RESEARCH ARTICLE

Gas Chromatography-Mass Spectrometry Analysis of Seed Extracts of *Annona squamosa*

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Abstract

The plant *Annona squamosa*, popularly known as custard apple, is a member of the Annonaceae family and is indigenous to South America and the West Indies. It is highly popular due to its folk medicinal as well as nutritional and ornamental value. The plant has many medicinal properties like antihypertensive, antidiabetic, antiparasitic, anticancer, antimalarial, antioxidant, and insecticidal. Seed extracts of this plant were prepared in petroleum-ether, chloroform, and acetone solvents separately by soxhlet extraction method. 15 chemicals were found in both the petroleum-ether and chloroform extracts after being analyzed using gas chromatography-mass spectrometry (GC-MS). Chromatograms of acetone extracts also revealed the existence of 23 main peaks.

Keywords: *Annona squamosa*, Annonaceae, Folk medicine, GC-MS, Phytochemicals

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Introduction

Medicinal plants or herbs are the paramount resource to obtain an assortment of drugs because of their slightest or no side effect to human health. Traditional remedies are used by the majority of people in developing nations. The *Annona squamosa* tree is frequently used as traditional medicine to treat a variety of maladies and human disorders (Al Kazman *et al*., 2022, Gajalakshmi *et al*., 2011). *A. squamosa* Linn, sometimes referred to as custard or sugar apple in English, Sharifa, and sitaphal in Hindi, is a member of the family Annonaceae (Narwade and Aher, 2019). The sugar apple is indigenous to South America and the West Indies (Pandey and Barve, 2011). Numerous Indian states have considerable *A. squamosa* cultivation (Ma *et al*., 2017). It is a tiny shrub or tree with a height of 3 to 8 meters. A leaf is lanceolate, measuring 6 to 17 cm in length and 3 to 5 cm in width. Fruits range in shape from round to heart-shaped, ovate to conical, and measure 5 to 10 cm in diameter with many numerous seeds. The seed is 1.3cm to 1.6cm elongated, rectangular, smooth, glossy, and deep brown or black in color (Chen *et al*., 2011).

Custard apples are a good source of K, Cu, Fe, vitamin B6, A and C (Bala *et al*., 2018). They also have a low-fat content. *A. squamosa* fruit are said to be a good tonic to improve blood and muscle power in Ayurveda (Ma *et al*., 2017). Tribes treat a variety of illnesses with various plant parts. Tribes employ *A. squamosa*'s young leaves for the treatment of diabetes. Additionally, leaves are employed as antispasmodic, insecticidal, and rheumatism and painful spleen treatments (Gajalakshmi *et al*., 2011). The root is a purgative and is also used to treat spinal marrow disease and dysentery (Narwade and Aher, 2019). The anti-diarrheal and anti-cancer properties of are shown by bark. Fruit pulp has been discovered to have a significant caloric value due to its increased sugar content (58% of dry mass), which also helps to reduce blood sugar levels (Gajalakshmi *et al*., 2011). Various food products use fruit pulp as a flavoring ingredient (Ma *et al*., 2017). Its seeds contain fish poison and are contraceptives. Combining seeds with flour exhibits an anti-lice action (Narwade and Aher, 2019). *A. squamosa* seed extract is also utilized to control lipid peroxidation and hyperthyroidism, therefore quercetin is present (Panda and Kar, 2007). The plant's leaves and stem showed antitumor action in a 50% ethanol extract (Bhakuni *et al*., 1972). Uses for seed oil in the soap and paint industries.

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Amino acids, alkaloids, acetogenins, steroids, flavonoids, terpenes, lipids, ascorbic acid, tannins, and vitamins are the phytochemicals linked to this plant (Narwade and Aher, 2019; Ma *et al*., 2017; Soni *et al*., 2018; Shiekh *et al*., 2021). Acetogenins extracted from seed have been shown to be *in-vitro* cytotoxic activity in opposition to human tumor cell lines. Diterpenes extracted from *A. squamosa* have anti-HIV and anti-platelet aggregation properties. Antimicrobial and pesticidal properties have been reported by isolated Flavonoids' from the plant (Chen *et al*., 2011). It is used as a pesticide, antitumor, antidiabetic, antioxidant, antilipidemic, and anti-inflammatory medication due to the presence of cyclic peptides (Marahatta *et al*., 2019; Thangaraj *et al*., 2017). The current work aims to screen the pharmaceutically significant bioactive compounds from *A. squamosa* seeds that significantly advance the nutraceutical and pharmacological research in light of its medical significance.

