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GC-MS PROFILING OF BIOACTIVE COMPOUNDS IN ETHANOL EXTRACT OF *Annona squamosa* L. LEAVES

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ABSTRACT

Annona squamosa L., commonly known as custard apple, is a medicinal plant used traditionally for the treatment of various ailments because of its antimicrobial, anti-inflammatory and antioxidant properties. Its leaves contain a wide array of phytochemicals with potential therapeutic benefits. In this study, ethanol was employed as the extraction solvent to isolate bioactive phytochemicals from the leaves of *A. squamosa*. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted to characterize the volatile and semi-volatile components present in the ethanol extract. Leaves of *A. squamosa* were collected, air-dried, pulverized, and extracted with ethanol. The resulting extract was subjected to GC-MS analysis to identify the phytochemical constituents based on retention time and spectral comparison with standard databases. A total of 21 compounds were identified. Major constituents included 1-methyl-2-pyrrolidinone (13.09%), caryophyllene (9.36%), neophytadiene (8.56%), cyclohexanone (7.43%), and ethanol, 2-butoxy (6.20%). Many of the identified compounds are known for their anti-inflammatory, antimicrobial, antioxidant, and insecticidal properties. The results suggest that the ethanol extract of *A. squamosa* leaves is a rich source of biologically active compounds, supporting its traditional use in herbal medicine. Further pharmacological and toxicological investigations are recommended.

Keywords: Annona squamosa, GC-MS, phytochemicals, caryophyllene, neophytadiene, ethanol extract, bioactive compounds.

INTRODUCTION

Plants are a prolific source of structurally diverse organic compounds, often classified into primary and secondary metabolites, with many serving vital roles in drug discovery and pharmacological development [1]. Medicinal plants, in particular, form the cornerstone of traditional healthcare systems worldwide and continue to contribute significantly to the global pharmaceutical sector [2].

According to the World Health Organization (WHO), medicinal plants are defined as those containing substances in one or more of their organs that can be used therapeutically or serve as precursors for drug synthesis [3,4]. The exploration of medicinal plants for novel bioactive compounds has gained traction, especially in the context of increasing resistance to synthetic drugs and the global pursuit of natural remedies.

Annona squamosa L., commonly known as custard apple, is an underutilized tropical fruit tree native to parts of Africa, Asia, and the Americas. While its fruits are widely consumed, the leaves have been traditionally employed to treat a variety of ailments including spasms, rheumatism, and infections [5]. Phytochemical investigations have revealed that the plant contains compounds such as flavonoids, alkaloids, terpenes, acetogenins, and steroids, which exhibit diverse pharmacological activities [6,7].

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Despite the known medicinal potential of *A. squamosa*, much of the existing literature has focused on aqueous and methanolic extracts. Ethanol, a polar solvent with lower toxicity and better extractive compatibility for both polar and moderately non-polar compounds, offers a more efficient and safer alternative for extracting a broader range of bioactives [8]. However, studies using ethanol as the primary extraction solvent for the phytochemical profiling of *A. squamosa* leaves remain limited, particularly with regard to gas chromatography–mass spectrometry (GC-MS)-based analysis.

Previous phytochemical studies on *A. squamosa* have largely been restricted to qualitative screenings or have used different plant parts (e.g., seeds, bark, and pulp), often employing methanol or aqueous solvents [9].

This study is novel in its use of ethanol to maximize the extraction of pharmacologically significant phytochemicals, followed by systematic GC-MS profiling to identify and quantify the chemical constituents. By doing so, the work seeks to uncover potentially novel compounds or higher yields of known compounds that could have been missed in previous solvent systems.

MATERIALS AND METHODS

Sample Collection and Identification

Fresh leaves of *Annona squamosa* L. were collected from the Botanical Garden within The Federal Polytechnic, Ilaro, Ogun State, Nigeria. The plant was taxonomically identified and authenticated by Dr. Nodza George at the Botany Unit, University of Lagos. A voucher specimen was deposited with the herbarium number 9750.

