IJPSR (2017), Vol. 8, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 November, 2016; received in revised form, 04 February, 2017; accepted, 17 February, 2017; published 01 June, 2017

DETERMINATION OF BIOACTIVE PHYTOCOMPONENTS FROM HYDROETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* (LINN.) LEAF BY GC- MS

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Keywords:
A. squamosa, Hydroethanolic extract, GC-MS technique, Herbal medicine

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ABSTRACT: *Annona squamosa* Linn.commonly called as custard apple, is an edible tropical fruit belongs to the family Annonaceae, is said to show varied medicinal effects, including insecticide, antiovulatory and abortifacient. Hence the present investigation was carried out to determine the phytochemical composition of *A. squamosa* leaf extract using Gas Chromatography–Mass Spectrometry technique, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. This paper shows the isolation and structural elucidation of the several new natural bioactive compounds from the leaf of *A. Squamosa* linn in hydroethanolic extract. GC-MS analysis expressed thirty analytes, among that two compounds were showed high peak level such as of 2-Butanone, 3-amino-4-phenyl-(10.21%) and (-)-1,2,3,4-Tetrahydroisoquinoline, 6,7-dimethoxy-2-methyl-1-(6.01%), Hence, the present study focused the leaf extract of *Annona squamosa* plant possessed promising source of bioactivity as well as justifying the use of this plant to treat many ailments in folk and herbal medicines.

INTRODUCTION: From ancient times onwards, plants have been one of the essential sources of medicines. It has been quite interest in plant based medicines, health care products, pharmaceuticals, nutrient supplements, cosmetic agents, and so forth. As per the WHO review 80% populations living in the third world countries depend solely on conventional medication for their essential human needs [1]. Plants are rich in secondary metabolites with varied pharmacological activities. Secondary metabolites are an important source with different structural arrangements and properties [2]. Distinguished examples of these compounds include flavonoids, phenols, phenolic glycosides, saponins and cyanogenic glycosides [3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(6).2539-44

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (6).2539-44

Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of medicinal plants as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant since it may serve as sources of antibiotic prototypes [5, 6]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [7].

Annona squamosa Linn. (Family: Annonaceae) commonly known as custard apple, is a small, woody, semi deciduos, ever green tree is cultivated throughout India for its fruits. Different parts of *Annona squamosa* Linn. are used in folkloric medicine for the treatment of various disease [8]. It is considered beneficial for cardiac disease, Antidiabetic, hyperthyroidism and Antioxidant. The root is considered as a drastic purgative [9]. An infusion of the leaves is considered efficacious in

prolapsusani of children, the crushed leaves are sniffed to overcome hysteria and fainting spells, and also used for wounds and ulcer. The leaves of the plants have been used as insecticide, anthelmintic, styptic, externally used as suppurant. Fruits of *Annona squamosa* helpful for antidysentric and bark is used for powerful astringent, vermifuge and antidysentric.

Annonaine, an alkaloid which is found in bark and leaves of *A. squamosa* [10]. A paste of seed powder has been applied to the head to kill lice. It is also used for destroying worm in the wound of cattles [11]. Leaf extract of *Annona squamosa* has Hypoglycemic and Antidiabetic properties. [12, 13] From the bark of *Annona squamosa*, a bioactive acetogenin with anticancer activity have been isolated [14, 15]. Flavonoids from leaves, aporphine, [16] alkaloids,[17] glycoside [18] and squamoline were isolated from this plant. Two acetogenins, annoreticuin and isoannoreticuin, isolated from the leaves, were found to be selectively cytotoxic to certain human tumours. Leaves and stems of *A. squamosa* also has dopamine, coclaurine, salsolinol and alkaloids [19-21].

Fruit is used for making milk beverages as well as ice creams. In the ayurvedic system of medicine, herbal extracts but not purified compounds have been used from centuries, because many constituents with more than one mechanism of action are considered to be beneficial. Since there is no relevant report on the phytoconstituents of *Annona squamosa* hydroethanol leaf extract, it was chosen for the study.

