GC-MS BASED PHYTOCONSTITUENTS PROFILING AND PHYTOCHEMICAL INVESTIGATION OF *ANNONA MURICATA* L.

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Abstract
Annona muricata L. is conventionally used to treat various ailments. This plant shows varied medicinally valuable effects like anti cancer, anthelmintic, antiparasitic, antipyretic, sedative, antispasmodic, nervine, hypotensive, anticonvulsant, digestive, anti diabetes, anti microbial,anti- inflammatory, anti- spasmodic and anti- dysenteric. parasiticide, anti- rheumatic and neurologic effects. In the present work investigate the leaf extract of Annona muricata L. was subjected to phytoconstituents and GC-MS analysis. The phytochemical qualitative analysis of A.muricata leaves exhibit the presence of carbohydrates, tannins, saponins, alkaloids, flavonoids, glycosides, quinines, phenols, terpenoids, cardiac glycosides, ninhydrin, caumarins, anthraquinones, steroids, phlobatannins and anthracyanine.The GC-MS analysis report shows the 22 compounds in the leaf ethanolic and hexane extract of A.muricata by comparing retention time and interpretation of their mass spectra. The leaves extract of plant, exhibited pharmacological significant agents.

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

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1. INTRODUCTION

Annona muricata Linn. commonly known as Laxman phal, a species of the genus *Annona* of the custard apple tree family, Annonaceae, which has edible fruit. The fruit is usually called soursop or graviola due to its slightly acidic taste when ripe. *Annona muricata* is native to the Caribbean and Central America but is now widely cultivated in some areas, becoming invasive in tropical and subtropical climates throughout the world.(Lans; 2006 and Hamizah *et al.*, 2012). All parts of the graviola tree have been used in traditional herbal medicine. Conventional herbal medicine proponents have attributed graviola with the properties: anthelmintic, antiparasitic, antipyretic, sedative, antispasmodic, nervine, hypotensive, anticonvulsant and digestive. Commonly, the leaves are used for headaches, insomnia, cystisitis, liver problems, diabetes and as anti- inflammatory, anti- spasmodic and anti- dysenteric. The decoction of the leaves have parasiticide, anti- rheumatic and neuralgic effects when used internally, while the cooked leaves , applied topically, fight rheumatism and abscesses (Orlando *et al*., 2010). Phyto is the Greek word for plant. Phytochemicals are natural bioactive and non-nutrient compounds that protect plants from bacterial and fungal infections (Dougharie*t al*., 2009 and Krishnaiahe*t al*., 2009). Plants produce phytochemicals to protect themselves but present research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each act differently (Kumar *et al*., 2009 and Afifye*t al*., 2011). Recently, bioactive phyto -compounds and their effects on human health have been intensively studied. In particular, a search for antioxidants and anticancer agents in vegetables, fruits, teas, spices and medicinal herbs has attracted great attention (Afifye*t al*., 2011). Action of extracted phytochemicals identifying the mode of action of anticancer agents of plant origin provides helpful information for their future use. Thus it is

important to screen the apoptotic potential of plants either in their crude extract form or as pure compounds (Tarapadar*et al*., 2001 and Wamidh, 2011). Due to their multiple intervention strategies, crude plant extracts have been proposed to prevent, arrest, or reverse the cellular and molecular processes of carcinogenesis (Neergheen*et al*., 2009 and Wamidh, 2011). The distribution of bioactive compounds differs according to the plant used, in various studies; different solvents were used to extract these compounds from different medicinal plants (Wamidh, 2011). Antioxidants are most useful for fighting cancer and other processes that potentially lead to diseases such as atherosclerosis, Alzheimer's, Parkinson's, diabetes, and heart disease (Valko *et al*.,2007, Joabe *et al*., 2010 and Aboul-Enein *et al*.,2012). Annonaceousacetogenins, from *A. muricata* found to be promising new anti-tumor and anticancer agent in numerous in vitro studies. These acetogenins demonstrated to be selectively toxic against various types of the cancerous cells without harming healthy cells (Riese*et al*., 1993, Wu *et al*., 1995 and Hamizah *et al*., 2012). *A .muricata* leaves extracts are potential to be developed as a novel alternative therapy for cervical cancer (Qorina*et al*., 2020).The present study explore the secondary metabolites of *A.muricata* and characterization of compound using GC-MS analysis to the presence of phytochemical constituents, to cure many disease and disorders.