Sample Preparation

The collected leaves were destalked, rinsed thoroughly with tap water followed by distilled water to remove dust and debris. The cleaned leaves were then air-dried at room temperature (25–28°C) for two weeks. Once completely dry, the leaves were pulverized into fine powder using a Qasa QBL-1861 blender (Qlink Electronics, China). The powdered sample was stored in clean, labeled amber bottles at room temperature until further use [10].

Preparation of Leaf Extract

A portion of the powdered leaves (50 g) was macerated in 500 mL of absolute ethanol for 72 hours at room temperature with intermittent stirring. The mixture was filtered using Whatman No. 42 filter paper, and the filtrate was concentrated under reduced pressure using a BUCHI Rotavapor R-300 rotary evaporator (Flawil, Switzerland) at 40°C. The concentrated ethanol extract was stored in a refrigerator at 4°C prior to GC-MS analysis [11].

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The phytochemical constituents of the ethanol extract were analyzed using an Agilent 7820A Gas Chromatograph coupled with an Agilent 5975C inert Mass Spectrometer equipped with a triple-axis detector and electron impact ionization source. A HP-5 capillary column (30 m × 0.32 mm × 0.25 µm film thickness, Agilent Technologies) coated with 5% phenyl methyl siloxane served as the stationary phase.

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The carrier gas was helium, maintained at a constant flow rate of 1.49 mL/min. One microlitre of the sample was injected in splitless mode with an injector temperature set at 300°C. The oven temperature was programmed from 40°C (1 min hold), ramped at 12°C/min to 300°C, and held for 10 minutes. The total run time was 32.667 minutes, with a 5-minute solvent delay.

The mass spectrometer operated in electron impact mode at 70 eV with an ion source temperature of 230°C, quadrupole temperature of 150°C, and transfer line temperature of 280°C. Data acquisition was performed in scan mode over the m/z range of 45–550 amu at a scan rate of 2.0 seconds.

Identification of Components

The mass spectra of the constituents were interpreted using reference libraries from the National Institute of Standards and Technology (NIST08s), WILEY8, and FAME databases. The names, molecular weights, and molecular formulas of the detected compounds were determined by comparing their spectral data with those in the reference libraries.

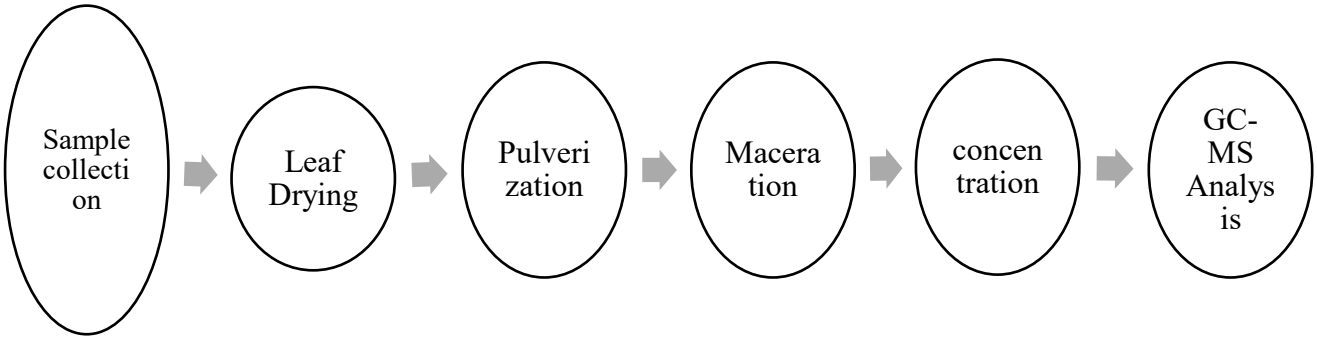

Figure 1. Experimental Workflow for GC-MS Analysis of *Annona squamosa* Leaves

RESULTS AND DISCUSSION

The GC-MS analysis of the extract revealed twenty-one major phytochemical constituents, predominantly comprising terpenoids and hydrocarbons, with varying biological activities.

Table 1 shows the major Compounds (≥1%) Identified in the Ethanol Extract of Annona squamosa Leaves while Fig 2 shows the chromatogram obtained from the crude extract of the *A. squamosa* leaf extract.

