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DETERMINATION OF PHYTOCONSTITUENTS IN *ANNONA RETICULATA* LINN. METHANOLIC LEAF EXTRACT USING GCMS

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ABSTRACT

The present investigation was carried out for the qualitative screening of major phytochemical groups and to identify specific bioactive compounds present in the methanolic leaf extracts of *Annona reticulata* Linn. The qualitative screening of phytochemical groups was done using the standard procedures described in Experimental Phytopharmacognosy and the identification of bioactive compounds by using GC-MS analysis. The qualitative phytochemical screening revealed the presence of phenolics, flavonoids, alkaloids, tannins, glycosides, terpenoids and carbohydrates. The GC-MS analysis identified 33 phytocompounds of which most of them were reported to have important biological activities. The study confirmed the medicinal property of *Annona reticulata* Linn. and suggest that further research works are to be carried out for the utilization of the active principles for future drug developments.

KEYWORDS: *Annona reticulata* Linn, GC-MS analysis, Phytoconstituents, Methanolic leaf extract.

INTRODUCTION

Secondary metabolites of plant origin are believed to play an important role as one of the major sources of bioactive principles in new drugs in the years to come because plants are relatively cheap source of biological material available for selecting the phytocompounds of incomparable structural diversity and desired biological activity, with minimum or no side effects. Further, the synthetic drugs which mostly exert their effects based upon single xenobiotic compounds while the pharmacological action of phytomedicine is often based on the additive or synergistic action of several phytocompounds acting at single or multiple target sites associated with a physiological process. This kind of synergistic or additive

pharmacological effect of natural medicine is not only effective in eliminating wide range of pathogenic organisms and reducing the chances of these organisms developing resistance or adaptive responses (Parekh, 2007)[1] but also has the ability to eliminate the problematic side effects associated with the predominance of a single synthetic compound in the body (Tyler, 1999).[2] In the light of the above, the use of medicinal plants as raw materials in the production of drugs is ever increasing and the screening of plant extracts has been of great interest to scientists for the discovery of new drugs which is effective in the treatment of several diseases. The present investigation is an attempt to analyze the pharmacological interests of the plant *Annona reticulate*, a small tree belongs to the family Annonaceae,. The plant leaves are known to be used in the treatment of cough and cold in children in the Indian traditional system of medicine. To our knowledge, no scientific chemical analysis has been previously reported on this plant. The investigation includes detection of major chemical groups using qualitative phytochemical screening and identification of important bioactive compounds present in the methanolic leaf extracts with the aid of GC-MS analysis.

MATERIALS AND METHODS

Collection and identification of plant material

Plant material of *Annona reticulata* is collected in fresh condition. The taxonomic identity of the plant was confirmed with the Dept of Botany, Dr. Bapuji salunkhe college Miraj.

Extraction of plant sample

Fresh and healthy leaf material of *Annona reticulate* is collected and washed thoroughly under running tap water. The collected material is then air dried under shade and then powdered. Then the suitable quantity of the powdered plant material is placed in soxhlet apparatus and subjected to extraction using methanol. Subsequently, the extract is filtered and the filtrate is then evaporated using vacuum evaporator under reduced pressure at ≤ 40°C temperature to dryness till constant weight is obtained. The crude dried extract obtained after evaporation is stored in desiccators for further studies.

Preliminary phytochemical analysis

Different biochemical tests were performed for establishing a preliminary and qualitative profile of various phytochemical groups present in the methanolic leaf extracts of *Annona reticulate*. Qualitative phytochemical tests were carried out using the standard procedures described in Experimental Phytopharmacognosy (Khadabadi *et al*., 2013).[3]

a) Detection of alkaloids

The methanolic extract of the sample drug is evaporated to dryness on a boiling water bath. The residue is dissolved in 2 N HCl and the mixture is filtered.

Mayer's test: One portion is mixed with 2ml of Mayer's reagent. The creamish precipitate indicates the presence of alkaloids.

Dragendroff's test: One portion is mixed with 2ml of Dragondroff's reagent. The reddish brown precipitate indicates the presence of alkaloids.

b) Detection of tannins and phenolic compounds

Lead Acetate Test: Add 3 ml of 10% lead acetate solution to the plant extract, a bulky white precipitate formed indicate the presence of tannins and phenolic compounds.

