Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2018; 10(5); 235-239
doi: 10.25258/phyto.10.5.9

ISSN: 0975-4873

Research Article

Phytochemical Investigation of Essential Oils from Petroleum Ether Extract of *Annona squamosa* Seeds by Gas Chromatography-Mass Spectroscopy

Patil A S, Doshi G M*

Department of Pharmacology, Vivekanand Education Society's College of Pharmacy, Chembur (E), Mumbai-400074

Received: 18th Nov 17, Revised 21st Apr, 18, Accepted: 12th May, 18; Available Online:25th May, 18

ABSTRACT

Objective: *Annona squamosa* (AS) commonly named as custard apple, is an edible tropical fruit belonging to the family Annonaceae. It has been known for numerous medicinal effects with a deep history of traditional use. Phytochemical analysis of the extract showed the existence of significant to reasonable amounts of phytoconstituents *viz;* steroids, flavonoids, saponins, glycosides, tannins, phenols, fixed oils and fats. Gas Chromatography–Mass Spectroscopy (GC-MS) studies were carried out on AS seeds petroleum ether extract to explicate phytoconstituents which may prove its effectiveness in near future in the treatment of various diseases. Methods: Present research studies encompasses identification of plant related constituents in AS seeds petroleum ether extract by using hyphenated techniques like GC-MS which when coupled gives a clear insight of such constituents. The compounds were identified by matching mass spectra with mass spectrum libraries. Results: There were five different compounds analyzed from AS seeds. The components present were oleic acid; hexadecanoic acid-2-hydroxy-1-(hydroxymethyl)ethyl ester; 9-Octadecenoic acid(Z), 2-hydroxy-1-(hydroxyl-methyl)ethyl ester; 9,12,15-Octadecatrienoic acid-, 2-[(trimethylsilyl)oxy]-1-[[(trimethyl-silyl)oxy]methyl]ethyl ester (Z,Z,Z) and *n*-hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester. Conclusion: In future, AS seeds may be subjected to different extraction and chromatographic techniques in order to check the presence of some additional phytoconstituents present in maximum proportion and their corresponding pharmacotherapeutic potential may be proven.

Keywords: *Annona squamosa*, Annonaceae, Gas chromatography-mass spectroscopy, oleic acid, *n*-hexdecanoic acid.

INTRODUCTION

Plants are found to be rich in secondary metabolites with numerous pharmacologically evident therapeutic potential. Secondary metabolites are significant sources along with distinct structural arrangements and properties[1]. Over the past century, the phytochemicals in plants have been scrutinized as a pivotal pipeline for herbal drug discovery[2]. Phytochemicals are naturally existing, biologically effective chemical compounds present in plants. In plants, phytochemicals act as a natural defence system for host plants that provide colour, aroma and flavour. Phytochemicals are protective and disease-preventing especially for few forms of cancer and heart diseases. The most valuable action of these phytoconstituents with respect to human beings is that they function as antioxidants reacting with the free oxygen molecules or free radicals in our bodies. Ethanobotanical benefits deliberated by plant based products have surpassed the chemical counter parts owing to their lesser side effects and more potent therapeutic effect. Natural products continue to play the most significant role in the drug discovery and development process. Hence, it is a demanding need of the hour to study the various pharmacologically relevant aspects of medicinal plants[3].

Plants with a long history of use in ethno medicine are considered to be having rich source of active phytoconstituents that offers benefits to mankind. One such plant with considerable traditional value is *Annona Squamosa* (Annonaceae)[2]. Annonaceae family is one of the large families that show many characteristic features such as antitumor, antioxidant, antimicrobial and many other bio-activities due to existence of secondary metabolite compounds[4]. The species of AS is a small evergreen tree reaching 6-8 meters (20-26 ft) tall, commonly found in deciduous forests, cultivated throughout India and other countries. It has been traditionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever and ulcer[5].

