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Annona glabra fruit extracts: Chemical profiling and their potential antimicrobial activity against pathogenic microbial strains

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Abstract

Annona glabra L. is known for its edible fruit and it has numerous medicinal and therapeutic benefits. The aim of this study is to evaluate the antimicrobial activity of different solvent extracts of *Annona glabra* fruits followed by chemical profiling of the most bioactive extracts. The antimicrobial activity was estimated using disc agar plate diffusion test and chemical characterization was achieved using GC-MS analysis. Results revealed that all tested extracts exhibited noticeable antimicrobial activities with remarkable consideration to ethyl acetate, *n*-butanol and methanolic extracts. Ethyl acetate extract showed antimicrobial inhibition zone values ranged from 10 to 16mm. Whereas, *n*-butanol extract exhibited antimicrobial inhibition zone values ranged from 10 to 18mm. In addition, the methanolic extract displayed antimicrobial inhibition zone values ranged from 8 to 21mm, all against *S. aureus*, *E. coli*, *C. albicans* and *A. niger*. GC-MS analysis showed that 3,5-decadiyne, 2,2-dimethyl- (24.93%), hexadecanoic acid, methyl ester (12.92%), and hexadecanoic acid, methyl ester (23.69%), were detected as main chemical ingredients in the ethyl acetate, *n*-butanol, and methanol extracts, respectively. *Annona* glabra fruits could be a good source of natural antimicrobial agents.

***Keywords*:** *Annona glabra*, solvent extracts, GC-MS, antimicrobial activity

1. Introduction

Recently, many microbial strains have shown strong and fierce resistance against antibiotics, which indicates a real threat to human life as a result of exposure to infectious diseases, so scientists have turned to searching for alternative pathways to eliminate microbial infection, including the discovery of drugs from natural sources as safe antimicrobial agents [1-4].

Natural products (NPs) have a long history as a vital source of naturally occurring antimicrobial agents. These sources include marine, plant and microbial extracts, in addition to their bioactive secondary metabolites as well as essential oils [5-13]. Moreover, numerous classes of NPs have been evaluated for their antimicrobial effects against different types of pathogenic microbial strains including gram negative & gram positive bacteria, fungi and yeast. In particular, plant extracts as well as their derived secondary metabolites showed promising antimicrobial activity, and this activity is attributed to the unique diversity in their chemical composition [5, 14-18]. The genus *Annona* belongs to the family Annonaceae, it comprises numerous species of tropical and subtropical species. Most *Annona* species are famous for their edible fruits [19,20]. Numerous parts of *Annona* species were utilized in traditional remedies to treat various diseases including cancer and infectious diseases [21]. *Annona glabra* is an edible plant; it's widely distributed in America and some regions in Africa. Several parts of

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the plant were utilized in traditional medicine to handle cancer and other health problems [22]. Pervious phytochemical studies on the plant led to the characterization and separation of numerous chemical ingredients including acetogenins [23], diterpenoids [24], peptides [25], alkaloids [26,27] , flavonoids [28], and phenolics [29]. Additionally, different parts of the plant have been evaluated for their antimicrobial [28], anticancer [24,30], and cytotoxicity activities [29]. Therefore, the current study aims to chemical characterization and *in vitro* antimicrobial activity evaluation of various extracts of *Annona glabra* fruits.

1. Materials and Methods

1.1. Plant materials

Annona glabra fruits were purchased from a local Market (Giza, Egypt) during August 2018. The crushes were removed, finely divided, and air dried.

1.2. Extraction and fractionation

Dry powdered fruits (300 g) were extracted via maceration using methanol (2L X 4). After 4 days, the extract was filtered using Whatman filter paper, and then was concentrated using Rotavapoure. The crude methanolic extract (45 g) was undergoing consecutive fractionation using different organic solvents like petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH [31, 32].

1.3. Evaluation of antimicrobial activity

The antimicrobial activity was estimated using disc agar plate test versus four tested microbes comprising *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* [33, 34].

1.4. Gas chromatography/mass spectrometry (GC/MS) analysis

GC-MS investigations of the most potent extracts were accomplished according to the described studies [35, 36].