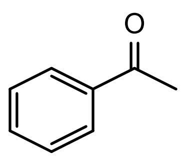
Material And Methods

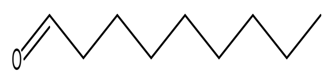
Collection of Sample

A. squamosa seeds were collected from Jaipur, Rajasthan (India) and were washed, cleaned and dried.

Table 1: Phytochemicals discovered by GC-MS in the petroleum-ether extract of the *A. squamosa* seed sample

Peak	*R. Time*	*M. weight*	*M. formula*	*Area%*	*Name*
1.	10.624	120	C_8H_8O	17.99	Acetophenone
2.	11.601	142	$C_9H_{18}O$	2.85	Nonanal
3.	15.345	164	$C_{10}H_{12}O_2$	1.42	Thymoquinone
4.	20.028	202	$C_{15}H_{22}$	1.81	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-m
5.	20.522	206	$C_{14}H_{22}O$	3.76	Phenol,3,5-bis(1,1-dimethylethyl)-
6.	21.834	292	$C_{17}H_{24}O_4$	2.49	Phthalicacid,4,4-dimethylpent-2-ylethyl ester
7.	25.869	278	$C_{16}H_{22}O_4$	1.63	1,2-Benzenedicarboxylic acid,bis(2-methyl propyl) ester
8.	28.011	222	$C_{16}H_{14}O$	3.85	2-Buten-1-one, 1,3-diphenyl-
9.	28.861	294	$C_{19}H_{34}O_2$	9.38	9,12-Octadecadienoic acid(Z,Z)-,methyl
10.	28.938	296	$C_{19}H_{36}O_2$	7.32	9-Octadecenoicacid, methylester, (E)-
11	29.250	256	$C_{16}H_{32}O_2$	2.37	Tetradecanoicacid,12-methyl-, methylester
12	29.670	322	$C_{21}H_{38}O_2$	9.40	n-Propyl9,12-octadecadienoate
13	29.738	310	$C_{20}H_{38}O_2$	7.20	(E)-9-Octadecenoicacidethylester
14	30.066	364	$C_{20}H_{35}F_3O_2$	16.48	9-octadecenoicacid, 2,2,2-trifluoroethylester
15	31.392	312	$C_{19}H_{36}O_3$	12.06	Glycidylpalmitate


1. Acetophenone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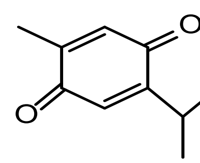
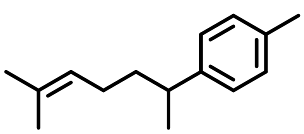
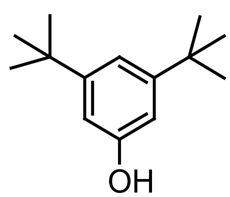
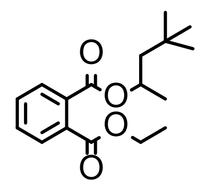
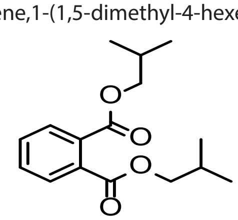
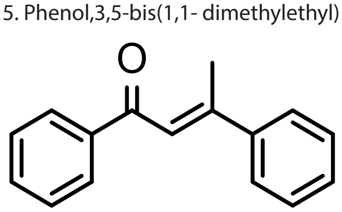

