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Research Article

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Chemical constituents and antimicrobial activity of different *Annona* species cultivated in Egypt

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ABSTRACT

Three Annona species, A. squamosa, A. cherimola and A. Abdel Razik were investigated for their volatile oils in leaves, qualitatively and quantitatively. GC/MS recorded that A. Cherimola contains 27.59% isocaryophylline. A. squamosa contains the highest percentage of copaene, α- pinene and β pinene in the following amounts, 17.06, 9.96 and 12.99 respectively. The oil showed potent antimicrobial activity against different pathogenic organisms. The investigation deals with the antioxidant content of the fixed and volatile oils. The fixed oil of the seeds contains higher amount of oleic and linoleic acids, where the the degree of the unsaturated fatty acids reaches over 70%.

Keywords: quantitative, qualitative analyses volatile, fixed oils, antimicrobial, antioxidant.

INTRODUCTION

Annona (Annonaceae) comprises approximately 162 species of trees and shrubs that are found predominantly in lowland tropical regions [1]. Previous chemical and pharmacological investigations of some species from this genus have indicated the presence of important bioactive compounds (alkaloids, acetogenins and terpenes) that exhibit various pharmacological activities, including cytotoxicity against tumor cell lines, antimicrobial and particularly trypanocidal properties [2-4].

Annona squamosa has been used in folk medicine. Its leaves are reported to possess stimulant, antispasmodic, sudorific, antihelmintic and insecticidal properties. A poultice of the leaves is used as a cataplasm over boils and ulcers to induce suppuration [5]. The search for trypanocidal and antiplasmodial compounds from annonaceous plants are going on for *A. squamosa* and specially from *Annona foetida* [6].

Annona fruits contain considerable amount of polyphenolic compounds [7]. These compounds are antioxidants that may help to prevent diseases associated with oxidative stress, such as cancer, atherosclerosis and neurodegenerative diseases [8-10]. In the body, free radicals derive from two sources: endogenous sources, e.g. nutrient metabolism and the ageing process, and exogenous sources, e.g. air pollution [11]. Free radicals can attack various substrates in the body and contribute to chronic disease development. For example, oxidatively modified LDL has been hypothesized to be a causative agent in the development of cardiovascular disease [12]. Oxidatively modified DNA may also play an important role in human carcinogenesis [13]. Usually the human body has its mechanisms for eliminating the free radicals by some of the nutrients in the diet that have antioxidant activities.

EXPERIMENTAL SECTION

1. MATERIALS

a. Plant Materials and Botanical description of *Annona* sp.

The present study was carried out on three species of *Annona* tree obtained from a private farm at Mansoriya, Giza Governorate, Egypt and identified by Dr. M. Gibali, Department of Taxonomy, Faculty of Science, Cairo University. Voucher specimen were deposited at the National Research Centre Herbarium under no 521, 522, 523. Growth parameters were measured as plant height, fruit weight, seed weight and different color of the leaves, and fruits, shapes, fig (1, 2 and 3).

Fig. 1. *Annona Squamosa* L.

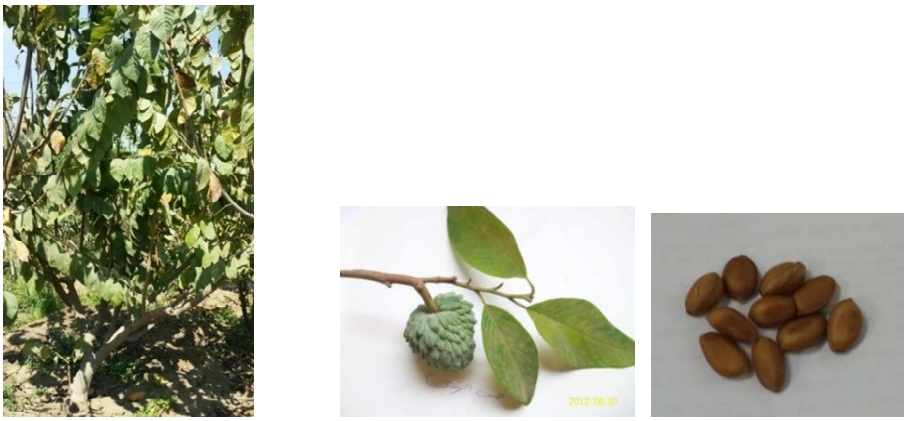


Fig. 2. *Annona Cherimola* M.

Fig. 3. *Annona Squamosa X Annona Cherimola* hybrid (locally traded under the cultiver Abdel Razik)

b. Phytochemical Investigation

1. Detection of sterols and/or triterpenes

The presence of unsaturated sterols and/or triterpenes were detected using acetic acid anhydride (0.3 mL) – sulphuric acid (0.5 mL) reagent and observed for the formation of a brown ring at the junction of the two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer indicate a positive test for steroids and triterpenoids respectively, as reported by Hanson [14].