2. Results and Discussion

2.1. *In vitro* antimicrobial activity

In the current study, the various extracts of *A. glabra* crushes were estimated for their antimicrobial potentials versus four tested microbes involving *S. aureus*, *E. coli*, *C. albicans* and *A. niger*. The results indicated that the inhibition zone values of the methanol extract are 18mm, 11mm, 21mm, and 8mm. While, methylene chloride extract exhibited inhibition zone values of 14mm, 0mm, 12mm, and 7 mm. Moreover, the ethyl acetate extract showed inhibition zone values of 16mm, 10mm, 14mm, and 10mm. The inhibition zone values for petroleum ether extract are 19mm, 0mm, 16mm, and 0mm. Also, *n*-butanol extract showed promising activity with inhibition zone values of 18mm, 10mm, 18mm, and 13mm. Furthermore, the water extract showed inhibition zone values of 15mm, 0mm, 14mm, and 0mm, all respectively versus *S. aureus*, *E. coli*, *C. albicans*, and *A. niger* **(Table 1 and Figure 1)**. The ethanolic extract of *A. muricata* seeds growing in Egypt displayed antimicrobial inhibition zone values of 11mm (*S. aureus*), 12mm (*E. coli*), 13mm (*P. aeruginosa*), and 0mm (*C. albicans*) [37]. The ethanolic extract of *A. muricata* fruits growing in Nigeria showed antimicrobial inhibition zone value of 11mm (*P. mirabilis*), 7mm (*K. pneumonia*), 12mm (*S. aureus*), 13mm (*Salmonella*), and 12mm (*E. coli*) [38]. The ethyl acetate fruit extract of *A. muricata* growing in Nigeria presented antimicrobial inhibition zone of 14mm (*E. coli*), 20mm (*S. aureus*), 4mm (*C. albicans*), and 5mm (*C. tropicalis*). However, the *n*-hexane extract exhibited inhibition zone values of 15mm (*E. coli*), 22mm (*S. aureus*), and 4mm (*S. typhi*) [39] . The ethanol extract of *A. muricata* fruit growing in Nigeria displayed antibacterial inhibition zone values versus three pathogenic strains including 17mm (*S.* aureus), 16mm (*K. pneumonia*), and 16mm (*P. aeruginosa*) [40] .

Table 1. The antimicrobial activity of different solvent extracts of *A. glabra* crushes

No.	Sample Name	Clear zone (ϕmm)			
		S. aureus	*E. coli*	*C. albicans*	*A. niger*
1	MeOH	18	11	21	8
2	CH_2Cl_2	14	0	12	7
3	EtOAc	16	10	14	10
4	Pet. ether	19	0	16	0
5	*n*-BuOH	18	10	18	13
6	H_2O	15	0	14	0

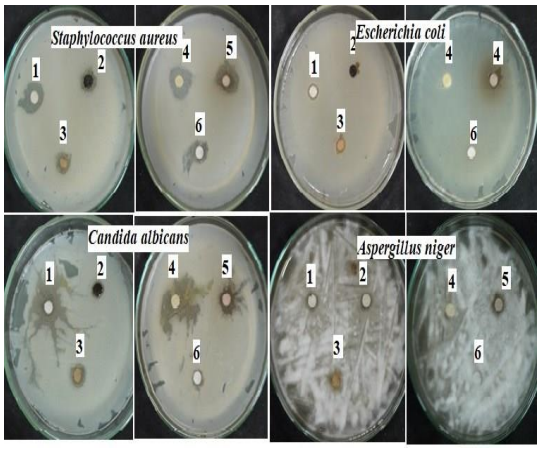

Fig. 1. Antimicrobial inhibition zones (mm) of various extracts from *A. glabra* fruits versus some tested microbes

2.2. GC-MS analysis

In the current study, three extracts were selected for their chemical characterization using GC-MS technique depends on retention time, and mass fragmentation profile. Thirty eight compounds

were observed in the ethyl acetate extract of *A. glabra* fruits accounting for 89.22% from the overall extract composition. 3,5-decadiyne, 2,2-dimethyl- (24.93%), hexadecanoic acid, methyl ester (7.16%), 9-eicosene, (E)- (5.94%), 3-eicosene, (E)- (5.54%), 9-octadecenoic acid (Z),-methyl ester (5.64%), cycloeicosane (3.52%), 1-hexadecene (3.27%), and 1-cyclohexene-1-methano l, à,2,6,6-tetramethyl- (3.14%) are the main identified ingredients **(Table 2, Fig. 2, 5)**. Furthermore, GC-MS analysis of the *n*-butanol extract led to the identification of forty one compounds representing 92.54% from the overall extract composition. The main identified ingredients are hexadecanoic acid, methyl ester (12.92%), (-)-spathulenol (8.79%), 9-octadecenoic acid (Z)-, methyl ester (8.14%), isoaromadendrene epoxide (5.23%), octadecanoic acid, methyl ester (4.71%), and kaur-16-en-19-ol (3.68%) **(Table 3, Fig. 3, 6)**. Moreover, the methanolic extract contains 41 compounds representing 95.12% from the total extract composition. The main detected constituents are hexadecanoic acid, methyl ester (23.69%), 9,12-octadecadienoic acid, methyl ester (14.10%), 9-octadecenoic acid (Z),- methyl ester (11.93%), (-)-spathulenol (8.89%), dihydrorimuene (8.24%), and methyl stearate (8.01%) **(Table 4, Fig. 4, 7)**.

