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# Characterization of the Leaf Essential Oil Composition of *Annona squamosa* L. from Foothills of North India

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## Abstract

The leaf essential oil composition of *Annona squamosa* L., collected from the lower region of Himalaya was investigated using gas chromatography-flame ionisation detector (GC-FID) and GC-mass spectrometry (GC-MS). A total of forty-three constituents, representing 88.6% of the total oil composition were identified. The essential oil was primarily composed of sesquiterpenoids (sesquiterpene hydrocarbons: 63.4% and oxygenated sesquiterpenes: 21.8%). Major constituents of the oil were (*E*)-caryophyllene (15.9%), γ-cadinene (11.2%), *epi*-*α*-cadinol (9.4%), (*Z*)-caryophyllene (7.3%), *γ*-muurolene (5.4%), *α*-humulene (5.2%), viridiflorene (5.0%), *α*-cadinol (3.9%), aromadendrene (2.9%), *δ*-cadinene (2.9%), *α*-cadinene (2.9%), (2*Z*,6*Z*)-farnesal (2.2%) and caryophyllene oxide (2.1%).

**Keywords:** *Annona squamosa*; Annonaceae; Essential oil composition; Sesquiterpenes

## Introduction

*Annona* L. (Annonaceae) comprises approximately 162 species of trees and shrubs that are found predominantly in lowland tropical regions [1]. *Annona squamosa* L., commonly known as a 'custard apple' or 'sharifa' is native to South America and the West Indies, but now it is cultivated throughout India for its nutritive fruits [2]. In Indian system of medicine, it is used as antitumour, diuretic and for wound healing. The leaves are anthelmintic and the powdered unripe fruits are taken internally in the form of paste with water for the treatment of diarrhoea and dysentery [3]. The plant is used in traditional medicines of several tropical countries to treat epilepsy, dysentery, cardiac problems, worm infection, constipation, haemorrhage, antibacterial, dysurea, fever and ulcer. The young leaves of the plant are used extensively for their antidiabetic activity [4]. Seeds of the plant are well known for killing head lice [5]. The dried unripe fruit powder is used to destroy vermin. A paste of seed powder has been used for killing worm in the wound of cattles [6]. The plant is also attributed with antifertility, abortifacient, antitumor and antimalarial activities [7-9]. An alkaloid, *p*-hydroxy benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, isolated from the leaves is reported to be act as cardiotonic [10]. A fraction of total root alkaloids is reported to be antihypertensive, antispasmodic, antihistaminic and a bronchodilator [11]. The leaf essential oil of the plant has been demonstrated potent trypanocidal and antimalarial activities [12].

The chemical composition of the essential oil of *A. squamosa* has been studied previously from different countries. It is evident from the literature that oil of *A. squamosa* is exist in six different chemotypic forms which are: (i) monoterpene hydrocarbon rich; (ii) oxygenated monoterpene rich; (iii) sesquiterpene hydrocarbon rich; (iv) oxygenated sesquiterpene rich; (v) oils with relative amounts of sesquiterpene hydrocarbons and oxygenated sesquiterpenes and (vi) oil containing sesquiterpene and diterpenoids compounds [13]. The leaf essential oil from Benin possessed isocaryophyllene, camphene, β-caryophyllene, *epi*-*α*-cadinol and *epi*-*α*-muurolol as major constituents [14]. The leaf essential oil rich in (*E*)-caryophyllene, germacrene D, bicyclogermacrene, (*Z*)-caryophyllene, *β*-elemene and *α*-humulene is reported from Brazil [12]. Major constituents of the leaf essential oil of *A. squamosa* from north Indian plains are (*E*)-caryophyllene, germacrene D, bicyclogermacrene, *β*-elemene, *γ*-cadinene, *α*-muurolol and aromadendrene [15]. The leaf oil from southern India is dominated by *β*-cedrene and *β*-caryophyllene [16]. The root essential oil of *A. squamosa* rich in *β*-caryophyllene, *α*-pinene, *α*-humulene and *α*-gurjunene is reported from Brazil [17]. Main components of the leaf oil from France are germacrene D, *β*-elemene, *α*- and *β*-pinene, sabinene, bicyclogermacrene, *τ*-cadinol and *τ*-muurolol, while the fruit oil contained spathulenol, bornyl acetate, germacrene D, borneol and verbenone [18]. The fruit oil contained *τ*-cadinol, *τ*-muurolol, spathulenol, *α*-copaene and *α*-terpineol is reported from Malaysia [19]. Moreover, the bark essential oil contain 1H-cycloprop(e)azulene, germacrene D, bisabolene, caryophyllene oxide, bisabolene epoxide and kaur-16-ene as main constituents [20].