3. Thymoquinone

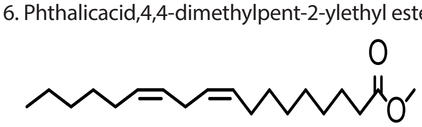

4. Benzene,1-(1,5-dimethyl-4-hexenyl)-4-m

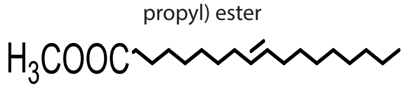

5. Phenol,3,5-bis(1,1- dimethylethyl)


6. Phthalicacid,4,4-dimethylpent-2-ylethyl ester

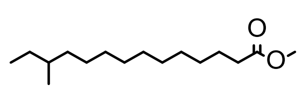

7. 1,2-Benzenedicarboxylic acid,bis(2-methyl propyl) ester

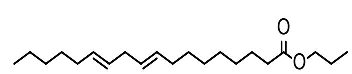

8. 2-Buten-1-one, 1,3-diphenyl-

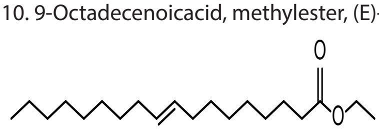

9. 9,12-Octadecadienoic acid(Z,Z)-,methyl

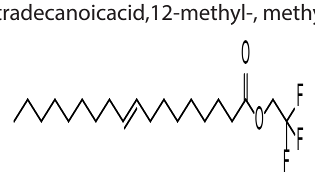


10. 9-Octadecenoicacid, methylester, (E)-


11. Tetradecanoicacid,12-methyl-, methylester


12. n-Propyl9,12-octadecadienoate


13. (E)-9-Octadecenoicacidethylester


14. 9-octadecenoicacid, 2,2,2-trifluoroethylester

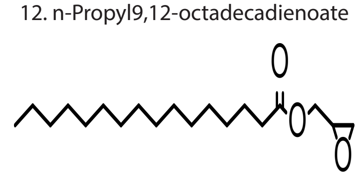

15. Glycidylpalmitate

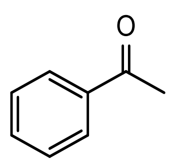
Fig. 1: Structures of phytochemicals determined by GC-MS in the petroleum-ether extract of an *A. squamosa* seed sample

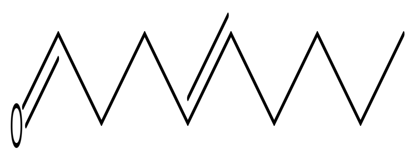
Table 2: Major Phytochemicals identified in petroleum-ether extract of *A. squamosa* seeds

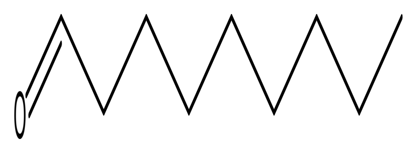
S. No.	*Phytochemical*	*Area %*	*MS Fragment -ions*
1.	Acetophenone	17.99	39, 51, 77, 105, 120
2.	9-octadecenoicacid,2,2,2-trifluoroethylester	16.48	41, 55, 69, 83, 97, 111, 125, 139, 155, 207, 222, 265
3.	Glycidylpalmitate	12.06	29, 43, 57, 69, 84, 98, 116, 129, 143, 154, 171, 185, 239, 269

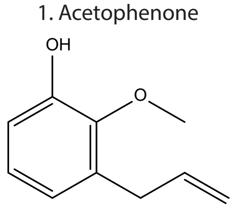
Table 3: Phytochemicals found in the *A. squamosa* seed sample's chloroform extract by GC-MS

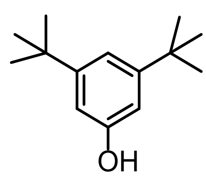
Peak	*R. Time*	*M. weight*	*M. formula*	*Area%*	*Name*
1.	10.628	120	C_8H_8O	7.63	Acetophenone
2.	11.317	142	$C_9H_{18}O$	0.36	4-Nonenal,(E)-
3.	11.600	164	$C_{10}H_{12}O_2$	1.24	Nonanal
4.	17.625	202	$C_{15}H_{22}$	0.50	Phenol,2-methoxy-3-(2-propenyl)-
5.	20.523	206	$C_{14}H_{22}O$	0.63	2,4-Di-tert-butylphenol
6.	25.876	292	$C_{17}H_{24}O_4$	0.49	1,2-Benzenedicarboxylic acid,bis(2-methyl propyl)ester
7.	26.698	278	$C_{16}H_{22}O_4$	0.96	Hexadecanoicacid, methylester
8.	27.160	222	$C_{16}H_{14}O$	2.07	Dibutylphthalate
9.	28.009	294	$C_{19}H_{34}O_2$	2.13	2-Buten-1-one, 1,3-diphenyl-
10.	28.861	296	$C_{19}H_{36}O_2$	1.88	9,12-Octadecadienoic acid(Z,Z)-,methyl ester
11	28.942	256	$C_{16}H_{32}O_2$	1.98	9-Octadecenoicacid(Z)-,methylester
12	29.250	322	$C_{21}H_{38}O_2$	1.11	Pentacosanoicacid, methylester
13	30.072	310	$C_{20}H_{38}O_2$	15.17	9-Octadecenoicacid,(E)-
14	30.762	364	$C_{20}H_{35}F_3O_2$	45.63	Hexadecanoicacid,2-hydroxy-1-(hydroxy methyl)-ethyl ester
15	31.390	312	$C_{19}H_{36}O_3$	18.21	Glycidylpalmitate

