Investigation of Cashew Apple Juice Constituents Through GC-MS Analysis

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Abstract

Cashew apple is a tropical fruit widely known for its rich phytochemical composition and traditional medicinal uses. This study aimed to analyze the bioactive compounds present in cashew apple juice using Gas Chromatography-Mass Spectrometry (GC-MS). The ethyl acetate extract of cashew apple juice revealed 46 bioactive constituents, including esters, alcohols, aldehydes, fatty acids, organic acids, siloxanes and flavonoids. The most abundant compounds were 3-Hydroxyflavone (12.21%), Carbonic acid, tetradecyl vinyl ester (7.86%), Tricosane (4.12%), 9-Octadecene (3.99%) and Benzamide (5.71%), which possess antioxidant, antimicrobial, anticancer, anti-inflammatory and antimutagenic properties. Other significant compounds, such as Phenol, 3,5-bis(1,1-dimethylethyl)-(2.64%), 4,6-di-tert-Butylresorcinol (3.05%) and Sulfurous acid, octadecyl 2-propyl ester (3.36%), enhance the juice's pharmacological potential. Additionally, the presence of esters, aldehydes and terpenes contributes to the aromatic and organoleptic characteristics, making the juice nutritionally valuable. The GC-MS analysis proved to be a powerful technique for identifying phytoconstituents, facilitating their potential utilization in pharmaceuticals, nutraceuticals, cosmetics and functional foods. The diverse bioactivities of these compounds suggest promising applications in the prevention and treatment of chronic diseases, emphasizing the need for further pharmacological and nutritional research. Future studies should focus on isolating and characterizing these compounds to explore their therapeutic efficacy.

Keywords: Anacardium Occidentale, Bioactive Compound, Cashew, Gas Chromatography-Mass Spectrum.

Introduction

Numerous cultures use plants for medicinal purposes and these botanical sources provide pharmaceutical companies with specific bioactive compounds essential for developing effective drugs. The availability of natural chemical constituents makes plants a crucial resource in the pharmaceutical industry [1]. Plants are great natural antioxidants, hence phytochemical studies on extracts containing their primary phytocompounds are crucial. Component analysis of bioactive phytochemicals derived from plants is preceded by an essential extraction process that plays a critical role in their acquisition and isolation [2]. Medicinal plants are significant sources for medicines, constituting about 25% of prescriptions in the pharmaceutical industry.

The chemicals in a plant sample are detected and determined using a combined analytical method known as gas chromatography-mass spectroscopy (GC-MS). Determining the proportion of particular active compounds found in herbs that are used in culinary, cosmetic, medical, environmental and forensic applications may prove to be an intriguing tool [3]. GC-MS is essential for the phytochemical analysis and chemotaxonomic study of medicinal plants containing physiologically active compounds. The gas chromatography mass spectrometry technique, is among the fastest, most reliable and effective methods for identifying various bioactive substances. These include alcohols, alkaloids, long chain hydrocarbons, esters, acids, steroids and compounds containing nitro and amino acids. The integration of specific detection methods with GC-MS has transformed these instruments into sophisticated tools for evaluating a wide array of substances [4, 5]. As stated by the World Health Organization, more than 80% of individuals in developing countries use traditional medicine, which will remain significant in the healthcare system.

In northeastern Brazil, the cashew tree is referred to as a cajueiro and the fruit is known as caju, which comes from the Indigenous term acaju or acajous, which means "to pucker the mouth" and refers to the astringent flavor of the fruit [6]. Portuguese traders brought the cashew plant to eastern Africa and India in the late 16th century, where it thrived on nutrient poor sandy soils and was used as a fast growing soil maintainer near the coast. [7]. After becoming a part of the environment, cashew trees were especially exceedingly resistant and drought tolerant, surviving for 30-40 years in the high tropical heat of these regions [8]. The low growing, gnarled branches of cashew trees can spread and grow into huge forests in the tropical lowlands of the West Indies, Central America and northern South America [9]. Because of its high value, the cashew tree is extremely important [10, 11].

Numerous biological properties, including antimicrobial, antioxidant, anticancer [12] and antimutagenic [13, 14] properties, have been reported for cashew apple. The cashew apple has tremendous economic and medical value. In ethnopharmacology, this species is mostly used to treat infectious and inflammatory disorders, as well as pain ailments, such as diarrhea, psoriasis, stomatitis, aphthae, bronchitis, intestinal discomfort, cramps, stomach diabetes, muscle weakness and toothache [15, 16]. In this present study, the bioactive compounds of Anacardium occidentale juice were examined.

