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Journal of Advanced Scientific Research

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ISSN
0976-9595
Short Communication

PROFILING OF PHYTOCHEMICALS IN *ANNONA RETICULATA* L. LEAF USING GC-MS ANALYSIS

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Received:13-12-2021; Accepted:13-04-2022; Published: 30-04-2022

 https://doi.org/10.55218/JASR.202213331

ABSTRACT

Annona reticulata L. belongs to the family Annonaceae and is native to West Indies, South and Central America. It is a medicinally important plant and mainly grown for its delicious and nutritious fruit. Fresh leaves were used to prepare ethanolic extract and further used in GC-MS analysis for isolation and identification of compounds present in them. GC-MS chromatogram showed 47 peaks showing the presence of 47 compounds. Vitamin E (28.73%) was present in highest percent and 2-Dodecylcyclobutanone in lowest percent (0.09%) with retention time 24.111minutes and 17.293 minutes respectively.

Keywords: *Annona reticulata* L., Bioactivity, Ethanol, GC-MS, Phytochemicals.

Abbreviations: GC-MS (Gas Chromatography Mass Spectroscopy), R.T (Retention Time), TNF-α (Tumour necrosis factor α), IL-6 (Interleukin 6), NO (Nitric Oxide), NFκB (Nuclear Factor kappa B), iNOS (Inducible nitric oxide synthase), P13k (Phosphoinositide 3-kinase), Akt (Protein kinase B), MAPK (Microtubule associated protein kinase).

1. INTRODUCTION

Annona reticulata L. belongs to family the Annonaceae, which consists of 130 genera and 2500 species; and *Annona* consists of 119 species [1-3]. It is commonly known as Custard apple, Bullock's heart, Sweetsop, and Ramphal. It is native to West Indies and South and Central America [4-7]. Trees are mainly known to grow in tropical and sub-tropical areas of the world. It is a small and fast-growing tree, which grows up to a height of 10 meters; its canopy is dense and large. The tree is deciduous in nature. Flowering takes place between March to July and fruiting takes place from August to January [6]. It is cultivated mainly for its sweet and nutritious fruit, which is rich in potassium, phosphorus, calcium, magnesium, iron, and vitamin C that helps in boosting immunity and preventing common cold and cough.

Globally it is cultivated in India, Bangladesh, Pakistan [5], Brazil, Malaysia, Philippines [8], Bhutan, China, Nepal, Africa, North America, Costa Rica, Mexico, Belize, Caribbean Island, South America, Bolivia, Ecuador, Peru and Venezuela [7]. In India, it is inhabited in states like Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Delhi, Jharkhand, Meghalaya, Orissa, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal, Punjab, Gujarat, Maharashtra, Karnataka and Kerala [6, 7].

The plant is medicinally important as it possesses enormous medicinal properties. The parts which are medicinally important are leaf, stem, stem bark, fruit, fruit peel, seeds, and roots. These plant parts are used ethnomedicinally as purgative, stimulant, anthelmintic, insecticidal and in the treatment of diarrhoea, dysentery, fever, indigestion, toothache, malaria, epilepsy, inflammation and useful in destroying lice. It also possesses some pharmacological properties like antioxidant activity, anti-cancer activity, antidiabetic activity, anti-inflammatory, anthelmintic, analgesic and CNS depressant properties [9].

Acetogenins are secondary metabolites exclusively found in family Annonaceae, which is a C32 or C34 fatty acid compound that is known to possess some pharmacological activities like antifeedant, antimicrobial, antiparasitic, antitumor, immuno-

suppressant, and pesticidal activities. Reticulatacin is a bioactive acetogenin isolated from *Annona reticulata* L. which shows prominent anti-tumour and anti-cancer activity [10].

The aim of this study is to identify the phytochemicals present in the ethanolic extract of *Annona reticulata* L. leaf through GC-MS analysis and their bioactivity by literature survey.

2. MATERIAL AND METHODS

The leaves of *Annona reticulata* L. were collected in the month of December from Ranchi, Jharkhand. Collected leaves were washed with water to remove the dust particles from the leaf surface then dried in shade and ground into fine powder using an electric grinder and stored in an airtight glass container in a dark place. The powdered sample was first suspended with methanol in the ratio 1:10 (10 g sample: 100 ml solvent) and left for 72hrs in an incubator shaker at 45°C and then filtered using Whatman filter paper no. 1. Filtered extract was then kept in a dark for 3days to evaporate the solvent completely and get dried extract. The dried extract was further resuspended in ethanol to make a final concentration of 385.8 mg/ml.