Now-a-days, the most wanted secondary metabolites studied by the research in annonaceous family plants are called as acetogenins. Acetogenins are large class of unique structurally homogeneous polyketide (C32-C34 fatty acid). The compounds are regarded as powerful cytotoxins. They have been reported for *in vivo* antitumor, antimalarial, anthelmintic, antiviral and anti-microbial properties[6]. AS is consumed as fresh fruit and part of the fruit production is marketed. But use of its seed and seed

*Author for Correspondence: gaurav.pharmacology@gmail.com

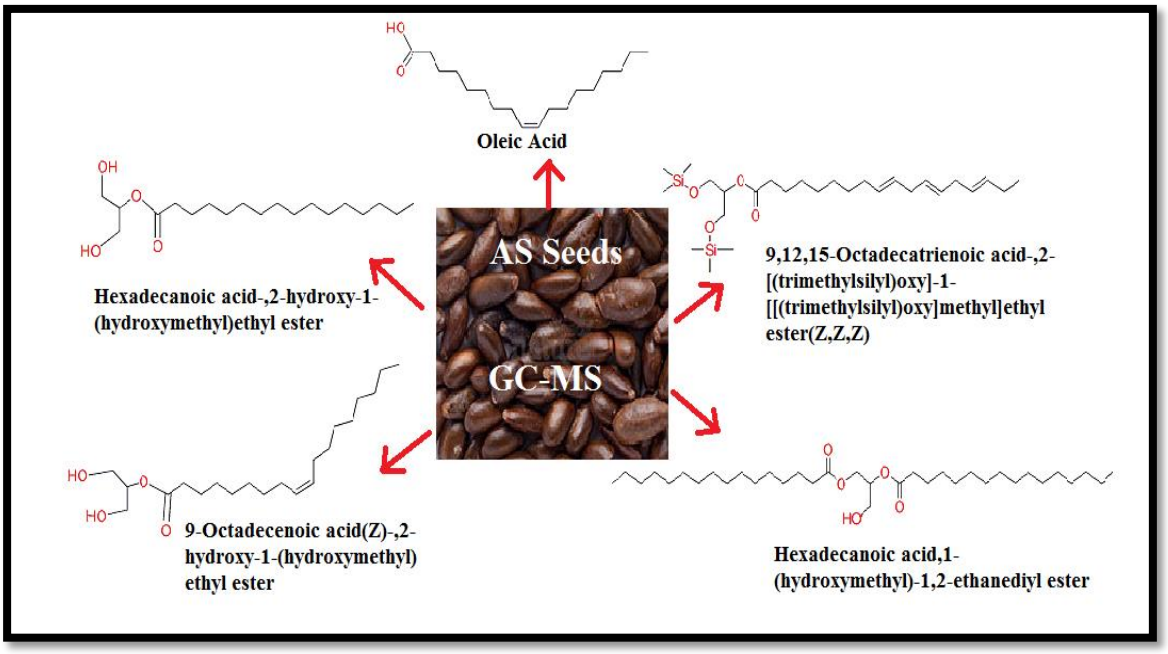

Figure 1: Chemical constituents from AS seeds petroleum ether extract by GC-MS.