Materials and Methods

Cashew Apple Collection and Juice Preparation

Fresh cashew apples were gathered during the harvesting period (April-June 2023) and rinsed with sterile water. Cashew apple was immediately placed in plastic boxes. The fruits were macerated in a mixer grinder without the use of water. The pulp was filtered a muslin cloth and clarified using 0.3 gelatin to eliminate the tannin content. After full extraction, the clear solution was kept at -20° C until further examination [17]. **Figure 1** shows the procedure for Cashew Apple collection, extraction and GCMS analysis.

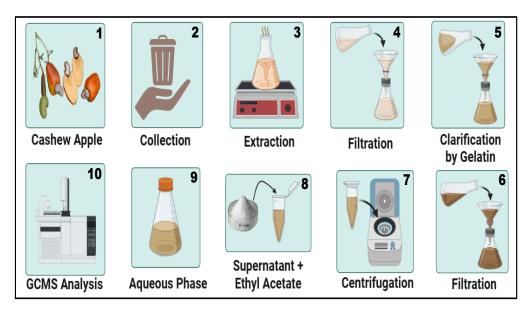


Figure 1. Stepwise Procedure for Cashew Apple Collection, Extraction and GC-MS Analysis

Analysis of Bioactive Substances in Cashew Apple Juice using GC-MS

The centrifugation of cashew apple juice was conducted at 10,000 rpm for 15-20 mins. The resulting supernatant was then transferred to a new microcentrifuge tube with care. A 2 mL aliquot of ethyl acetate was incorporated into the supernatant and the resultant mixture was vigorously mixed before being allowed to separate at room temperature. The following separation, the aqueous phase was then extracted from the ethyl acetate layer. The extract thus obtained underwent GC-MS analysis to identify and characterize the unique bioactive compounds present in the sample [18].

A Shimadzu 2010 plus instrument, equipped with an AOC-20i autosampler and a gas chromatograph linked to a mass spectrometer, was used for the GC-MS analysis of the ethyl acetate extract from cashew apple juice. An RTX 5Ms column (0.32 mm diameter, 30 m length, 0.50 μ m thickness) was used in the electron impact mode at 70 eV. The carrier gas, helium (99%), was delivered at a steady rate of 1.00 mL/min. Sample injection was conducted in split mode at a 30:1 ratio. The ion source temperature was established at 200 °C, the oven at 50 °C and the injector at 250 °C. The mass spectra of particles between 40 and 450 Da were acquired at 70 eV with a scan interval of 0.5 s. The overall duration of the GC runs was 27.00 mins. The relative percentage of each component was determined by dividing the average peak area by the total area. The Turbo Mass Ver 5.2.0 software was used to manage the mass spectra and chromatograms.

Identification of Components

The analysis of the GC-MS data was analyzed using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 spectral patterns. The spectra of the unidentified components were meticulously compared to those known components listed in the NIST collection. The components in the test materials were identified based on their nomenclature, molecular weights and structural features [19].

Results

Therapeutic plants serve as a rich source of novel pharmaceuticals, with numerous contemporary drugs derived indirectly from their constituents. These botanical resources have supplied a range of components that are crucial for treating various illnesses and health conditions. The examination and isolation of plant materials are fundamental to the advancement, enhancement and quality

assurance of herbal preparations. Furthermore, investigations into medicinal plants enhance our knowledge of plant-based toxins, thereby protecting both human and animal populations against naturally occurring harmful substances. Hence, this study focused on identifying the bioactive compounds present in the ethyl acetate extract of cashew apple juice through GC-MS analysis.

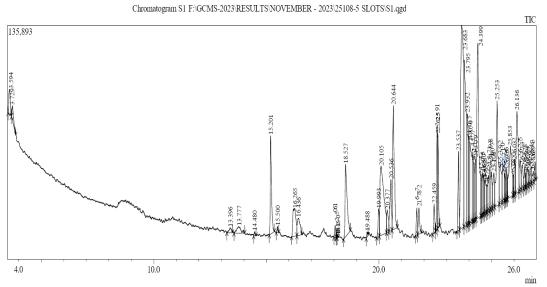


Figure 2. CAJY-P - Cashew Apple Juice Yellow Panruti Ethyl Acetate Extract's GC-MS Spectrum

The chemical compositions of isolates secondary metabolites were identified and the separated chromatograph were compared using the NIST library. The Cashew apple juice yellow Panruti (CAJY-P) ethyl acetate GC-MS chromatogram showed 46 compounds with different peak areas and retention times. All compounds have been documented, including their molecular formulas, molecular weights, peaks area and retention times. The corresponding 3D structures are presented in **Table 1** and **Figure 2**.