Ferric Chloride Test: Add few drops of neutral 5% ferric chloride solution to the plant extract, blue black to blue green colour indicates the presence of tannins and phenolic compounds.

c) Detection of glycosides

Legal's test: Mix 2ml of pyridine + sodium nitropruside with 1ml plant extract, pink or red colour indicates the presence of glycosides.

d) Detection of terpenoids

Liebermann-Burchard's Test: Mix 2ml of the plant extract with 1ml $CHCl_3$ and 1ml acetic unhydride and then add one drop of H_2SO_4, blue-green to red-orange colour indicate the presence of terpenoids.

Salkowski test: Dissolve 1-2mg of the test sample in 1ml of $CHCl_3$ and add 1ml of concentrated H_2SO_4. The chloroform layer shows red colour and acid layer shows green fluorescence indicate the presence of terpenoids.

e) Detection of Saponins

Foam test: Shake vigorously the aqueous solution of test sample, the form produced is stable for 15 minutes or more indicates the presence of saponins.

f) Detection of flavonoids

Shinoda test: Add magnesium powder and few drops of concentrated HCl or H_2SO_4 into 2ml of the test solution, appearance of orange, red, purple, pink to magenta colour indicate the presence of flavonoids.

Alkaline reagent test: Add few drops of sodium hydroxide solution to the test solution, appearance of yellow colouration and which turn colourless on addition of few drops of dilute acid indicate the presence of flavonoids.

g) Detection of carbohydrates

Molisch's test: Mix 1ml of Molisch's reagent with 2ml of extract solution and add 1ml of concentrated H2SO4, appearance of red to violet ring at the junction of the two liquids indicates the presence of carbohydrates.

Benedict's test: Mix 2ml of Benedict's reagent with 2ml of extract solution and boil in a water bath, formation of red, yellow or green colour or precipitate indicates the presence of carbohydrates.

h) Detection of Proteins

Biuret Test: Mix 2ml of extract solution with 2ml of Biuret reagent, appearance of violet to pink colour indicates the presence of proteins.

Millon's Test: Mix 2ml of extract solution with 2ml of Millon's reagent and then boil, appearance of red colour indicates the presence of proteins.

GC-MS screening for specific bioactive compounds

GC-MS screening of methanolic leaf extracts of *Annona reticulata* are carried out using GC Agilent Technologies (Model – 5975C) system interfaced to a mass spectrometer (GC-MS) instrument (MS 7890A) employing the following conditions: column DB5-MS fused silica capillary column (30 X 0.25 mm ID X 0.25 mm film thickness, composed of 5% Phenyl, 95% Dimethyl Polysiloxane), operating in electron impact mode at 70 eV, helium (99.999%) is used as carrier gas at a constant flow of 1 mL/min, injector temperature 250°C; ion-source temperature 150°C. The oven is programmed with initial temperature 40°C for 5 min, with an increase of 5°C/min, to 280°C hold for 10 Min. Mass spectra is taken at 70 eV, a scan interval of 0.2 s and fragments are scanned from 50 to 550 Da. (Jiji & Subin, 2017).[4] Total GC running time was 57 minutes. The constituents were identified after comparison with those available in the Computer Library (NIST ver. 2.1) attached to the GC-MS instrument and reported.

RESULTS AND DISCUSSION

The preliminary phytochemical screening in the methanolic leaf extract of *Annona reticulata* showed the presence of phytochemical groups like alkaloids, phenolics, glycosides,

terpenoids, flavonoids, tannins and carbohydrates while other groups like saponins and proteins analyzed were not detected in the methanolic extract. The presence of above said bioactive in the leaf component of *Annona reticulata* is a clear indication of therapeutic values of the plant which may be utilized in the treatment of some human ailments. The details of preliminary phytochemical screening are shown in the table 1

Table 1: Preliminary phytochemical screening of methanolic leaf extract of *Annona reticulate.*