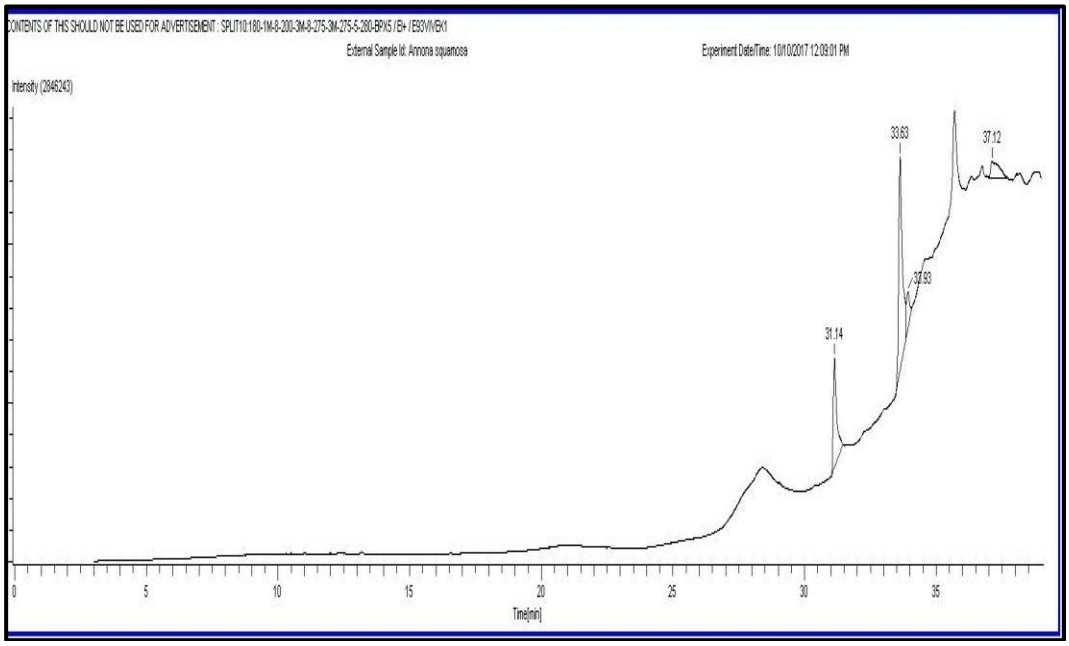

Figure 2: GC-MS spectrum of AS seeds.

oil is not popular in India either as animal feed, edible oil, or as a source of biodiesel. Thus, we chose to analyze the seeds of AS for oil content and to determine the chemical composition of the seed oil obtained from custard apple seeds, in order to explore the possible future uses of the oil[7]. In recent times, the research on plants as medicines has been focusing on isolation and characterization of bioactive constituents and secondary metabolites. In this study, the gas chromatography-mass spectrometry (GC-MS) methods is used because of their capability in (i) identifying and quantifying volatile and semi-volatile organic compounds, (ii) determining the molecular weights and elemental compositions of unknown organic compounds, and (iii) determining the structural of unknown organic compounds in complex mixtures. Furthermore, it is hope that it could provide the preliminary observations to select crude plant extracts with potentially useful properties for further chemical investigations[6]. In the present work, we identified and confirmed the structures of the constituents by GC-MS from the petroleum ether extracts of AS seeds.

MATERIALS AND METHODS

Part A: Collection and Authentication

The AS seeds were collected from Raigad district, Maharashtra state and air dried at room temperature.

Table 1: Various components and their fragments in AS extract.

Sr. No.	Retention Time (Fig. No.)	Molecular formula (Identity)	m/z	Synonyms
1	28.39 (3)	$C_{18}H_{34}O_2$ (1)	282	Oleic acid; 9-Octadecenoic acid(z), cis-Oleic acid; cis-9-Octadecenoic acid; Z-9-Octadecenoic acid; cis-Octadec-9-enoic acid; Oleinic acid; Pamolyn; Emersol 211; Emersol 220 white oleic acid; Oleine7503; pamolyn 100; 9,10-Octadecenoic acid; Red oil; Vopcolene 27; δ- 9-cis-Oleic acid; cis-δ-9-Octadecenoate; neo-Fat 90-04; neo-Fat 92-04
2	31.14 (4)	$C_{19}H_{38}O_4$ (2)	330	Hexadecanoic acid-, Palmitin- 2-mono; 2-hydroxy-1-(hydroxymethyl)ethyl ester; Palmitic acid beta-monoglyceeride; 2-Hexadecanoyl glycerol; 2-Monopalmitin; 2-Monopalmitoyl-sn-glycerol; 1,2,3-Propanetriol 2-hexandecanoyl ester; 2-Hydroxy-1-(hydroxymethyl)ethyl palmitate, Glycerol beta-palmitate.
3	33.63 (5)	$C_{21}H_{40}O_4$ (3)	356	9-Octadecenoic acid(Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester; Olein -2-mono; β-Monoolein,Glycerol 2-monooleate; 2-Monoolein; 2-Monooleoylglycerol; 2-Oleoyl glycerol ether; 2-Oleoylglycerol; 2-Hydroxy-1-(hydroxymethyl)ethyl(9Z)-9-octadecenoate.
4	33.93 (6)	$C_{27}H_{52}O_4Si_2$ (4)	496	9,12,15-Octadecatrienoic acid-,2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester(Z,Z,Z); 1,2-[(Trimethylsilyl)oxy]-1-([(trimethylsilyl)oxy]methyl)ethyl(9E,12E,15E)-9,12,15-octadecatrienoate.
5	35.7 (7)	$C_{35}H_{68}O_5$ (5)	568	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester; Palmitin-1,2-di; Dipalmitin; Glycerol 1,2-dipalmitate; 1,2-Dipalmitin; 1,2-Dipalmitoylglycerol; 2-Hydroxy-1-[(palmitoyloxy)methyl]ethyl palmitate.

The dried seeds were authenticated by Agharkar Research Institute, Pune.

The sample was stored in an air tight container at 6°C for GC-MS studies.