Peak	Name	R. Time	Area%	Molecular formula	Molecular formula	Molecular weight (g/mol)	Biological activity
1.	2,3- Butanediol, [R-(R*,R*)]-	3.594	0.74	C4H10O2	H · O · H	90.12	Antimicrobial activity
2.	2,3- BUTANEDIO L	3.729	0.47	C4H10O2	H ^O H HO ^H HO ^H	90.12	Antimicrobial activity
3.	1,4-Dioxane- 2,6-dione	13.396	0.24	C4H4O4	0 0 0	116.07	Antimicrobial Activity, Antioxidant Activity
4.	2-BUTENE- 1,4-DIOL	13.777	0.58	C4H8O2	H ₀ H	88.11	Antimutagenic Activity

Table 1. Chemical Compositions of CAJY-P Ethyl Acetate Extract Analysis by GC-MS

5.	CYCLOPROP ANE	14.480	0.09	СЗН6	\triangleright	42.08	Antimicrobial Activity, Anticancer Activity
6.	Phenol, 3,5- bis(1,1- dimethylethyl) -	15.201	2.64	C14H22O	●-E	206.32	Antioxidant, antimicrobial activity.
7.	Oxalic acid, dineopentyl ester	15.500	0.12	C12H22O4		230.30	Antimicrobial Activity, Antimutagenic Activity, Anti- inflammatory Activity
8.	2,4- DIMETHYL- 1-HEPTENE	16.265	1.81	С9Н18		126.24	Antimicrobial, antioxidant activity.
9.	Phthalic acid, butyl pent-2- en-4-yn-1-yl ester	16.436	1.00	C17H18O4	n − − − − − − − − − − − − − − − − − − −	286.32	Antimicrobial, antioxidant activity
10.	DOCOSANE	18.061	0.25	C22H46	~~~~~~	310.6	Antimicrobial Activity
11.	Trifluoroethan ol, TBDMS derivative	18.125	0.04	C8H17F3O si	F F F	214.30	Antimicrobial Activity
12.	3- HEXADECEN E, (Z)-	18.527	3.06	C16H32		224.42	Antimicrobial, antioxidant activity, Anti- inflammatory Activity
13.	TETRADECA NOIC ACID, 12-METHYL-, METHYL ESTER	19.993	0.68	C16H32O2	~~~~~ ^l .	256.42	Antimicrobial activity
14.	Benzamide	20.105	5.71	C7H7NO	H-Z O	121.14	Anticancer, anti- inflammatory activity

15.	BUTANOIC ACID, 1,1- DIMETHYLE THYL ESTER	20.377	0.76	C8H16O2		144.21	Antimicrobial Activity, Anti- inflammatory Activity
16.	1,2,3- BENZOTRIA ZIN-4(3H)- ONE, 3- ETHYL-	20.536	1.61	C9H9N3O		175.19	Anticancer and antimicrobial properties.
17.	9- OCTADECEN E, (E)-	20.644	3.99	C18H36	**************************************	252.5	Antimicrobial Activity, Antioxidant Activity
18.	2- PROPYLDEC AN-1-OL	21.687	0.56	C13H28O	H.O.	200.36	Antimicrobial activity
19.	1-Octanol, 2- nitro-	21.782	0.74	C8H17NO 3	H H H H H	175.23	Antimicrobial Activity, Antioxidant Activity, Antimutagenic Activity, Anti- inflammatory Activity
20.	4-(([(E)-1- PHENYLETH YLIDENE] AMINO) OXY)-2H- CHROMEN- 2-ONE	22.459	0.60	C17H13N O3		279.29	Anticancer and anti- inflammatory activity
21.	Formic acid, undecyl ester	22.591	2.20	C12H24O2		200.32	Antimicrobial activity
22.	HEPTANE, 3- ETHYL-5- METHYL-	22.625	1.49	C10H22		142.28	Antimicrobial Activity
23.	2-Bromo dodecane	23.537	1.56	C12H25Br	8	249.23	Antimicrobial activity