Therefore the study was initiated to determine the compounds present in the Hydroethanol leaf extract of *Annona squamosa* with the help of Gas Chromatography-Mass Spectroscopy technique, which may reveal an insight in its use in folk medicine.

MATERIALS AND METHODS:

Collection of Sample: During the months from November to January, fresh leaves of *Annona squamosa* plant was collected.

Authentification of the Plant Material: The taxonomic identification of the plant material was authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, India. A voucher specimen is maintained in plant anatomy research centre, Chennai (PARC/2009/456). Fresh leaves of *Annona squamosa* was used for phytochemical analysis.

Preparation of Leaf Extract: Air dried powder was macerated with 70:30 hydroethanol and stored for 72 hours in ice cold condition. After 72 hours the extracts were filtered through a Whattman filter paper No. 42 (125 mm) and the organic layer was allowed to evaporate. The resulted dark green extracts were concentrated using a rotary evaporator with a water bath set at 40 °C. The concentrated crude extracts were lyophilized into paste (5 and 15 g respectively) and were taken for further investigation.

Gas Chromatography- Mass Spectrum Analysis (GC-MS): GC-MS technique was used in this study to identify the phytocomponents present in the *Annona Squamosa* leaf extract. GC-MS technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column of 30 m length, 0.25mm diameter and 0.25 μm thickness and composed of 100% Dimethyl poly siloxane. For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2μl was employed. Injector temperature was 200 °C and Ionsource temperature was 200 °C.

The oven temperature was programmed from 70 °C (isothermal for 2 min.), with an increase of 300 °C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver. 2.53.

Identification of Components: Interpretation of mass spectrum GC-MS was conducted using the

database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more than 62000 spectral patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was ascertained.

RESULTS AND DISCUSSION:

GC-MS Analysis: In the present study, thirty compounds have been identified from hydroethanol extract of the leaves of *Annona squamosa* by GC-MS analysis. The chromatogram obtained by hydroethanol fraction of *Annona squamosa* leaf extract was shown in **Fig. 1**. The active principle, area of the peak, Concentration (%) and Retention Time (RT) are presented in **Table 1** and the Bioactivity of phytocomponents are presented in **Table 2**. **Fig. 2** shows the structure of identified phytochemical compositions. The prevailing compounds were 2-Butanone, 3-amino-4-phenyl-(10.21%), (-)-1,2,3,4-Tetrahydroisoquinoline, 6,7-dimethoxy-2-methyl-1-(6.01%), Octadecanoic acid (2.90%), Resorcinol(2.37%), n-Hexadecanoic acid (2.62%), Oleic acid(1.64%), Phenol(1.11%), 9, 12, 15 - Octadecatrienoic acid (Z,Z,Z)-, 2-Methoxy-4-vinylphenol(0.86%), dl-à-Tocopherol (0.63%).

9,12,15-Octadecatrienoic acid, (Z,Z,Z) has the property of anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties [22]. n-Hexadecanoic acid has the property of antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities [23]. Oleic acid are found in plants and animals are used to produce hormone-like substances that regulate a number of functions including blood clotting, blood pressure, blood lipid levels, immune responses and the inflammation responses to injury infection [24]. The study concludes that the extraction of hydroethanol produces number of active constituents responsible for many biological activities. So the compounds can be used for the development of folklore medicines and further experiments need to be undertaken to determine its bioactivity and to elute novel active compounds from *Annona squamosa* plants which may be created a new way to treat many incurable diseases. (Dr. Duke's Phytochemical and Ethnobotanical Database).