Chemical examination of fresh fruits of *A. glabra* led to separation and characterization of kaurane diterpenoid, acetogenins, steroids, and oxoaporphine [41] . On the other side, the volatile oil of *A. glabra* comprises main constituents like β-caryophyllene (21.5%) germacrene D (17.7%), α-cadinol (5.4%) and β-elemene (5.2%) [19]. In addition, β-gurjunene (42.49%) was observed as a main ingredient in the essential oil of *A. glabra* [21]. Moreover, urs-12-ene, squalene, and clionasterol were observed as main components in the *n*-hexane, ethyl acetate and methanol leaves extracts of Nigerian *A. muricata*, respectively [42] . GC-MS analysis of different solvent extracts from leaves of Indonesian *A. muricata* showed that β-sitosterol, and neophytadiene were observed as main components in the *n*-hexane extract, while protocatechuic acid was identified as a main ingredient in the ethyl acetate extract, also the methanol extract includes main ingredients like arabinitol, pentakis-*O*-(trimethylsilyl)-, erythritol, malic acid, L-proline,1-(trimethylsilyl)-, trimethylsilyl ester, DL-malic acid, *O*-(trimethylsilyl)-, bis(trimethylsilyl)ester, alpha-D-glucopyranose, myo-inositol, 1,2,3,4,5,6-hexakis-*O*-(trimethylsilyl)-, trimethylsilyl ether of glycerol, and mannoic acid,2,3,5,6-tetrakis-*O*-(trimethylsilyl)-, lactone. Additionally the aqueous extract was mainly constituted of p-coumaric acid, arabinitol, pentakis-*O*-(trimethylsilyl)-, glucopyranose, pentakis-*O*-trimethylsilyl, and niacin [43].

GC-MS analysis of the seed hexane extract from Turkish *A. muricata* led to characterization of main components like 9-octadecenoic acid, pentadecanoic acid, 9,12-octadecadienoic acid (*Z,Z*), (3-β)-stigmast-5-en-3-ol, (*Z*)-9-octadecenamide, octadecanoic acid, (*E*)-nerolidol, and (*E,E*)-2,4-decadienal [44] . Moreover, chemical investigation of Nigerian *A. muricata* seed methanol extract via GC-MS analysis led to characterization of hexadecanoic acid, 2,6-dimethyl-1,7-octadien-3-ol, 9-octadecanoic acid, and nonadecanoic acid [45] .

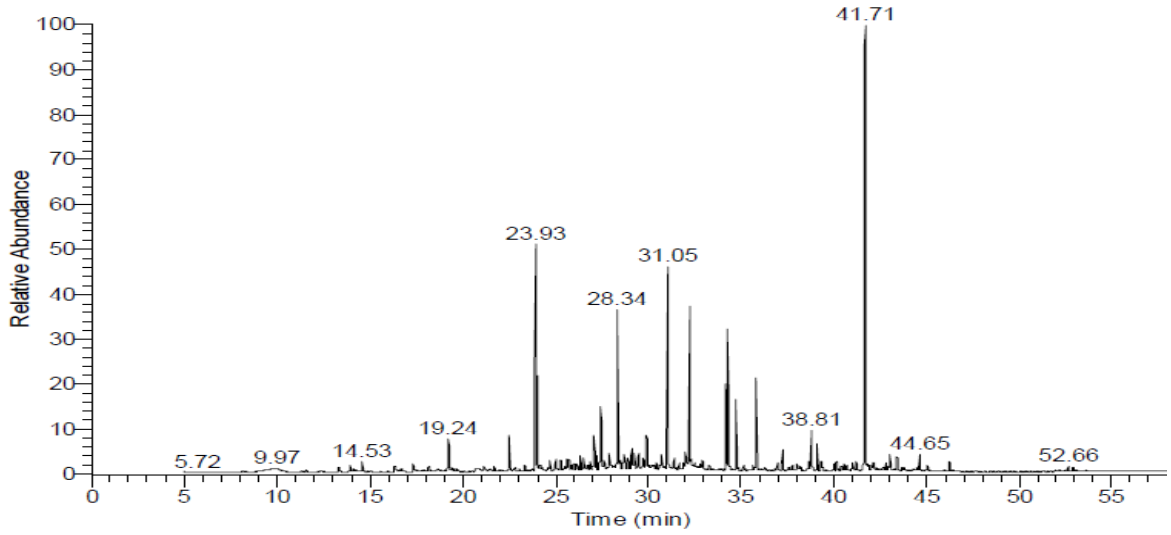

Fig. 2. GC-MS chromatogram of the ethyl acetate extract of *A. glabra* fruits.