2. Essential oils:

2.1. Determination of essential oil content in different species leaves parts:

Leaves of the three *Annona* trees collected during November, 2013 were used for the determination of volatile oil content. The volatile oil of each fresh sample was extracted by the water distillation method (for 3 hrs.) using Clevenger's apparatus (Guenther, 1953). The resulted essential oil of each species was separately dehydrated with anhydrous sodium sulphate and kept in deep freezer until GC/MS analysis. Distillation was done in triplicate and the mean values of the oil content (%) were recorded.

2.2. Identification of the chemical composition of oils:

The components of *Annona* sp. leaves essential oils were identified by GC/MS analyses. The essential oil samples were fractionated using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications: Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 40 ^{o}C for 1 min; rising at 4.0 ^{o}C /min to 160 ^{o}C and held for 6 min; rising at 6 ^{o}C /min to 210 ^{o}C and held for 1min. The injector and detector were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

3. Determination of total lipid concentration.

2 gm of air-dried fine powder of each *Annona sp.* seeds were extracted by petroleum ether 40-60^{o}C using Soxhlet apparatus till complete extraction. The solvent was evaporated by rotary evaporator at 40 ^{o}C till dryness then the residue was kept in vacuum desiccator till constant weight and the concentration of total lipids were calculated [15].

Identification of unsaponifiable matter and fatty acids

3.1. Separation of unsaponifiable matter and fatty acids

One mL oil of the three species were saponified with methanolic KOH (20 mL, 10%) at 80˚C for 3 hr under reflux. The unsaponifiable matter of each oil was extracted with ether (4 x 10 mL), washed several times with distilled water, dried over anhydrous sodium sulphate. Then the solvent was evaporated and the unsaponifiable matter was quantified (g) and kept for further analysis.

3.2. Separation of saponifiable matter and fatty acids

The soap solution of the three oils were acidified with HCl (10%), the liberated fatty acids were extracted with ether (3 x 30 mL), washed several times with distilled water till acid free, dried over anhydrous sodium sulphate. The solvent was evaporated and the fatty acids were quantified [16].

3.3. Identification of the unsaponifiable matter

The unsaponifiable matter of the three *Annona* sp. seeds were identified using GC (Central Services Lab. NRC), with the following conditions:

Hewlett Packard HP 6890 apparatus equipped with HP-1 methyl siloxane capillary column (0.25 mm x 30 m), using flame ionization detector (FID), and nitrogen was used, as carrier gas. Nitrogen, hydrogen and air gases were set at flow rates 30, 30 and 300 mL/min, respectively. Oven temperature was programmed from 70-280˚C at a rate 8˚C / min. Temperatures of detector and injectors were 300 and 250˚C, respectively.

The hydrocarbon and sterol compounds were identified by comparing the relative retention times of the separated components with those of available standard materials injected under the same conditions. The quantitative estimation of each compound was based on the area of the recorded peak area.

3.4. Preparation of fatty acid methyl esters

Methyl esters of fatty acids were prepared by refluxing 10 mg of the liberated fatty acids with 10 mL (2%) of H_2SO_4 in anhydrous methanol for 5 h in a water bath at 90˚C [17].

The fatty acid methyl esters were extracted with pet. ether (10 mL/each). The pet-ether extract was treated with diluted sodium bicarbonate solution to remove the acidity, washed several times with distilled water, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure [18].

3.5. Identification and quantitative determination of fatty acids by GC/MS
The fatty acid methyl esters were identified using GC/MS stand in Central Services Lab. NRC under the following conditions:

Pye Unicam PU 4550 apparatus equipped with a coiled glass column (4 mm x 1.5 m, i.d.), packed with diatomite – C (100-120 mesh) and coated with 10% polyethylene glycol adipate "PEGA", with flame ionization detector (FID). Nitrogen was used as a carrier gas, hydrogen and air gases flow rates were at 30, 33 and 330 mL/min, respectively. Oven temperature was programmed from 70 to 190˚C, increased by 10˚C/min. Temperatures of detector and injector were 300 and 250˚C, respectively.

Fatty acids were identified by comparing the relative retention time of each peak with those of standard fatty acid methyl esters injected under the same conditions.

4. Antimicrobial activity
Antimicrobial assays were conducted using the disc-agar method [19] against diverse sets of microorganisms include gram positive (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*), gram negative (*Pseudomonas aeruginosa*), *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans*. Both bacterial strains were grown on nutrient agar medium, while, the fungal and yeast strains were grown on malt medium. The filter paper discs (9 mm) were soaked in each volatile oils (50 µL) and dried at room temperature under sterilized conditions. The paper discs were placed on inoculated agar plates and incubated for 24 h at 37°C for bacteria and 48 h (30°C) the fungi.

5. Antioxidant
The DPPH (1,1-diphenyl-2-picrylhydrazyl, 250mM) radical scavenging model of Shimada *et al.* [20] was used. The mixture was shaken vigorously for 1min then left to stand for 60 min in the dark. Scavenging capacity was measured spectrophotometrically at 517 nm. Inhibition (%) was plotted against the extract concentration in the reaction system. The percentage inhibition of the DPPH radical was calculated according to the following formula:

% Inhibition = [(A control −A sample) / A control] X 100

Where A is absorbance at 517nm.

