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A HIGH PRECISION PHYTOCHEMICAL STUDIES OF *ANNONA MURICATA L.* BY LCMS STUDIES

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ABSTRACT

Phytochemical studies helps to reveal the chemical nature of secondary metabolites which is pharmacologically effective for many of the ailments. Conventional methods are much time consuming and be less efficient to reveal the chemistry of the phytochemical constituents. High performance liquid chromatography coupled with mass spectrometry (LC/MS) can be an efficient tool for the accurate and efficient phytochemical studies. *Annona muricata* are traditionally used to treat a variety of ailments including inflammations, bacterial infections, herpes virus infections, and cancer. The alcoholic extract of leaves was subjected to phytochemical evaluation by high performance liquid chromatography coupled with mass spectrometry method (LC/MS) for the studies of various phytoconstituents. The LC-MS Analysis revealed 29 constituents of which 17 of the compounds were identified and matched to the Metwin 2.0 library data**.** The current study suggests that ethanol extract of leaves of *Annona muricata* have revealed the presence of many phytochemicals that assures the therapeutic efficacy of the *Annona muricata.*

KEYWORDS: *Annona muricata*; Ethanolic extracts; LC-MS; Phytochemicals.

1. INTRODUCTION

Annona muricata (soursop) belongs to the genus Annona of the custard apple tree family and has age-long traditional use as medicinal plant.[1] *Annona muricata* (Linn) belongs to the family Annonaceae comprising approximately 130 genera and 2,300 species. It is an upright, evergreen tree that grows between 5 to 7 m in height, with large, smooth dark green leaves. The plant is indigenous to the warmest tropical areas of South and North America.[2] The plant has been reported to contain nutrients like vitamins B and C, phosphorus, iron and calcium. These nutrients are thought to be complimentary in the traditional use of the plant. The bark, leaves and seeds possess diverse biological activities and they are traditionally used in the treatment of infectious and chronic non-communicable diseases such as diabetes, hypertension and inflammation. The leaves have been demonstrated to be hepatoprotective, antispasmodic and anti-diabetic. The fruit has been reported to possess antimicrobial, antitumor and antiviral effects.[1] *Chan et al* investigated the anti-arthritic activity of leaves in complete Freund's adjuvant (CFA)-induced arthritis in rats. Oral administration of the extract reduced the edema in a dose-dependent manner after two weeks of administration.[3] Ethyl acetate extract of *A. muricata* leaves has found against colon cancer cells (HT-29 and HCT-116) and lung cancer cells (A549).[4,5]

Gouemo and colleagues investigated the effect of the ethanol extract of the leaves against pentylenetetrazol-induced tonic-clonic seizures in mice.[6] Intraperitoneal injection of streptozotocin-induced diabetic Wistar rats with the methanol extract of *A. muricata* leaves (100 mg/kg) for two weeks significantly reduced their blood glucose concentration.[7] The seeds and leaves of the plant are reported to possess enzymatic antioxidants, including catalase and superoxide dismutase, and non-enzymatic antioxidants, including vitamin C and E.[8] Oral treatment in rats with *A. muricata* ethanol leaf extracts significantly reduced carrageenan-induced edema in rat paws by 79% in a dose-dependent manner, exhibiting its anti-inflammatory activities.[9] In the present investigation, phytochemical studies of the ethanol extract of *A. muricata* leaves, which is extracted by hot extraction were subjected to Liquid chromatography - Mass spectroscopy (LCMS) and the results were compiled.

MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of Annona Muricata Leaves

Fresh leaves of *Annona muricata was* collected in the month of October from nearby premises of Cherthala at Alappuzha District .of Kerala. Collection was done at early morning in order to obtain maximum constituents. The collected leaves were identified in our laboratory.

2.2 Extraction of leaves of Annona muricata

The leaves of *Annona muricata* were shade dried for three weeks. The leaves were washed with water and cut into small pieces, drying was done at room temperature, and the dried leaves were pulverized into coarse powder in a mechanical grinder, passed through 40# mesh sieve. 150 g of powdered leaves were extracted using 500 ml ethanol using soxhlet apparatus for 10 hour, obtained crude extracts were evaporated to dry and was stored in refrigerator in amber colored bottles for further investigations. A part of this extract is subjected to phytochemical studies.

