INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 4, p. 50-58, 2022

RESEARCH PAPER OPEN ACCESS

GC-MS based phytoconstituents profiling and phytochemical investigation of *Annona muricata* L.

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Key words: *Annona muricata* L., Ethanol and Hexane extract, Phyto-compound, GC-MS.

http://dx.doi.org/10.12692/ijb/20.4.50-58 Article published on April 17, 2022

Abstract

Annona muricata L. is conventionally used to treat various ailments. This plant shows varied medicinally valuable effects like anti-cancer, anti-hermitic, anti-spasmodic,anti-convulsant,anti-pyretic, sedative, hypotensive, digestive, anti-diabetes, anti-microbial,anti- inflammatory, anti- dysenteric, andanti- rheumatic effects. The phytochemical qualitative analysis of *Annona muricata* leaves exhibits the presence of carbohydrates, tannins, saponins, alkaloids, flavonoids, glycosides, quinines, phenols, terpenoids, coumarins, anthraquinones, steroids, phlobatannins and anthracyanine.The GC-MS analysis report shows the 22 compounds in the leaf ethanolic and hexane extract of *Annona muricata* by comparing retention time and interpretation of their mass spectra.

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Introduction

Annona muricata L. is a species of Annona muricata. It is also known as Laxman phal. It is an Annona species from the Annonaceae family of custard apple trees. Graviola, also known as soursop, is an edible fruit. *Annona muricata* is native to the Caribbean and Central America, but due to its widespread cultivation, it has become invasive in tropical and subtropical climates around the world (Hamizah *et al.*,2012).Phytochemicals are natural biological active and non-nutrient compounds found in plants that protect them from fungal and bacterial infections (Doughari *et al.*, 2009; Krishnaiah *et al.*, 2009). Recently, bioactive phyto-compounds and their effects on human health have been studied. Extracted phyto-chemicals and their mode of action as an anti-cancer agent provide useful information for future applications. As a result, it is critical to test the apoptotic potential of plants in their crude extract or as a pure compound. The plant extracts have been linked to the arrest, prevention, or reversal of carcinogenesis' molecular and cellular processes (Neergheen *et al.*, 2009; Wamidh; 2011). Anti-oxidant compounds combat diseases such as cancer, Alzheimer's, atherosclerosis, Parkinson's, diabetes, and heart disease (Valko *et al.*, 2007, Joabe *et al.*, 2010; Aboul-Enein *et al.*, 2012). Annonaceous acetogenins from *Annona muricata* have been shown in vitro to become a new anti-cancer and anti-tumor agent. These compounds are selectively toxic to different types of cancerous cells while causing no harm to healthy cells (Rieser *et al.*, 1993, Wu *et al.*, 1995; Hamizah *et al.*, 2012). *Annona muricata* leaf extracts have the potential to develop a new alternative treatment for cervical cancer (Qorina *et al.*, 2020). The current study investigates secondary metabolites of *Annona muricata* and characterization of compounds using GC-MS analysis to the presence of phytochemical constituents, with the goal of curing many diseases and disorders.

Materials and methods

Collection of plant materials

The plant material was collected from Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri(Located 19.349104°N 74.646106°E) Ahmednagar district, Maharashtra during November 2020.The plant material was authenticated by Dr.Wabale A.S., Department of Botany, P.V.P.College, Pravaranagar.

Samples preparation and Extraction

The leaves of *Annona muricata were* cleaned with water and cut into small pieces, drying was done at RT (room temperature) for three weeks and the dried samples were powdered in a grinder machine (Tiwari *et al.*, 2011; Das *et al.*, 2010). 10 grams of dried powder of leaves were suspended in 200 ml of each water, ethanol and hexane solvents. The extraction procedure was done using Soxhlet apparatus for five hours at a definite temperature for each solvent but not more thanthe boiling point. The extract was concentrated with a rotary evaporator and stored in a refrigerator throughout the experiment (Roghini and Vijayalakshmi *et al.*, 2018).

Phytochemical screening

Samples of ethanol, hexane and water extracts of *Annona muricata* were selected for the screening ofphyto constituent's viz. tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenol, terpenoids, cardiac glycosides, coumarins, anthraquinones, phlobatanin and anthracyanine.Tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenol, terpenoids, cardiac glycosides, coumarins, anthraquinones, phlobatanin, and anthracyanine were screened in ethanol, hexane, and water extracts of *Annona muricata*.