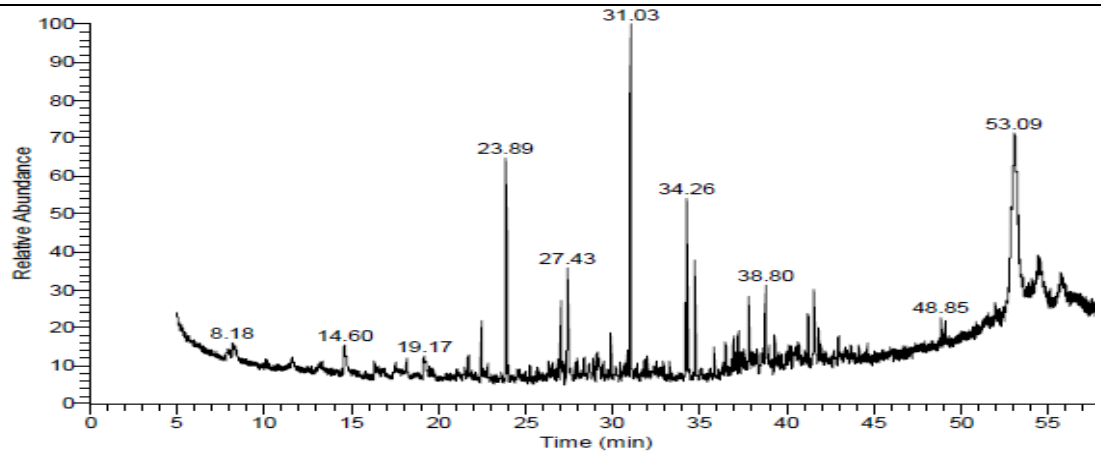

Fig. 3. GC-MS chromatogram of the *n*-butanol extract of *A. glabra* fruits.

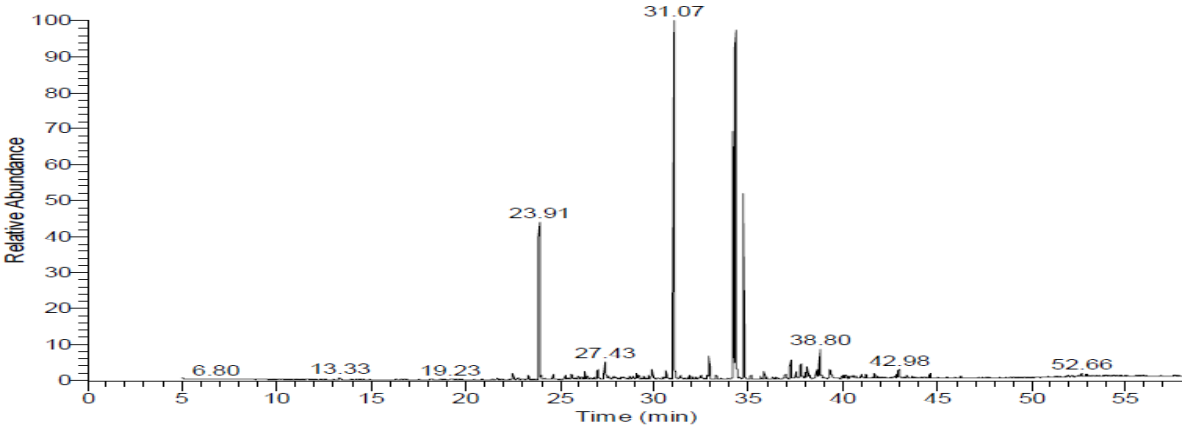

Fig. 4. GC-MS chromatogram of the methanolic extract of *A. glabra* fruits.

Table 2: Chemical compositions of the ethyl acetate extract *A. glabra* fruits

R_t	No.	Compound Name	Area%	M.F.	*m/z*
14. 54	1	1-Butene	0.48	C_4H_8	220
19. 24	2	*n*-Tridecan-1-ol	1.22	$C_{13}H_{28}O$	200
22. 67	3	Cyclotetradecane	1.34	$C_{14}H_{28}$	196
23. 93	4	(-)-Spathulenol	0.27	$C_{15}H_{24}O$	220
24. 02	5	1-Hexadecene	3.27	$C_{16}H_{32}$	224
24. 69	6	Isoaromadendrene epoxide	0.43	$C_{15}H_{24}O$	220
25. 28	7	3- (4-Isopropylphenyl) -2-methylpropionaldehyde	0.54	$C_{13}H_{18}O$	190
26. 34	8	Aromadendrene oxide-(1)	0.55	$C_{15}H_{24}O$	220
27. 06	9	1H-Cycloprop [e] azulen-7-ol ‹decahydro-1‹1‹7-trimethyl-4-methylene- ‹[1ar- (1aα ‹4aα ‹7β ‹7aβ ‹7bα)] -	1.81	$C_{15}H_{24}O$	220
27. 19	10	Benzene, (1-butyloctyl)-	0.53	$C_{18}H_{30}$	246
27. 45	11	1-Cyclohexene-1-methano l, à,2,6,6-tetramethyl-	3.14	$C_{11}H_{20}O$	168
27. 88	12	7,10-Pentadecadiynoic acid	0.69	$C_{15}H_{22}O_2$	234
28. 33	13	9-Eicosene, (E)-	5.94	$C_{20}H_{40}$	208
28. 67	14	Benzene, (1-methylundecyl)-	0.94	$C_{18}H_{30}$	246
28. 90	15	Cedren-13-ol ‹8-	0.52	$C_{15}H_{24}O$	220
29. 13	16	(7,7-Dimethyl-1,4-dioxo-2,3,4,5,6,7-hexahydro-1H -inden-2-yl)acetic acid	0.92	$C_{12}H_{18}$	162