24.	3- Hydroxyflavon e	23.683	12.21	C15H10O3		238.24	Antioxidant, anticancer and anti- inflammatory activity
25.	1-Phenyl-3-(2- pyridyl)-5- pyrazolone	23.932	2.91	C14H11N3 O		237.26	Anti- inflammatory and analgesic properties.
26.	Benzamide, 4- methyl-	24.005	1.93	C8H9NO	H-Z	135.16	Anticancer activity
27.	2-Thia- 1,3,6,11- tetraaza- cyclopenta[a]a ntracene	24.050	3.40	C12H6N4S		238.27	Anticancer and antimicrobial properties.
28.	1-Hydroxy-2- methylanthraq uinone	24.230	1.22	C15H10O3	O,H	238.24	Antioxidant and antimicrobial properties
29.	Carbonic acid, tetradecyl vinyl ester	24.399	7.86	C17H32O3	0 0	284.4	Antioxidant and antimicrobial properties, Anti- inflammatory Activity
30.	4-METHYL- N-[2- (PHENYLAC ETYL)PHEN YL] BENZAMIDE	24.660	0.88	C22H19N O2		329.4	Anticancer and anti- inflammatory activity.
31.	Benzamide, 3,4- dimethoxy-N- isobutyl-	24.795	0.50	C13H19N O3		237.29	Anti- inflammatory properties.

32.	1,1,3,3,5,5- Hexamethyl- 1,5-bis(2- methylpropoxy) trisiloxane	24.938	1.14	C14H36O4 Si3		352.69	Antimicrobial Activity, Anti- inflammatory Activity
33.	CYCLOTRISI LOXANE, HEXAMETH YL-	25.108	1.07	C6H18O3S i3		222.46	Antimicrobial Activity
34.	TRICOSANE	25.253	4.12	C23H48	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	324.6	Antimicrobial Activity
35.	Benzestrol, 2TMS derivative	25.510	1.37	C26H42O2 Si2		442.8	Antimicrobial Activity, Antioxidant Activity
36.	Benzamide,4- methoxy-N-[4- (1- methylcyclopr opyl) phenyl]-	25.595	0.50	C18H19N O2		281.3	Antimicrobial Activity, Anticancer Activity, Anti- inflammatory Activity
37.	d-Prolyl-d- proline, n- propoxycarbon yl-, propyl ester	25.695	0.44	C17H28N2 O5		340.4	Antioxidant Activity
38.	4,6-di-tert- Butylresorcino l	25.853	3.05	C14H22O2	H.O.	222.32	Antimicrobial Activity, Antioxidant Activity, Antimutagenic Activity, Anticancer Activity, Anti- inflammatory Activity
39.	METAZACH LOR	25.980	0.32	C14H16Cl N3O		277.75	Antimicrobial Activity

40.	4-Methyl-2,4- bis(p- hydroxyphenyl) pent-1-ene, 2TMS derivative	26.032	0.84	C24H36O2 Si2	SI O	412.7	Antimicrobial Activity, Antioxidant Activity, Anti- inflammatory Activity
41.	Sulfurous acid, octadecyl 2- propyl ester	26.136	3.36	C21H44O3 S		376.6	Antimicrobial Activity, Anti- inflammatory Activity
42.	ARSENOUS ACID, TRIS(TRIME THYLSILYL) ESTER	26.275	0.97	C9H27AsO 3Si3	t X	342.49	Antimicrobial Activity,
43.	Benzoic acid, 4-methyl-2- trimethylsilylo xy-, trimethylsilyl ester	26.373	0.80	C14H24O3 Si2		296.51	Antimicrobial Activity, Antioxidant Activity
44.	2,4,6- Cycloheptatrie n-1-one, 3,5- bis- trimethylsilyl-	26.530	0.23	C13H22OS i2	Si Si	250.48	Antioxidant Activity
45.	ACETOPHEN ONE, 4'- (TRIMETHYL SILOXY)-	26.667	0.37	C11H16O2 Si		208.33	Antimicrobial Activity, Anticancer Activity, Anti- inflammatory Activity
46.	Penta siloxane, dodecamethyl-	26.863	0.33	C12H36O4 Si5	SI O SI O SI O SI	384.84	Anti- inflammatory Activity

The GC-MS analysis of the ethyl acetate extract obtained from cashew apple juice identified a major bioactive compound at 12.21 %. The major bioactive compounds present in the samples were 3-Hydroxyflavone (12.21%), Carbonic acid, tetradecyl vinyl ester (7.86%), Tricosane (4.12%) and 9-octadecene, (E) (3.99%). In the isolation experiments, further attention was paid to compounds with larger peak area. The amount of a component in the combination is correlated with the peak area.