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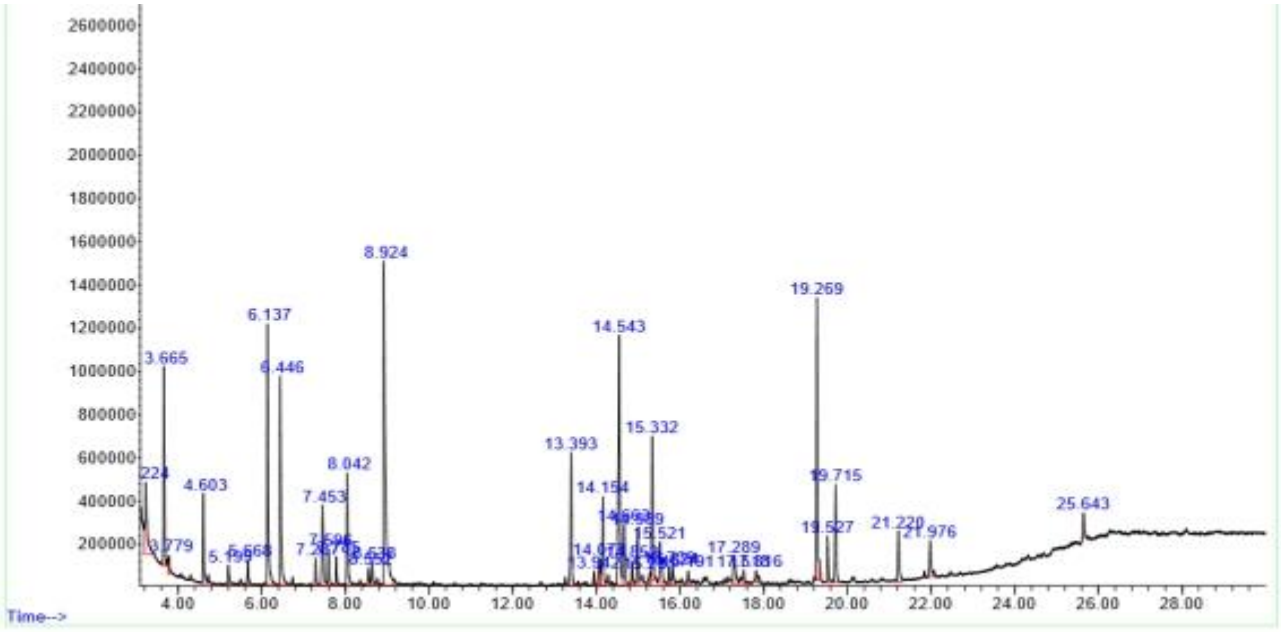

Figure 2: Chromatogram obtained for ethanol fraction of *Annona squamosa* leaf extract

Table 1. Major Compounds (≥1%) Identified in the Ethanol Extract of Annona squamosa Leaves

Peak No.	Compound Name	Retention Time (min)	Molecular Formula	Area (%)
1	Methyl isobutyl ketone	3.224	$C_6H_{12}O$	3.30
2	Toluene	3.665	C_7H_8	4.55
3	Acetic acid, butyl ester	4.603	$C_6H_{12}O_2$	2.38
4	Cyclohexanone	6.137	$C_6H_{10}O$	7.43
5	Ethanol, 2-butoxy	6.446	$C_6H_{14}O_2$	6.20
6	Benzene, 1-ethyl-2-methyl	7.453	C_9H_{12}	3.19
7	Mesitylene (combined)	7.596 / 8.042	C_9H_{12}	4.32
8	1-Methyl-2-pyrrolidinone	8.924	C_5H_9NO	13.09
9	α-Terpinene	13.393	$C_{10}H_{16}$	4.17
10	Cyclohexane, 1-ethenyl-1-methyl-	14.154	$C_{10}H_{16}$	2.91

