

Phytochemical Profiling, GC–MS Analysis and Antioxidant Potential of***Annona squamosa* Leaf Extract**

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Abstract

Annona squamosa, a medicinal plant widely used in traditional medicine is known for its diverse pharmacological properties. In this study, the leaves of *Annona squamosa* were used to determine the total phenolic and flavonoid content as well as antioxidant

activity of methanol crude extracts. The phenolic content of methanol extract of *Annona squamosa* was 186.49 μ g/100mg was expressed as gallic acid equivalent. The flavonoid content of methanol extract of *Annona squamosa* was 306.25 μ g/100mg was expressed as catechin equivalents. The antioxidant activity of extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The methanol extract exhibited a notable antioxidant activity with a value of 221.80 μ g/mL, indicating its moderate free radical scavenging potential. GC-MS analysis of the extract revealed the presence of several bioactive compounds, including major constituents with potential pharmacological significance. This study highlights its potential applications in the development of natural product for pharmaceutical and therapeutical uses.

Key words : *Annona squamosa*, Phytochemicals, GCMS, Antioxidant activity

Introduction

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients [1]. Medicinal plants have a long history of usage in traditional medicine systems as sources of therapeutic remedies. They have served as valuable resources for the discovery and development of bioactive compounds with diverse pharmacological activities. The rich biodiversity of medicinal plants offers a wide array of chemical constituents that possess potential health benefits [2]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [3]. The treatment and prevention of several illnesses have showed promise when using the bioactive substances obtained from medicinal plants [4].

Annona squamosa is one of the garden plant that comes from the Annonaceae family known as custard apple [5]. It has been reported to possess a wide variety of pharmacological activities and is used in traditional applications [6]. The extracts from different parts of the tree have shown extraordinary medicinal properties such as anti-insecticidal, antiulcer, anti-microbial, anti-fertility and wound healing properties [7].

Free radicals play a crucial role in the development of tissue damage in pathological events. Antioxidants are chemical compounds which have the ability to quench the free radicals and thereby it prevents the human body against various diseases. Plants are the rich sources of antioxidants which contain secondary metabolites such as phenolic and flavonoid compounds commonly which act as antioxidants with redox and metal chelating properties [8].

Materials and methods:

Plant collection

The fresh leaves of *Annona squamosa* were collected from Thovalai, Kanyakumari District. That plant leaves were washed with tap water to remove soil and unwanted dust particles. Then the leaves were shaded, dried, and then grinded in the form of powder and stored in air tight bottles.

Preparation of extracts

The powdered plant leaves (25g) were soaked with 250ml of Methanol in maceration process for 3 days. The mixture was filtered through Whatman filter paper no. 42. and leave it for evaporation. After evaporation the remaining extracts were used for next tests [9].

Phytochemical quantitative analysis

Quantitative estimation of phenolic compounds

The total phenolics content in methanol solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, different concentrations of the 1 ml of the extract were mixed with 0.4 ml FCR (diluted 1:10v/v). After 5 min 4 ml of 7% sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using Gallic acid solutions as standard (20 to 200 $\mu\text{g}/\mu\text{l}$).

Quantitative estimation of flavonoids

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1 ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5% sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract) [10].

GCMS Analysis

GCMS investigation of the volatile compounds from *Annona squamosa* has been executed using an Agilent 8890 system comprising an AOC-20i auto-sampler and a gas chromatograph connected to a Mass spectrometer (GCMS) fitted out with an Elite-5MS (5% diphenyl /95% dimethyl polysiloxane) glued a capillary column (30 × 0.25µm ID × 0.25µm df). For GCMS finding, an electron ionization system was worked in electron stimulus mode and with ionization energy of 70 eV. Helium gas (99.99%) was applied as a carrier gas at an unceasing flow rate of 1.2 ml/min, and an injection volume of 1µl was engaged (a split ratio of 15:1). The injector temperature was preserved at 250°C, the ion source temperature was 230°C, the oven temperature was planned from 350°C, with an escalation of 5°C/min to 300°C/5 min. Mass spectra were booked at 70 eV; a scan intermission of 0.5 sec. and fragments from 45 to 450 Da. The solvent delay was 3 min, and the total GC/MS running time was 53.5 min. The comparative proportion quantity of each constituent was calculated by equating its average peak area to the total areas. The mass detector applied in this examination was Turbo-Mass Gold-Perkin-Elmer, and the software implemented to knob mass spectra and chromatograms was a Turbo-Mass ver-5.2.28-29 [11].

Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of methanol extract of *Annona squamosa* (10-160µg/ml) were evaluated through a free radical scavenging effect on 1,1-diphenyl-2-

picrylhydrazyl (DPPH) radical. 2ml of DPPH solution were added to both standard and test tubes. 100 μ l of test sample and standard solution of various concentration (10,20,40,80, and 160 μ g/ml) and were added to respective tubes. Then stand for 30 mins incubation. After incubation, observe the colour change and measured the absorbance of each tubes at 517nm. Calculate the % of inhibition of each concentration with the formula.

$$\% \text{ of inhibition} = (1 - A_s/A_c) \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance in the presence of the sample extract or standard [12].

Results and discussion

Quantitative analysis of phytochemicals

The amount of total phenol and flavonoid content are presented in the table 1. The total phenolic and flavonoid contents of *Annona squamosa* were determined to be 186.49 μ g/100 mg and 306.25 μ g/100 mg, respectively, indicating a substantial presence of bioactive compounds with potential antioxidant properties."

Table 1: phenolic and flavonoid content of Methanolic leaves extract of *Annona squamosa*

Test	Result
Total phenolic content	186.49 μ g/100mg
Total flavonoid content	306.25 μ g/100mg

GCMS

GCMS chromatogram analysis of the methanolic extract of *Annona squamosa*(figure1)showed twentyeightpeaks and have been identified after comparison of the mass spectra with NIST libraries (Table2), indicating the presence of sixteen phytocomponents. Of the sixteen compounds identified, the most prevailing compounds were 1,4-Eicosadiene (33.39%),3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.69%), Bicyclo[2.2.1]heptane-2,3-diol, 1, 7,7-trimethyl (8.02%), Chloro-methyl-

methoxy-amine(5.15%). These bioactive compounds may collectively contribute to the pharmacological potential of the extract.

Table 3 presents the biological activities of the Phytocomponents identified in the *Annona squamosa* extract through GC-MS analysis. The compounds exhibited various pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, and anticancer activities as reported in previous studies.

