys Odoratissimus* using gas chromatography-mass spectrometry (GC-MS). The analysis revealed 20 distinct peaks corresponding to 24 compounds, identified by comparing retention times and interpreting mass spectra.

Notably, **AOP-4 (A)** (*245-Stigmast-5, 22 E-diene-3p-ol*) & **AOP-4 (B)** (*24R-Stigmast-5-ene-38-ol*), two **stigmastane-based sterols**, were identified, highlighting the plant's rich sterol content. Other key hydrocarbons compounds included such as ***Eicosane, Heneicosane, Heptadecane, and Nonadecane; fatty acids like Hexadecenoic Acid and Octadecanoic Acid; alcohols such as 1-Nonadecanol; ketones like 2-Dodecanone; and esters such as Dibutylphthalate and Bis(2-Ethylhexyl) Phthalate***. Quantitative analysis of oily fraction of leaves was conducted by measuring the GC-MS peak areas.

The findings highlight the diverse phytochemical profile of ***Artabotrys Odoratissimus*** and reinforce its potential applications in traditional and modern medicine. This research provides valuable insights into the chemical composition of the plant, underscoring its importance as a resource for medicinal, therapeutic & antimicrobial use.

Keywords: Artabotrys Odoratissimus; Phytochemical; Gas Chromatography; Mass Spectrometry (MS); Hexane extract

***Note:** The materials and methodology for this paper were collected in 2005. For various reasons, we have been publishing this manuscript.

1. Introduction

In India, medicinal plants are deeply woven into the fabric of traditional medicine and serve as a vital resource for countless tribal communities that rely on these natural remedies for various health issues. Among these plants is Artabotrys odoratissimus, commonly known as Kantili Champa or Nag Champa, which belongs to the Annonaceae family. This remarkable plant is recognized for its large, woody climbing vines and clusters of fragrant flowers that emit an aroma reminiscent of a ripe jackfruit. The blossoms, which can be found blooming year-round, especially flourish during the warm summer months and wet rainy season.

Artabotrys odoratissimus Scientific classification [(1)]

- Kingdom: Plantae
- Clade: Angiosperms
- Clade: Magnoliids
- Order: Magnoliales
- Family Annonaceae
- Genus Artabotrys
- Species: A. Odoratissimus

Figure 1. Image of Artabotrys Odoratissimus

Artabotrys Odoratissimus plays an important role in traditional healing practices. In the Malaya Archipelago, for example, a decoction made from its leaves is traditionally used to address cholera, showcasing its medicinal value. Additionally, numerous scientific studies have highlighted its potential health benefits, including antifertility and antimicrobial properties, affirming its significance in herbal medicine.

In this study, we employed gas chromatography-mass spectrometry (GC-MS) as an advanced analytical technique to meticulously isolate and analyze 20 distinct compounds derived from the leaves of Artabotrys odoratissimus. This method allowed us to achieve precise separation of complex chemical components, providing a deeper understanding of the plant's bioactive properties.

2. Materials And Method

A. Plant material

Leaves of Artabotrys Odoratissimus (totaling 8 kg) were collected from parks in Ujjain city and the university campus. The samples were authenticated by experts at the Institute of Environment Management and Plant Science, Vikram University, Ujjain. A voucher specimen was deposited in the herbarium of the School of Studies in Botany, Vikram University, Ujjain, India.

The collected leaves were shade-dried, finely powdered, and subjected to sequential extraction using n-hexane and benzene in a Soxhlet apparatus for 62–72 hours each. The solvents were removed under reduced pressure, yielding solid extracts. Given the significant yield of the hexane extract, it was selected for further analysis in this study.

B. Extraction & Isolation

The hexane extract of the leaves of Artabotrys Odoratissimus was prepared by extracting shade-dried, powdered material using a Soxhlet extractor, followed by solvent removal under vacuum using a rotary film evaporator, yielding 250 g of extract. The extract was qualitatively analyzed using thin-layer chromatography (TLC) on silica gel G. TLC revealed 8–10 spots when developed with hexane and a hexane-benzene mixture (9:1, v/v) as solvent systems.

Subsequently, the extract was fractionated using alumina grade III column chromatography with elution performed using solvents in an increasing order of polarity. Column fractions were monitored by TLC, and those with similar compositions were pooled and concentrated. This process yielded two pure compounds, designated as AOP-2 and AOP-20.

Further separation of specific fractions using rechromatography with hexane-benzene mixtures (8:2, v/v, 6:4, v/v, and 2:8, v/v) resulted in the isolation of three additional pure compounds: AOP-5, AOP-4(A), and AOP-4(B). Due to the limited quantity of individual fractions, alternative separation techniques were not feasible. Therefore, gas chromatography-mass spectrometry (GC-MS) was employed for detailed compound analysis.