2.3 Phytochemical screening of ethanol extract of Annona muricata leaves

The ethanol extract thus obtained is subjected to various phytochemical tests in order to determine the presence of various chemical compounds such as sterols, alkaloids, carbohydrates, aminoacids, saponins, glycosides, terpenoids etc in accordance with those standard chemical test described.[10,11,12]

2.4 LCMS analysis

LC-MS analysis was carried out on a Liquid Chromatographic system comprising a liquid Chromatograph interfaced to a Mass Spectrometer (LC-MS) instrument; employing the following conditions. Liquid Chromatography were done by reverse phase method using C-18 column using the mobile phase at a speed rate of 10avp. The mobile phase used was water: methanol in the ratio 50:50. The 2l electronic spray ionization /APCI ionization was used in both positive and negative mode. The injection volume is 10microlitre at a flow rate of 2ml/min. The phenomenex rp18 column with a dimension of 25cm*2.5mm was used at a column temperature of 25^0C. The LC detection was done at a wavelength of 254 nm and a m/z range of 50-800 for negative and 50-950 for positive. The 'class vp' integrated software was used. The compounds were then identified by using (Metwin2.0) library data of the corresponding compounds and the specifications were mentioned in table 1.

3 RESULTS AND DISCUSSIONS

3.1 Phytochemical screening of ethanol extract of Annona muricata leaves

The ethanol extract were subjected for a preliminary phytochemical studies by conventional method and the report were compiled in table 2. The presence and absence were reported as (+) and (-).

3.2 LCMS studies

Phytochemical analysis by LC-MS analysis of the ethanol extract of leaves of *Annona muricata* revealed the presence of different sterols, aminoacids heterocyclic compounds. From the analysis, 29 compounds have been elucidated for the first time in this study on *Annona muricata*, of which 17 compounds were effectively matched with Metwin 2.0 library and identified compounds were mentioned in table 3. The secondary metabolites of plant be effective in the management of various ailments. Many of the phytoconstituents of *Annona muricata* has anti-inflammatory, hepatoprotective, and anticancer activities. The possible pharmacological activities of the identified constituents were correlated with the indications of *Annona muricata* leaves as depicted in Table 4.

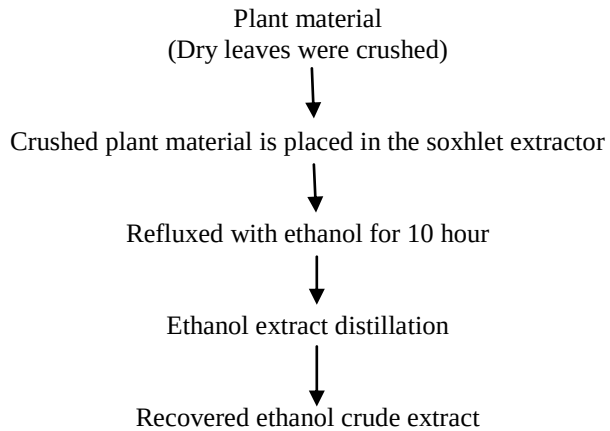

Fig. 1: Flow Chart depicting the flow chart of extraction of the plant material.

Table 1: Operating Conditions for LCMS Studies.

Liquid Chromatography column	reverse phase c-18
Pump	speed 10 avp
Mobile phase	water: methanol(50:50)
Ionization mode	2lectronic spray ionization/APCI
Mode	both positive and negative
Injection volume	10microlitre
Flow rate	2 ml/min
Column temperature	25^0c
Column	Phenomenex rp 18
Column dimension	25 cm x2.5 mm
LC detection	254 nm
m/z range	50-800 for negative and 50-950 for positive
Soft ware	class vp integrated
Library	Metwin 2.0

Table no. 2: Conventional Phytochemical analysis of ethanol extract.

Phytochemical compounds	Observation
Alkaloids	_
Carbohydrates	+
Saponins	+
Glycosides	+
Terpenoids	_
Flavanoids	_
Coumarins	_
Sterols	+
Phenols	-
Phytosterols	+
Quinones	_
Proteins	_

Abbreviations (+) present (-) absent

Table 3: Identified phyto-components in the ethanolic leaves extract of *Annona muricata* by matching with mass spectra of Metwin library 2.

