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Phytochemical Screening, GC - MS Analysis, Antibacterial and Antioxidant Activity of Seeds Oil of *Annona Squmosa* L. Sudanese Medicinal Plant

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Abstract

The objective of this the study was examine Phytochemical analysis of *A. squmosa* ethanolic extracts and investigating the chemical constituent's and to assess it potential antibacterial and antioxidant activities of Sudanese *A. squmosa* seeds oil. Using cold maceration and Soxhlet method to extract the *A. squamosa* seeds, where DPPH assay and paper disc diffusion assay were employed to evaluate the antioxidant and antibacterial activities respectively. The results showed that Alkaloids, Flavonoid, Carbohydrate, Saponins, Triterpene, Streol, Tannins and phenolic compounds were present in the *A. squmosa* seeds ethanolic extracts where GC-MS analysis detected Twenty-one components in *the A. squmosa* seeds oil. Five of them are major namely, Hexadecanoic acid (18.48), Heptadecanoic acid Methyl margarate (1.32%), 9,12-octadecadienoic acid (Z, Z) (19.29%), 9 - octadecenoic acid (Z) (34.01%), Methyl stearate (17.12%), Cis-11-Eicosenoic acid (1.14%) and Eicosanoic acid (3.28%.). The DPPH assay, showed moderate antioxidant potential (50 ± 0.09 compared with standard 93± 0.01; the antibacterial showed showed high inhibitory effect against *Pseudomonas aeruginosa* (16mm), *Bacillus subtilis* (15mm), *Candida albicans* (15mm) moderate against *Escherichia coli* (13mm) and *Candida albicans*.

Keywords

Phytochemical Screening, GC-MS Analysis, Antibacterial and Antioxidant Activities

1. Introduction

The importance of the bioactive materials of plants in medicine and agriculture has stimulated significant interest in the bioactivities of substances [1]. *Annona squamosa* L. (Annonaceae) is a fruit tree with a long history of traditional uses. *A. squamosa* is an evergreen plant mainly located in tropical and subtropical regions. The fruits of A. squamosa, are extensively used to prepare candies, ice creams and beverages. A wide range of ethno-medicinal uses has been related to different portions of *A. squamosa*, such as tonic, apophlegmatisant, cool medicine, abortient and heart sedative. Numerous research projects on *A. squamosa* have found that it has anticancer, anti-oxidant, antidiabetic, antihypertensive, hepatoprotective, antiparasitic, antimalarial, insecticidal, microbicidel and molluscicidal activities [2]. *Annona squamosa* L., which is commonly known as sugar apple, custard apple, sweet sop, sweet apres and sitaphal, is a member of Annonaceae family, comprising approximately 135 genera and 2300 species [3, 4]. The birthplace of *A. squamosa* is not clear. It is a semi-deciduous tree widely distributed in tropical South America and in the West Indies. The Spaniards probably carried seeds from the New World to the Philippines and the Portuguese were assumed to introduce the sugar apple to southern India before 1590 [5]. Nowadays, it is cultivated in tropical and sub-tropical regions worldwide [6]. *A. s*quamosa is an ever-green tree reaching 3–8m in height. Leaf oblong lanceolate or lanceolate,

6–17 cm long and 3–5 cm wide, alternately arranged on short petioles; bark thin, gray; flower greenish, fleshy, drooping, extra-axillary, more on leafy shoot than on the older wood and tending to open as the shoot elongates; fruit can be round, heart-shaped, ovate or conical, 5–10 cm in diameter, with many round protuberance; seeds 1.3–1.6 cm long, oblong, smooth, shiny, blackish or dark brown [7]. In the south of China, seed extraction was used as a folkloric remedy for "malignant sores" (cancer) [8]. In traditional Indian, Thai, and American medicine, the leaves are used in a decoction to treat dysentery and urinary tract infection [9]. In Mexico, the leaves are rubbed on floors and put in hens' nests to repel lice [5] and used in diarrhea. *A. squamosa* is used as an insecticidal, an antitumor agent, and an anti-diabetic, antioxidant, anti-lipidomics and anti-inflammatory agent which has been characterized due to the presence of the cyclic peptides. In addition, the crushed leaves were sniffed to overcome the hysteria and fainting spells, and they were also applied on the ulcers and wounds. A leaf decoction was taken in the case of dysentery [10]. Previous studies showed that *A. squamosa* has many secondary metabolisms and some biological activities [11]. There are many bioactive compounds were isolated from the root extract of this plant [12]. Based on above knowledge the present study was designed to investigate the antioxidant properties and antibacterial activities of oil from Sudanese *Annona squamosa* seeds.