GC-MS analysis was carried out in Shimadzu QP-2010 Plus with Thermal Desorption System TD 20. The carrier gas used was Helium at the flow rate of 16.3 mL/min and column flow rate of 1.21 mL/min. The amount of sample used was 6µl, and the mode of injection was split with a temperature of 260°C. The column oven temperature was set at 100°C. The total running time of GC-MS was 35 minutes. For determining the phytoconstituents the obtained retention time and mass weight were compared with the GC-MS spectra database of online Wiley library and NIST (National Institute of Standard and Technology).

For identifying the bioactivity of the phytoconstituents, online literature surveys were done using popular search engines like Google Scholar and PubMed.

3. RESULTS AND DISCUSSION

In GC-MS analysis of ethanolic extract of *Annona reticulata* L. Leaves, a total of 47 compounds were identified from chromatogram peaks (Fig. 1). Out of these 47 compounds, vitamin E showed the highest peak area % of 28.73% with a retention time of 24.111 minutes and compound 2-Dodecylcyclobutanone showed the least peak area % of 0.09% with a retention time 17.293 minutes. The retention time, peak area percentage, compound name, molecular formula, molecular weight, and structure of phytochemicals identified in the GC-MS chromatogram are given in Table 1 and bioactivity of some compounds in Table 2. Compounds that were present in the majority in the ethanolic extract are Neophytadiene (17.54), Phytol (8.69), Vitamin E (28.73), and gamma-Sitosterol (13.25) (Fig.2).

Some of phytochemicals identified in GC-MS analysis have bioactivities like antibacterial, antidiabetic, hepato-protective, cardioprotective, anti-viral, antioxidant, anti-tumor, anti-inflammatory, immunostimulant, nematicide, insecticide, and antiaging properties which have been given in Table 2. Neophytadiene is known to significantly modulate the expression of TNF-α, IL-6, IL-10, IL1β, NF-κB, iNOS, PI3k/Akt, and MAPK confirming its anti-inflammatory, antioxidant and cardioprotective properties [11]. Hexadecanoic acid, methyl ester and n-Hexadecanoic acid are fatty acid derivatives that possesses antioxidant, nematicide, insecticide, lubricant, antiandrogenic, hemolytic, hypocholesterolemic [12], anti-inflammatory, anti-oxidant, antiandrogenic, and hypocholesterolaemia activities [13]. Phytol is an acyclic diterpene alcohol which has antitubercular activity against *Mycobacterium tuberculosis*, antinociceptive, anticonvulsant, antioxidant and anti-inflammatory activity [14]. Squalene a triterpene has anti-oxidant, antibacterial, immuno-stimulant, anticancer pesticide, sunscreen, chemo-preventive properties [15]. Alpha-Tocospiro B has antioxidant, antidiabetic, anti-inflammatory, and cytotoxic properties [16]. Gamma-Tocopherol inhibits human cancer cell cycle and possesses anticancer, antioxidant, antitumor, anti-inflammatory, hypocholesterolemic, cardioprotective activity [17]. Stigmasta-5, 22-dien-3-ol, acetate,(3.beta.) is source of synthetic progesterone and exhibits antihepatotoxic, antiviral, antioxidant, cancer preventive, hypocholesterolemic activity [18]. Vitamin E has antiaging, antidermatitic, antidiabetic, antioxidant, antitumor, cancer preventive, immunostimulant, analgesic, anti-inflammatory, anti-leukemic, hepatoprotective, hypocholesterolaemia, antiulcerogenic, vasodilator, antispasmodic, anti-bronchitic, anticoronary, skin protective properties [19,20]. Ergost-5-en-3-ol, (3.Beta., 24R)- is a phyto-sterol useful in liver disease, jaundice, arthrosclerosis and possesses antioxidant, anti-cancerous properties [18]. Gamma-Sitosterol is a steroid that exhibits antidiabetic activity by decreasing blood glucose level and increasing plasma insulin level [21]. Compound Lup-20(29)-en-3-one is triterpenoid, earlier reported in

Erica multiflora is known for its melanogenesis stimulating activity by increasing the tyrosinase enzyme expression [22]. Thus Lup-20(29)-en-3-one can be considered as possible agent for the treatment of hypopigmentation diseases like vitiligo.

