



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, CH₂Cl₂, 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) | | | |
|-----|---------------------------------|------------------|----------------|--------------------|-----------------|
| | | <i>S. aureus</i> | <i>E. coli</i> | <i>C. albicans</i> | <i>A. niger</i> |
| 1 | MeOH | 18 | 11 | 21 | 8 |
| 2 | CH ₂ Cl ₂ | 14 | 0 | 12 | 7 |
| 3 | EtOAc | 16 | 10 | 14 | 10 |
| 4 | Pet. ether | 19 | 0 | 16 | 0 |
| 5 | <i>n</i> -BuOH | 18 | 10 | 18 | 13 |
| 6 | H ₂ O | 15 | 0 | 14 | 0 |

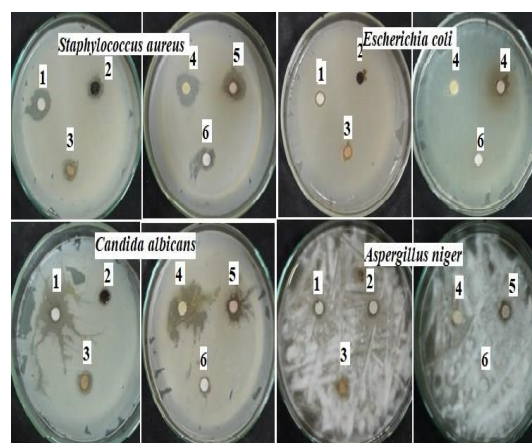


Fig. 1. Antimicrobial inhibition zones (mm) of various extracts from *A. glabra* fruits versus some tested microbes

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 1, 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%), dihydromuene (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-octadecenamamide, 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].

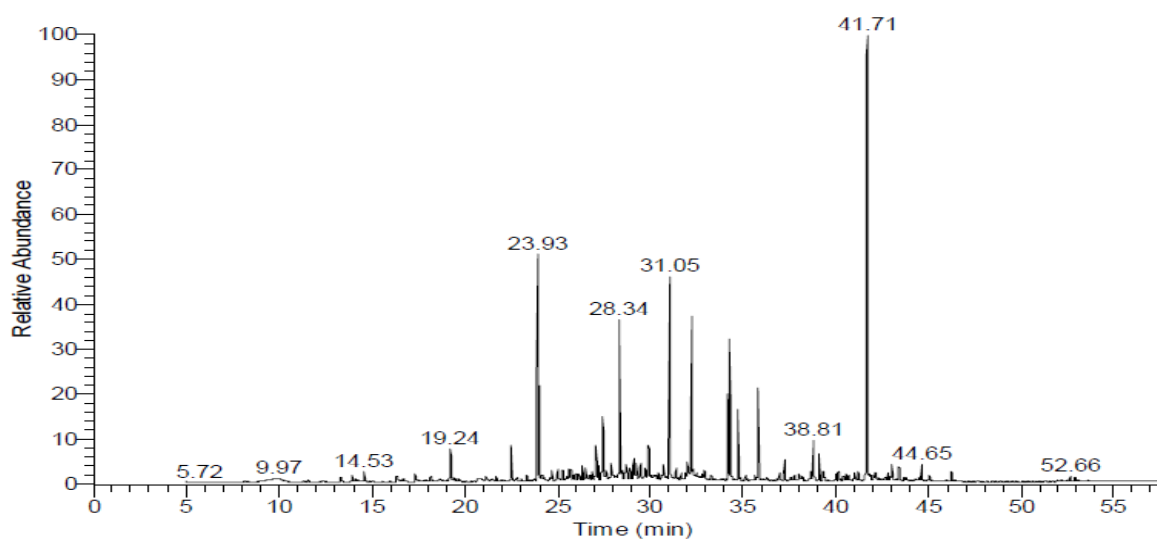


Fig. 2. GC-MS chromatogram of the ethyl acetate extract of *A. glabra* fruits.