RESULTS AND DISCUSSION

1. Botanical description of three species of *Annona* trees.
Tables (1) compile the data of parameters of the three species of *Annona* taken through the growth season October, 2014 including the three *Annona* species.

Table (1) shows that the plant height of *A. cherimola* plant (2.5 to 3m), *A. squamosa* (1-1.5m), and Hybrid between the two species *A. Cherimola* X *A. squamosa* called cultivar Abdel Razik (2 to 2.5m) and weight of the fruit of the three species *A. Cherimola* (500-800g), *A. squamosa* (300-500g) and cultivar Abdel Razik (1000-1500g).

Table 1. Botanical description of *Annona* sps grown in Egypt

Parameter	A. Cherimola	A. squamosa	A. Cherimola x A. squamosa Hybrid
Egyptian Name	Hindy	Balady	Abdel Razik
length of plant	2.5-3 m	1-1.5 m	2-2.5
Length of fruits	8 cm	7 cm	12-15 cm
Diameter of fruits	6 cm	5 cm	10-15
Weight of fruit	500 -800 g	300-500 g	1000-1500 g
Color of fruits	Dark green	Light green	Green yellowness
Color of leaves	green	green	Light green
Color of seeds	brown	Black brown	Dark brown
Weight of 100 seeds	11.3627g	5.658g	7.128g

the data in table (1) showed that Hindy species *A. cherimola* is the tallest tree reached 2.5-3 meters followed by Abdel Razik variety 2-2.5 meter and Balady variety is 1-1.5m in height.

Hybrid Abdel Razik beers all the good and high characters, length of fruit, diameter of fruit, its weight which reached the following 10-15cm, 1-1.5kg, the two other species attain less figures than Abdel Razik. in which Balady species shows the least, while Hindy came in the middle range. On the other hand Hindy species possess the highest weight of 100g seeds.

2. The Essential oils.

2.1. Essential oil contents through the growth season.

The following table (2) compiled the volatile oils content of the three species included in the present study. Abdel Rasik variety leaves contains the highest oil content than the other two species. Oil content was more higher in October and December in the three species of Annona as evidently seen from Table (2). Significant differences were markedly found between oil content of the three species studied. The leaves of the three species of *Annona* tree contained the highest amount of essential oil (0.48g) on October in cultivar Abdel Razik, compared with the other two species; *A. Squamosa* and *A. Cherimola* 0.096 ml and 0.156 ml, respectively. Statistical analysis showed significant differences in oil content between the three species of *Annona* tree during growth period from January to December.

Table 2.The concentration of volatile oil from fresh leaves of *Annona* species (ml/ 25g fresh)

Months	*A. Cherimola*	*A. squamosa*	*A. Cherimola* x *A. squamosa* Abdel Razik
Jan.	0.008^{f}	0.002^{f}	0.012^{f}
Mar.	0.015^{e}	0.018^{e}	0.125^{e}
Jun.	0.048^{d}	0.024^{d}	0.16^{d}
Aug.	0.08^{c}	0.03^{c}	0.28^{c}
Oct.	0.156^{a}	0.096^{a}	0.48^{a}
Dec.	0.098^{b}	0.05^{b}	0.36^{b}

- *Statistical analysis is carried out by one way analysis of variance (ANOVA), Co-stat Computer Program.*
- *Unshared letters between brackets are significant values between groups at p<0.0001.*

2.2. Chemical composition of the essential oil of the three species of *Annona* tree leaves.

According to GC/MS analysis, the identified constituents of the essential oil are presented in Table (3). The essential oils of *Annona* species leaves contained 98 compounds represent 100% of the total oil constituents identified from of hybrid Abel Razik (LA) oil, 73.3% of *A. squamosa* (LB) oil, and 91.78% *A. cherimola* (LH) oil. The main constituents of the essential oil identified with GC/MS are β-Pinene (12.90%), α-copaene (17.06%) and Isocaryphyllene (27.59%) in LA, while α-copaene (21.78%) and caryophyllene (13.99 %) are found in LH.

The results in Table (3) show that the essential oil of *Annona* sp. leaves contained hydrocarbons of (94.11%), (55.57%) and (82.51%) in LA, LB and LH respectively. The oxygenated compounds comprise to (5.92%), (17.73) and (8.84) as main chemical groups in LA, LB, LH in the same order.

Considering the chemical constituents of the essential oil obtained from leaves of *Annona squamosa* tree grow in brazil, Meira et al [21] reported on the occurrence of sesquiterpenes as major constituents ranging from 10.8% bicyclogermacrene to 27.4 % (E)-caryophyllene.

These values of sesquiterpenes found in Brazilian *Annona* are greatly higher than that found in *Annona* grown in Egypt. The differences occurred may be attributed to genetic factors and climatic ones, soil type, or to environmental conditions and geographical origin.