- **Gas Chromatography (GC) operating parameters**

GC utilizes a glass column with an inner diameter of 5.5 mm × 4 mm, packed with Carbowax 20M (10% on Chromocarb W) and treated with dimethyl chlorosilane (DMCS) to enhance separation. Nitrogen gas served as the carrier gas, flowing at a rate of 40 ml/min, facilitating the movement of the analytes through the column. As the sample traveled through the column, it was subjected to a temperature program that gradually increased from 70 to 200 °C at a rate of 4 °C/min. This temperature gradient helps efficiently separate compounds based on their volatility, allowing more volatile substances to be eluted first. The detector and injection port temperatures were maintained at 300 °C and 200 °C, respectively, to ensure optimal detection and injection of the analytes.

- **Mass Spectrometry (MS) Operating parameters**

In the mass spectrometer, the ionization voltage was set to 70 eV with an ionization current of 100 µA, facilitating the generation of ions from the separated compounds as they exited the GC. The ion source temperature was maintained at 225 °C to ensure efficient ionization. The accelerating voltage was set to 1.33 kV, which helped increase the kinetic energy of the ions for better detection. The resolution of MS was set to 1000, enabling the system to effectively distinguish between closely related compounds. The scan speed was 3 s per scan decade, allowing for thorough analysis of the mass spectrum throughout the run. Mass spectra were recorded at this scan speed and resolution, capturing a comprehensive range of molecular ions. MS data were processed using a Kratos DS-50 data processing system, and helium was used as the carrier gas within the GC, providing higher efficiency and improved sensitivity during the analysis.

Overall, a combination of GC and MS techniques provides a powerful method for the identification and quantification of compounds in complex mixtures.

- **Identification of AOP-1**
  - **Physical State:** Semi-solid
  - **TLC Solvent System:** Hexane

The GC–MS analysis of AOP-1 revealed the presence of 24 peaks, corresponding to 24 compounds. Of these, 20 compounds were identified through retention time comparison and mass spectral interpretation. The hexane

fraction, designated as AOP-1, exhibited three broad spots with streaking during TLC examination. Due to the limited quantity of AOP-1 and unsuccessful attempts to separate it via column chromatography, it was directly analyzed using GC-MS.

The quantitative estimation of each compound was conducted by measuring the area under each peak, using a computer connected to the GC-MS instrument.

Table

Peak no.	Retention time (sec.)	Area%	Mol.Ion peak m/z	Base peak	Other Important fragments	Name of compounds
1.	5.26	2.61	M^+281	71	57, 85, 99, 113, 127	Eicosane
2.	5.63	6.82	M^+296	71	71,85,99,113,127	Heneicosane
3.	5.71	2.44	M^+268	57	71,85,99,113,99	1-Methyl Nonadecane
4.	5.80	2.85	M^+282	71	57,85,99,113,127	Heptadecane
5.	6.25	2.74	M^+281	149	191,57,104,85,76	Dibutylphthslate
6.	7.24	3.57	M^+296	71	57,85,99,113,127	3-Methylheneicosane
7.	7.49	2.1	M^+281	57	69,83,125,97,111	Eicosane-3-Ene
8.	7.61	6.1	M^+281	57	71,85,97,113,127	2-Methyl eicosane
9.	7.69	1.93	M^+310	71	57,85,99,113,127	Docosane
10.	7.77	1.74	M^+310	57	71,99,133,127,155,141	10-Methyl Heneicosane
11.	9.22	2.07	M^+268	57	71,85,113,127,99	Nonadecane
12.	10.95	0.866	M^+355	71	57 ,85 ,113 ,127 ,141	Tetracosane
13.	11.20	2.36	M^+478	57	113 ,155, 127, 169 ,197	Tetratriacontane
14.	12.40	8.55	M^+256	43	73, 60, 129, 87 ,115	Hexadecenoic Acid
15.	14.26	2.52	M^+284	43	73 ,60 ,57 ,129, 83	Octadecanoic Acid
16.	16.88	2.30	M^+279	149	57,43, 167, 71, 139	Bis (2-Ethyl, Hexyl) Phthalate
17.	19.83	15.84	M^+336	57	43, 69, 83, 97, 111	1-Docosene
18.	19.96	6.33	M^+223	71	43, 59, 85, 99, 127	2- Dodecanone
19.	22.38	12.74	M^+281	57	43, 77, 97, 83, 111	1-Nonadecanol
20.	22.51	13.59	M^+207	57	79,85, 99, 113,154	2,7,10-Trimethyldodecane.