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	2,4-bis(1-methylethenyl)			
11	Caryophyllene	14.543	$C_{15}H_{24}$	9.36
12	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	14.663	$C_{15}H_{24}$	2.35
13	Humulene	14.989	$C_{15}H_{24}$	1.80
14	Germacrene D	15.332	$C_{15}H_{24}$	4.26
15	1,5-Heptadiene, 2,5-dimethyl-3-methylene	15.521	$C_{10}H_{16}$	2.05
16	Bicyclo[4.4.0] dec-1-ene, 2-isopropyl-5-methyl-9-methylene	17.289	$C_{15}H_{24}$	1.46
17	Neophytadiene	19.269	$C_{20}H_{38}$	8.56
18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.527	$C_{20}H_{40}O$	1.49
19	1-Hexadecyne	19.715	$C_{16}H_{30}$	3.04
20	Hexadecatetraen-3-ol, tetramethyl (E,E)	21.220	$C_{20}H_{34}O$	1.95
21	Phytol	21.976	$C_{20}H_{40}O$	1.37

Table 2: Selected Isolated Compounds from *Annona squamosa*, Their Classes, Sources, and Literature References

S/N	Compound	Chemical Class	Plant Source	Reference
1	Copaene	Sesquiterpene	Leaf	This study
2	Caryophyllene	Sesquiterpene	Leaf	This study
3	Germacrene D	Sesquiterpene	Leaf	This study

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4	Neophytadiene	Diterpene	Leaf	This study
5	α-Farnesene	Sesquiterpene	Leaf	This study
6	Phytol	Acyclic diterpenoid alcohol	Leaf	This study
7	Bicyclogermacrene	Sesquiterpene	Leaf	This study
8	1-Methyl-2-pyrrolidinone	Lactam	Leaf	This study
9	D-Limonene	Monoterpene	Leaf	This study
10	Bis(2-ethylhexyl) phthalate	Phthalic acid ester	Leaf	This study
11	Annolipoxy	Fatty acid	Seeds & Fruit pulp	Sultana, 2008
12	Palmitone	Fatty acid ester	Waxes	Shanker et al., 2007
13	Dimethoxyisoquinolone	Alkaloid	Twig	Soni et al., 2012
14	Esquamosan	Alkaloid	Leaf	Di Giulio et al., 2023

The GC-MS profiling of the ethanol extract of *Annona squamosa* leaves revealed a diverse range of phytochemicals with potential pharmacological significance. A total of 21 major compounds (≥1% peak area) were identified (Table 1), which fall into different chemical classes such as sesquiterpenes, diterpenes, monoterpenes, fatty acid esters, and lactams (Table 2). The most abundant constituents were 1-methyl-2-pyrrolidinone (13.09%), caryophyllene (9.36%), and neophytadiene (8.56%). Most of the bioactive compounds predominant in *A. squamosa* leaves are Sesquiterpene hydrocarbons such as α-Terpinene (4.17%), Caryophyllene (9.36%), Humulene (1.80%), and so on. Al-Nemari [20] assessed the bioactive compounds present in the methanolic extracts of *A. squamosa* leaves and reported the presence of α-copaene (2.12%), humulene (1.15%) and phytol (2.22%) among others. 1-Methyl-2-pyrrolidinone (13.09%, RT: 8.924 min) was the most abundant compound identified in the extract. This compound, though more commonly known as a solvent, has demonstrated antimicrobial and anti-inflammatory properties in plant extracts [12]. Its significant presence supports the traditional use of *A. squamosa* for inflammatory conditions. Caryophyllene (9.36%, RT: 14.543 min) is the most prominent sesquiterpene. Caryophyllene, a natural bicyclic sesquiterpene and constituent of many essential oils is noted for its anti-inflammatory activity. It also helps to relieve anxiety and pain, reduce cholesterol, prevent osteoporosis, and treat seizures [13]. It is widely known for its anti-inflammatory and analgesic properties and is found in various essential oils such as clove, black pepper, and *Cannabis sativa* [14].

Another significant constituent, Neophytadiene (8.56%, RT: 19.269 min), is a diterpenoid with reported anti-inflammatory and antimicrobial properties. It is often isolated from leaf waxes and certain medicinal plants [15] Its presence supports

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the traditional use of *A. squamosa* for treating pain and infections. Medicinal plants leave containing Neophytadiene are used in the treatment of headaches, rheumatism, and some skin diseases, whereas Neophytadiene has shown analgesic, antipyretic, anti-inflammatory, and antioxidant properties. [12,16].