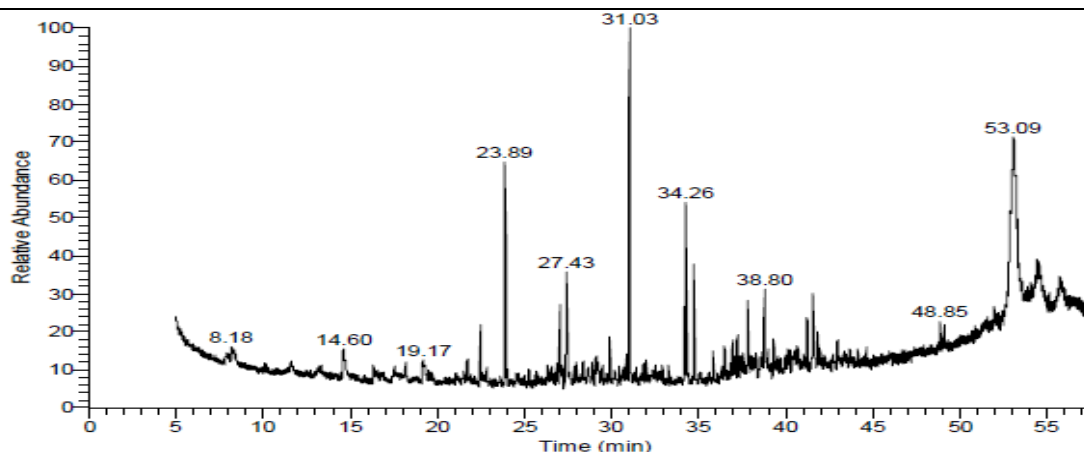


Fig. 3. GC-MS chromatogram of the *n*-butanol extract of *A. glabra* fruits.

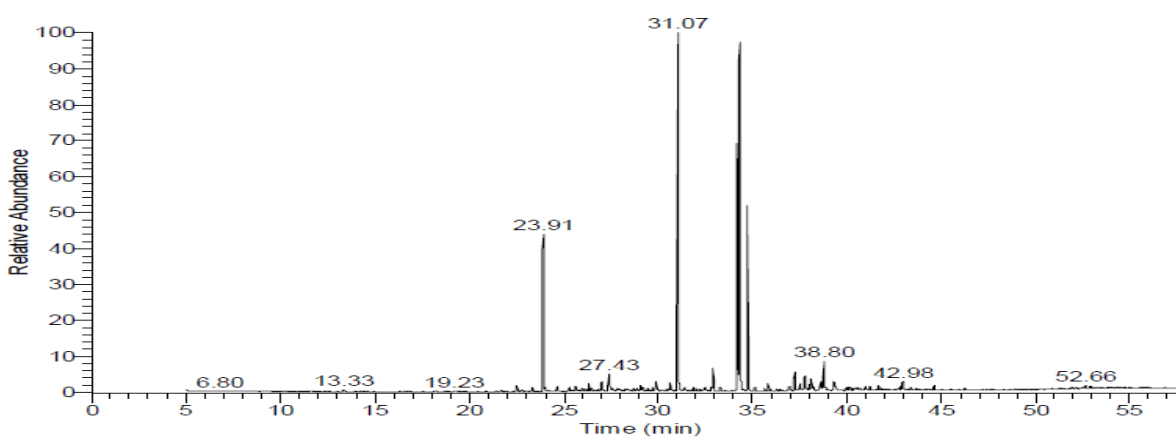


Fig. 4. GC-MS chromatogram of the methanolic extract of *A. glabra* fruits.

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 ₄ H ₈ | 220 |
| 19. 24 | 2 | <i>n</i> -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- (1α '4α '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 1, 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-methyl-1-ethyl)-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,17à)- | 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 | 6à- 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)-, (4à)- | 0.62 | C ₂₂ H ₃₄ O ₃ | 346 |
| Total % | | | 89.22% | | |