Discussion

The GC-MS analysis of cashew apple juice provided valuable insights into its complex chemical composition, revealing various bioactive compounds. This study identified several key compounds that contribute to the nutritional and aromatic profile of juice, including organic acids, esters, alcohols, aldehydes and fatty acids. These compounds play crucial roles in the flavor, aroma and potential health benefits of cashew apple juice.

In this study, the CAJY-P ethyl acetate GC-MS chromatogram revealed 49 compounds with different peak areas and retention times. In similar studies, Sivagurunathan *et al.*, analyzed the GC-MS analysis of successive extracts such as chloroform and NaOH extracts of cashew apple. The results revealed multiple phenolic compounds. Sample 1 (S1) showed 8 compounds likewise sample 2 showed 7 compounds, sample 3 showed 5 compounds and sample 4 showed 7 compounds [20].

According to Oloniya *et al.*, the GC-MS chromatograms of samples A (undefatted cashew kernel flour), B (cold pressed defatted cashew kernel flour) and C (hot pressed defatted cashew kernel flour) were examined. Sample A (undefeated cashew kernel flour) contained 42 bioactive compounds, hot-pressed defatted cashew kernel flour contained 36 bioactive compounds and hot pressed cashew kernel flour contained 33 bioactive compounds, according to GC-MS [21].

An ethanol extract of *A. occidentale* was subjected to GC-MS analysis by Ghosh *et al.*, two compounds, methyl ester and 14,17octadecadienoic acid, can heal wounds and cause skin scaling. GC-MS analysis confirmed the antioxidant, anti-inflammatory, antifibrotic and vasodilatory qualities of hexadecanoic acid,16 methyl ester [22].

Using high resolution gas chromatography, 41 compounds were identified in the cashew apple juice sample. Esters comprise majority of volatile substances in cashew apple beverages, followed by alcohols, acids and aldehydes. Some ethyl esters, such as ethyl decanoate, ethyl lactate, ethyl hexanoate, ethyl butyrate and ethyl 3-methyl butyrate are the main constituents [23].

Priyadharshini *et al.*, investigated the active principles present in the methanolic extract of A. occidentale L. through GC-MS analysis. The analysis of the methanolic extract of the CA juice revealed the presence of 11 bioactive phytocomponents. The results indicated the identification of the following compounds: 2,3dihydroxy propyl ester, trans-13-octadecenoic acid and 9-octadecenoic acid (Z)-methyl ester [18].

This study presents a novel approach by employing advanced GC-MS analysis to identify a broader range of bioactive compounds in cashew apple juice. Unlike previous studies that focused on specific extracts or a limited number of compounds, this research identified 46 bioactive compounds in a single extraction process. Key compounds such as 3-Hydroxyflavone, Carbonic acid tetradecyl vinyl ester and Tricosane were found to be abundant and these compounds have demonstrated antioxidant, antimicrobial. anti-inflammatory anticancer, and antimutagenic properties. This work provides a detailed analysis of the juice's chemical composition and highlights its significant therapeutic potential. The results suggest that cashew apple juice could be a valuable source for developing pharmaceuticals, nutraceuticals and functional foods. Future research will aim to isolate these compounds and explore their individual therapeutic applications, paving the way for more targeted and effective treatments based on natural bioactive substances.

Conclusions

The GC-MS analysis of cashew apple juice revealed a diverse array of chemical compounds that contributed to its rich nutritional profile, distinctive flavor and potential health benefits. There were more phenolic compounds and a modest number of flavonoids in the fruits of A. occidentale. The pharmaceutical sector has experienced a reduction in the use of natural products, primarily due to the incompatibility between high-throughput screening methods and conventional natural product extract libraries. In further studies, individual active compounds will be isolated by chromatographic methods and the fractions will be assessed independently by FTIR and NMR to determine the nature compounds, structure and functional group of the compound to be transformed into a new active medicament.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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