29. 49	17	3,5-Decadiyne, 2,2-dimethyl-	24.93	$C_{19}H_{36}O_2$	296
29. 73	18	Aromadendrene oxide-(2)	0.42	$C_{15}H_{24}O$	220
29. 91	19	3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)]decane-9,10-diol	1.61	$C_{15}H_{26}O_2$	238
30. 01	20	Caryophyllene oxide	0.35	$C_{15}H_{24}O$	220
30. 71	21	5,7-Dioxatetracyclo[7.4.0.0(3,10)0.(4,8)]tridecane, 2-methylene-11-(1-methy lethyl)-1,6,6-trimethyl	0.82	$C_{18}H_{28}O_2$	276
31. 05	22	Hexadecanoic acid, methyl ester (CAS)	7.16	$C_{17}H_{34}O_2$	270
31. 42	23	9,12,15-Docosatetraenoic acid, methyl ester	0.64	$C_{23}H_{38}O_2$	346
32. 00	24	Cis-Z-a-Bisabolene epoxide	0.77	$C_{15}H_{24}O$	220
32. 25	25	3-Eicosene, (E)-	5.54	$C_{20}H_{40}$	280
34. 19	26	9,12-Octadecadienoic acid(Z,Z)-, methyl ester	2.97	$C_{19}H_{34}O_2$	294
34. 28	27	9-Octadecenoic acid (Z),- methyl ester (CAS)	5.64	$C_{19}H_{36}O_2$	296
34. 73	28	Methyl stearate	2.72	$C_{19}H_{38}O_2$	298
35. 82	29	Cycloeicosane	3.52	$C_{24}H_{50}O$	354
37. 25	30	Podocarp-7-en-3-one, 13β-methyl-13-vinyl-	0.83	$C_{20}H_{30}O$	286
38. 81	31	Biformene	1.66	$C_{20}H_{32}$	272
39. 10	32	*n*-Tetracosanol-1	0.98	$C_{24}H_{50}O$	354
39. 32	33	Androstan-17-ol, 2,3-epoxy,-(2à,3à,5à,17á)-	0.61	$C_{19}H_{30}O_2$	290
40. 15	34	n-Propyl5,8,11,14,17-eicosapenta enoate	0.48	$C_{23}H_{36}O_2$	344
43. 02	35	6á- Hydroxytestosterone	0.78	$C_{19}H_{28}O_3$	304
43. 42	36	Kaur-16-en-18-oic acid, methyl ester, (4β)-	0.59	$C_{21}H_{32}O_2$	316
44. 21	37	Dihydro-isosteviol methyl ester	0.35	$C_{21}H_{34}O_3$	334
44. 65	38	Kauran-18-al, 17-(acetyloxy)-, (4á)-	0.62	$C_{22}H_{34}O_3$	346
Total %			**89.22%**		

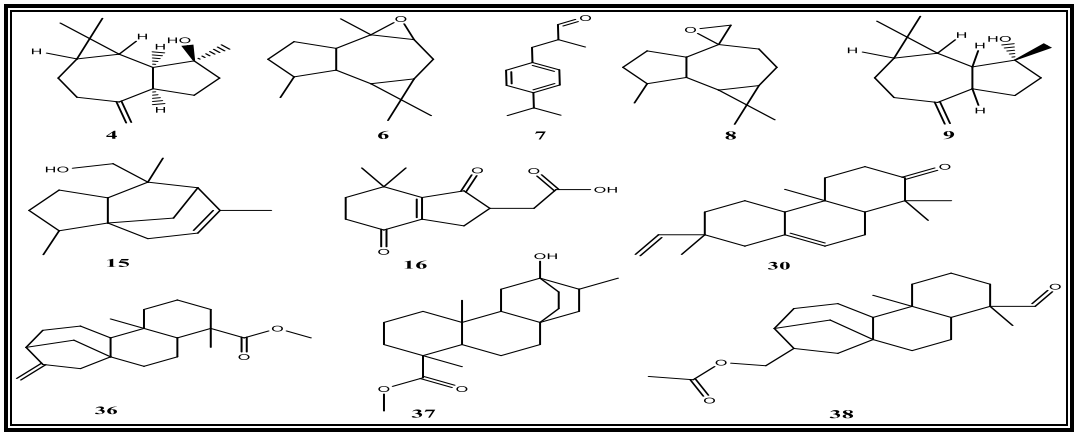

Fig. 5. Chemical structures of some selected identified compounds in the ethyl acetate extract

Table 3: Chemical compositions of the *n*-butanol extract of *A. glabra* fruits

R_t	No.	Compound Name	Area%	M.F.	*m/z*
19. 23	1	Tridecanol	1.12	$C_{13}H_{28}O$	220
23. 89	2	(-)-Spathulenol	8.79	$C_{15}H_{24}O$	220
26. 85	3	6,9,12-Octadecatrienoic acid, methyl ester	1.10	$C_{19}H_{32}O_2$	292
27. 03	4	Soaromadendrene epoxide	1.16	$C_{15}H_{18}O_3$	246
27. 17	5	Ambrosin	1.78	$C_{13}H_{20}O$	192
27. 43	6	Isoaromadendrene epoxide	5.23	$C_{15}H_{24}O$	220
27. 96	7	Alloaromadendr enoxid-(1)	0.96	$C_{15}H_{24}O$	220
28. 38	8	(E)-4-(5',5'-Epoxymethano-1',2',2'-trimethyl-6'-oxo-1'-cyclohexyl)-3-buten-2-one	1.13	$C_{14}H_{20}O_3$	236