2. MATERIALS AND METHODS

1. Collection of plant materials

The plant material was collected from The Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri (Located 19.349104°N 74.646106°E) Ahmednagar district, Maharashtra during November 2020. A voucher specimen of plant has been deposited in the herbarium of Department of Botany, P.V.P. College, Pravaranagar. The plant was identified with the help of accessible literature.

2. Samples preparation and Extraction:

The leaves of *A.muricata* washed with water and cut into small pieces, drying was done at room temperature for three weeks, and the dried samples were powdered (Tiwari *et al*., 2011& Das *et al*., 2010). 10 gm of dried powder of Leaves were suspended in 200 ml of water, ethanol and hexane solvents. Extraction was done using Soxhlet apparatus for five hours at a definite temperature for each solvents but not exceeding the boiling point. The extract was concentrated with rotary evaporator and preserved in refrigerator in glass bottle throughout the experiment. (Roghini and Vijayalakshmi*et al*., 2018).

3. Phytochemical screening of *Annona muricata*:

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

3.1. Carbohydrates Test: 2 ml of extract was treated with 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid which resulted in the formation of purple or reddish color confirmed presence of carbohydrates. (Roghini and Vijayalakshmi *et al*., 2018).

3.2. Tannins Test: In 1 ml of extract, 2 ml of 5% ferric chloride was added. Appearance of Dark blue or greenish black indicates the presence of tannins. (Roghini and Vijayalakshmi *et al*. 2018).

3.3. Saponins Test: In 2 ml of extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of 1 to 2cm layer of foam that indicated the presence of saponins. (Roghini and Vijayalakshmi *et al*., 2018).

3.4. Alkaloids Test: To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then 1 to 2 drops of Mayer's reagent were added. Presence of green color or white precipitate reveals the presence of alkaloids. (Roghini and Vijayalakshmi *et al*., 2018).