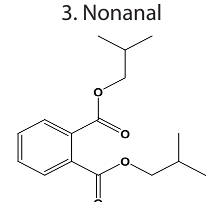

1. Acetophenone

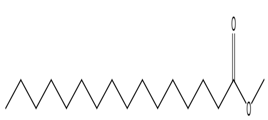

2.4-Nonenal,(E)-

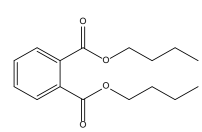

3. Nonanal

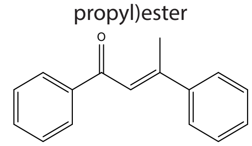

4.Phenol,2-methoxy-3-(2-propenyl)-

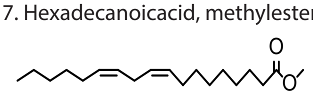

5. 2,4-Di-tert-butylphenol

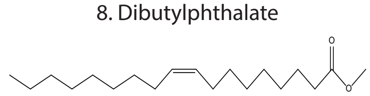

6. 1,2-Benzenedicarboxylic acid,bis(2-methyl propyl)ester

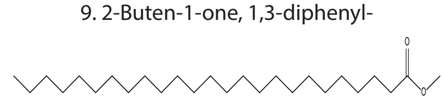

7. Hexadecanoicacid, methylester

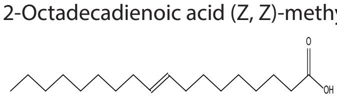

8. Dibutylphthalate

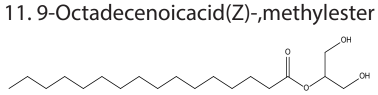

9. 2-Buten-1-one, 1,3-diphenyl-


10. 9,12-Octadecadienoic acid (Z, Z)-methyl ester


11. 9-Octadecenoicacid(Z)-,methylester


12. Pentacosanoicacid, methylester


13. 9-Octadecenoicacid(E)-


14. Hexadecanoicacid,2-hydroxy-1-(hydroxy methyl)-ethyl ester

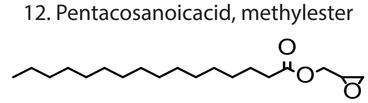
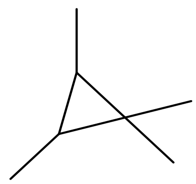

15. Glycidylpalmitate

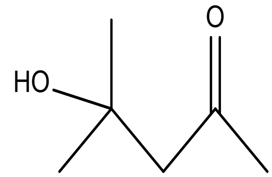
Fig. 2: Phytochemicals' structures were determined by GC-MS analysis of the *A. squamosa* seed sample's chloroform extract

Table 4: Significant phytochemicals found in *A. squamosa* seed extract in chloroform

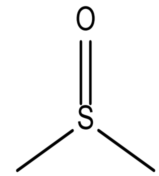
S. No.	*Name*	*Area%*	*MS Fragment –ions*
1.	Hexadecanoic acid,2-hydroxy-1-(hydroxy methyl)-ethyl ester	45.63	29, 43, 57, 69, 74, 98, 112, 134, 147, 239, 257
2.	Glycidylpalmitate	18.21	29, 43, 57, 69, 84, 98, 116, 129, 143, 154, 171, 185, 239, 269
3.	9-Octadecenoicacid,(E)-	15.17	27, 41, 55, 69, 83, 97, 112, 127, 151, 180, 222, 264, 282



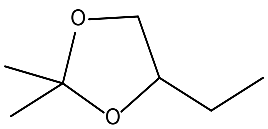
1. Cyclopropane, 1,1,2,3-tetramethyl-



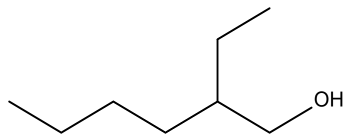
2. 2-Pentanone,4-hydroxy-4-methyl-



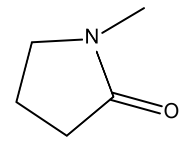
3. DimethylSulfoxide



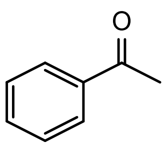
4. 1,3-Dioxolane-4-methanol, 2,2-dimethyl-