28. 70	9	4a,7-Methano-4aH-napht h[1,8a-b]oxirene, octahydro-4,4,8,8-tetram ethyl-	1.10	$C_{15}H_{24}O$	220
28. 87	10	Ledene oxide-(II)	1.07	$C_{15}H_{24}O$	220
29.12	11	9,12,15-Octadecatrienoic acid	1.81	$C_{28}H_{40}O_4$	440
29.26	12	(Z)-9-Tetracosene-1,24-di ol	1.26	$C_{24}H_{48}O_2$	368
29. 90	13	Aromadendrene oxide-(2)	2.12	$C_{15}H_{24}O$	220
30. 70	14	Pregn-4-ene-1,20-dione, 12-hydroxy-16,17-dimeth yl-	1.01	$C_{23}H_{34}O_3$	358
30. 87	15	11-Octadecenal spectrum disagrees	1.54	$C_{18}H_{34}O$	266
31.03	16	Hexadecanoic acid, methyl ester	12.92	$C_{17}H_{34}O_2$	270
31. 98	17	2-[4-methyl-6-(2,6,6-trime thylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-e n-1-carboxaldehyde	0.94	$C_{23}H_{32}O$	324
34. 17	18	9,12-Octadecadienoic acid, methyl ester, (E,E)-	2.85	$C_{19}H_{34}O_2$	294
34. 26	19	9-Octadecenoic acid (Z)-, methyl ester	8.14	$C_{19}H_{36}O_2$	296
34. 72	20	Octadecanoic acid, methyl ester	4.71	$C_{19}H_{38}O_2$	298
35. 82	21	Methyl6-cis,9-cis,11-trans-octad ecatrienoate	1.62	$C_{19}H_{32}O_2$	292
36. 12	22	Estran-3-one, 17-hydroxy-, (5à,17á)-	1.25	$C_{18}H_{28}O_2$	276
36. 49	23	Androstan-2-one, (5à)-	1.43	$C_{19}H_{30}O$	274
36.75	24	Androstane-3,11-diol, (3á,5à,11á)-	1.86	$C_{19}H_{32}O_2$	292
36. 97	25	5à,14á-Androstane, 16à,17à-epoxy-	1.68	$C_{19}H_{30}O$	274
37. 23	26	Podocarp-7-en-3-one, 13á-methyl-13-vinyl-	1.70	$C_{20}H_{30}O$	286
37. 42	27	Androstan-3-one, 17-(acetyloxy)-, (5α,17β)-	1.63	$C_{21}H_{32}O_3$	332
37. 82	28	Preg-4-en-3-one, 17à-hydroxy-17á-cyano-	3.12	$C_{20}H_{27}NO_2$	313
38. 79	29	Kaur-16-en-19-ol	3.68	$C_{20}H_{32}O$	288
39. 31	30	Cyclooctenone, dimer (CAS)	2.22	$C_{16}H_{24}O_2$	248
40. 43	31	1-Phenanthrenecarboxyli c acid, 7-ethenyl-1,2,3,4,4a,4b,5, 6,7,8,10,10a-dodecahydr o-1,4a,7-trimethyl-, methyl ester, [1R-(1à,4aá,4bà,7à,10aà)]	0.99	$C_{21}H_{32}O_2$	316
40. 67	32	1-Heptatriacotanol	1.00	$C_{37}H_{76}O$	536
41.23	33	17-Pentatriacontene	2.66	$C_{35}H_{70}$	490
41. 55	34	Ergost-22-en-3-ol, (3á,5à,22E,24R)-	3.14	$C_{28}H_{48}O$	400
41.63	35	Diisooctyl-phthalate	1.34	$C_{24}H_{38}O_4$	390
41.83	36	Isosteviol methyl ester	1.59	$C_{21}H_{32}O_3$	332
42. 29	37	Dihydro-isosteviol methyl ester	1.76	$C_{21}H_{34}O_3$	334
42. 99	38	1-Naphthalenepropanol, à-ethyldecahydro-5-(hydr oxymethyl)-à,5,8a-trimet hyl-2-methylene-,	0.97	$C_{20}H_{36}O_2$	308
43. 09	39	1-Phenanthrenecarboxyli c acid, tetradecahydro-7-(2-metho xy-2-oxoethylidene)-1,4a,8-trimethyl-9-oxo-, methyl ester, [1S-(1à,4aà,4bá,8á,8aà,10aá)]-	1.46	$C_{22}H_{32}O_5$	376
43. 39	40	3,19:5,6-Diepoxyandrostane, 17-acetoxy-4,4-dimethyl-3á-methoxy	0.93	$C_{24}H_{36}O_5$	404
43. 67	41	Bufa-20,22-dienolide, 14,15-epoxy-3,11-dihydro xy-, (3á,5á,11à,15á)-	1.16	$C_{24}H_{32}O_5$	400
Total %			**92.54%**		