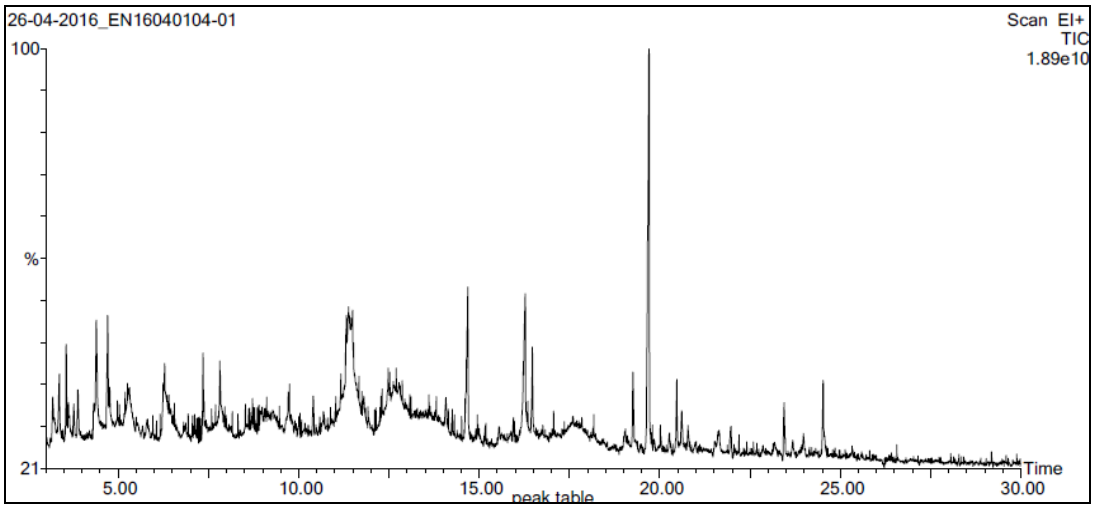


FIG. 1: CHROMATOGRAM OBTAINED FOR HYDROETHANOL FRACTION OF *ANNONA SQUAMOSA* LEAF EXTRACT

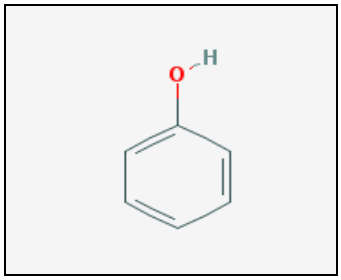
TABLE 1: THE PHYTOCHEMICAL COMPOSITION OF HYDROETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAF

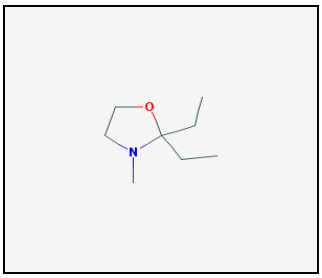
S. no	RT	Name of the Compound	Molecular Formula	Molecular Weight g/mol	Peak%
1	3.190	Phenol	C_6H_6O	94.11	1.11
2	3.371	Oxazolidine, 2,2-diethyl-3-methyl-	$C_8H_{17}NO$	143.22668	1.02
3	4.398	Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-	$C_8H_{12}O$	124.1803	1.93
4	6.283	Resorcinol	$C_6H_6O_2$	110.11064	2.37

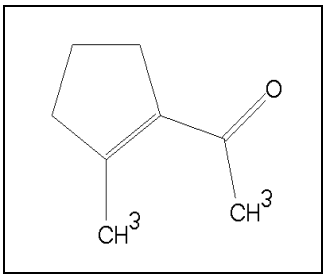
5	7.351	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.1745	0.86
6	7.812	Phenol, 3,4-dimethoxy-	$C_8H_{10}O_3$	154.1632	1.15
7	11.167	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-	$C_{15}H_{24}O$	220.3505	0.87
8	11.377	2-Butanone, 3-amino-4-phenyl-	$C_{10}H_{12}O$	148.20168	10.21
9	11.669	Dinocap	$C_{18}H_{24}N_2O_6$	364.39	0.65
10	12.614	Oleic Acid	$C_{18}H_{34}O_2$	282.46136	1.64
11	12.783	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-	$C_{13}H_{20}O_2$	208.2967	1.46
12	12.871	1H-1,2,3-Triazole	$C_2H_3N_3$	69.06532	0.64
13	14.686	n-Hexadecanoic acid	$C_{16}H_{32}O$	256	2.62
14	16.273	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.4296	2.90
15	16.471	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	0.85
16	17.598	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester,	$C_{28}H_{44}O_4$	444.64656	1.70
17	19.698	(-)-1,2,3,4-Tetrahydroisoquinoline, 6,7-dimethoxy-2-methyl-1-	$C_{12}H_{17}NO_2$	207.26888	6.01
18	23.445	dl-à-Tocopherol	$C_{29}H_{50}O_2$	430.7061	0.63