2. Materials and Methods

2.1. Plant Material

Annona squamosa (Gishta) seeds were collected in February 2018 from the Blue Nile state, Eldamazin, Sudan, and was identified at the herbarium of the Aromatic and Medicinal Plants Research Institute.

2.2. Preparation of Ethanolic Extracts

100 g of seeds were extracted three times with ethanol at room temperature (each 3days × 500ml), then the extract was filtered through the Buchner funnel. The ethanol was evaporated on a steam-bath and dried under reduced pressure to get10g. The residue was stored at 4°C in the dark for subsequent experiments. Phytochemicals screening for the active constituents was carried out using the methods described by [9, 13]. Mayer's, Wanger's, Hagar's, Drogndroff's reagents test (for alkaloids), $AlCl_3$, NH_4OH, KOH and Mg/H_2SO_4 test (for Flavonoids), Molisch's, Fehling, Barfoed, and Iodine test (for carbohydrates), water test (for saponins), Liberman test (for Triterpen/ Sterol), Ferric chloride and Aluminum chloride test (for tannins)

2.3. Extraction of Seed Oil

Soxhlet method [14] was used to extract the *A. squamosa* seeds. n-Hexane (boiling point, 60–70 C) was employed as the extraction solvent. Extraction was performed for 10h and the hexane was separated from the oil by distillation. Traces of Hexane were evaporated at 100 C in an air oven for 1 h. The oil was cooled in a desiccator to room temperature (25 C), put into a dark glass bottle, and stored at 4 C for further analysis.

2.4. GC.MS Method

The qualitative and quantitative analysis of the sample was carried out by using GC MS technique model (GC/MS-QP2010-Ultra) from japan "Simadzu Company, with capillary column (Rtx-5ms -30 m × 0.25 mm ×0.25 µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60 C with rate 10 C /min to 300 c as final temperature degree, the injection port temperature was 300c, the ion source temperature was 200 c and the interface temperature was 250 c. The sample was analyzed by using scan mode in the range of m/z 40 – 550 charge to ratio. Identification of component for the sample was achieved by comparing their retention times and mass fragmentation patent with those available in the library, the National Institute of Standards and Technology (NIST). Results were recorded.

2.5. Antibacterial Assay

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured. The results were interpreted in commonly used terms; >9mm: inactive; 9 - 12mm: partially active; 13-18mm: active; <18mm: very active).

2.6. DPPH Radical Scavenging Assay

The DPPH radical scavenging was determined according to the method of [15]. with some deification. In 96 wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µM). the test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All test and analysis were run in triplicate.

3. Results and Discussions

The preliminary phytochemical screening of of seeds of Annona squmosa in methanol showed the presence of various metabolic compounds like Alkaloid, carbohydrate, flavonoid, saponins, triterpenes, sterol, and tannins. The compounds have shown affirmative and strong response in methanol as presented

in Table 1.

Table 1. *Phytochemical screening of seeds ethanolic extracts.*

No	Constituents	Test	Results
1.	Alkaloids	Mayer's, Wanger's, Hagar's, Drogndroff's reagents	++++
2.	Flavonoid	$AlCl_3$	++++
		NH_4OH	++++
		KOH	++++
		Mg/H_2SO_4	-
3.	Carbohydrate	Molish's	+++
		Fehling	+++
		Barfoed	+++
		Iodine	-
4.	Saponins	Water	++++
5.	Triterpen/ Sterol	Liberman	+++/+
6.	Tannins, phenolic	Ferric chloride, Aluminum chloride	++

(+++)- Heavy; (++)-Medium; (+)-Low; (-)-indicates absent.