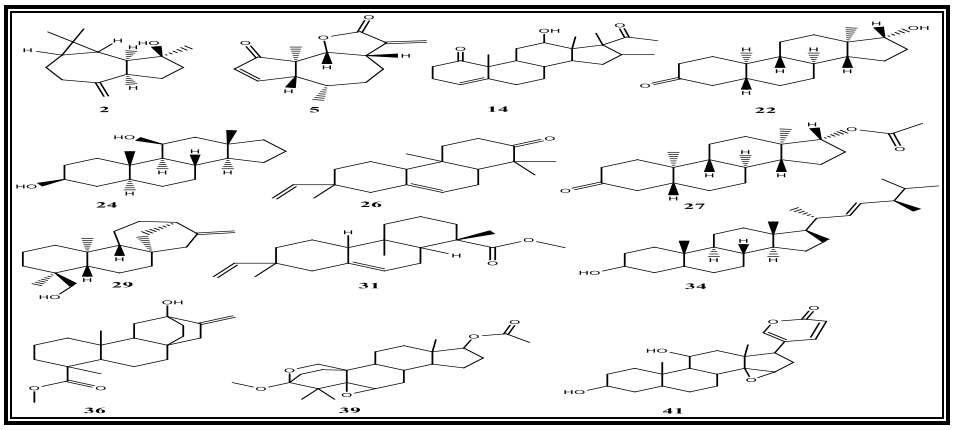

Fig. 6. Chemical structures of some selected identified compounds in *n*-butanol extract

Table 4: Chemical compositions of the methanolic extract of *A. glabra* fruits

R_t	No.	Compound Name	Area%	M.F.	*m/z*
23. 91	1	(-)-Spathulenol	8.89	$C_{15}H_{24}O$	220
24. 03	2	1,2,3,4,4a,5,6,8a-octahyd ro-4a,8-dimethyl-2-(2-pro penyl)-	0.34	$C_{15}H_{24}O$	220
24. 67	3	2,5-Octadecadiynoic acid, methyl ester	0.39	$C_{19}H_{30}O_2$	290
25. 30	4	tau-Cadinol	0.27	$C_{15}H_{26}O$	222
25. 62	5	Aromadendrenepoxide	0.28	$C_{15}H_{24}O_2$	220
26. 33	6	Ledene oxide	0.43	$C_{15}H_{24}O$	220
27. 03	7	Caryophyllene oxide	0.76	$C_{15}H_{24}O$	220
29. 06	8	Pentadecanoic acid, methyl ester	0.26	$C_{16}H_{32}O_2$	256
30. 64	9	9-Hexadecenoic acid, methyl ester, (Z)-	0.65	$C_{10}H_{12}O_2$	268
31. 07	10	Hexadecanoic acid, methyl ester	23.69	$C_{17}H_{34}O_2$	270
31. 26	11	Octadecanoic acid methyl ester	1.09	$C_{19}H_{38}O_2$	298
31. 41	12	Androsta-1,4-dien-3-one17-hydroxy-17-methyl,- ,(17à)-	0.28	$C_{20}H_{28}O_2$	300
32. 53	13	Calarene	0.30	$C_{15}H_{24}$	204
32. 92	14	Heneicosanoic acid, methyl ester	1.43	$C_{19}H_{38}O_2$	340
33. 29	15	Kaur-16-ene ‹(8β ‹13β)	0.27	$C_{20}H_{32}$	272
34. 20	16	9,12-Octadecadienoic acid, methyl ester	14.10	$C_{19}H_{34}O_2$	294
34. 31	17	9-Octadecenoic acid (Z),- methyl ester (CAS)	11.93	$C_{19}H_{36}O_2$	296
34. 74	18	Methyl stearate	8.01	$C_{19}H_{38}O_2$	298
35. 54	19	Doconexent	0.44	$C_{23}H_{36}O_2$	328
35. 84	20	Methyl7,10,13,16,19-docosapent aenoate	0.71	$C_{23}H_{36}O_2$	344
36. 97	21	Dihydrorimuene	8.24	$C_{20}H_{34}$	354
37. 24	22	Naphthalene, decahydro-1,1,4a-trimeth yl-6-methylene-5-(3-met hyl-2,4-pentadienyl)-, [4aS-(4aà,5à,8aá)]-	1.07	$C_{20}H_{32}$	272
37. 34	23	Podocarp-7-en-3-one, 13β-methyl-13-vinyl-	1.16	$C_{20}H_{30}O$	286
37. 54	24	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	0.49	$C_{19}H_{36}O_3$	312
37. 79	25	Octadecanoic acid, 9,10-dihydroxy-, methyl ester (CAS)	1.32	$C_{19}H_{38}O_4$	330
38. 01	26	6-Acetylbenzo[b]naphtho[2,3-e]-[1,4]-dioxin	1.23	$C_{18}H_{12}O_3$	276
38. 12	27	13,16-Octadecadiynoic acid, methyl ester	0.56	$C_{25}H_{42}O_2$	374
38. 21	28	6,9,12,15-Docosatetraenoic acid, methyl ester	0.26	$C_{23}H_{38}O_2$	346
38. 62	29	Bicyclo[2.2.1]heptan-2-ol, 2-allyl-1,7,7-trimethyl-	0.42	$C_{13}H_{22}O$	194
38. 80	30	[17-(14-C]-18-hydroxyaphidicol-16-ene	1.07	$C_{20}H_{32}O$	288
39. 01	31	Androstan-3-one17-(acetyloxy)-, (5à,17á)-	0.42	$C_{21}H_{32}O_3$	332