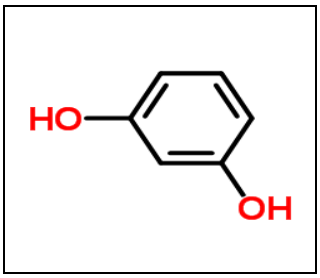
TABLE 2: BIOACTIVITY OF PHYTOCOMPONENTS IDENTIFIED IN THE HYDROETHANOL EXTRACTS OF *ANNONA SQUAMOSA* (LINN.) LEAF BY GC-MS

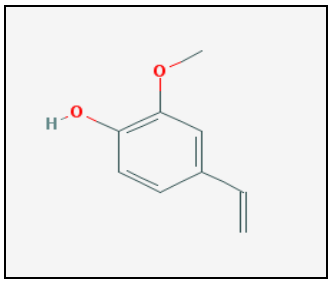
S.no	RT	Name of the Compound	Biological Activity
1	3.190	Phenol	Production of phenolic resins Production of caprolactam and bisphenol A Slimicide Adhesives and Sealants
2	3.371	Oxazolidine, 2,2-diethyl-3-methyl-	Not intended for therapeutic purpose
3	4.398	Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-	Not intended for therapeutic purpose
4	6.283	Resorcinol	Dermatological treatments such as acne and related skin conditions Keratolytic, Antipruritic and Antiseptic Personal Care Products
5	7.351	2-Methoxy-4-vinylphenol	Used as a flavoring agent Natural aroma of buckwheat
6	7.812	Phenol, 3,4-dimethoxy-	Not intended for therapeutic purpose
7	11.167	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-	Not intended for therapeutic purpose
8	11.377	2-Butanone, 3-amino-4-phenyl-	Not intended for therapeutic purpose
9	11.669	Dinocap	Used as a fungicide and acaricide.
10	12.614	Oleic Acid	Used in the preparation of oleates and lotions, Pharmaceutical solvent Adhesives and sealant chemicals Lubricants and lubricant additives Process regulators Surface active agents Plasticizers and Personal care products
11	12.783	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-	Not intended for therapeutic purpose
12	12.871	1H-1,2,3-Triazole	Not intended for therapeutic purpose
13	14.686	n-Hexadecanoic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
14	16.273	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Adhesives and sealant chemicals Agricultural chemicals (non-pesticidal) Finishing agents Lubricants and lubricant additives Surface active agents Arts, Crafts, and Hobby Materials
15	16.471	Octadecanoic acid	Surfactant and softening agent Production of detergents, soaps, and cosmetics common lubricant