The comparison between the reported constituents of leaves *Annona* sp. essential oil and those obtained in this work, indicate that there were qualitative and quantitative differences. In this respect Meira et al [21] mentioned that the major sesquiterpenes were (E)-caryophyllene (27.4%), germacrene D (17.1%), bicyclogermacrene (10.8%), bicyclogermacrene (39.0%), spathulenol (14.0%) and α-phellandrene (11.5%).found in *A. squamosa* leaves grown in Brazil. The differences in chemical constituents between three *Annona* sps. volatile oil may be due to genetic factors [21].

Table 3. The main constituents of the essential oil of *Annona* sp.

peak No.	[a]Identification constituents	SI[b]	RSI[c]	Rt	% components in *Annona* sp.			MS (M/e)	
					LA	LB	LH	Chemical formula	Molecular weight
1	α-Phellandrene	884	886	4.64	0.15	-	0.13	$C_{10}H_{16}$	136
2	α-Pinene	921	921	4.84	9.96	2.13	6.47	$C_{10}H_{16}$	136
3	Camphene	916	916	5.29	0.31	1.55	0.19	$C_{10}H_{16}$	136
4	β-Pinene	922	922	6.05	12.90	-	8.2	$C_{10}H_{16}$	136
5	α-Myrcene	905	905	6.31	0.64	-	0.37	$C_{10}H_{16}$	136
6	D-Limonene	923	924	7.63	3.20	0.55	1.85	$C_{10}H_{16}$	136
7	Eucalyptol	894	896	7.77	-	-	0.07	$C_{10}H_{18}O$	154
8	α-Ocimene	944	944	8.20	0.92	-	-	$C_{10}H_{16}$	136
9	3-Carene	862	916	8.22	-	-	0.11	$C_{10}H_{16}$	136
10	γ -Terpinene	907	908	8.65	0.13	-	0.21	$C_{10}H_{16}$	136
11	Cyclohexene, 4-methyl-3-(1-methylethylidene)-	903	909	9.63	-	-	0.26	$C_{10}H_{16}$	136
12	1,6-Octadien-3-ol,3,7-dimethyl-	924	924	10.28	0.48	-	0.49	$C_{10}H_{18}O$	154
13	Terpinen-4-ol	897	898	13.64	-	-	0.13	$C_{10}H_{18}O$	154
14	13-Tetradece-11-yn-1-ol	721	723	16.18	-	0.2	-	$C_{12}H_{19}NO_3$	208
15	Bornyl acetate	928	929	17.90	0.21	-	-	$C_{12}H_{20}O_2$	196
16	3-Oxatricyclo[4.1.1.0(2,4)]octane, 2,7,7-trimethyl-	954	957	18.94	-	0.34	-	$C_{10}H_{16}O$	152
17	γ-Elemene	826	833	19.65	0.20	0.28	-	$C_{15}H_{24}$	204
18	2-Carene	834	897	19.82	8.27	-	2.88	$C_{10}H_{16}$	136
19	α-Cubebene	917	921	20.30	0.17	-	0.31	$C_{15}H_{24}$	204
20	α-ylangene	899	899	21.24	0.22	0.19	0.23	$C_{15}H_{24}$	204
21	β-Cubebene	897	917	21.53	0.82	-	-	$C_{15}H_{24}$	204
22	(-)-á-Bourbonene	805	808	21.84	0.10	0.35	0.19	$C_{15}H_{24}$	204
23	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	906	906	22.15	2.72	5.86	2.91	$C_{15}H_{24}$	204
24	Isocaryophillene	931	931	22.72	0.16	27.59	1.61	$C_{15}H_{24}$	204
25	α-Guaiene	843	845	22.84	-	0.62	-	$C_{15}H_{24}$	204
26	Caryophyllene	960	960	23.36	5.34	0.86	13.99	$C_{15}H_{24}$	204
27	γ-Elemene	874	885	23.80	0.18	-	0.17	$C_{15}H_{24}$	204
28	Aromandendrene	907	907	24.13	0.59	-	0.59	$C_{15}H_{24}$	204
29	(-)-Aristolene	878	878	24.28	0.64	0.78	0.18	$C_{15}H_{24}$	204
30	Isoledene	860	869	24.50	0.10	-	-	$C_{15}H_{24}$	204
31	γ-Muurolene	861	863	24.60	0.21	-	-	$C_{15}H_{24}$	204
32	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-	866	874	24.62	-	0.32	-	$C_{15}H_{25}$	205
33	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	873	877	24.75	-	1.53	-	$C_{15}H_{26}$	206
34	Humulene	867	877	24.87	2.03	-	-	$C_{15}H_{24}$	204
35	γ-Muurolene	852	859	25.02	0.14	-	0.24	$C_{15}H_{24}$	204
36	1H-Cyclopropa [a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1, 1,7,7a-tetramethyl-, [1aR-(1aà,7à, 7aà,7bà)]	845	855	25.31	0.26	0.38	-	$C_{15}H_{24}$	204
37	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	898	903	25.58	0.25	-	0.55	$C_{15}H_{24}$	204
38	Ylangene	870	871	25.66	-	1.27	-	$C_{15}H_{25}$	205
39	γ-Cadinene	919	920	25.69	-	-	2.13	$C_{15}H_{26}$	206
40	Alloaromadendrene	883	888	25.73	1.95	-	-	$C_{15}H_{24}$	204
41	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	902	904	25.85	-	0.90	-	$C_{15}H_{25}$	205
42	α-copaene	866	866	25.93	17.06	-	21.78	$C_{15}H_{24}$	204
43	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	860	873	26.04	0.32	-	-	$C_{15}H_{24}$	204
44	Cadina-3,9-diene	919	923	26.16	0.77	-	-	$C_{15}H_{24}$	204
45	α-Selinene	900	901	26.28	3.09	0.7	4.52	$C_{15}H_{24}$	204
46	α-Elemene	900	901	26.52	2.96	0.53	4.63	$C_{15}H_{24}$	204
47	α-Muurolene	905	905	26.67	0.20	0.58	0.36	$C_{15}H_{24}$	204
48	Cadina-1(10),4-diene	882	884	26.80	1.53	-	0.73	$C_{15}H_{24}$	204
49	Butylated Hydroxytoluene	847	857	26.88	-	0.2	-	$C_{15}H_{24}O$	220
50	p-Menth-3-ene, 2-isopropenyl-1-vinyl-, (1S,2R)-(-)-	874	879	27.00	1.64	-	1.11	$C_{15}H_{24}$	204
51	γ-Muurolene	906	909	27.26	1.00	-	-	$C_{15}H_{24}$	204
52	β.-Copaene	857	865	27.43	2.35	-	-	$C_{15}H_{24}$	204
53	trans-calamenene	815	833	27.65	-	0.2	-	$C_{15}H_{22}$	202
54	o-Menth-8-ene, 4-isopropylidene-1-vinyl-	812	817	27.78	3.16	-	0.59	$C_{15}H_{24}$	204
55	Naphthalene, 1,2,3,4,6,8a-hexahydro-1-isopropyl-4,7-dimethyl-	908	917	28.04	0.28	-	0.53	$C_{15}H_{24}$	204