Part B: Extraction

Fresh seeds, free from any visible contamination were collected and air dried under normal environmental condition and homogenized to coarse powder.

200 g of grinded seeds were then soaked and macerated in 1000 ml of petroleum ether for 7 days at room temperature. Extracts were then filtered by using Whatman filter paper no.1.

Extracts were concentrated and stored in the refrigerator at 4°C for future use.

Part C: Gas Chromatography - Mass spectroscopy

The instrument used in the experimentation purpose was Joel, USA with model of Accu Time of Flight GCV.

The column details comprises of Capillary (Type), Semistandard nonpolar (Class), 30m/60m(length) with diameter of 0.25 mm. The mobile phase waspetroleum ether and The Carrier used was Helium with heat rate of2k/min to 3k/min.

The libraries used wereNIST 2.0 f and Fine, NIH, EINECS, TSCA, RTECS, HODOC, IRDB, LIB for identification and interpretation of compounds.

RESULTS AND DISCUSSION

The gas chromatogram obtained from the seed extracts of AS showed several peaks representing different chemical compounds. In the present study, the principal compounds present in AS were predominantly saturated and unsaturated fatty acids or their esters consisting of oleic acid; Hexadecanoic acid-, 2-hydroxy-1-(hydroxymethyl)ethyl ester; 9-Octadecenoic acid(Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester; 9,12,15-Octadecatrienoic acid-, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester (Z,Z,Z) and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester. The general procedure for the extraction is using light petroleum ether since the solvent helps to separate the compounds by suitable chromatography techniques. Gas Chromatography is considered as vital technique to interpret essential oils. In the current research study, 5 compounds were identified by GC-MS technique from AS petroleum ether extract.

Oleic acid (1) with m/z 282 and fragment ions of 41, 55, 69, 83, 97, 111, 185, 213, 264 and 282 was seen most commonly from seeds of *Annona squamosa*. Hexadecanoic acid-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (2) with m/z 330 and fragment ions of 43, 57, 74, 84, 98, 112, 134, 154, 168, 213, 239, 257, 299, 330 along with 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3) with m/z 356 and fragment ions of 41, 55, 69, 81, 98, 109, 123, 137, 151, 165, 180, 207, 221, 235, 264, 280, 307, 325 and 9, 12, 15-Octadecatrienoic acid-, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester(*Z,Z,Z*) (4) with m/z 496 and fragment ions of 41, 55, 73, 103, 133, 149, 169, 191, 221, 149, 281, 195 respectively are seen prominently. Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester (5) with m/z 568 and fragment ions of 43, 57, 73, 83, 98, 116, 129, 157, 185, 213, 239, 256, 299, 313, 331, 367, 423, 451, 507 was characterized. All the components and their fragments are depicted in Table 1.

CONCLUSION

Current research article states the identification of five compounds by GC-MS. Some of the important

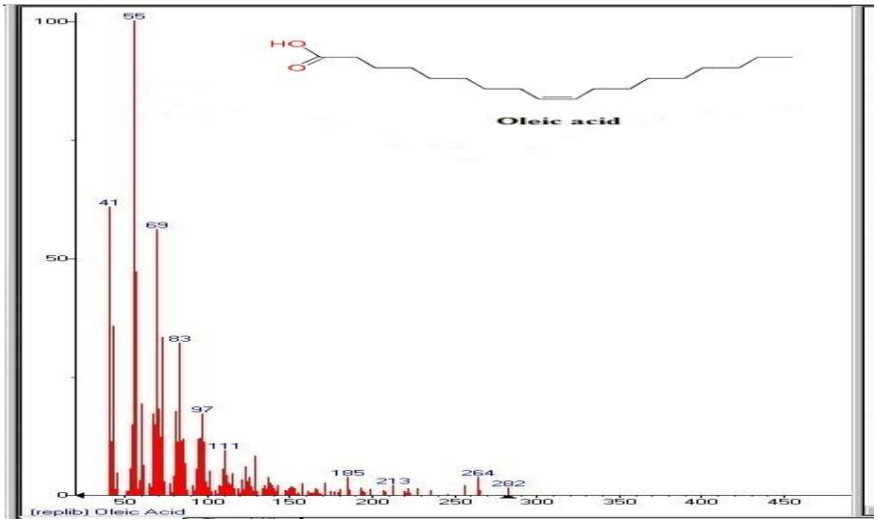

Figure 3: Mass spectrum showing presence of Oleic acid (1) in AS extract.