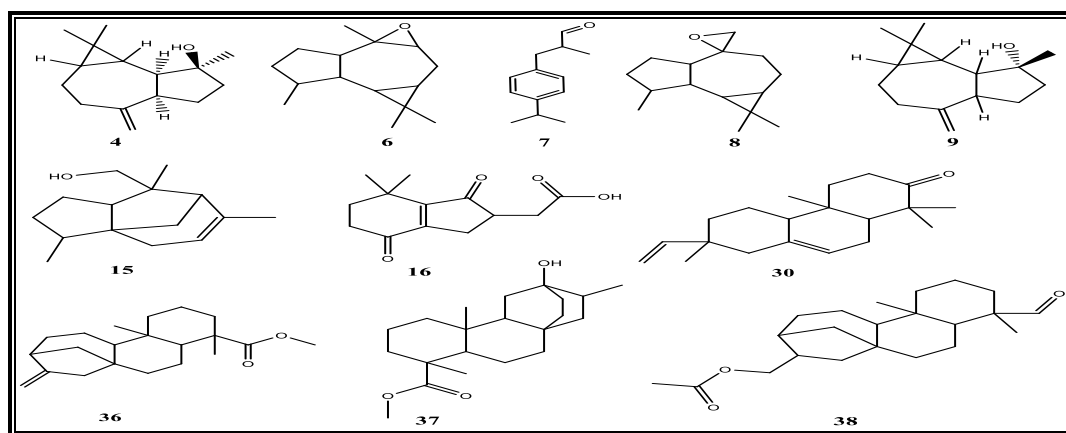


Fig. 5. Chemical structures of some selected identified compounds in the ethyl acetate extract

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

| R _i | No. | Compound Name | Area% | M.F. | <i>m/z</i> |
|----------------|-----|---|-------|--|------------|
| 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'-Epoxy-methano-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-naphthalen[1,8a-b]oxirene, octahydro-4,4,8,8-tetraethyl- | 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-diol | 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-dimethyl- | 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-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-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 | Methyl 6-cis,9-cis,11-trans-octadecatrienoate | 1.62 | C ₁₉ H ₃₂ O ₂ | 292 |
| 36. 12 | 22 | Estran-3-one, 17-hydroxy-, (5 α ,17 α)- | 1.25 | C ₁₈ H ₂₈ O ₂ | 276 |
| 36. 49 | 23 | Androstan-2-one, (5 α)- | 1.43 | C ₁₉ H ₃₀ O | 274 |
| 36. 75 | 24 | Androstane-3,11-diol, (3 α ,5 α ,11 α)- | 1.86 | C ₁₉ H ₃₂ O ₂ | 292 |
| 36. 97 | 25 | 5 α ,14 α -Androstane, 16 α ,17 α -epoxy- | 1.68 | C ₁₉ H ₃₀ O | 274 |
| 37. 23 | 26 | Podocarp-7-en-3-one, 13 α -methyl-13-vinyl- | 1.70 | C ₂₀ H ₃₀ O | 286 |
| 37. 42 | 27 | Androstan-3-one, 17-(acetyloxy)-, (5 α ,17 β)- | 1.63 | C ₂₁ H ₃₂ O ₃ | 332 |
| 37. 82 | 28 | Preg-4-en-3-one, 17 α -hydroxy-17 α -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-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl-, methyl ester, [1R-(1 α ,4 α ,4b α ,7 α ,10 α)] | 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, (3 α ,5 α ,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, α -ethyldecahydro-5-(hydroxymethyl)- α ,5,8a-trimethyl-2-methylene-, | 0.97 | C ₂₀ H ₃₆ O ₂ | 308 |
| 43. 09 | 39 | 1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoethylidene)-1,4a,8-trimethyl-9-oxo-, methyl ester, [1S-(1 α ,4 α ,4b α ,8 α ,8 α ,10 α)]- | 1.46 | C ₂₂ H ₃₂ O ₅ | 376 |
| 43. 39 | 40 | 3,19:5,6-Diepoxystropane, 17-acetoxy-4,4-dimethyl-3 α -methoxy | 0.93 | C ₂₄ H ₃₆ O ₅ | 404 |
| 43. 67 | 41 | Bufo-20,22-dienolide, 14,15-epoxy-3,11-dihydroxy-, (3 α ,5 α ,11 α ,15 α)- | 1.16 | C ₂₄ H ₃₂ O ₅ | 400 |
| Total % | | | 92.54% | | |