56	γ-Muurolene	868	879	28.18	0.23	0.33	0.21	$C_{15}H_{24}$	204
57	Selina-3,7(11)-diene	887	901	28.31	-	0.26	-	$C_{15}H_{24}$	204
58	Cadala-1(10),3,8-triene	675	797	28.4	-	0.24	-	$C_{15}H_{22}$	202
59	Hedycaryol	866	869	28.76	0.46	1.05	0.13	$C_{15}H_{26}O$	222
60	Isoaromadendrene epoxide	737	751	29.00	-	0.18	-	$C_{15}H_{24}O$	220
61	γ-Elemene	906	908	29.02	5.84	-	1.35	$C_{15}H_{24}$	204
62	(-)-Spathulenol	868	871	29.83	-	-	0.19	$C_{15}H_{24}O$	220
63	Caryophyllene oxide	883	887	30.00	-	-	0.29	$C_{15}H_{24}O$	220
64	(-)-Globulol	810	841	30.15	-	0.24	-	$C_{15}H_{26}O$	222
65	α-ylangene	803	829	30.43	0.10	-	-	$C_{15}H_{24}$	204
66	Viridiflorol	780	792	30.54	-	0.85	-	$C_{15}H_{26}O$	222
67	Ledol	826	834	30.59	0.17	-	1.35	$C_{15}H_{26}O$	222
68	trans-á-Ionone	761	767	31.04	-	0.27	0.1	$C_{13}H_{20}O$	192
69	Cubenol	834	859	31.53	0.16	0.17	0.44	$C_{15}H_{26}O$	222
70	Humulane-1,6-dien-3-ol	820	849	31.54	-	-	0.11	$C_{15}H_{26}O$	222
71	Selina-6-en-4-ol	856	871	31.67	0.17	-	-	$C_{15}H_{26}O$	222
72	Epiglobulol	870	871	31.75	0.40	0.86	0.44	$C_{15}H_{26}O$	222
73	Ledene oxide-(II)	784	784	31.87	0.31	0.3	0.21	$C_{15}H_{24}O$	220
74	8-epi-.gama.-eudesmol	859	872	31.94	0.13	0.3	-	$C_{15}H_{26}O$	222
75	Eremophila-1(10),11-diene	805	816	31.96	-	-	0.81	$C_{15}H_{24}$	204
76	Spathulenol	820	824	32.26	0.16	-	-	$C_{15}H_{24}O$	220
77	.tau.-Cadinol	900	906	32.37	1.02	5.54	1.75	$C_{15}H_{26}O$	222
78	.tau.-Muurolol	847	864	32.64	0.10	-	0.48	$C_{15}H_{26}O$	222
79	α-Cadinol	860	867	32.86	1.91	2.8	2.1	$C_{15}H_{26}O$	222
80	Globulol	849	857	33.01	0.11	-	0.27	$C_{15}H_{26}O$	222
81	6-epi-shyobunol	819	834	34.27	-	-	0.08	$C_{15}H_{26}O$	222
82	Aromadendrene, dehydro-	834	796	37.88	0.12	1.25	0.1	$C_{15}H_{22}$	202
83	Isolongifolene, 9,10-dehydro-	817	837	38.74	-	-	1.28	$C_{15}H_{22}$	202
85	Cycloisolongifolene,8,9-dehydro-	849	862	39.02	0.22	2.45	0.31	$C_{15}H_{22}$	202
86	α-Vatirenene	848	874	39.61	0.68	3.16	0.43	$C_{15}H_{22}$	202
87	Ledene oxide-(II)	771	778	39.78	-	0.19	-	$C_{15}H_{24}O$	220
88	cis-Z-à-Bisabolene epoxide	718	726	41.13	0.13	-	0.09	$C_{15}H_{22}O$	218
89	Andrographolide	784	826	42.15	-	0.96	-	$C_{20}H_{30}O_5$	350
90	2,6,11,15-Tetramethyl-hexadec a-2,6,8,10,14-pentaene	762	774	43.23	-	0.44	-	$C_{20}H_{32}$	272
91	10,12-Tricosadiynoic acid	749	751		-	0.57	-	$C_{24}H_{40}O_2$	360
92	geranyl-à-terpinene	752	779	43.69	-	1.17	-	$C_{20}H_{32}$	272
93	Oxirane,	646	724	44.96	-	0.43	-	$C_{30}H_{50}O$	426
94	Calarene epoxide	784	814	46.7	-	1.31	-	$C_{15}H_{24}O$	220
84	Isoaromadendrene epoxide	787	822	46.80	-	-	0.12	$C_{15}H_{24}O$	220
95	Alloaromadendrene oxide-(1)	786	817		-	0.35	-	$C_{15}H_{24}O$	220
96	1-Heptatriacotanol	738	740	47.75	-	0.3	-	$C_{37}H_{76}O$	536
97	Alloaromadendrene oxide-(2)	735	760	48.57	-	0.29	-	$C_{15}H_{24}O$	220
98	2H-Pyran, 2-(7-heptadecynyloxy)tetrahyd ro-	712	756	49.05	-	0.21	-	$C_{22}H_{40}O_2$	336
	Total Identification				100.03	73.3	91.35		
	unidentification compound				0	26.7	8.65		
	oxygenated compounds				5.92	17.73	8.84		
	non-oxygenated compounds				94.11	55.57	82.51		