A review of literature revealed that there is no report on the essential oil composition of *A. squamosa* growing in the foothill region of north India. Therefore, in this research, leaf essential oil composition of *A. squamosa* collected from foothills of Uttarakhand has been investigated using gas chromatography and gas chromatography-mass spectrometry.

## Materials and Methods

### Plant material and isolation of essential oil

Fresh leaves of *A. squamosa* were collected from the experimental field of CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Pantnagar, Uttarakhand in the month of November (Figure 1). The plant material was authenticated at the Botany Department, CIMAP Research Centre Pantnagar by one of the

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**Figure 1:** Twigs of *Annona squamosa* showing leaf, flower and fruit.

authors (AC). The experimental site is located between coordinates 29.02˚ N, 79.31˚ E and an altitude of 243 m in foothills of north India. Isolation of the essential oil from shade dried leaves was carried out by hydrodistillation for 3 h using a Clevenger's type apparatus. Isolated oil was dried over anhydrous $Na_2SO_4$ and stored at 4°C until further analyses.

## Gas chromatography (GC/FID)

Gas chromatography-flame ionization detection (GC-FID) was performed for quantification of the essential oil constituents. GC analysis of the essential oil was carried out on a PerkinElmer AutoSystem XL gas chromatograph, equipped with DB-5 capillary column (60 m × 0.32 mm i.d., film thickness 0.25 µm) and flame ionization detector (FID). The oven column temperature ranged from 70-250°C, programmed at 3°C $min^{-1}$, with initial and final hold time of 2.0 min, using $H_2$ as carrier gas at 10 psi constant pressure, a split ratio of 1:35, an injection size of 0.03 µL neat, and injector and detector temperatures were maintained at 250°C and 280°C, respectively.

## Gas chromatography-Mass spectrometry (GC/MS)

Gas chromatography-mass spectrometry (GC-MS) was performed for identification of the essential oil constituents. GC-MS analysis of the essential oil was carried out on a Clarus 680 GC interfaced with a Clarus SQ 8C mass spectrometer of PerkinElmer fitted with Elite-5 MS fused-silica capillary column (5% phenyl polysiloxane, 30 m × 0.25 mm internal diameter, film thickness 0.25 µm). The oven temperature program was from 60°C to 240°C, at 3°C $min^{-1}$, and programmed to 270°C at 5°C $min^{-1}$. Injector temperature was 250°C; transfer line and source temperatures were 220°C; injection size 0.03 µL neat; split ratio 1:50; carrier gas He at 1.0 mL $min^{-1}$; ionization energy 70 eV; mass scan range 40-450 amu.

## Identification of essential oil constituents

Characterization of the essential oil constituents was carried out on the basis of retention index (RI), calculated using a homologous series of *n*-alkanes ($C_8$-$C_{30}$, Supelco Bellefonte, PA, USA) under identical experimental conditions; mass spectra library search (NIST/EPA/NIH, version 2.0 g, and Wiley registry of mass spectral data 9th edition) and by comparing the mass spectral and retention data with literature [21]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

## Statistical analysis

To examine whether the essential oil constituents identified are useful in reflecting the chemical relationships between different compositions, their contents (%) were subjected to hierarchical cluster analysis using average method [22]. This software computes the hierarchical clustering of a multivariate dataset based on dissimilarities. The derived dendrogram depicts the grouping of chemical compositions as per their chemical constituents.