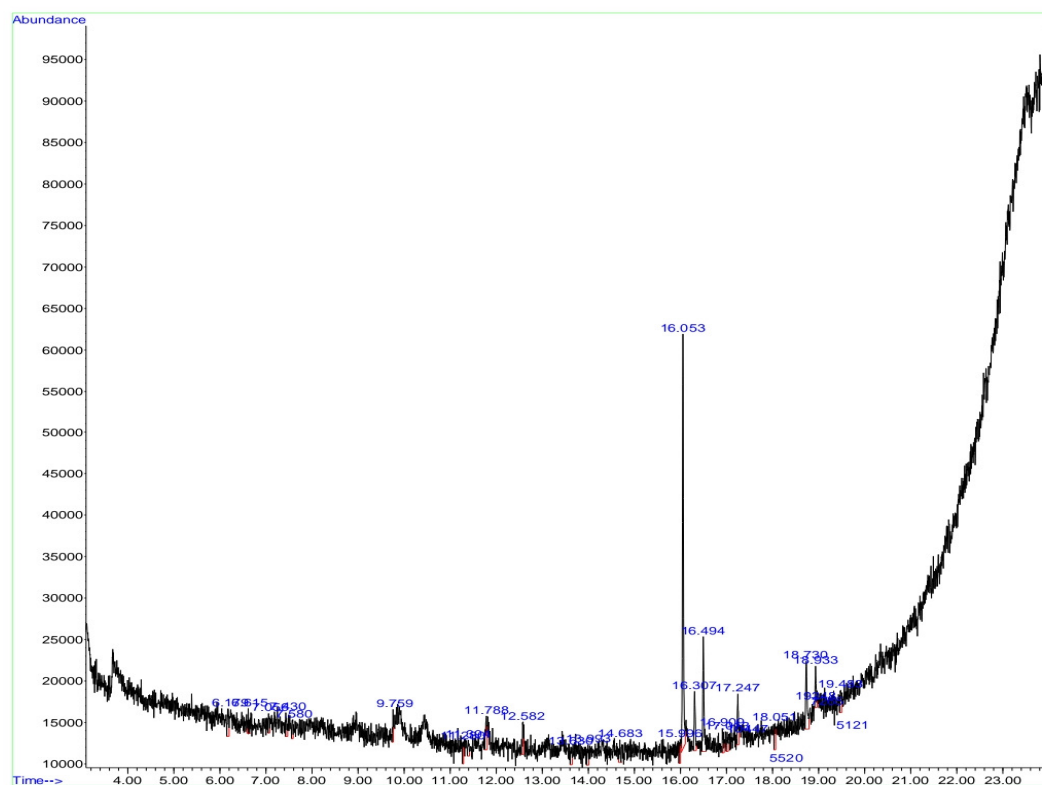


Figure 1: GC-MS Chromatogram of *Annona squamosa* methanolic extract

Table 2: Phytocomponents identified in the methanolic extract of *Annona squamosa* by GC-MS

S.N o	Retention time (Min)	Name of the Compound	Molecular formula	Molecular weight	Peak %
1.	6.179	Hydrazinecarbothioamide, 2-cyclopentylidene	C ₆ H ₁₁ N ₃ S	157.24	2.88
2.	6.615	D-Fructose, diethyl mercaptal, pentaacetate	C ₁₈ H ₂₈ O ₁₁ S ₂	484.54	1.62
3.	7.056	Indane-1,3-dione, 1-thiosemicarbazone	C ₁₀ H ₉ N ₃ O ₂ S	235.26	1.45
4.	7.580	Morpholine, 4-methyl-, 4-oxide	C ₅ H ₁₁ NO ₂	117.15	1.50
5.	9.759	2-Amino-4,6-diphenyl-6H-1,3,5-thiadiazine	C ₁₄ H ₁₂ N ₂ S	240.33	1.48
6.	11.280	4,6-Dimethylthiopicolinamide	C ₈ H ₁₀ N ₂ OS	182.24	1.51
7.	11.394	Phenol, 2,4-dibromo	C ₆ H ₄ Br ₂ O	251.90	1.49
8.	11.788	Hydrazinecarbothioamide, 2-(phenylmethylene)	C ₈ H ₉ N ₃ S	179.24	3.73
9.	13.630	Benzene-1,2-diol, 4-(2-guanidinothiazol-4-yl)	C ₁₀ H ₁₀ N ₄ O ₂ S	250.28	1.78
10.	16.053	1,4-Eicosadiene	C ₂₀ H ₃₈	278.51	33.39
11.	16.307	Chloro-methyl-methoxy-amine	C ₂ H ₆ ClNO	95.53	5.15
12.	16.494	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.53	10.69
13.	18.051	Phenol, 2,6-dichloro-4-nitro	C ₆ H ₃ Cl ₂ NO ₃	208.00	2.30
14.	18.730	Bicyclo[2.2.1]heptane-2,3-diol, 1, 7,7-trimethyl-,	C ₁₀ H ₁₈ O ₂	170.25	8.02
15.	18.933	2-Butanone, (2,4-		250.21	3.03

		dinitrophenyl)hydrazone	C ₁₀ H ₁₀ N ₄ O ₄		
16.	19.483	2-(3,4-Dimethyl-.alpha.-thiosemicarbazobenzyl)benzoic acid	C ₁₆ H ₁₆ N ₄ O ₂ S	328.39	2.13