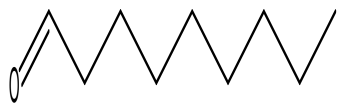
5. 1-Hexanol,2-ethyl-



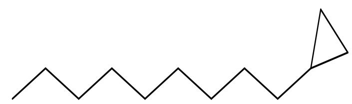
6. 2-Pyrrolidinone, 1-methyl-



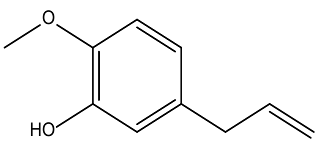
7. Acetophenone



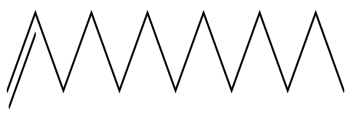
8. Nonanal



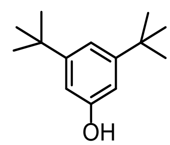
9. Cyclopropane, nonyl-



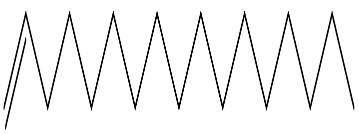
10. 3-Allyl-6-methoxyphenol



11. 11-Tridecene

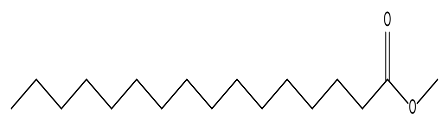


12. 2,4-Di-tert-butylphenol

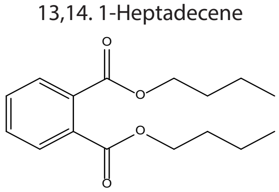


13,14. 1-Heptadecene

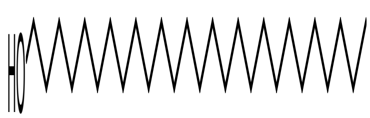
15. 1,3,12-Nonadecatriene



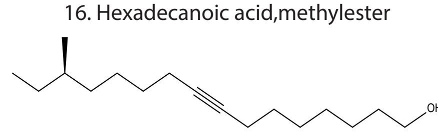
16. Hexadecanoic acid,methylester



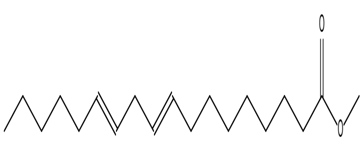
17. Dibutylphthalate



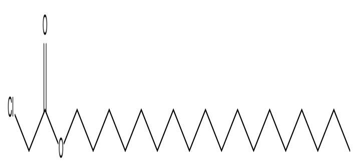
18. 1-Heptacosanol



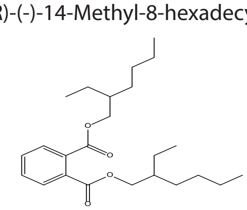
19. (R)-(-)-14-Methyl-8-hexadecyn-1-ol



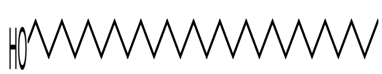
20. 9,12-Octadecadienoicacid (Z,Z)-, methylester



21. Acetic acid,chloro-octadecylester



22. Bis(2-ethylhexyl) phthalate



23. 1-Heptacosanol

Fig. 3: Phytochemicals discovered by GC-MS in the acetone extract of *A. squamosa* seed sample

Table 5: Phytochemicals discovered by GC-MS in the acetone extract of *A. squamosa* seed samples