## Results and Discussion

The shade dried leaves of *A. squamosa* yielded 0.13% essential oil on hydrodistillation. The resulting essential oil was subjected to GC-FID and GC-MS analyses. Altogether, forty-three constituents, representing 88.6% of the total oil composition were identified. The detailed results are summarised in Table 1. The essential oil composition was mainly dominated by sesquiterpenoids (sesquiterpene hydrocarbons: 63.4% and oxygenated sesquiterpenes: 21.8%) (Figure 2). Major sesquiterpenoid constituents of the oil were (*E*)-caryophyllene (15.9%), γ-cadinene (11.2%), *epi*-α-cadinol (9.4%), (*Z*)-caryophyllene (7.3%), γ-muurolene (5.4%), α-humulene (5.2%), viridiflorene (5.0%), α-cadinol (3.9%), aromadendrene (2.9%), δ-cadinene (2.9%), α-cadinene (2.9%), (2*Z*,6*Z*)-farnesal (2.2%), caryophyllene oxide (2.1%), spathulenol (1.9%), β-elemene (1.9%), humulene epoxide II (1.8%), δ-elemene (1.5%) and α-copaene (1.3%). However, notable monoterpenoids constituents of the oil were *p*-cymene (0.8%), limonene (0.8%) and bornyl acetate (0.5%). The structures of the major constituents of the oil are presented in Figure 3.

The leaf essential oil composition of *A. squamosa* has been investigated earlier from different countries (Table 2). Major constituents of the Brazilian oil were (*E*)-caryophyllene (27.4%), germacrene D (17.1%), bicyclogermacrene (10.8%), (*Z*)-caryophyllene (7.3%), β-elemene (6.2%) and α-humulene (5.7%) [12]. Two distinct compositions were described from Egypt. First one was dominated by carvone (24.9%), diacetyl (9.3%) and linalool (7.7%) [23], while