- **Research Graph**

3. Results & Discussion

Comprehensive gas chromatography-mass spectrometry (GC-MS) analysis of AOP revealed a total of twenty-four distinct peaks, each indicating the presence of a unique compound. Through careful examination of retention times and interpretation of mass spectra, researchers successfully identified 20 of these compounds.

Peak-1: Eicosane

The mass spectrum showed a pattern typical of a long-chain hydrocarbon. The base peak was at **m/z 71**, which is common for hydrocarbons like **eicosane** (a 20-carbon alkane). **Phytochemical**: Long-chain aliphatic hydrocarbon (eicosane).

Peak-2: Henicosane

This spectrum showed a long-chain hydrocarbon with a base peak at **m/z 57**, indicating it was **henicosane** (a 21-carbon alkane). **Phytochemical**: Long-chain hydrocarbon (henicosane).

Peak-3: 1-methyl-nonadecane

The fragmentation pattern was similar to that of saturated hydrocarbons, with the base peak at **m/z 57**. This was identified as **1-methyl-nonadecane** (a 19-carbon alkane with a methyl group). **Phytochemical**: Alkane with methyl group (1-methyl-nonadecane).

Peak-4: Heptadecane

This mass spectrum showed a typical fragmentation for long-chain hydrocarbons, with a base peak at **m/z 71**. The compound was identified as **heptadecane** (a 17-carbon alkane). **Phytochemical**: Long-chain hydrocarbon (heptadecane).

Peak-5: Dibutyl Phthalate

The base peak was at **m/z 149**, suggesting it was **dibutyl phthalate**, a compound formed by the cleavage of ester bonds. This is a common plasticizer. **Phytochemical**: Ester (dibutyl phthalate).

Peak-6: 3-methyl-henicosane

The spectrum showed a long-chain hydrocarbon with the base peak at **m/z 71**, confirming **3-methyl-henicosane** (a 21-carbon alkane with a methyl group). **Phytochemical**: Methylated alkane (3-methyl-henicosane).

Peak-7: Eicosane-3-ene

This spectrum showed the base peak at **m/z 69**, formed by allylic cleavage. The compound was identified as **eicosane-3-ene**, a hydrocarbon with a double bond at position 3. **Phytochemical**: Unsaturated hydrocarbon (eicosane-3-ene).

Peak-8: 2-methyl-eicosane

The fragmentation pattern matched that of a long-chain hydrocarbon with the base peak at **m/z 57**. The compound was identified as **2-methyl-eicosane** (a 20-carbon alkane with a methyl group at position 2). **Phytochemical**:

Methylated alkane (2-methyl-eicosane).

Peak-9: Docosane-13

The spectrum was similar to that of saturated long-chain hydrocarbons, with a base peak at **m/z 71**, confirming **docosane-13** (a 22-carbon alkane). **Phytochemical**: Long-chain hydrocarbon (docosane-13).

Peak-10: 10-methyl-henicosane

The mass spectrum showed the base peak at **m/z 57**, suggesting **10-methyl-henicosane** (a 21-carbon alkane with a methyl group at position 10). **Phytochemical**: Methylated alkane (10-methyl-henicosane).

Peak-11: Nonadecane

The spectrum showed a base peak at **m/z 57**, with fragmentation patterns typical of long-chain saturated hydrocarbons. This compound was identified as **nonadecane** (a 19-carbon alkane). **Phytochemical**: Long-chain hydrocarbon (nonadecane).

Peak-12: Tetracosane

The spectrum showed a base peak at **m/z 71**, confirming **tetracosane** (a 24-carbon alkane). **Phytochemical**: Long-chain hydrocarbon (tetracosane).

Peak-13: Tetratriacontane

The spectrum showed a base peak at **m/z 57**, indicating **tetratriacontane** (a 34-carbon alkane). **Phytochemical**: Long-chain hydrocarbon (tetratriacontane).

Peak-14: Hexadecanoic Acid (Palmitic Acid)

The spectrum showed peaks at **m/z 60** (McLafferty rearrangement) and **m/z 73** (α-cleavage), with a base peak at **m/z 43**, confirming the compound as **hexadecanoic acid** (palmitic acid, a common saturated fatty acid). **Phytochemical**: Fatty acid (hexadecanoic acid).

Peak-15: Octadecanoic Acid (Stearic Acid)

This spectrum showed similar fragmentation to **hexadecanoic acid**, with peaks at **m/z 60** and **m/z 73**, and a base peak at **m/z 43**, identifying the compound as **octadecanoic acid** (stearic acid, another common fatty acid). **Phytochemical**: Fatty acid (octadecanoic acid).

Peak-16: Bis(2-ethylhexyl) Phthalate

The spectrum showed the base peak at **m/z 149**, confirming the compound as **bis(2-ethylhexyl) phthalate**, a plasticizer commonly used in PVC production. **Phytochemical**: Ester (bis(2-ethylhexyl) phthalate).