Peak	*R. Time*	*M. weight*	*M. formula*	*Area%*	*Name*
1	4.065	98	C_7H_{14}	1.49	Cyclopropane, 1,1,2,3-tetramethyl-
2	4.911	116	$C_6H_{12}O_2$	25.89	2-Pentanone,4-hydroxy-4-methyl-
3	5.195	78	C_2H_6OS	0.56	DimethylSulfoxide
4	7.267	132	$C_6H_{12}O_3$	0.81	1,3-Dioxolane-4-methanol, 2,2-dimethyl-
5	9.625	130	$C_8H_{18}O$	0.87	1-Hexanol,2-ethyl-
6	9.953	99	C_5H_9NO	1.13	2-Pyrrolidinone, 1-methyl-
7	10.628	120	C_8H_8O	11.92	Acetophenone
8	11.601	142	$C_9H_{18}O$	1.36	Nonanal
9	13.880	168	$C_{12}H_{24}$	3.75	Cyclopropane, nonyl-
10	17.626	164	$C_{10}H_{12}O_2$	0.74	3-Allyl-6-methoxyphenol
11	18.307	182	$C_{13}H_{26}$	4.10	1-Tridecene
12	20.526	206	$C_{14}H_{22}O$	3.51	2,4-Di-tert-butylphenol
13	21.844	238	$C_{17}H_{34}$	3.59	1-Heptadecene
14	24.887	238	$C_{17}H_{34}$	2.48	1-Heptadecene
15	26.155	262	$C_{19}H_{34}$	2.66	1,3,12-Nonadecatriene
16	26.695	270	$C_{17}H_{34}O_2$	1.65	Hexadecanoicacid,methylester
17	27.157	278	$C_{16}H_{24}O_4$	2.17	Dibutylphthalate
18	27.601	396	$C_{27}H_{56}O$	3.62	1-Heptacosanol
19	27.985	252	$C_{17}H_{32}O$	9.29	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
20	28.863	294	$C_{19}H_{34}O_2$	1.40	9,12-Octadecadienoicacid (Z,Z)-,methylester
21	28.942	346	$C_{20}H_{39}ClO_2$	3.03	Aceticacid,chloro-,octadecylester
22	29.811	390	$C_{24}H_{38}O_4$	5.24	Bis(2-ethylhexyl) phthalate
23	30.064	396	$C_{27}H_{56}O$	8.76	1-Heptacosanol

Table 6: Significant phytochemicals found in *A. squamosa* seed extract in acetone

S.N.	*Name*	*Area%*	*MS Fragment -ions*
1.	2-Pentanone,4-hydroxy-4-methyl-	25.89	27, 37, 43, 59, 73, 83, 101
2.	Acetophenone	17.99	39, 51, 77, 105, 120
3.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	9.29	41, 55, 67, 81, 95, 109, 121, 135, 150, 166

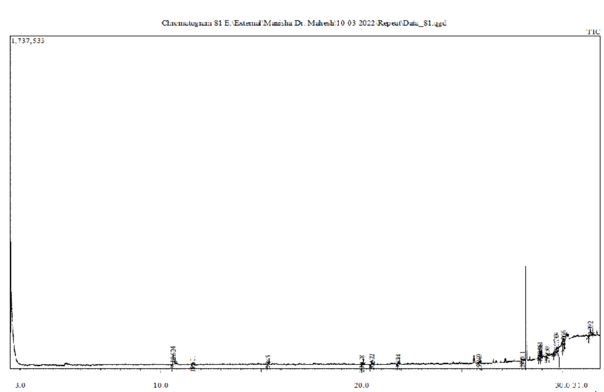


Fig. 4: GC-MS Spectrum of Petroleum ether extract of *A. squamosa* seeds.

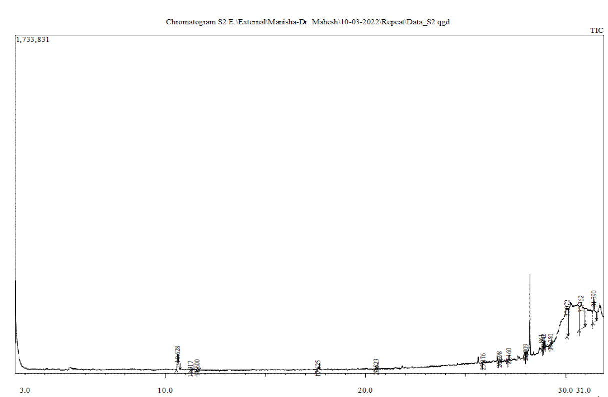


Fig. 5: GC-MS Spectrum of Chloroform extract of *A. squamosa* seeds

Preparation of Extracts

A grinder was used to reduce the dried seeds of *A. squamosa* to a fine powder. Then, the powder (100 g) was put in a soxhlet apparatus and successively extracted for 12 hours on a water bath with 500 mL petroleum ether, chloroform, and acetone. To eliminate impurities, the aforementioned extracts were