Table 3: Bioactivity of Phytocomponents identified in the Methanolic extract of *Annona squamosa* by GC-MS

S.No	Name of the Compound	Activity
1.	Hydrazinecarbothioamide, 2-cyclopentylidene	anticancer, antioxidant, antimicrobial [13]
2.	D-Fructose, diethyl mercaptal, pentaacetate	Antioxidant, Antimicrobial, Anti-inflammatory activity [14]
3.	Indane-1,3-dione, 1-thiosemicarbazone	Anticancer, Antimicrobial, Antioxidant [15]
4.	Morpholine, 4-methyl-, 4-oxide	Antimicrobial, Antifungal, Antioxidant [16]
5.	2-Amino-4,6-diphenyl-6H-1,3,5-thiadiazine	antibacterial, antifungal, antitumor, anti-inflammatory, and antioxidant [17]
6.	4,6-Dimethylthiopicolinamide	anticancer activity [18]
7.	Phenol, 2,4-dibromo	antibacterial, antifungal, and antioxidant activities [19]
8.	Hydrazinecarbothioamide, 2-(phenylmethylene)	antibacterial, antifungal, antitubercular, anticancer, and antioxidant properties [20]

9.	Benzene-1,2-diol, 4-(2-guanidinothiazol-4-yl)	NAF
10.	1,4-Eicosadiene	antioxidant, anti-inflammatory, and antimicrobial properties [21]
11.	Chloro-methyl-methoxy-amine	antibacterial and cytotoxic potential [22]
12.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory effects [23]
13.	Phenol, 2,6-dichloro-4-nitro	NAF
14.	Bicyclo[2.2.1]heptane-2,3-diol, 1, 7,7-trimethyl-,	anti-inflammatory, analgesic, and antimicrobial activities [24]
15.	2-Butanone, (2,4-dinitrophenyl)hydrazone	antimicrobial and antioxidant activities [25]
16.	2-(3,4-Dimethyl-.alpha.-thiosemicarbazobenzyl)benzoic acid	NAF

NAF- No Activity found

Antioxidant activity

The antioxidant potential of the methanol extract of *Annona squamosa* was evaluated using the DPPH radical scavenging assay. The percentage inhibition increased with increasing concentrations of the extract (Table 4). The highest inhibition (34.60%) was observed at 160 µg/mL concentration, while the lowest (0.82%) was recorded at 10 µg/mL. The IC₅₀ value, representing the concentration required to scavenge 50% of the DPPH radicals, was found to be 221.80 µg/mL, indicating moderate antioxidant potential.

The antioxidant property of the extract may serve as a moderate contributing factor to the pharmacological potential of the plant in the management and treatment of oxidative stress-related disorders[26].

Table 4: Antioxidant activity of *Annona squamosa* leaves by DPPH assay

Concentration of the sample	Percentage of inhibition
10µg/ml	0.82
20µg/ml	2.53
40µg/ml	12.44
80µg/ml	20.45
160µg/ml	34.60
IC50	221.80

Conclusion

This investigation has given the chemical composition of *Annona squamosa* using quantitative analysis and GC-MS techniques. In the present study 16 chemical constituents have been identified from methanolic extract of *Annona squamosa* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The presence of these bioactive compounds in *Annona squamosa* lends credence to its use by the human community. It could be concluded that *Annona squamosa* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance.

The evaluation of antioxidant activities in our study indicates that *Annona squamosa* leaves is the main source of phenolic compounds and flavonoids and it is regarded as sources of natural antioxidants.

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential

sources of new compounds of therapeutic value and as sources of lead compounds in drug development. From this study it can be concluded that the *Annona squamosa* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds.

ACKNOWLEDGEMENT:

The author wishes to thank Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, Tamil Nadu, India.

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