Germacrene D (4.26%, RT: 15.332 min) and α-Terpinene (4.17%, RT: 13.393 min) are both known for their antioxidant and antimicrobial activities. Germacrene D has also been reported to exhibit insecticidal properties, contributing to the plant's defense mechanisms [17]. α-Terpinene, a monoterpene, is commonly found in citrus and *Melaleuca* species and is recognized for its radical scavenging capacity [18].

Although, Humulene (1.80%, RT: 14.989 min), Phytol (1.37%, RT: 21.976 min), and Copaene (0.42%, RT: 13.942 min) were detected in lower amounts, their known antioxidant and antimicrobial properties enhance the overall therapeutic potential of the extract. These findings align with reports from similar studies [19,20] and support the continued exploration of *A. squamosa* as a source of natural drug candidates. They are known for their diverse pharmacological activities. Humulene and copaene are common sesquiterpenes with anti-inflammatory and antibacterial properties, while phytol, a diterpene alcohol, is a precursor in the biosynthesis of vitamins E and K and possesses antioxidant and anticancer activities [21,22]. Although phytol was present at only 1.37%, its known antioxidant and antibacterial properties merit attention. It has demonstrated activity against *Staphylococcus aureus* in the leaf extract of *Lantana camara* [20]. The presence of phytol enhances the medicinal profile of the extract and validates its potential use in traditional therapies.

D-Limonene (0.74%, RT: 8.638 min), a ubiquitous monoterpene found in citrus peel oils, has been extensively studied for its antioxidant, anti-inflammatory, and chemopreventive effects [23]. D-Limonene present in the ethanol extract of *A. squamosa* leaves (0.74%) is a monoterpene that is found in grapefruit (95%), tangerine (94%), orange (91%), and lemon (65%) [24]. D-limonene is also present in citrus essential oils but the most common food source of limonene is orange peel oil, which is about 90%–95% D-limonene by weight [25]. Limonene is frequently used as a dietary supplement and as a fragrance ingredient for cosmetics products. Because it is considered to be safe, D-limonene is engaged in the food industry as a flavouring agent [26]. Other notable compounds include cyclohexanone (7.43%), which has antimicrobial properties, and ethanol, 2-butoxy (6.20%), a glycol ether that may contribute synergistically to the biological activities of the extract.

Table 2 shows some isolated compounds, the class of compounds and the source in *A. squamosa* plant. It has been proposed that germacrene D plays a role as a precursor of various sesquiterpenes such as cadinenes and selinenes [27,28]. It has also been suggested that germacrene D, by itself may have deterrent effects against herbivores and it has been reported to have insecticidal activity against mosquitoes [29], as well as repellent activity against aphids [30] and ticks [31].

The diversity and prevalence of terpenoids in the analyzed extract support its potential therapeutic relevance, particularly in antimicrobial, antioxidant,

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and anti-inflammatory applications. These findings validate the ethnopharmacological use of the plant and highlight its prospects for future pharmaceutical exploration. Compared to the work by Al-Nemari [20], who identified *α-copaene* and *phytol* in *A. squamosa* methanolic extracts, the present ethanol-based study provides a broader profile of bioactives, including germacrene D and neophytadiene, indicating that solvent choice significantly impacts extract composition.

CONCLUSION

This study has demonstrated that *Annona squamosa* L. leaves contain a diverse range of bioactive compounds, as revealed by GC-MS analysis. 21 distinct phytochemicals were identified in the ethanol extract, with significant concentrations of caryophyllene, neophytadiene, and 1-methyl-2-pyrrolidinone, which are known to possess anti-inflammatory, antioxidant, and antimicrobial properties. The presence of these compounds provides scientific support for the traditional medicinal use of *A. squamosa* leaves. These findings contribute to the growing evidence base for the pharmacological potential of this plant and highlight its relevance in the development of herbal-based therapeutic agents. Future research should focus on bioactivity-guided isolation, in vivo testing, and formulation into therapeutic products.

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