a Identification was based on comparison of their GC/MS spectra and RI with those of the internal (computer) NIST library and those described by Adams (2007). RI^b (retention index) obtained on a capillary column DB-5MS based on the basis of n-alkanes, and calculated according to the method of Van Den Dool and Kratz [22] . RI^c according to Adams [23].

3. Lipoidal matter of *Annona* species.

The total lipids contents of seeds obtained from the three *Annona* species are given in Table (4). The oil contents of *A. Cherimola* amounted to 21.86%, and 18.9%, in *A. squamosa* seeds while hybrid Abel Razil amounted to 20.26%.

Table 4. The concentration of total lipid,unsaponifiable and saponifiable compounds of different seeds from*Annona* species (g/100g Dw)

	A. Cherimola	***A. squamosa***	***A. Cherimola* x *A. squamosa* Abdel Razik**
Total Lipids	21.86	18.9	20.26
Unsaponifiable matter	17.95	19.99	25.96

Saponifiable matter	81.26	79.21	73.17

The values obtained from the Egyptian *Annona* species hold true with those obtained by Mariod, *et al*. [24], They reported that the oil content of seeds obtained from *A. squamosa* ranged from 17.5 to 26.8%.

3.1. Qualitative and quantitative analysis of unsaponifiable matter.

The unsaponifiable lipid fraction is a potential source of bioactive components such as phytosterols, triterpenoids, and various hydrocarbons.

The data obtained from GC analyses of the unsaponifiable matter of the three *Annona* species are presented in Table (5) the highest content of the hydrocarbon identified are C20 eicosane 34.79 %, C22 Docosane, 45.24 and 54.1% for *A. squamosa* and hybrid Abdel Razik respectively. Phytosterols were found in traces or in small concentration in the three species.

Also, the results reveal the presence of hydrocarbons ranging from C_{14} to $C_{30,}$ of which tricosane, heptadecane, dotriacontane, eicosane and hexadecane are the most predominant components. Dealing with sterols, the unsaponifiable matter of most *Annona* species contained Campasterol, stigmasterol and β-sitosterol.

The major phytosterol in *Annona* squamosa seeds is β-Sitosterol (3.42%) while stigmasterol and campasterol are the major sterols in *A. squamosa* (2.15 and 3.42% respectively). These results go parallel with results obtained by Ragasa *et al.*, [25] they found that, β-sitosterol was the most prevalent phytosterol, about 9 mg/100 g in *Annona muricata* seeds.