Sl.No	Phytochemicalconstituents	Chemical test(s)	Results		
			R1	R2	R3
1	Alkaloids	Dragendroff's test	+	+	+
		Mayer s test	+	+	+
2	Phenolics	Lead Actetate test	+	+	+
		Ferric Chloride test	+	+	+
3	Saponins	Foam test	-	-	-
4	Glycosides	Legal's test	+	+	+
5	Terpenoids	Salkowski test	+	+	+
		Liebermann-Burchard's test	+	+	+
6	Flavonoids	Shinoda test	+	+	+
		Alkaline reagent test	+	+	+
7	Tannins	Ferric Chloride test	+	+	+
		Lead Actetate test	+	+	+
8	Carbohydrates	Benedict's test	+	+	+
		Molisch s test	+	+	+
9	Proteins and Amino acids	Biuret test	-	-	-
		Millon' s test	-	-	-

The GC-MS analysis carried out in the methanolic leaf extract of *Annona reticulata* showed the presence of thirty three bioactive compounds with different percentage peak area. The peak number, name of bioactive compounds, retention time (RT), % peak area, molecular formula and molecular weight are shown in the table 2. The major bioactive compounds identified in the methanolic leaf extract include n-Hexadecanoic acid; cis-13-Octadecenoic acid; Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]-; .psi.,.psi.-Carotene, 7,7',8,8', 11,11',12,12', 15,15'-decahydro-; (-)-Spathulenol; p-Dioxane-2,3-diol; Vitamin E; Aromadendrene oxide-(2); Caryophyllene oxide; Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-; Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-; Phytol; Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-; 1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)-; 9-Octadecenoic acid, methyl ester, (E)- and 12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-

(1R*,3E,7E,11R*)]-. Most of the major as well as minor phytocompounds identified in the present study is reported to have interesting biological activities (Table 3).

Table 2: Details of bioactive compounds identified in the methanolic leaf extract of *Annona reticulata* by GC-MS analysis.

Peak	RT (min)	Compound name	Molecularformula	Molecularweight (g/mol)	Peak area%
1	6.095	Benzene, [(methylsulfinyl)methyl]-	$C_8H_{10}OS$	154.229	1.609
2	6.714	p-Dioxane-2,3-diol	$C_4H_8O_4$	120.104	5.256
3	12.233	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	C10H16	136.234	2.971
4	24.559	Copaene	C15H24	204.357	1.166
5	24.94	Cyclohexane,1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-, [1S-(1α,2β,4β)]-	C15H24	204.351	6.695
6	25.728	Caryophyllene	C15H24	204.357	1.083
7	27.506	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-	C15H24	204.351	3.500
8	28.199	.tau.-Cadinol	C15H26O	222.372	1.216
9	28.305	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	C15H22	202.335	3.213
10	28.556	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	C15H24	204.351	1.902
11	29.718	(-)-Spathulenol	C15H24O	220.356	5.607
12	29.801	Caryophyllene oxide	C15H24O	220.356	4.046
13	30.458	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	C15H24O	220.356	2.113
14	30.91	(-)-Spathulenol	C15H24O	220.356	1.840
15	31.588	(-)-Spathulenol	C15H24O	220.356	3.005
16	31.833	1H-Cycloprop[e]azulen-7-ol, decahydro-1, 1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	C15H24O	220.350	1.416
17	32.702	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	C15H24O	220.356	1.761
18	32.081	Aromadendrene oxide-(2)	C15H24O	220.35	4.879
19	33.608	Acetic acid, 2,6,6-trimethyl-3-methylene-7-(3-oxobutylidene)oxepan-2-yl ester	C16H24O4	280.364	1.459
20	34.5	1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)-	C6H12O4	148.157	2.467
21	34.944	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	C15H24O2	236.177	1.386
22	35.072	1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)-	C6H12O4	148.157	1.304
23	36.728	Isoaromadendrene epoxide	C15H24O	220.35	1.399

24	37.089	Hexadecanoic acid, methyl ester	C17H34O2	270.457	1.939
25	37.988	n-Hexadecanoic acid	C16H32O2	256.43	7.989
26	40.275	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294.479	1.736
27	40.403	9-Octadecenoic acid, methyl ester, (E)-	C19H36O2	296.495	2.271
28	40.606	Phytol	C20H40O	296.539	2.799
29	41.14	2-Fluorobenzoic acid, 2-tetrahydrofurylmethyl ester	C12H13O3	224.231	1.251
30	41.272	cis-13-Octadecenoic acid	C18H34O2	282.468	7.510
31	41.687	Octadecanoic acid	C18H36O2	284.484	1.930
32	55.391	.psi.,.psi.-Carotene,7,7',8,8',11,11',12,12',15,15'-decahydro-	C40H66	546.968	6.208
33	56.652	Vitamin E	C29H50O2	430.717	5.078