Sl. No	Compound Name	Chemical formula	Molecular mass	IUPAC name of matched compound with Metwin library 2
1	Valine	$C_5H_{11}NO_2$	117.05	(2S)-(2)-amino-3-methyl butanoic acid
2	Vanillin	$C_8H_8O_3$	152.15	4-hydroxy-3-methoxybenzaldehyde
3	Indole acetic acid	$C_{10}H_9NO_2$	175.19	2-(1H-indol-3-yl)acetic acid
4	Hydroxyl L tryptophan	$C_{11}H_{12}N_2O_3$	220.23	2amino-3-(5hydroxy-1-H indol-3-yl) propanoic acid
5	Flavidin	$C_{15}H_{12}O_3$	240.26	9,10dihydro-5H-phenanthrol[4,5-bed]pyran-2,7diol
6	B-alanine	$C_3H_7NO_2$	89.1	Alpha amino propionicacid
7	Cycloeudesmol	$C_{15}H_{26}O$	222.37	2[(1aR,3aR,7S,7aR)-3a,7-dimethyl-2,3,4,5,6,7-hexahydro-1-H-cyclopropa[i]inden-1-a-yl]propan-2-ol
8	Alpha cyclocostunolide	$C_{15}H_{20}O_2$	232.33	(3aS,5aR,9aR,9bS)-5a,9-dimethyl-3-methylidene-4,5,6,7,9a,9b-hexahydro-3-aH-benzo[g][i]benzofuran-2-one
9	Methyl phenol	C_7H_8O	108.14	3-methylphenol
10	Parasorbic acid	$C_6H_8O_2$	112.13	(2S)-2-methyl-2,3-dihydropyran-6-one
11	Aspartic acid	$C_4H_7NO_4$	133.11	(2S)-2-aminobutanedioic acid
12	Methoxy cinnamaldehyde	$C_{10}H_{10}O_2$	162.19	(E)-3-(2-methoxyphenyl) prop-2-enal
13	Lupeol	$C_{30}H_{50}O$	426.73	(1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a,5hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11, 11b,12,13,13a,13bhexadecahydrocyclopenta[a]chrysen-9-ol
14	Campesterol	$C_{28}H_{48}O$	400.68	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9, 11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a] phenanthren-3-ol
15	Trigonelline chloride	$C_7H_8ClNO_2$	173.60	1-methylpyridin-1-ium-3-carboxylicacid chloride
16	Amino-B-oxalylaminopropionic acid	$C_5H_8N_2O_5$	176.13	3-[(carboxy carbonyl)amino]alanine
17	7-Methoxy coumarin	$C_{10}H_8O_3$	176.17	7-methoxychromen-2-one

Table 4: Possible reported biological actions of identified compounds.

Sl.no	Identified compound	Possible Medicinal use
1	Valine	A dietary essential amino acid, valine is required for optimal growth of children[13]
2	Vanillin	Vanillin is now used more often than natural vanilla extract as a flavouring agent ,neuroprotective against huntingtons disease, global ischemia, antioxidant, anti-inflammatory[14]
4	Hydroxyl –L tryptophan	Used in insomnia, anxiety and migraine[16]
5	Flavidin	Antioxidant[17]
6	B-alanine	To increase intramuscular carnosine, which play role in ability of muscle tissue to contract[18]
7	Cycloeudesmol	Antibiotic[19]
8	Alpha cyclocostunolide	Anti-proliferative and induce apoptosis in a variety of human lumar cells Anti-inflammatory antifungal and anti-viral properties, modify immune responses antitumor[20]
9	Methyl phenol	In the production of phenolic resins, tricresylphosphate, organic intermediates[21]
10	Parasorbic acid	Boost the immune system, prevent certain cancers and bacterial infections[22]
11	Aspartic acid	Plays a vital role in neuro-endocrine system, helps in the secretion of prolactin, luteinizing hormone and growth hormone It supports good liver health Assists the function of RNA & DNA[23]
12	Methoxy cinnamaldehyde	Anti-proliferative effect & induce apoptosis[24]
13	Lupeol	Anti-inflammatory, Antitumor Antiviral, Antiprotozoal[25]
14	Campesterol	Decrease LDL, lower the risk of heart diseases[26]
15	Trigonelline chloride	Hypolipidemic, hypoglycemic, Neuro protective, antimigrane, sedative Antibacterial, antiviral, antitumor activities[27]
16	Amino B oxalyl amino propionic acid	Neuroexitatory[28]
17	7-methoxy coumarin	Antinociceptive, anti-inflammatory, Hepatoprotective, antioxidant[29]

4 CONCLUSION

The phytochemical evaluation of the Annona muricata leaves by LC/MS method was a rapid and elaborative method for the screening of phytochemicals. The important phytconstituents detected reveals the presence of various secondary metabolites like sterols, saponins, amino acids etc. The current study suggests that ethanolic extract is used for many ailments. This helps to make it as a future medicine as good pharmacophore.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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