In GC-MS analysis of chloroform extract of *Annona reticulata* L. Leaf, 13 phytochemicals were reported [23], in another study, 12 phytochemicals were identified from different fractions of chloroform, ethyl acetate, methanol and hydro-alcoholic leaf extract [24]; compounds identified in these two studies are completely different from the compounds identified in present study except two compounds i.e., Phytol and n-Hexadecanoic acid. In GC-MS analysis of methanol root extract [25] and seed extract [26], a total of 46 and 11 compounds were identified respectively and they were also different from the present study.

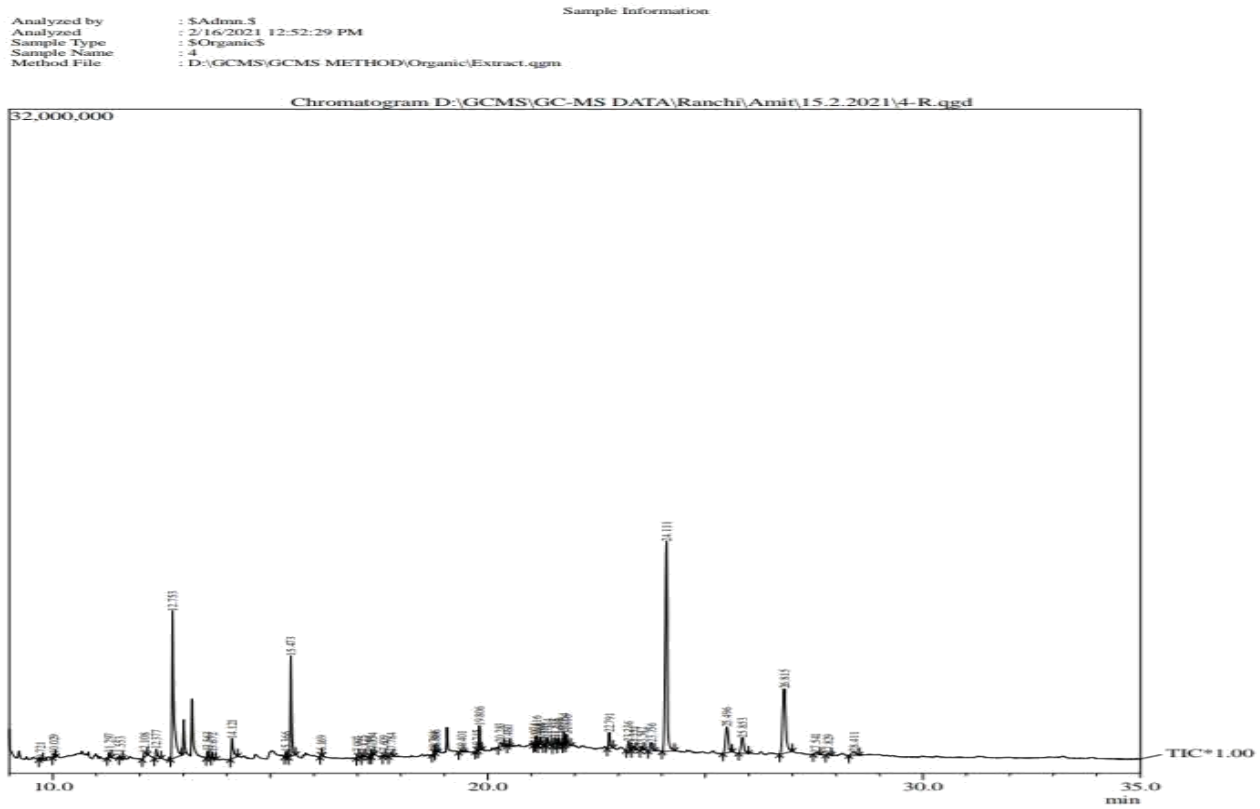
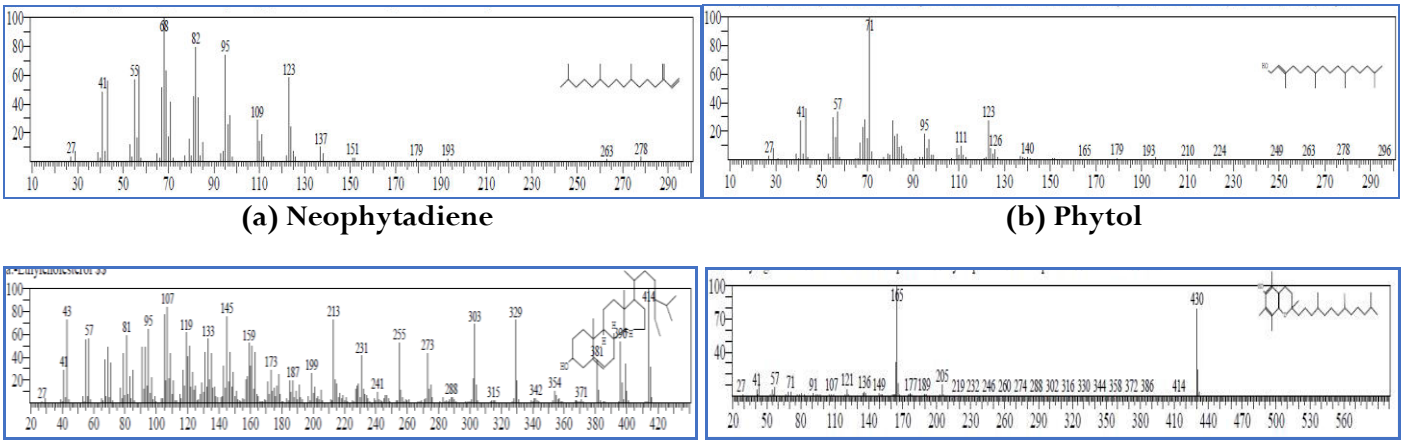