The present investigation revealed that *Annona reticulata* leaves contain many phytochemical groups and specific compounds which have various bioactivities such as antimicrobial, antioxidant, anticancer and anti-inflammatory. The phytochemical groups like alkaloids, terpenoids, flavonoids, phenolics, glycosides, tannins etc are good sources of antioxidant and antimicrobial compounds which have received more attention for their potential role in prevention of human diseases and the presence of these chemical groups in *Annona reticulata* indicates strong radical scavenging property of the plant and the ability to prevent oxidative damage (Jang *et al*., 2009; Ndukwe & Ikpeama, 2013).[5,6] The study revealed strong antimicrobial property of the plant as these constituents are known to be potentially toxic to the growth and development of wide range of pathogenic microorganisms (Funatogawa *et al*., 2004; Okwu & Josiah, 2006).[7,8] Terpenoids, glycosides, flavonoids, tannins and alkaloids are further reported to have anti-inflammatory, anti allergic and anticancer activity (Prieto *et al*., 1999; Priyanga et al., 2014).[9,10]

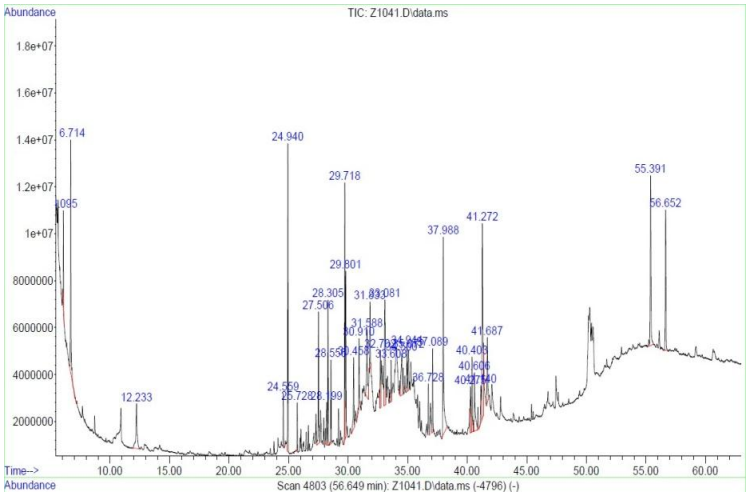
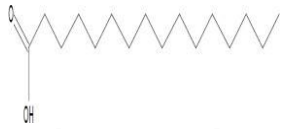
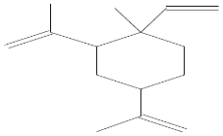
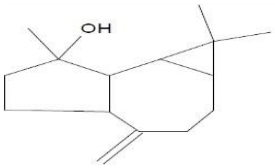
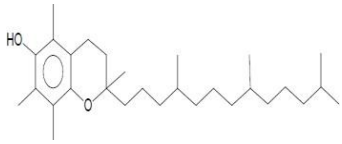
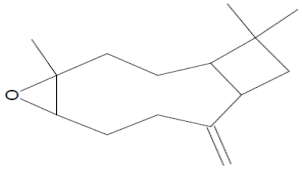
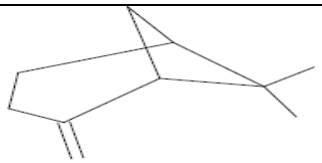
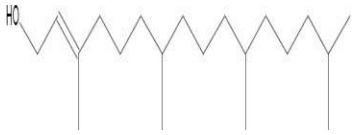
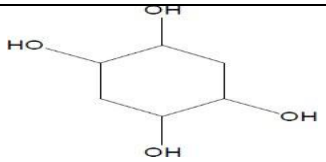