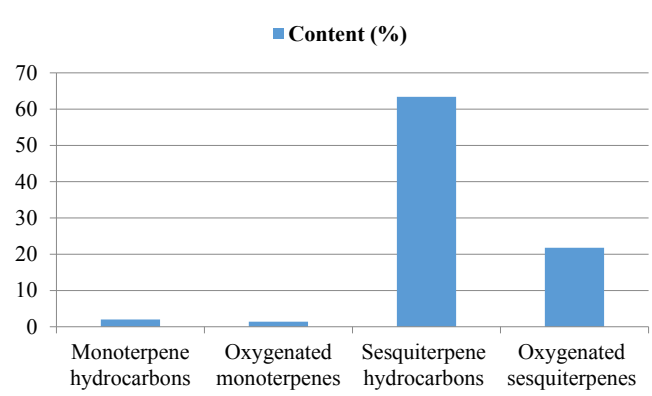


**Figure 2:** Class composition of the leaf essential oil of *Annona squamosa* collected from foothills of north India.

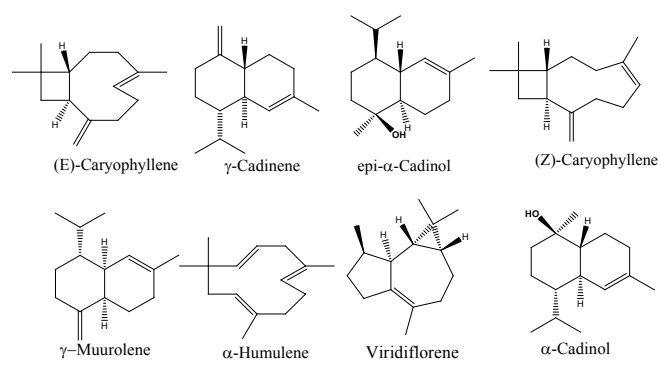


**Figure 3:** Structures of major constituents of *Annona squamosa* leaf essential oil.

| S. No. | Compound | RI[a] | RI[b] | Content (%) |
|---|---|---|---|---|
| | *α*-Pinene | 927 | 932 | 0.1 |
| | Myrcene | 983 | 988 | 0.1 |
| | *α*-Phellandrene | 996 | 1002 | t |
| | *p*-Cymene | 1018 | 1020 | 0.8 |
| | Limonene | 1022 | 1024 | 0.8 |
| | 1,8-Cineole | 1024 | 1026 | t |
| | (*Z*)-*β*-Ocimene | 1028 | 1032 | t |
| | (*E*)-*β*-Ocimene | 1040 | 1044 | 0.1 |
| | *γ*-Terpinene | 1051 | 1054 | 0.1 |
| | Terpinolene | 1082 | 1086 | t |
| | Linalool | 1094 | 1095 | 0.2 |
| | Camphor | 1137 | 1141 | 0.3 |
| | Terpinen-4-ol | 1171 | 1174 | 0.2 |
| | Myrtenol | 1191 | 1194 | 0.2 |
| | Bornyl acetate | 1279 | 1284 | 0.5 |
| | δ-Elemene | 1331 | 1335 | 1.5 |
| | Cyclosativene | 1364 | 1369 | t |
| | *α*-Copaene | 1369 | 1374 | 1.3 |
| | *β*-Bourbonene | 1382 | 1387 | t |
| | *β*-Elemene | 1385 | 1389 | 1.9 |
| | (*Z*)-Caryophyllene | 1402 | 1408 | 7.3 |
| | (*E*)-Caryophyllene | 1412 | 1417 | 15.9 |
| | Aromadendrene | 1434 | 1439 | 2.9 |
| | *α*-Humulene | 1448 | 1452 | 5.2 |
| | *γ*-Muurolene | 1472 | 1478 | 5.4 |
| | Germacrene D | 1474 | 1480 | t |
| | *β*-Selinene | 1484 | 1489 | t |
| | Viridiflorene | 1490 | 1496 | 5.0 |
| | *α*-Selinene | 1492 | 1498 | t |
| | *α*-Muurolene | 1494 | 1500 | t |
| | *γ*-Cadinene | 1508 | 1513 | 11.2 |
| | δ-Cadinene | 1516 | 1522 | 2.9 |
| | *trans*-Calamenene | 1518 | 1521 | t |
| | *α*-Cadinene | 1532 | 1537 | 2.9 |
| | *α*-Calacorene | 1538 | 1544 | t |
| | Elemol | 1542 | 1548 | t |
| | Spathulenol | 1572 | 1577 | 1.9 |
| | Caryophyllene oxide | 1576 | 1582 | 2.1 |
| | Globulol | 1584 | 1590 | 0.5 |
| | Humulene epoxide II | 1604 | 1608 | 1.8 |
| | *epi*-*α*-Cadinol | 1634 | 1638 | 9.4 |
| | *α*-Cadinol | 1648 | 1652 | 3.9 |
| | (2Z,6Z)-Farnesal | 1676 | 1683 | 2.2 |
| | **Class composition** | | | |
| | Monoterpene hydrocarbons | | | 2.0 |
| | Oxygenated monoterpenes | | | 1.4 |
| | Sesquiterpene hydrocarbons | | | 63.4 |
| | Oxygenated sesquiterpenes | | | 21.8 |
| | Total identified (%) | | | 88.6 |

**Table 1:** Leaf essential oil composition of *Annona squamosa* from Uttarakhand, India.

other composition was characterised by higher amount of *β*-gurjunene (42.49%), viridiflorene (6.68%), aromadendrene (5.49%), *γ*-muurolene (5.72%) and *allo*-aromadendrene epoxide (5.31%) [24]. Moreover, germacrene D (15.7%), *β*-elemene (12.0%), sabinene (8.8%) and *α*-pinene/*β*-pinene (8.1%) were major constituents of the leaf oil from France [18]. Further, major constituents of the leaf oil from north Indian plains were (*E*)-caryophyllene (22.9%), germacrene D (21.3%), bicyclogermacrene (8.5%), *β*-elemene (7.8%), *γ*-cadinene (6.7%) and *α*-muurolol (5.7%) [15]. On the other hand, leaf oil from southern India was characterised by higher amounts of *β*-cedrene (23.3%), *β*-caryophyllene (14.1%), (*E*,*E*)-farnesol (7.0%) and cadina-1,4-diene (6.9%) [16].