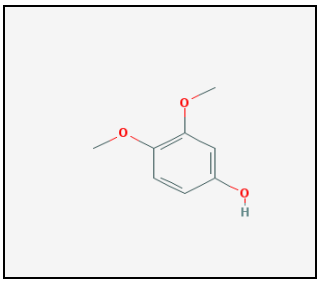

Phenol

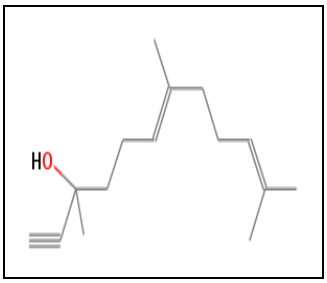

Oxazolidine, 2,2-diethyl-3-methyl-

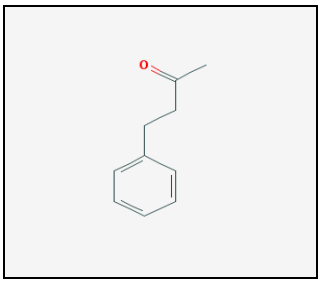

Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-

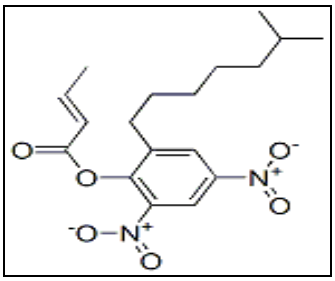

Resorcinal

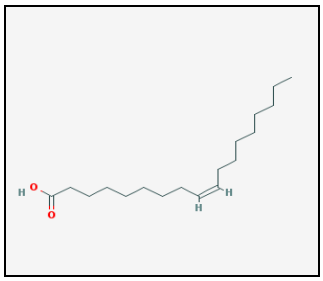

2-Methoxy-4-vinylphenol

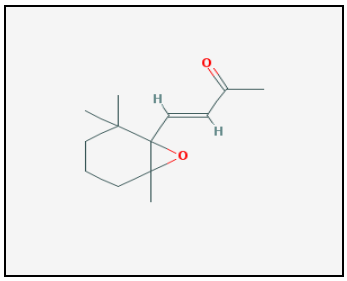

Phenol, 3,4-dimethoxy-

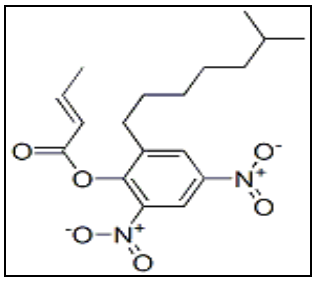

6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-

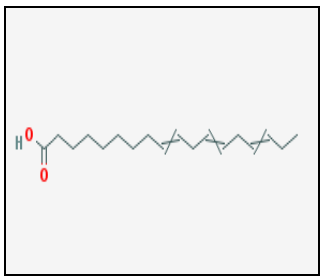

2-Butanone, 3-amino-4-phenyl-

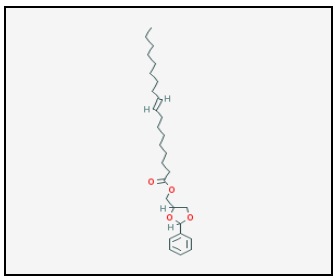

Dinocap

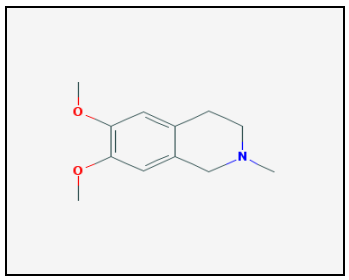

Oleic Acid


Acetic acid, 2-(2,2,6-trimethyl-7-


1H-1,2,3-Triazole oxa-bicyclo[4.1.0]hept-1-yl)-


9,12,15-Octadecatrienoic acid, (Z,Z,Z)-


9-Octadecenoic acid, (2-phenyl -1,3- dioxolan-4-yl) methyl ester,


(-)-1,2,3,4-Tetrahydroiso quinoline,6,7-dimethoxy -2-methyl-1-

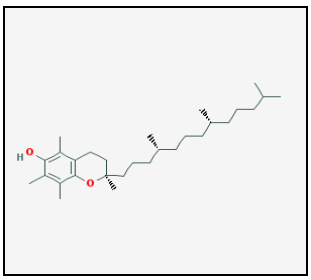

dl-à-Tocopherol

FIG. 2: THE STRUCTURES OF IDENTIFIED PHYTOCHEMICAL COMPOSITIONS

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How to cite this article:
Hemalatha S, Amudha P, Bharathi NP and Vanitha V: Determination of bioactive phytocomponents from hydroethanolic extract of *Annona squamosa* (Linn.) Leaf by GC- MS. Int J Pharm Sci Res 2017; 8(6): 2539-44.doi: 10.13040/IJPSR.0975-8232.8(6).2539-44.