Fig. 1: Chromatogram of GC-MS analysis

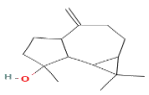
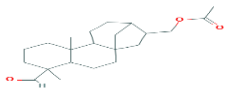
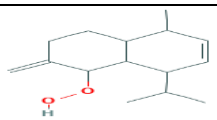
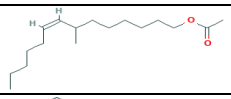
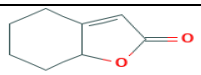
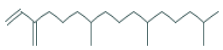
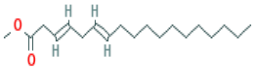
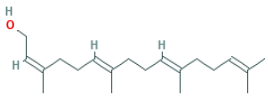
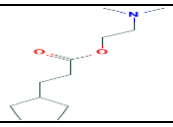
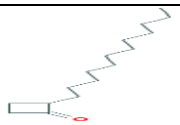
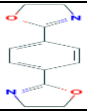
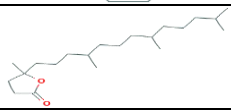


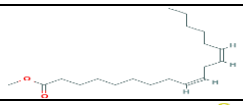
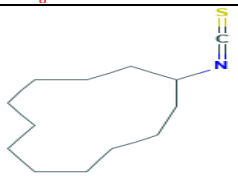
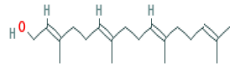
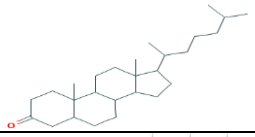
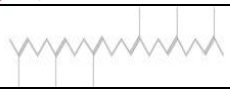
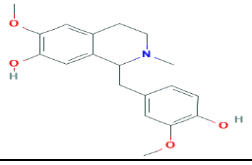
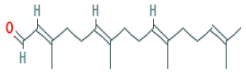
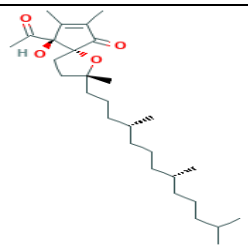
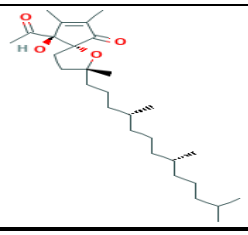
(a) Neophytadiene **(b) Phytol**

(c) Vitamin E **(d) Gamma-Sitosterol**

Fig. 2: (a)-(d) Individual spectra of compounds present in high percent

Table 1: Phytochemicals Identified in GC-MS Analysis of Ethanolic Extract of *Annona reticulata*L. Leaf