Figure 1: GC-MS chromatogram of methanolic leaf extract of *Annona reticulate.*

 n-Hexadecanoic acid (% peak area-7.989)	 Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]- (% peak area-6.695)	 (-)-Spathulenol-(% peak area-5.607)
 Vitamin E (% peak area-5.078)	 Caryophyllene oxide (% peak area-4.046)	 Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-, (1S)-(% peak area- 2.971
 Phytol (% peak area- 2.799)	 1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)- (% peak area- 2.467)	 9-Octadecenoic acid, methyl ester, (E)- (% peak area-2.271)

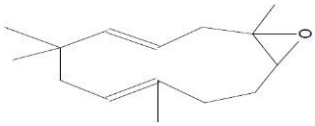
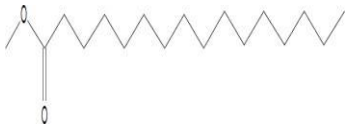
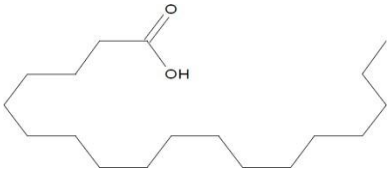
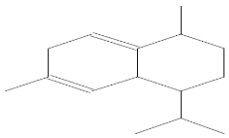
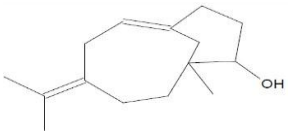
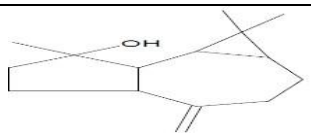
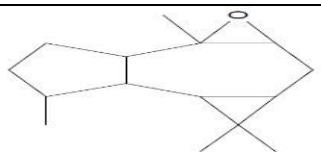
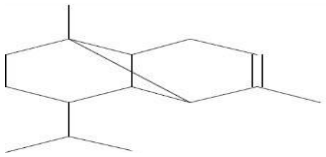
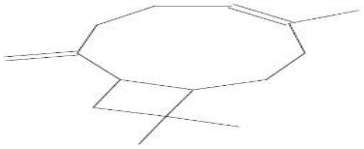
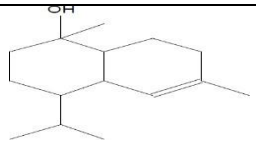
 12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl-,[1R-(1R*,3E,7E,11R*)]- (% peak area- 2.113)	 Hexadecanoic acid, methyl ester (% peak area- 1.939)	 Octadecanoic acid (% peakarea-1.93)
 Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- (% peak area-1.902)	 7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene (% peak area-1.761)	 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1aα,4aα,7β,7aβ,7bα)]- (% peak area-1.416)
 Isoaromadendrene epoxide (% peak area-1.399)	 Copaene (% peak area-1.166)	 Caryophyllene (% peak area-1.083)
	 .tau.-Cadinol (% peak area-1.216)	

Figure 2: Structure of important compounds identified in the methanolic leaf extracts of *Annona reticulate.*

The investigation further confirmed the pharmacological properties of *Annona reticulata* through the identification of several specific bioactive compounds in the leaf component by GC-MS analysis (Table 3 and Figure 2). The identification of n-Hexadecanoic acid; Vitamin E; 1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)-; 9-Octadecenoic acid, methyl ester, (E)-; Hexadecanoic acid, methyl ester; Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-; 7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene; Copaene and Caryophyllene in the leaf component indicates the plant possess antioxidant property. The presence of bioactive compounds which have antimicrobial property such as Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]-;Caryophyllene oxide; (-)-Spathulenol; Phytol; 12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-; Octadecanoic acid; Isoaromadendrene epoxide; 1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1aα,4aα,7β,7aβ,7bα)]-; .tau.-Cadinol; 1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)-; 9-Octadecenoic acid, methyl ester, (E)- and Caryophyllene confirms the antimicrobial property of the plant. The antimicrobial property may be attributed to the ability of these compounds to disrupt microbial phospholipid cell membrane (Cowan, 1999).[11] The present investigation also noticed that compounds like Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-,(1S)-; Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-; Caryophyllene; 12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-; 1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)- and Vitamin E identified in *Annona reticulata* also reported to possess anticancer or anti-inflammatory or both activities and this may be attributed to the antioxidant or antimicrobial properties of these compounds. The GC-MS analysis further revealed that majority of the phytocompounds identified belongs to sesquiterpenes, a biologically significant class of terpenes which act as phytoalexins, anti-herbivory and attractants of insect pest predators. Sesquiterpenes are usually produced in plants in response to microbial and insect pest attack (Holopainen, 2004).[12] There are research reports on the direct or indirect biological activity of sesquiterpene compounds in terms of combating human diseases and this include cancer (Zhang *et al*., 2005),[13] microbial diseases (Barbara *et al*., 2009),[14] inflammation (Meratate et al., 2016)[15] and cardiovascular diseases (Wong & Menendez, 1999).[16] The effect of sesquiterpene compounds in cardiovascular diseases may be attributed to their ability to relax smooth muscle tissue by inhibiting iNOS up-regulation and consequently increasing levels of NO (Martin *et al*., 2013).[17]