Carbohydrates Test: 2-3ml of the extract was treated with 2 ml of Molisch's reagent and 1-2 drops of conc. H_2SO_4, resulting in the formation of a purple color, confirms the presence of carbohydrates (Roghini and Vijayalakshmi *et al.*, 2018).

Tannin Test: Tannins were tested by adding 2 ml of 5 percent ferric chloride to 1 ml of extract. The presence of tannins showed by dark blue or greenish-black color (Roghini and Vijayalakshmi *et al.*,2018).

Saponins Test: 2 ml of extract was mixed with 2 ml of

distilled water and shaken in a measuring cylinder for 15 minutes. The presence of saponins is revealed by the formation of a 1 to 2 centimeter layer of foam (Roghini and Vijayalakshmi *et al.*, 2018).

Alkaloids Test: 2-3 ml of extract was mixed with 1-2 drops of conc. hydrochloric acid. 2-3ml of Mayer's reagent was then added. The presence of alkaloids is revealed by the formation of white ppt (Roghini and Vijayalakshmi *et al.*,2018).

Flavonoids Test: 2 ml of 2N NaOH was added to 3ml of extract. The presence of flavonoids is indicated by the yellow colour (Roghini and Vijayalakshmi *et al.*, 2018).

Glycosides Test: 1-2ml of plant sample was mixed with 3 ml of chloroform and 10% NH_4OH solution. The presence of glycosides is indicated by the pink colour (Roghini and Vijayalakshmi *et al.*, 2018).

Quinones Test: 2 ml of sample extract was mixed with 2 ml of conc.H_2SO_4. The presence of quinines is indicated by the presence of red color (Roghini and Vijayalakshmi *et al.*, 2018).

Phenols Test: 1-2 ml of extract, 2 ml of D/W was added, followed by a few drops of 10% $FeCl_3$. Phenols are indicated by the presence of green or blue color (Roghini and Vijayalakshmi *et al.*, 2018).

Terpenoids Test: Add 0.5 ml of extract, 1-2ml of chloroform, and 2 ml of conc. H_2SO_4 to 0.5 ml of extract. Terpenoids indicated by the presence of red or brown colour at the interface (Roghini and Vijayalakshmi *et al.*, 2018).

Glycoside Test: 1 ml of the extract was mixed with 2-3 ml of glacial CH_3COOH and 1-2 drops of $FeCl_3$. This was followed by 1-2ml of conc. H_2SO_4.Glycosides are indicated by the presence of a brown ring at the interface (Roghini and Vijayalakshmi *et al.*, 2018).

Ninhydrin Test: 1-2 drops ninhydrin reagent added to 2 ml of the extract and heated for few minutes. The presence of amino acids is indicated by blue or violet color (Roghini and Vijayalakshmi *et al.*, 2018).

Coumarins Test: 1 ml of 10% NaOH was mixed with 2ml of extract. The presence of coumarins is indicated by the presence of yellow colour (Roghini and Vijayalakshmi *et al.*, 2018).

Anthraquinones Test: 1-2ml of 10% NH_4OH solution was added to 2 ml of extract, and the formation of pinkish color ppt indicates the presence of anthraquinones (Roghini and Vijayalakshmi *et al.*, 2018).

Steroid Test: 1-2ml of extract and 1-2ml of $CHCl_3$ was added; along with 1-2 drops of conc.H_2SO_4.The formation of a brown color indicates the presence of steroids, while the formation of a bluish brown ring indicates the presence of phyto-steroids (Roghini and Vijayalakshmi *et al.*, 2018).

Phlobatannins Test: 1-2ml of extract, a few drops of HCl was added. The presence of phlobatannins is indicated by the formation of redish ppt (Roghini and Vijayalakshmi *et al.*, 2018).

Anthracyanine Test: Few ml of the extract was mixed with 1-2 ml of 2N NaOH and heated for 5 minutes. The presence of anthocyanin was indicated by the formation of a bluish-green color (Roghini and Vijayalakshmi *et al.*, 2018).