3.5. Flavonoids Test: To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Yellow color indicates the presence of flavonoids. (Roghini and Vijayalakshmi *et al*., 2018).
3.6. Glycosides Test: To 2 ml of extract, 3ml of chloroform and 10% ammonia solution were added. Pink color indicates presence of glycosides. (Roghini and Vijayalakshmi *et al*., 2018).
3.7. Quinones Test: To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Red color indicates presence of quinones. (Roghini and Vijayalakshmi *et al*., 2018).
3.8. Phenols Test: 2 ml of distilled water followed by few drops of 10% ferric chloride were added to 1ml of the extract. Green or blue color reveals presence of phenols. (Roghini and Vijayalakshmi *et al*., 2018).
3.9. Terpenoids Test: 0.5 ml of the extract was treated with 2 ml of chloroform and conc. sulphuric acid. Red or brown color appear at the interface indicates the presence of terpenoids. (Roghini and Vijayalakshmi *et al*., 2018).
3.10. Cardiac Glycoside Test: To 0.5 ml of the extract, 2 ml of glacial acetic acid and few drops of ferric chloride were added. This was layered with 1 ml of conc. sulphuric acid. Brown ring appear at the interface shows the presence of cardiac glycosides. (Roghini and Vijayalakshmi *et al*., 2018).
3.11. Ninhydrin Test: To 2 ml of the extract few drops of 0.2% ninhydrin reagent was added and heated for 5 min. Blue or violet color shows the presence of amino acids. (Roghini and Vijayalakshmi*et al*., 2018).
3.12. Coumarins Test: 1 ml of 10% sodium hydroxide was added to 1ml of the extract. Yellow color indicates the presence of coumarins. (Roghini and Vijayalakshmi*et al*., 2018).
3.13. Anthraquinones Test: To 1 ml of extract few drops of 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones. (Roghini and Vijayalakshmi*et al*., 2018).
3.14. Steroid Test: To 1 ml of extract equal volume of chloroform was added and a few drops of concentrated sulphuric acid were added appearance of brown ring indicates the presence of steroids and formation of bluish brown ring indicates the presence of phytosteroids. (Roghini and Vijayalakshmi *et al*., 2018).
3.15. Phlobatannins Test: Few drops of 2% hydrochloric acid were added to 1ml of the extract. Formation of red color ppt indicates the presence of phlobatannins. (Roghini and Vijayalakshmi *et al*., 2018).
3.16. Anthracyanine Test: To 1 ml of the extract 1 ml 2N sodium hydroxide was added and heated for 5 min at 100 °C. Appearance of bluish green color indicated the presence of anthocyanin. (Roghini and Vijayalakshmi *et al*., 2018).
4. Gas chromatography–Mass spectrometry (GC-MS) analysis:
Ethanol and Hexane fraction of *Annonamuricata* leaf extracts were taken for GC-MS analysis. The analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the 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 carrier gas at a constant flow of 1 ml per minute and an injection volume of 1 micro liter was employed. The oven temperature was programmed from 70ºC with an increase of 6ºC/min to 260ºC, then 5ºC/min to 280ºC. Mass spectra taken at 70eV; Total gas column running time is 37.52min. The relative % amount of each constituent was calculated by comparing its average peak area to the total areas. Software urged to handle mass spectra and chromatograms was a Thermo GC-Trace Ultra Ver 5.0 (Shibula*et al.*, 2015).

3. RESULTS AND DISCUSSION

1. Qualitative phytochemical analysis

The phytochemical analysis of A. muricata summarized in table number one (Table 1). The phytochemical analysis of distilled water

extract revealed the presence of secondary metabolites like carbohydrates, saponins, glycosides, quinones and terpenoids. Ethanol extract revealed the presence of secondary metabolites like carbohydrate, Tanins, alkaloids, quinones, phenols, terpenoids, cardiac glycosides, ninhydrin, steroids and anthracyanins while hexane extract reveals the presence of carbohydrates, alkaloids, flavonoids, quinones, terpenoids, cardiac glycosides and caumarins. This finding is in agreement with findings of Roghini and Vijayalakshmi., (2018). They proved that phytochemicals such as alkaloids, carbohydrates, saponins, reducing sugars, flavonoids, phenols, proteins, tannins, terpenoids and glycosides especially found abundant in ethanolic extract than other extracts.

2. Gas chromatography–Mass spectrometry (GC-MS) analysis:

2.1. GC-MS analysis of Ethanol Extract

Total ion chromatogram (TIC) of the ethanolic extract showed the GC-MS profile of the identified compounds (Table 2, Fig. 1). Twelve compounds were identified in ethanol fraction of *Annonamuricata* 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, Cyclohexanepropionic acid and 4-oxo-, ethyl ester. Based on the structure of annonaceousacetogenins from *A. muricata*, the presence of hydrofurans and epoxides in the sample were detected by GC-MS analysis.

2.2. GC-MS analysis of Hexane Extract

The total ion chromatogram (TIC) of the hexane extract, showed the GC-MS profile of the compounds (Table 3, Fig. 2). Nine compounds were identified in hexane fraction of *Annonamuricata* 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 1: Qualitative phytochemical analysis of different extracts of *Annona muricata*