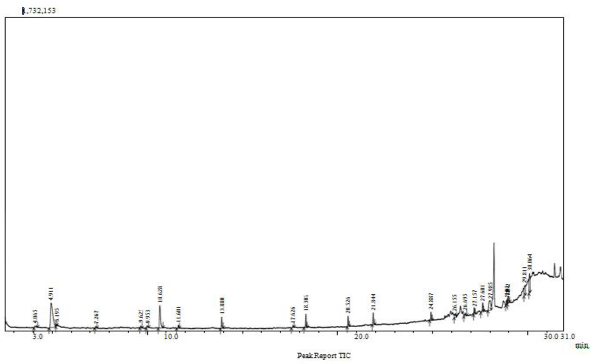


Fig. 6: GC-MS Spectrum of Acetone extract of *A. squamosa* seeds.

filtered through the Whatman No. 41 paper (Merck, Mumbai, India). A rotating evaporator uses a vacuum to remove extra solvent. The GC-MS analysis of the concentrated extracts was then performed.

Instruments and Chromatographic Conditions

For routine compound analysis, gas chromatography with mass spectroscopy are a preferable technology. In this investigation, 1.5 µl of *A. squamosa* petroleum ether, chloroform, and acetone extracts were employed independently for the GC-MS analysis in order to identify diverse phytochemical components. The Manipal University CAF Center in Jaipur used the GC-MS technology. The following circumstances were used during analysis utilising a gas chromatography unit Shimadzu GCMS-QP2020 instrument: equipped with the Rxi-5 capillary column (30 m in length x 0.25 µm in thickness x 0.25 mm in diameter); He gas was used as carrier gas and an injection volume of 1.5µL was used (split ratio of 1.0) with column flow rate 1.18 mL/min; total flow rate 7.4 mL/min; Injection Temp. 250°C; Ion source temp. 250°C; Interface temp. 250°C; Pressure at column inlet 66.8 kPa. Initial column oven Temp. was 50ºC, held for 02 minutes; finally programmed to 250ºC at a rate of 8ºC/min, then held for 02min, total program time 31.92 minutes. The method of electron-impact ionization was applied. All information was gathered by gathering full scan mass spectra between 40 and 550 m/z with a scan speed of 2000. Components are identified Different retention durations used by the mass spectrometer to detect the components allowed for the identification of various components. The software that was coupled to it created a plot of intensity against retention time known as a chromatogram. By comparing the data with the software library NIST17 and standard mass spectra, the chemicals in the graph are identified. The components of the test materials' names, molecular weights, and structures were determined.

Result And Discussion

Chromatograms of the petroleum ether, chloroform, and acetone extracts identified 15, 15, and 21 chemicals, respectively. Acetophenone (17.99%) had the highest content in the pet-ether extract's GC/MS profiles, followed by 9-octadecenoic acid (16.48%), 2, 2, 2-trifluoroethylester (16.48%), and glycidyl palmitate (12.06%). Phytoconstituents detected in the chloroform seed extract of the plant using gas chromatography-mass spectrometry are hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl)-ethyl ester (45.63%), glycidyl palmitate (18.21%) and 9-Octadecenoicacid, (E)- (15.17%). Twenty-one phytochemicals identified in the acetone extract. Major compounds are 2-Pentanone, 4-hydroxy-4-methyl (25.89%), acetophenone (11.92%), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (9.29%) (Tables 1-6 and Figs 1-6).

Conclusion

This study investigated the effect of solvents with different polarities on the phytochemical compounds derived from *Annona squamosa*. The solvents included petroleum ether, chloroform and acetone. The majority of the compounds were extracted with acetone solvent.

In the present study *Annona Squamosa* seeds have been shown to have various secondary metabolites that possess many pharmacological properties. The GC-MS analysis showed the presence of various phytochemical constituents that contribute to the activities like antifungal, antibacterial, antioxidant, anticancer, anti-inflammatory, anti-diabetic, insecticidal and other activities. Hence, the presence of phytochemicals is responsible for their therapeutic effect. Therefore, investigation is required for the possible development of novel drugs using some of the bioactive compounds found in *A. squamosa*. Overall this method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetics, drugs, pharmaceuticals or the food industry.

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Author's Contribution

Authors Manisha and M.C. Sharma jointly designed and performed experimental work it was compiled as a manuscript to publication in the journal.

Conflict Of Interest

None

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