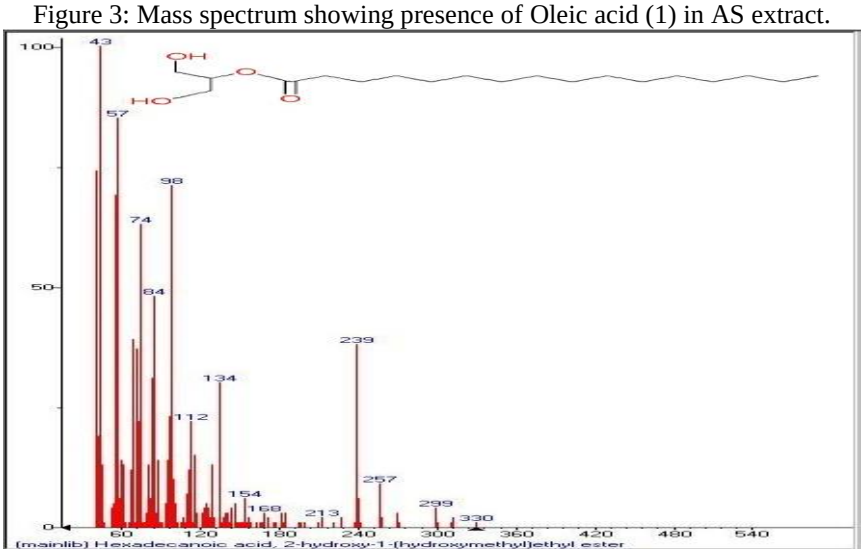

Figure 4: Mass spectrum showing presence of Hexadecanoic acid-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (2) in AS extract.

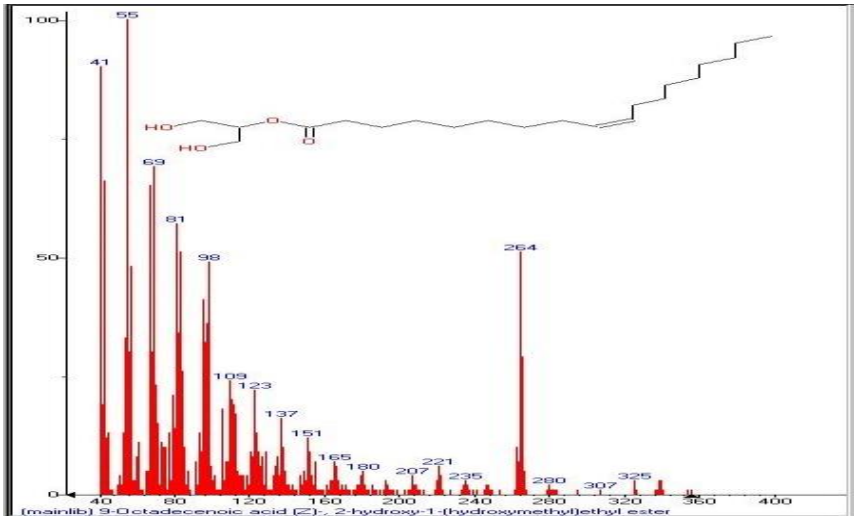

Figure 5: Mass spectrum showing presence of 9-Octadecenoic acid (*Z*)-,2-hydroxy-1-(hydroxymethyl)ethyl ester (3) in AS extract.

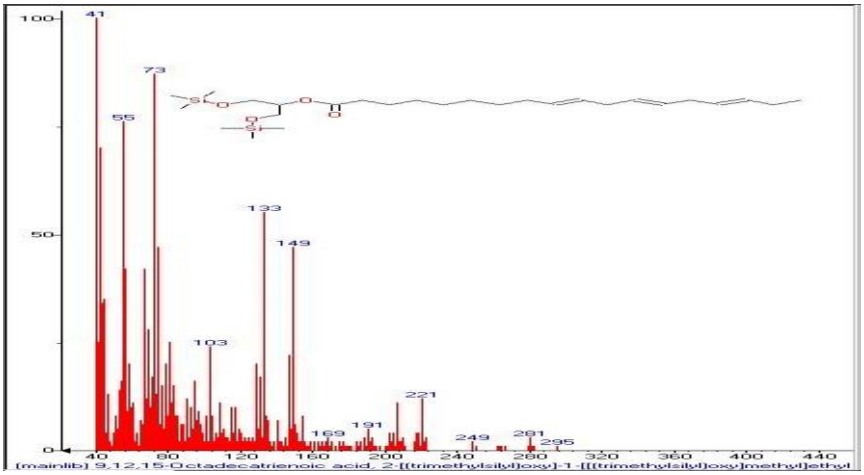

Figure 6: Mass spectrum showing presence of 9,12,15-Octadecatrienoic acid-,2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester(Z,Z,Z) (4) in AS extract