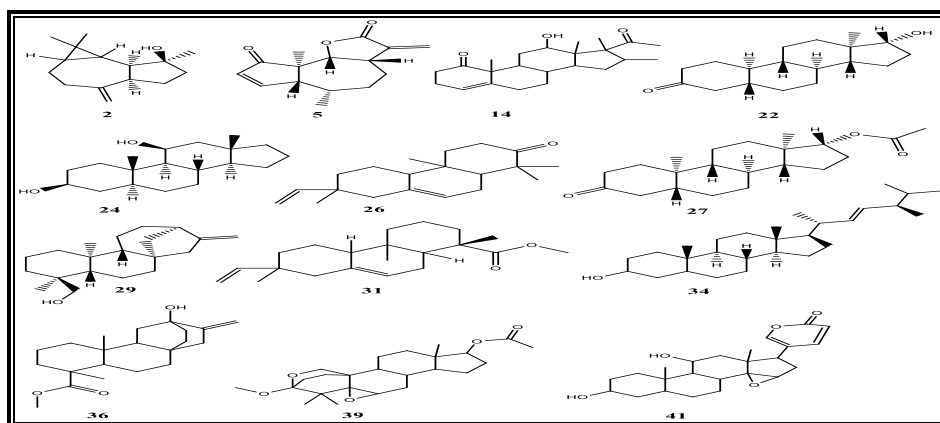


Fig. 6. Chemical structures of some selected identified compounds in *n*-butanol extract

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 ₁₆ H ₃₂ 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-one-17-hydroxy-17-methyl-, (17 α)- | 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 (8 β , 13 β) | 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-(4a α ,5 α ,8a α)]- | 1.07 | C ₂₀ H ₃₂ | 272 |
| 37. 34 | 23 | Podocarp-7-en-3-one, 13 β -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-one-17-(acetyloxy)-, (5 α ,17 α)- | 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-methyl-ethyl)-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,8á,9á,10á,12á) | 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,5á)- | 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% | | |

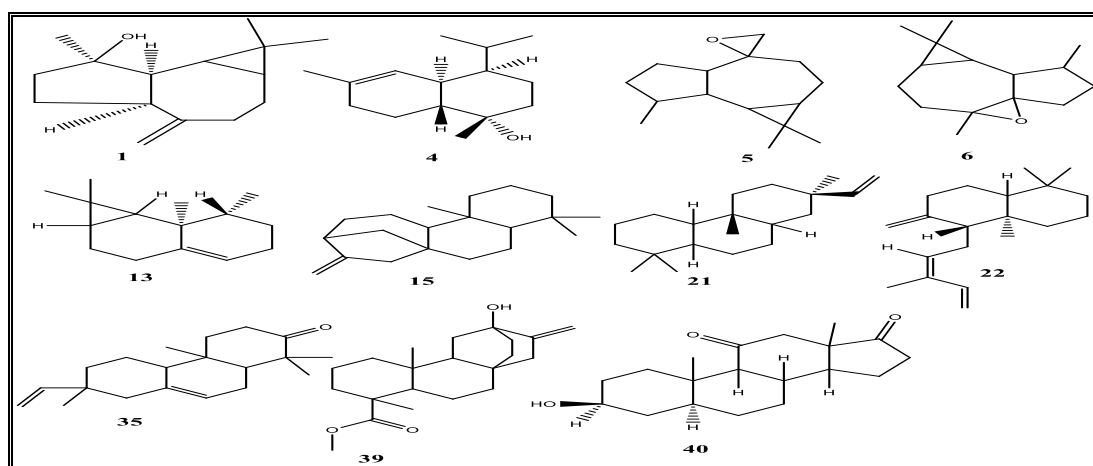


Fig. 7. Chemical structures of some selected identified compounds in methanolic extract

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.

CH₂Cl₂: Dichloromethane.

EtOAc: Ethyl acetate.

n-BuOH: n-butanol.

MeOH: Methanol.

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