Table 3: Bioactivity of compounds identified in the GC-MS analysis of methanolic leaf extract of *Annona reticulate.*

Name of compound	Bioactivity**
Benzene, [(methylsulfinyl)methyl]-	Antibacterial, antiasthmatics, antiarthritis, antipsychotics
p-Dioxane-2,3-diol	Anticancer, antidote, pancreaprotective, antiasthmatic
Bicyclo[3.1.1]heptane,6,6-dimethyl-2methylene-,(IS)-	Anti-inflammatory, sedative, anticancer, antibacterial
Copaene	Antioxidant
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-	Anti-tumor, antibacterial, anti-inflammatory, analgesic, fungicidal
Caryophyllene	antibacterial, anti-inflammatory, antioxidant activities, anticancer activities
Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]- .tau.-Cadinol	No activity reported Antimicrobial, antibactrial
Naphthalene,1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	No activity reported
Naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	Antioxidant, pest repellent orattractant
(-)-Spathulenol	Antimicrobial, antibacterial
Caryophyllene oxide	Antimicrobial, antibacterial
12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	Anti-tumor, antibacterial, antiinflammatory, analgesic, fungicidal
1H-Cycloprop[e]azulen-7-ol, decahydro-1, 1,7-trimethyl-4-methylene-,[1ar-(1aα,4aα,7β,7aβ,7bα)]-	Antimicrobial, antibacterial
7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1] undec-1-ene	Antioxidant
Aromadendrene oxide-(2)	Nitric-Oxide-Synthase-Inhibitor
Aceticacid,2,6,6-trimethyl-3-methylene-7-(3-oxobutylidene) oxepan-2-yl ester	Anti-tumor, anticancer, acidifier, acidulant
1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)-	Antioxidant, antimicrobial, anti-inflammatory
Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	Anti-inflammatory
1,2,4,5-Cyclohexanetetrol, (1α,2α,4α,5β)-	No activity reported
Isoaromadendrene epoxide	Antibacterial, insecticidal
Hexadecanoic acid, methyl ester	Antioxidant, hypocholesterolemic, nematicide
n-Hexadecanoic acid	Antioxidant, nematicide, hemolytic, hypocholesterolemic
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anti-cancer
9-Octadecenoic acid, methyl ester, (E)-	Antioxidant, antimicrobial
Phytol	Antimicrobial, anti-inflammatory, anticancer
2-Fluorobenzoic acid, 2-tetrahydrofurylmethyl ester	Acidifier, acidulant,
cis-13-Octadecenoic acid	Acidifier, acidulant

Octadecanoic acid	Antimicrobial
.psi.,.psi.-Carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro-	No activity reported
Vitamin E	Antioxidant, anticancer, antitumor, antidote, expectorant

CONCLUSION

The present investigation conclude that the leaf component of *Annona reticulata* possess strong medicinal value due to the presence of several bioactive principles which have antioxidant, antimicrobial, anticancer and anti-inflammatory properties. The study further would like to conclude that, many major and minor compounds present in the leaf components of *Annona reticulata* are sharing certain common biological activities and therefore the various major as well as minor phytocompounds are to be taken into consideration to account for their additive and synergistic effects. The present authors believe that the information reveled about the biologically active principles present in the leaf component of *Annona reticulata* will be useful for researchers and scientists who are involved in new active compound profiling and development of drugs against various diseases. The study suggested isolation, characterization and purification of different bioactive compounds and to conduct necessary experiments on their biological activities for safety and confirmation.

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