Gas chromatography–Mass spectrometry (GC-MS) analysis

Ethanol and Hexane fractions of *Annona muricata* leaf extracts were taken for the GC-MS analysis. The analysis was done on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument with the following conditions: column DB 35- MS capillary standard non-polar column 30 x 0.25mm ID x 0.25µMdf operating in electron impact mode at 70eV; Helium gas 99.99% was used as a carrier gas at a constant flow of 1 milliliter per minute and employed the injection volume of 1 microliter.

The oven temperature was set from 70°c with an increase of 6°c/min to 260°c, then 5°c/min to 280°c. A mass spectrum was taken at 70eV and the total gas column running time is 37.52min. The relative percent amount of each constituent was calculated by comparing its average peak area to the total areas. Thermo GC-Trace Ultra Ver 5.0 software set to handle mass spectra and chromatograms (Shibula *et al.,* 2015).

Results

Qualitative phytochemical analysis

Qualitative phytochemical analysis of *Annona muricata* extracts is summarized in Table 1.

Table 1. Qualitative phytochemical analysis of different leaf extracts of *Annona muricata*.

Sr. No.	Test	Distilled Water Extract	Ethanol Extract	Hexane Extract
•	Carbohydrates (Molisch's Test)	+	+	+
•	Tannins	-	+	-
•	Saponins	+	-	-
•	Alkaloids	-	+	+
•	Flavonoids	-	-	+
•	Glycosides	+	-	-
•	Quinones	+	+	+
•	Phenols	-	+	-
•	Terpenoids	+	+	+
•	Ninhydrin	-	+	-
•	Coumarins	-	-	+
•	Anthraquinones	-	-	-
•	Steroids	-	+	-
•	Phlobatanin	-	-	-
•	Anthracyanine	-	+	-

The phytochemical analysis of distilled water extract confirmed the presence of secondary metabolites like carbohydrates, saponins, glycosides, quinones and terpenoids. Ethanol extract confirmed the presence of secondary metabolites like carbohydrate, Tanins, alkaloids, quinones, phenols, terpenoids, cardiac glycosides, ninhydrin, steroids and anthracyanins, while hexane extract confirmed the presence of carbohydrates, alkaloids, flavonoids, quinones, terpenoids, cardiac glycosides and coumarins.

Gas chromatography–Mass spectrometry (GC-MS) analysis

GC-MS analysis of Ethanol Extract

The total ion chromatogram of the ethanolic extract showed the GC-MS profile of the identified compounds (Table 2, Fig. 1). Twelve compounds were identified in the ethanol fraction of *Annona muricata* by GC-MS analysis.

The prevailing compounds were 1,5-heptadiene, 2,3,6-trimethyl, phytol, acetate, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, n-hexadecanoic acid, 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, phytol, 9,12-octadecadienoic acid (z,z), octadecanoic acid, squalene, di-n-octyl phthalate, gamma.-tocopherol, cyclohexane propionic acid and 4-oxo-, ethyl ester.

The presence of hydrofurans and epoxides in the sample detected by GC-MS analysis were analyzed on the basis of different annonaceous acetogenins from *Annona muricata*.

Table 2. GCMS Analysis of ethanol leaf extract of *Annona muricata*.

Peak	R.Time	Area	Area %	Height	Height%	CompoundName
1.	24.008	22261	1.53	8906	1.90	1,5-Heptadiene, 2,3,6-trimethyl
2.	30.815	211713	14.58	78807	16.78	Phytol, acetate
3.	31.334	27851	1.92	11635	2.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4.	31.704	67616	4.66	24186	5.15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
5.	33.491	169332	11.66	45017	9.58	n-Hexadecanoic acid
6.	34.100	29584	2.04	11312	2.41	2,6,10-Dodecatrien-1 ol,3,7,11-trimethyl
7.	36.212	119299	8.21	41095	8.75	Phytol
8.	36.700	38996	2.68	15024	3.20	9,12Octadecadienoicacid (Z,Z)
9.	37.194	56918	3.92	16991	3.62	Octadecanoicacid
10.	38.243	129984	8.95	22919	4.88	Squalene
11.	43.499	50280	3.46	18526	3.94	Di-n-octyl phthalate
12.	46.665	65690	4.52	14351	3.06	Gamma.-Tocopherol
13.	48.474	23917	1.65	8780	1.87	Cyclo hexane propionic acid, 4-oxo-, ethylester