S. No.	R. T.	Peak area %	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Structure
1	9.721	0.21	TETRANEURIN - A-DIOL	$C_{15}H_{20}O_5$	280	
2	10.029	0.26	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-m	$C_{15}H_{24}O$	220.35	
3	11.297	0.50	KAURAN-18-AL, 17-(ACETYLOXY)-, (4.BETA.)-	$C_{22}H_{34}O_3$	346.5	
4	11.553	0.14	Murolan-3,9(11)-diene-10-peroxy	$C_{15}H_{24}O_2$	236.35	
5	12.108	0.73	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.4	
6	12.377	1.21	2(4H)-Benzofuranone, 5,6,7,7A-tetrahydro-6-	$C_8H_{10}O_2$	138.16	
7	12.753	17.54	Neophytadiene	$C_{20}H_{38}$	278.5	
8	13.563	0.53	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	$C_{18}H_{30}O$	262.4	
9	13.672	0.43	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	
10	14.121	2.61	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	
11	15.366	0.39	3,6-Octadecadienoic acid, Methyl ester	$C_{19}H_{34}O_2$	294.5	
12	15.473	8.69	Phytol	$C_{20}H_{40}O$	296.539	
13	16.169	0.10	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl	$C_{20}H_{34}O$	290.5	
14	Fg17.007	0.16	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}N_2$	213.32	
15	17.125	0.16	Octatriacontylpentafluoropropionate	$C_{41}H_{77}F_5O_2$	697.0	
16	17.293	0.08	2-Dodecylcyclobutanone	$C_{16}H_{30}O$,	238.41	
17	17.354	0.27	Oxaz, 2,2'-(1,4-butanediyl) BIS[4,5-dihydro	$C_{12}H_{12}N_2O_2$	216.24	
18	17.609	0.11	4,8,12,16-Tetramethyl-heptadecan-4-olide	$C_{21}H_{40}O_2$	324.5	

19	17.784	0.52	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	$C_{18}H_{30}O$	262.4	
20	18.764	0.12	Carbonic acid, octadecyl vinyl ester	$C_{21}H_{40}O_3$	340.5	
21	18.806	0.20	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.5	
22	19.401	0.31	Cyclododecyl isothiocyanate	$C_{13}H_{23}NS$	225.40	
23	19.745	0.15	(2Z,6E,10E)-3,7,11,15-Tetramethyl-2,6,10,14-hexa	$C_{20}H_{40}O$	296.5	
24	19.806	1.90	trans-Geranylgeraniol	$C_{20}H_{34}O$	290.5	
25	20.283	0.71	Tetratetracontane	$C_{44}H_{90}$	619.2	
26	20.460	0.09	Chloroacetic acid, undecyl ester	$C_{13}H_{25}ClO_2$	248.79	
27	21.074	0.09	Cholestan-3-one, methoxime	$C_{27}H_{46}O$	386.7	
28	21.116	0.57	Squalene	$C_{30}H_{50}$	410.7	
29	21.186	0.15	7- -4-methox Isoquinolinol, 1,2,3,4-tetrahydro-1-[(3-hydroxy	$C_{19}H_{23}NO_4$,	329.4	
30	21.310	0.14	(2E,6E,10E)-3,7,11,15-tetramethyl-2,6,10,14-hexa	$C_{20}H_{32}O$	288.5	
31	21.374	0.79	.alpha.-Tocospiro B	$C_{29}H_{50}O_4$	462.7	
32	21.538	1.01	.alpha.-Tocospiro B	$C_{29}H_{50}O_4$	462.7	

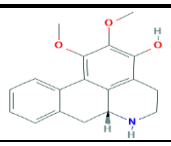
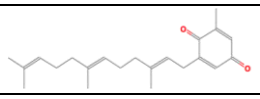
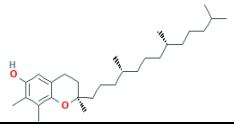
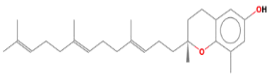
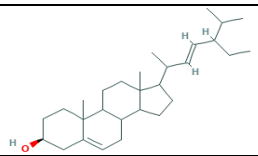
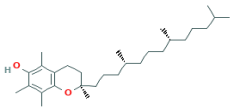
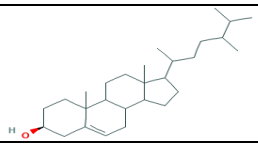
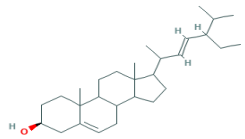
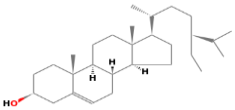
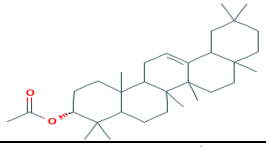
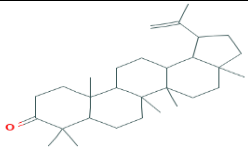
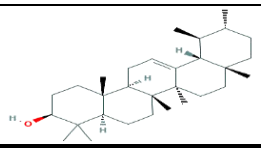
33	21.643	0.77	4H-dibenzo[DE,G]quinolin-1-OL, 5,6,6A,7-tetra	$C_{18}H_{19}NO_3$	297.3	
34	21.754	0.92	Tetracosane	$C_{24}H_{50}$	338.7	
35	21.816	1.07	1-Heptacosanol	$C_{27}H_{56}O$	396.7	
36	22.791	1.75	Senkyunone	$C_{22}H_{30}O_2$	326.4724	
37	23.236	1.02	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416.7	
38	23.351	0.48	(R)-2,8-Dimethyl-2-((3E,7E)-4,8,12-trimethyltrideca-3,7,1	$C_{27}H_{40}O_2$	396.6053	
39	23.542	0.36	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	$C_{29}H_{48}O$	412.7	
40	23.756	1.25	1-Eicosanol	$C_{20}H_{42}O$	298.5	
41	24.111	28.73	Vitamin E	$C_{29}H_{50}O_2$	430.7	
42	25.496	4.492	Ergost-5-en-3-OL, (3.beta.,24R)-	$C_{28}H_{48}O$	400.7	
43	25.853	2.97	Stigmasta-5,22-dien-3-OL, (3.beta.,22E)-	$C_{29}H_{48}O$	412.7	
44	26.815	13.25	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.7067	
45	27.541	0.42	Olean-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.8	
46	27.829	0.28	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424.7	
47	28.411	0.93	alpha.-Amyrin	$C_{30}H_{50}O$	426.7	