Table 5. GC/MS analysis of unsaponifiable matter of different seeds from *Annona* species

			Relative percentage (%)		
	No. carbon atom	**Rt**	***A. Cherimola***	***A. squamosa***	***A. Cherimola* x A. squamosa Abdel Razik**
	C10	**8.22**	**3.6**	-	-
Tetradecane	C14	10.17	4.2	0.355	0.62
Pentadecane	C15	12.09	0.37	0.504	0.59
Hexadecane	C16	13.42	0.93	3.72	0.42
Heptadecane	C17	14.26	0.61	1.31	4.66
Octadecane	C18	15.27	1.61	2.32	7.92
Nonadecane	C19	17.125	7.73	12.38	3.83
Eicosane	C20	18.25	34.79	11.21	10.23
Heneicosane	C21	19.501	11.48	2.23	2.45
Docosane	C22	20.177	27.61	45.24	54.1
Tricosane	C23	22.557	0.87	2.7124	3.17
Tetracosane	C24	24.105	0.83	1.0588	2.53
Pentacosane	C25	24.464	3.02	1.524	0.79
Hexacosane	C26	25.411	0.33	0.546	0.45
Heptacosane	C27	26.015	0.97	1.92	1.36
Octacosane	C28	27.4	-	2.435	1.85
Nonacosane	C29	28.33	-	1.71	1.38
Triocontane	C30	29.114	-	0.545	0.32
Tetraocontane	C31	29.679	-	0.63	0.14
Cholesterol	C27	31.271	-	0.33	0.161
Campasterol	C28	33.178	-	1.5	0.34
Stigmasterol	C29	33.873	-	2.15	0.51
β-Sitosterol	C29	35.263	1.02	3.42	0.9

Rt (Retention time)

3.2. Qualitative and quantitative analysis of fatty acids (saponifiable matter).

Table (6) represents the fatty acid methyl esters fractionated in different *Annona* species. GC is used in this investigation for the qualitative and quantitative determination of individual fatty acid. Fatty acid (C18:1 Oleic acid) amounted to 51.15, 51.33 and 46.54 for *A. cherimola*, *squamosa* and Abdel Rasik respectively followed by C18: 2 then C18:3 in the three mentioned species.

Table 5 shows that in *A. cherimola*, *A. squamosa,* and cultivar Adel Razik seeds, the saturated fatty acids amounted to 19.98, 26.69, and 23.46% respectively. Palmitic acid has been identified as the major saturated fatty acid (18.7, 18.40 and 20.27 %). in the three species respectively.

The total fatty acid of *Annona* spp. under study contained high percentage of unsaturated fatty acids, compared to the low concentration of saturated ones. *Annona* spp. Seed oil represents a promising plant oil, which is composed of an average of 77% poly- and monounsaturated fatty acids, responsible for its value as nutritive edible oil.

Table 6. GC analysis of fatty acid methyl esters of different *Annona* species

Fatty acid	No. carbon atom	Rt	Relative percentage (%)		
			A. Cherimola	*A. squamosa*	*A. Cherimola* x *A. squamosa* Abdel Razik
Capric	(C10:0)	7.69	-	-	0.14
Undecylic	(C11:0)	9.61	-	-	0.21
Lauric	(C12:0)	10.86	-	-	0.21
Myristic	(C14:0)	12.23	-	-	0.33
Palmitic	(C16:0)	15.55	18.7	18.40	20.27
Stearic	(C18:0)	17.48	1.28	8.29	2.05
Oleic	(C18:1)	19.52	51.15	51.33	46.54
Linoleic	(C18:2)	20.45	16.04	20.82	23.26
Linolenic	(C18:3)	21.72	10.19	0.70	1.89
3.cis-11Eicosenoic	(C20:1)	22.48	-	-	0.71
Eicosadienoic A	(C22:1)	24.68	-	0.44	1.22
Lignoceric	(C24:0)	27.03	-	-	0.25
Nervonic	(C24:1)	28.22	-	-	0.33
Cis-4,7,10,13,16,19Docosahexaenoic	(C22:6)	29.06	0.35	-	0.96
Mono unsaturated fatty acids			**51.15**	**51.33**	**46.54**
Poly unsaturated fatty acids			**26.23**	**21.52**	**25.15**
Unsaturated fatty acids			**78.66**	**73.29**	**73.95**
Saturated fatty acids			**19.98**	**26.69**	**23.46**
USFA/SFA ratio			**3.93**	**2.74**	**3.13**

Rt (Retention time)

As shown in Table (6) the major fatty acids varied according to *Annona* species seeds. In the case of *A. cherimola* the major unsaturated fatty acid is oleic comprising (18:1, 51.1%), followed by Linoleic acid (18:2, 16%) then linolenic (18:3, 10%), while in *A. squamosa* seeds the higher fatty acids is oleic acid (51.33%)followed by linoleic acid (20%), then traces of linolenic acid. In the case of cultivar Abdel Razik oleic acid (46%) is also the major followed by linoleic acid(18:2, 32%).