To compare the composition of the investigated *A. squamosa* essential oil with earlier reported oil compositions [12,15,16,18,23,24], twenty-four selected components (amount ≥ 5.0%) of different oils were subjected to the hierarchical cluster analysis. The derived dendrogram clearly showed dissimilarity based on the percentages of

the constituents present in the different oil compositions. The examined oil from foothill region of Uttarakhand was quite different from the oils of other regions, hence made a separate group in the hierarchical cluster analysis (Figure 4).

Though the examined essential oil was rich in sesquiterpenoid compounds, there were substantial quantitative and qualitative variations when compared with previous reports from India and other parts of the world. Noteworthy observation is the fact that compounds such as carvone, diacetyl, cadina-1,4-diene, *β*-cedrene, *β*-gurjunene, bicyclogermacrene, which were the major constituents in previous studies [15,16,18,23,24] could not be detected in this investigation. Moreover, germacrene D, a major constituent of various previous studies on *A. squamosa* leaf oil [12,15,18] was present in only trace amount in the examined oil. This may be attributed to several factors such as climatic condition, season, and age of the plant, genotype, and processing procedures.

## Conclusions

In this study, leaf essential oil composition of *A. squamosa* was analysed from foothills of north India. The essential oil was dominated by sesquiterpenoids (85.2%) with (*E*)-caryophyllene (15.9%), *γ*-cadinene (11.2%), *epi*-*α*-cadinol (9.4%), (*Z*)-caryophyllene (7.3%), *γ*-muurolene (5.4%), *α*-humulene (5.2%) and viridiflorene (5.0%) as major constituents. The examined oil showed considerable dissimilarity in chemical composition with previously reported leaf essential oil compositions from other regions.

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| County | Major Constituents | Reference |
|---|---|---|
| Brazil | (*E*)-Caryophyllene (27.4%), germacrene D (17.1%), bicyclogermacrene (10.8%), (*Z*)-caryophyllene (7.3%), β-elemene (6.2%), α-humulene (5.7%) | [12] |
| Egypt | β-Gurjunene (42.49), viridiflorene (6.68%), aromadendrene (5.49%), γ-muurolene (5.72%), allo-aromadendrene epoxide (5.31%) | [24] |
| Egypt | Carvone (24.9%), diacetyl (9.3%), linalool (7.7%) | [23] |
| India | (*E*)-Caryophyllene (22.9%), germacrene D (21.3%), bicyclogermacrene (8.5%), β-elemene (7.8%), γ-cadinene (6.7%), α-muurolol (5.7%) | [15] |
| India | β-Cedrene (23.3%), β-caryophyllene (14.1%), (*E*,*E*)-farnesol (7%), cadina-1,4-diene (6.9%), allo-aromadendrene (5.5%), calamenene (5.1%) | [16] |
| France | Germacrene D (15.7%), β-elemene (12.0%), sabinene (8.8%), bicyclogermacrene (6.0%), τ-cadinol (5.5%) | [18] |