Peak-17: 1-Docosene

This mass spectrum showed the base peak at **m/z 41**, suggesting an unsaturated long-chain compound. The compound was identified as **1-docosene** (a 22-carbon alkene). **Phytochemical**: Unsaturated hydrocarbon (1-

docosene).

Peak-18: 2-Dodecanone

The spectrum showed the base peak at **m/z 71**, confirming **2-dodecanone** (a 12-carbon ketone). **Phytochemical**: Ketone (2-dodecanone).

Peak-19: 1-Nonadecanol

The base peak at **m/z 57** suggested **1-nonadecanol** (a 19-carbon alcohol). **Phytochemical**: Alcohol (1-nonadecanol).

Peak-20: 2,7,10-Trimethyldodecane

The spectrum showed the base peak at **m/z 57**, identifying **2,7,10-trimethyldodecane** (a 12-carbon alkane with three methyl groups). **Phytochemical**: Trimethylated alkane (2,7,10-trimethyldodecane).

Key Phytochemicals:

- **Sterols:** (e.g. AOP-4 (A): 245-Stigmast-5, 22 E-diene-3p-ol, AOP-4 (B): 24R-Stigmast-5-ene-38-ol)
- **Alkanes** (e.g., eicosane, henicosane, docosane, tetracosane)
- **Methylated Alkanes** (e.g., 1-methyl-nonadecane, 2-methyl-eicosane)
- **Fatty Acids** (e.g., hexadecanoic acid, octadecanoic acid)
- **Alcohols** (e.g., 1-nonadecanol)
- **Ketones** (e.g., 2-dodecanone)
- **Phthalates** (e.g., dibutyl phthalate, bis(2-ethylhexyl) phthalate)
- **Unsaturated Compounds** (e.g., 1-docosene, eicosane-3-ene)

The identified compounds include a diverse array of hydrocarbons and chemical derivatives, such as eicosane, a straight-chain alkane essential in various industrial applications; heneicosane, known for its waxy properties; and 1-Methyl Nonadecane, which can be utilized in specialty chemicals.

Other notable compounds include Heptadecane and Dibutylphthalate, which have applications in plastic and fragrance industries. The analysis also identified 3-Methylheneicosane and Eicosane-3-Ene, each contributing to the complexity of the sample. Additionally, 2-Methyl eicosane and docosane have been noted for their potential industrial uses, while 10-Methyl Hen eicosane and nonadecane are recognized for their importance in various chemical processes.

Furthermore, Tetracosane and Tetratriacontane enrich the sample with longer-chain hydrocarbons, whereas compounds such as Hexadecenoic Acid and Octadecanoic Acid are known for their beneficial biological properties, particularly in medicinal contexts. Bis (2-Ethyl Hexyl) Phthalate and 1-Docosene were also highlighted, demonstrating their significance in synthetic applications. Lastly, 2-Dodecanone, 1-Nonadecanol, and 2,7,10-Trimethyldodecane round out the list, showing a range of compounds that are not only recognized but also hold considerable potential for medicinal purposes, underscoring their importance in ongoing research and development

4. Conclusion

For the first time, the **phytochemical composition** of *Artabotrys Odoratissimus* (R.Br.) leaves have been elucidated, identifying several **novel compounds**, including **hydrocarbons**, **fatty acids**, **alcohols**, **ketones**, **esters**, and **sterols**, all contributing to the plant's diverse **biological activities**. Key compounds such as **Eicosane**, **Heneicosane**, **Heptadecane**, and **Nonadecane** exhibit **anti-inflammatory** and **antimicrobial** properties, while **Hexadecenoic Acid** and **Octadecanoic Acid** are significant for their **antioxidant**, **anti-inflammatory**, and **antimicrobial** effects, also playing a role in **cell membrane integrity**. The alcohol **1-Nonadecanol** is noted for its **wound-healing** and **antimicrobial** properties, and **2-Dodecanone** is recognized for its **antimicrobial** activity. **Dibutylphthalate** and **Bis(2-Ethylhexyl) Phthalate**, typically used as plasticizers, also show potential **antimicrobial** effects. Additionally, the **sterols AOP-4 (A)** (*245-Stigmast-5, 22 E-diene-3p-ol*) and **AOP-4 (B)** (*24R-Stigmast-5-ene-38-ol*) possess **anti-inflammatory**, **antioxidant**, and **antimicrobial** properties, critical for maintaining **cell membrane integrity**. These findings underscore the **therapeutic potential** of *Artabotrys odoratissimus*, especially in **antimicrobial**, **anti-inflammatory**, and **antioxidant** applications, with further research on its constituents currently underway.

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