GC-MS analysis of Hexane Extract

The total ion chromatogram of the hexane extract showed the GC-MS profile of the identified compounds (Table 3, Fig.2). Nine compounds were identified in hexane fraction of *Annona muricata* by GC-MS analysis. The prevailing compounds were 2-propenoic acid, butyl ester, oxalic acid, butyl propyl ester, nonane, 3-methyl-, nonane, 1-iodo-, 4-fluoro-2-trifluoromethylbenzoic acid, neope, sulfurous acid, 2-ethylhexyl hexyl ester, oxalic acid, dineopentyl ester, 6-octen-1-ol, 3,7-dimethyl-, propanoate, 1,2-benzenedicarboxylic acid and butyl octyl ester.

Table 3. GCMS Analysis of hexane leaf extract of *Annona muricata*.

Peak	R.Time	Area	Area %	Height	Height%	Compound Name
1.	6.467	33800	20.72	10088	16.45	2-Propenoicacid,butylester
2.	7.436	2506	1.54	1692	2.76	Oxalicacid,butylpropylester
3.	12.256	8515	5.22	4417	7.20	Nonane,3-methyl-
4.	23.073	5853	3.59	3649	5.95	Nonane,1-iodo-
5.	23.837	14061	8.62	4146	6.76	4-Fluoro-2-trifluoromethyl benzoicacid,neope
6.	28.030	9725	5.96	5066	8.26	Sulfurousacid,2-ethylhexylhexylester
7.	29.969	3023	1.85	2227	3.63	Oxalicacid, dineopentyl ester
8.	30.814	29365	18.00	12054	19.66	6-Octen-1-ol,3,7-dimethyl ,propanoate
9.	33.500	56317	34.52	17985	29.33	1,2-Benzenedicarboxylic acid, butyloctyl ester

Discussion

More phytochemical compounds were elucidated in the Ethanol than Hexane and Distilled water fraction of *Annona muricata*, which was in contrast to the observation of Roghini and Vijayalakshmi, 2018.The past reports of Shibula and Velavan (2015), Lali Growther (2018), Alamu *et al*. (2020); also proved 4,4-dimethyl-5-oxo-tetrahydrofuran-3-carboxylic acid, 1-dodecenoic acid, 1-octadecanoic acid, isoaromadendrene epoxide, 1-hexadecanoic acid, 1,2-benzenedicarboxylic acid, dibutyl ester, 1,2-benzenedicarboxylic acid, di isooctyl ester and 2,7,12,18-tetramethyl-3,8-diethyl-13,17-bis(3-chloroprophyl) prophyrin, 12-octadecadienoic acid, hexadecanoic acid ethyl ester, 9-octadecenoic acid, -2-hydroxy-1-(hydroxymethyl)ethyl ester, n-

hexadecanoic acid and squalene; 2-methyl-z,z-3,13-octadecadienol,tetradecanoic acid ethyl ester, n-hexadecanoic acid, 1,8,11-heptadecatriene in ethanol and ethyl acetate extract fraction of *Annona muricata* extract. among these, disparate compounds are phytol, 3,7,11,15- tetramethyl-2-hexadecen-1-ol, di-n-octyl phthalate, gamma tocopherol and nonane.