**Table 2:** Leaf essential oil composition of *Annona squamosa* reported from different counties.

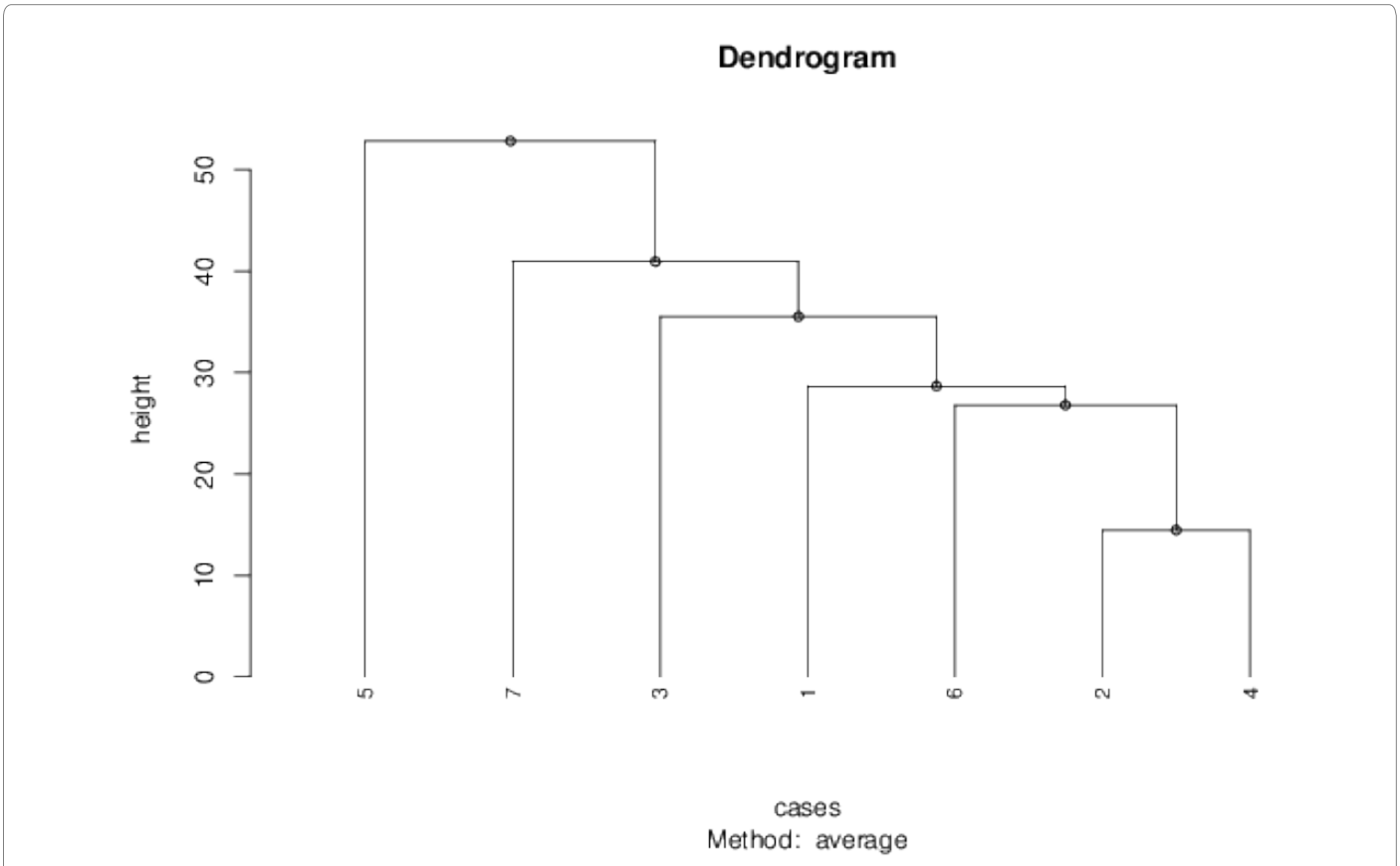


**Figure 4:** Hierarchical cluster analysis of the leaf essential oil composition of *Annona squamosa*. 1: Foothills, north India (present study); 2: north Indian plain [15]; 3: southern India [16]; 4: Brazil [12]; 5: Egypt [24]; 6: France [18]; 7: Egypt [23]. For detail compositions of the oils see Tables 1 and 2.

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