Sr. No.	Test	Distilled Water Extract	Ethanol Extract	Hexane Extract
1.	Carbohydrates (Molisch's Test)	+ + +	+ + +	+ + +
2.	Tannins	- - -	+ + +	- - -
3.	Saponins	+ + +	- - -	- - -
4.	Alkaloids	- - -	+ + +	+ + +
5.	Flavonoids	- - -	- - -	+ + +
6.	Glycosides	+ + +	- - -	- - -
7.	Quinones	+ + +	+ + +	+ + +
8.	Phenols	- - -	+ + +	- - -
9.	Terpenoids	+ + +	+ + +	+ + +
10.	Cardiac Glycosides	- - -	+ + +	+ + +
11.	Ninhydrin	- - -	+ + +	- - -
12.	Caumarins	- - -	- - -	+ + +
13.	Anthraquinones	- - -	- - -	- - -
14.	Steroids	- - -	+ + +	- - -
15.	Phlobatanins	- - -	- - -	- - -
16.	Anthracyanine	- - -	+ + +	- - -

Table 2: GCMS Analysis of ethanol extract of *Annona muricata*

Peak	R.Time	Area	Area%	Height	Height%	Name
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	9,12-Octadecadienoicacid(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	Cyclohexanepropionicacid, 4-oxo-, ethylester

Table 3: GCMS Analysis of hexane extract of *Annona muricata*

Peak	R.Time	Area	Area%	Height	Height%	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-trifluoromethylbenzoicacid,neope
6.	28.030	9725	5.96	5066	8.26	Sulfurousacid,2-ethylhexylhexylester
7.	29.969	3023	1.85	2227	3.63	Oxalicacid,dineopentylester
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,butyloctylester

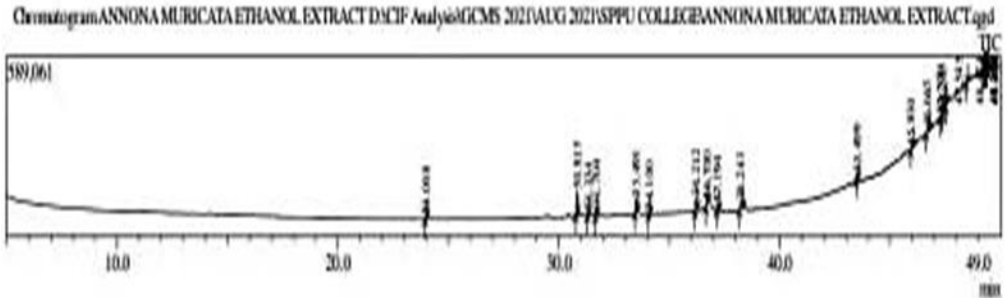

Fig: 1 GCMS Chromatogram of ethanol extract of *Annona muricata*

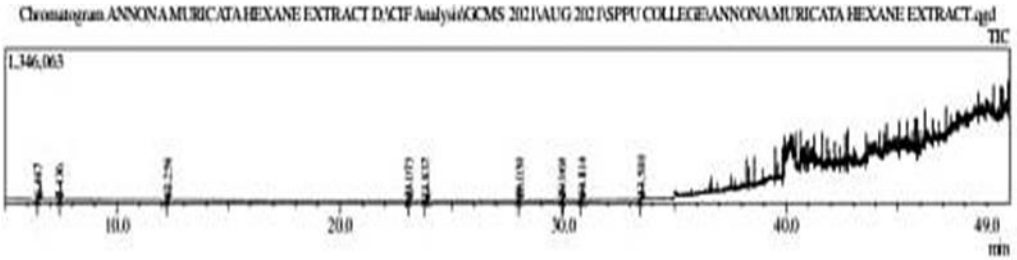

Fig: 2 GCMS Chromatogram of hexane extract of *Annona muricata*

4. CONCLUSION

Phytochemical screening reveals the presence of 5 phytoconstituents in water extract, 10 phytoconstituents in ethanol extract and 7 phytoconstituents in hexane extract. This finding proved variation in phytochemicals because of variation in solvent solubility and ethanolic extract as a potential source of phytochemicals. GCMS analysis proved presence of 13 compounds in ethanol extract and 9 compounds in hexane extract. The presence of various phytobioactive compounds in this plant is responsible for the pharmaceutical properties. Therefore, recommended as a plant of phytopharmaceutical importance.

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