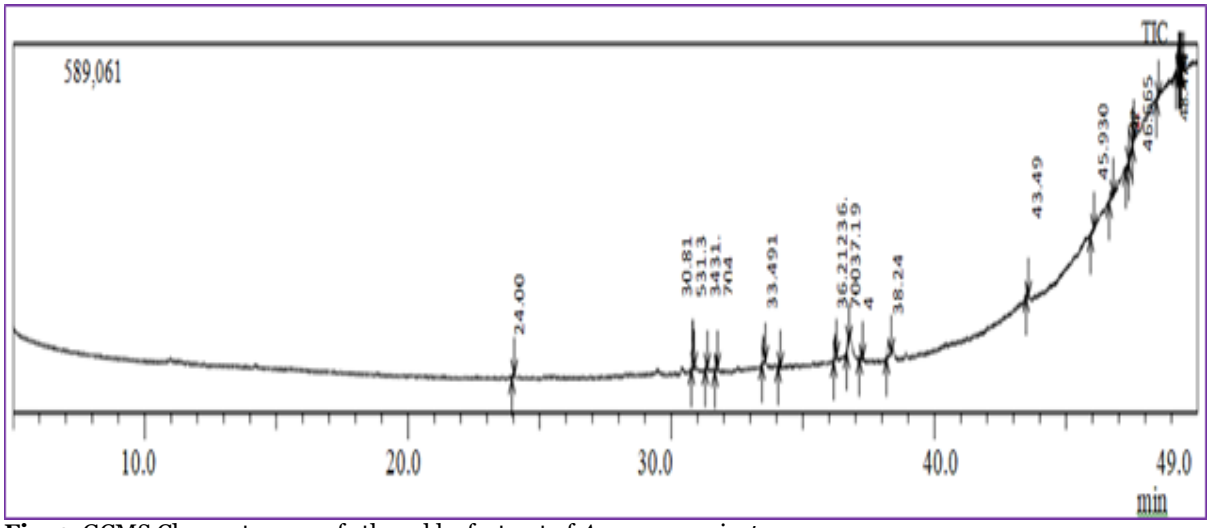

Fig. 1. GCMS Chromatogram of ethanol leafextract of *Annona muricata*.

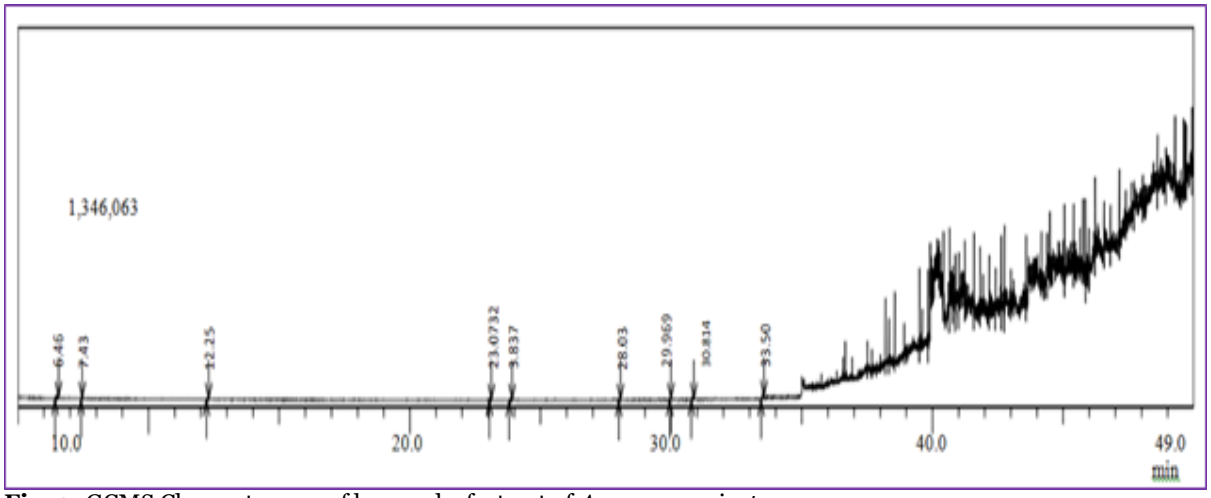

Fig. 2. GCMS Chromatogram of hexane leafextract of *Annona muricata*.

Conclusion

The presence of 5 phytoconstituents in water extract, 10 phytoconstituents in ethanol extract, and 7 phytoconstituents in hexane extract is revealed by phytochemical screening. This discovery demonstrated variation in phytochemicals as a result of solvent solubility variation and ethanolic extract as a potential source of phytochemicals. The presence of 13 compounds in ethanol extract and 9 compounds in hexane extract was confirmed by GC-MS analysis. The pharmaceutical properties of this plant are due to the presence of various phyto-bioactive compounds.

Acknowledgement

The authors would like to express their gratitude to the Council of Scientific and Industrial Research (CSIR), Human Resource Development Group, New Delhi, for their financial assistance in carrying out this research. The author would also like to thank the Central Instrumentation Facility at SPPU Pune.

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