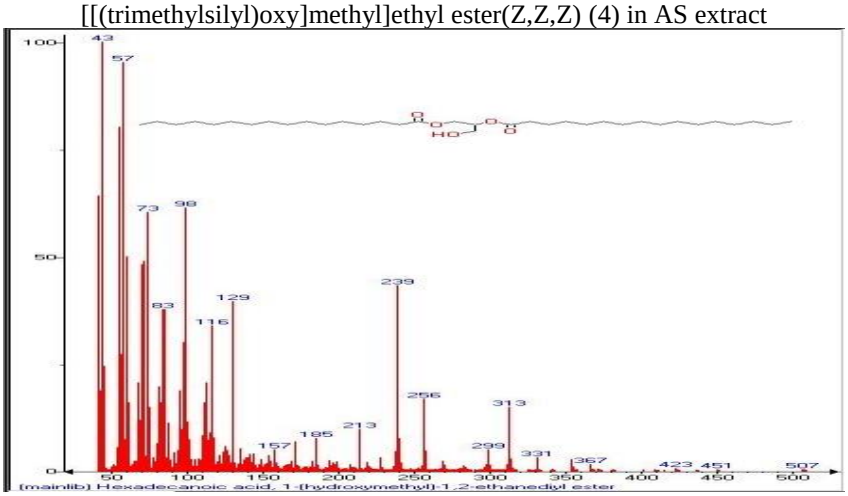

Figure 7: Mass spectrum showing presence of Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester (5) in AS extract

ethnopharmacological constituents that were found from the extract were Oleic acid, *n*-hexadecanoic acid-, 2-hydroxy-1-(hydroxymethyl) ethyl ester.

ACKNOWLEDGEMENT

We would like to acknowledge the college management and Dr. Supriya Shidhaye who provided us all the facilities to do this work. We would also like to acknowledge Indian Institute of Technology, Mumbai, for their help in analysis.

CONFLICT OF INTEREST

We declare no conflict of interest.

REFERENCES

1. S. Hemalatha, P. Amudha, N. Pushpa Bharathi, V. Vanitha. Determination of bioactive phytocomponents from hydroethanolic extract of *Annona squamosa* (linn.) leaf by GC- MS. Int. J. Pharm. Sci. Res 2017; 8(6): 2539-2544.
2. Moghadamtousi SZ, Fadaeinasab M, Nikzad S , Mohan G , Mohd Ali H, Kadir HA. *Annona muricata* (Annonaceae): A Review of its traditional uses, isolated Acetogenins and Biological Activities. Int. J. Mol. Sci. 2015; 16(7), 15625-15658.
3. Biba VS, Lakshmi S, Dhanya GS, Remani P. Phytochemical analysis of *Annona squamosa* seed extracts. Int. Res. J.Pharm. App. Sci 2013; 3(4):29-31.
4. Saripalli HR, Dixit PK. Studies on Morphological Features and Biological Activities of the Genus Annona of Ethiopia, N. E. Africa with a Special Emphasis on Graviola: A Review. Int. J Sci. Res 2016; 5(2) : 821-827.
5. Biba VS, Amily A, Sangeetha S, Remani P. Anticancer, antioxidant and antimicrobial activity of annonaceae family. WJPPS 2014; 3(3):1595-1604.
6. Antari AGNP, Wayan MI, Tika NI. Identification of ethyl acetate fraction components of srikaya (*annona squamosa*) seed extract by gas chromatography-mass spectrometry. e-J of Visvitalis Chemistry Ganesha Univ. Educ. Chem. Edu. Dept 2014; 2(1); 28-39.
7. Rana VS. Fatty Oil and Fatty Acid Composition of *Annona squamosa* Linn. Seed Kernels. Int. J. Fruit Sci 2015; 15 (1):79–84.