Table 2: Identified phytochemicals with their bioactivities

S. No.	Compound	Compound Class	Bioactivity
1	Neophytadiene	Diterpene	Anti-Inflammatory, Antibacterial [11]
2	Hexadecanoic acid, methyl ester	Fatty acid	Antioxidant, Nematicide, Insecticide, Lubricant, Anti-androgenic, Hemolytic, Hypocholesterolemic [12]
3	n-Hexadecanoic acid	Fatty acid	Anti-inflammatory, anti-oxidant, antiandrogenic, hypocholesterolaemia [13]
4	Phytol	Acyclic diterpene alcohol	Antitubercular activity against Mycobacterium tuberculosis, Antinociceptive, anticonvulsant, antioxidant, anti-inflammatory [14, 27]
5	Squalene	Triterpene	Anti-oxidant, Antibacterial, Immunostimulant, Anticancer Pesticide, Sunscreen, Chemopreventive[15]
6	alpha.-Tocospiro B	Tocopherol	Antioxidant, Antidiabetic, Anti-Inflammatory, And Cytotoxic [16]
7	gamma.-Tocopherol	Tocopherol	Inhibits human cancer cell cycle, Anticancer, Antioxidant, Antitumor, Anti-inflammatory, Hypocholesterolemic, Cardioprotective[17]
8	Stigmasta-5, 22-dien-3-ol, acetate, (3.beta.)-	Sterol	Synthetic Progesterone, Antihepatotoxic, Antiviral, Anti-oxidant, Cancer preventive, Hypocholesterolemic [18]
9	Vitamin E	Alpha Tocopherol	Antiaging, Antidermatitic, Antidiabetic, Antioxidant, Antitumor, Cancer Preventive, Immunostimulant, Analgesic, Anti-Inflammatory, Antileukemic, Hepatoprotective, Hypocholesterolaemia, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary, skin protection[19,20]
10	Ergost-5-en-3-ol, (3.beta.,24R)-	Sterol	Liver disease, Jaundice, Arthrosclerosis, Antioxidant, anti-cancerous properties [18]
11	gamma-Sitosterol	Sterol	Antidiabetic [21,28]
12	Lup-20(29)-en-3-one	Triterpenoid	Antidiabetic**,** stimulates melanogenesis[22]

4. CONCLUSION

GC-MS profiling of *Annona reticulata* L. leaves showed the presence of 47 phytochemicals that indicates its richness as an ethnomedicinal plant. Phytochemicals identified in present study were reported to possess various bioactivity and pharmacological activities, thus individual isolation of these biologically active compounds and study on their individual as well as combined effect two or more compounds will help in knowing their role as bioactive compound.

5. ACKNOWLEDGEMENTS

Authors are thankful to AIRF, JNU, New Delhi for GC-MS analysis and Dr. (Prof.) Jyoti Kumar, Head of Department, University Department of Botany, Ranchi University, Ranchi, Jharkhand for providing lab facilities to conduct this research.

Conflict of interest

Authors have no conflict of interest.

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