In the present investigation, among the identified fatty acids, oleic acid (C18:1) is predominant, then came in the second order linoleic acid (C18:2). These findings are in agreement with those determined in *A. squamosa* seed oil by Mariod *et al.* [22]. They reported that, *A. squamosa* seed oil, contained the following fatty acids palmitic, stearic, oleic and linoleic acids at the following percentages. 15.6%, 10.6%, 49.2% and 22.3%, respectively.

In the meantime, saturated fatty acid values are less than the values of monounsaturated as well as polyunsaturated fatty acids in Annona sp. seed oil.

Depending upon the above mentioned results, we can conclude that, oils derived from *A. cherimola*, *A. squamosa* , and cultivar Adel Razik were characterized by relatively high portions of unsaturated fatty acids than saturated ones and these oils represents a promising plant oil which may be a source of nutritive edible oil.

The antimicrobial activity due to the effect of the essential oils of different *Annona* are shown in Table (7) *A. cherimola* was the most effective oil, exhibiting a significant antimicrobial activity against most of the microorganisms tested, probably due to a synergistic effect of compounds present. The antimicrobial activity of these oils might be attributed to its mono and sesquiterpenes content.

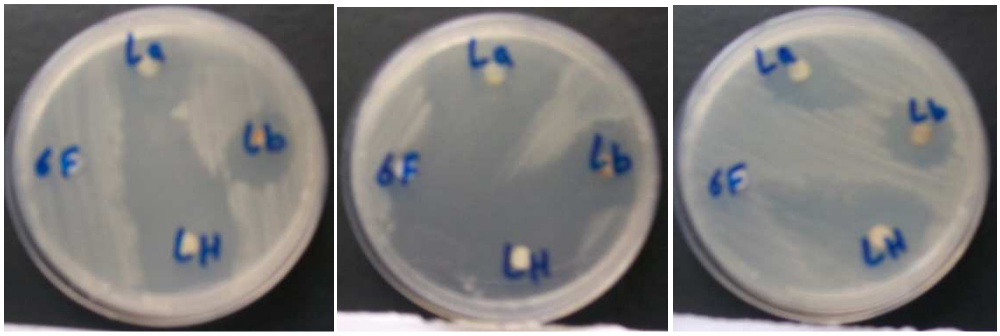
Table 7. Antimicrobial activities of volatile oil of *Annona* species (diameter of inhibition zone in mm)

Sample (100µl/disc)	Diameter of inhibition zone (mm)					
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus cereus*	*Saccharomyces cerevisiae*	*Cabdida albicans*

cultivar Adel Razik	25	20	22	19	27	26
A. squamosa	22	21	26	31	24	25
A. cherimola	30	26	28	35	32	30

Table (7) reveal that volatile oil of *A. cherimola* possesses higher antimicrobial activity against of microorganisma tested than the other two volatile oils, which induce different antimicrobial activity.

Bacillus cereus ***Bacillus Subtilis*** ***Cabdida albicans***



pseudomonas aeruginosa ***Saccharomyces cerevisiae*** ***Staphylococcus aureus***

Antioxidant activities reflected by the DPPH assay have demonstrated that the volatile oil of *A. cherimola* is relatively the best antioxidant agent when compared with other sample but still showed lower antioxidant activity than Vit C and Trolox at the concentrations tested.

Table 8. Antioxidant activity (%) against DPPH radical at different concentrations

Sample	DPPH Mean EC50 (µg/ml)
Fixed oil of *A. Cherimola* x *A. squamosa* Abdel Razik	>300
Fixed oil *A. squamosa*	>300
fixed oil of *A. Cherimola*	>300
unsap of *A. Cherimola* x *A. squamosa* Abdel Razik	>300
unsap of *A. squamosa*	>300
unsap of *A. Cherimola*	>300
sap of *A. Cherimola* x *A. squamosa* Abdel Razik	>300
sap of *A. squamosa*	>300
sap of *A. Cherimola*	>300
volatile oil of *A. Cherimola* x *A. squamosa* Abdel Razik	>300
volatile oil of *A. squamosa*	>300
volatile oil of *A. Cherimola*	241.76 ± 13.40
vit C	56.03 ± 1.80
trolox	34.45 1.80

**The EC50 values of the antioxidants calculated by GraphPad Prism*

Regarding DPPH_ assay, the EC_{50} is the antioxidant concentration required to obtain a 50% radical inhibition. Through the present study we provide the EC_{50} values through a mathematical calculation whereby they generated in table (8) Each extract was assayed at five different concentrations, within the range of 5–300 ug/ml. The concentrations were chosen according to their antiradical capacity towards the DPPH_ radical.

The results obtained for total antioxidant capacity were reported as ascorbic acid and trolox equivalents. The total antioxidant capacity of the two standards were 56.03 and 34.45 µg/ml. Few information about total antioxidant activity of different extracts of the three species were observed. The volatile oil of *A. Cherimola* in 241.76 ug/ml is the best extract.

In conclusion, our results show that the essential oils of *A. cherimola* are potent agents against most of the microorganisms either gram negative or positive and has an antioxidant activity.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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