39. 31	32	10,13-Eicosadienoic acid, methyl ester	0.49	$C_{21}H_{38}O_2$	322
39. 61	33	Trans-Geranylgeraniol	0.86	$C_{20}H_{34}O$	290
39. 98	34	Dihydroisopimaric acid methyl ester	0.46	$C_{21}H_{34}O_2$	318
40. 01	35	1,2,3,3a,4,5,6,8,9,9a,10,10 a-dodecahydro-7-(1-methy lethyl)-1,9a-dimethyl-4- methylene	0.36	$C_{20}H_{30}O$	286
40. 14	36	Kauren-18-ol, acetate, (4á)- (CAS)	0.22	$C_{22}H_{34}O_2$	330
41. 21	37	Atis-16-ene, (5á,8à,9á,10à,12à)	0.33	$C_{20}H_{32}$	272
41. 67	38	cis-5,8,11,14,17-Eicosapentaenoic acid	0.39	$C_{20}H_{30}O_2$	302
42. 71	39	Isosteviol methyl ester	0.57	$C_{21}H_{32}O_3$	332
42. 99	40	Androstane-6,17-dione ,3-hydroxy-, (3á,5à)-	0.36	$C_{19}H_{28}O_3$	304
44. 64	41	Dihydro-isosteviol methyl ester	0.34	$C_{21}H_{34}O_3$	334
Total %			**95.12%**		

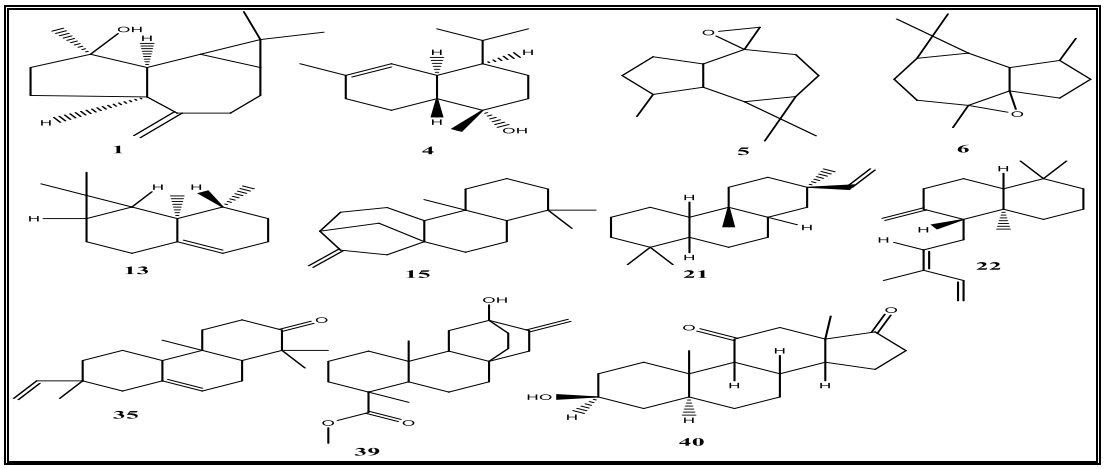

Fig. 7. Chemical structures of some selected identified compounds in methanolic extract

3. Conclusion

This study proved that various extracts of Annona glabra fruit exhibited noticeable in vitro antimicrobial activity against some tested microbes comprising Staphylococcus aureus, Escherichia coli, Candida albicans and Aspergillus niger. Further, it provides evidence that the antimicrobial activity of the tested extracts could be due to the co-activity between their major and minor constituents. In conclusion, these extracts could be considered an effective therapy to treat infectious diseases. Therefore, Annona glabra fruit could be used as a good source of naturally occurring antimicrobial remedies.

4. Financial support and sponsorship

Nil.

5. Conflicts of interest

There is no conflict of interest.

6. Data availability

All data generated or analyzed during the study are included in this article

7. Disclosure statement

There are no conflicts to declare

8. Ethical approval

Nil.

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Abbreviations

GC-MS: Gas chromatography–mass spectrometry.
M.F.: Molecular formula.
m/z: mass-to-charge ratio.
R_t: Retention time.
CH_2Cl_2: Dichloromethane.
EtOAc: Ethyl acetate.
***n*-BuOH**: *n*-butanol.
MeOH: Methanol.

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