The chemical constituents of *A. squmosa* seeds oil were analyzed by GC-MS, revealed the presence of twenty one components. The GC-MS running time was 22.30 minutes, and twenty-one components were detected. The GC-MS chromatogram is presented in Figure 1. The name of active compounds with their Retention Time (RT), and peak area are presented in the Table 2. The spectra of the compounds are matched with Wiley 9.0 and NIST libraries.

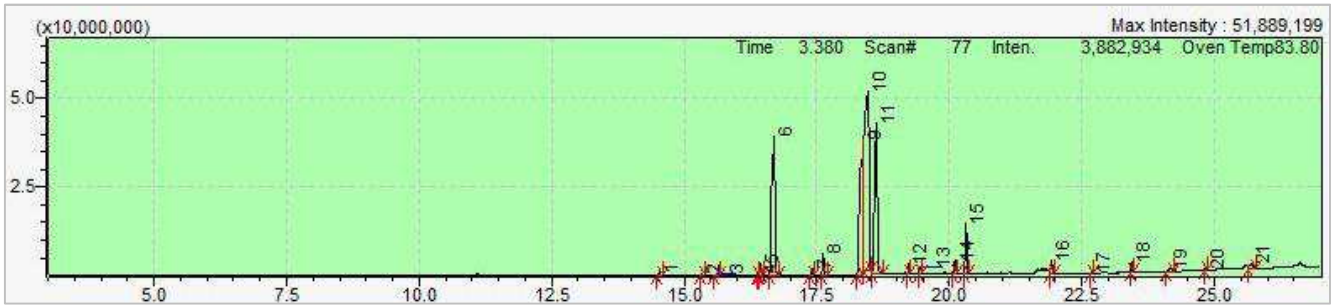

Figure 1. *The typical GC chromatogram of Annona squmosa seeds oil.*

Table 2. *The chemical constituents of seeds oil of A. squmosa.*

Peak report TIC				
Peak	**R.T**	**Area**	**Area %**	**Name**
1	14.536	1591941	0.19	Methyl tetradecanote, Myristic acid
2	15.344	340541	0.04	5-octadecenioc acid, methyl ester
3	15.610	973990	0.11	Pentadecanioc acid, methyl ester
4	16.403	1231218	0.14	7-Hexadecenoic acid, methyl ester (Z)
5	16.446	5415804	0.63	9-Hexadecenoic acid, methyl ester (Z)
6	16.698	158441738	18.48	Hexadecanoic acid, methyl ester
7	17.410	2845835	0.33	Cis- 10- Heptadecenoic acid, methyl ester
8	17.621	11303805	1.32	Heptadecanoic acid, methyl ester
9	18.375	165354795	19.29	9,12 octadecadienoic acid (Z, Z), methyl ester
10	18.473	291486670	34.01	9 - octadecenoic acid (Z), methyl ester
11	18.629	146726293	17.12	Methyl stearate
12	19.233	4722664	0.55	Cis – 10-Nonadecenoic acid, methyl ester
13	19.450	3642625	0.42	Nonadecanoic acid, methyl ester
14	20.111	9776306	1.14	Cis-11-Eicosenoic acid, methyl ester
15	20.315	28138544	3.28	Eicosanoic acid, methyl ester
16	21.92	7685312	0.90	Docosanoic acid, methyl ester
17	22.693	1014568	0.12	Tricosanoic acid, methyl ester
18	23.431	5473463	0.64	Tetracosanoic acid, methyl ester
19	24.157	4430505	0.52	Pentacosanoic acid, methyl ester
20	24.833	1063079	0.12	Hexacosanoic acid, methyl ester
21	25.699	5525170	0.64	A-Neogammacer-22 (29)-en-3-one
		857184866	100	

Five compounds namely 7- Hexadecanoic acid, methyl ester, heptadecanioc acid, methyl ester, 9, 12 octadecadienoic acid (Z, Z), methyl ester, 9- octadecenioc acid, methyl ester, and methyl stearate were found to be major in *Annona squmosa* seeds oil. Many minor constituents were also identified such as: Cis-11-Eicosenoic acid, methyl ester and

Eicosanoic acid, methyl ester.

Main constituents are: 7- Hexadecanoic acid, methyl ester, appeared at 16.698 min in the GC chromatogram with peak aria 18.48%, it was produced molecular ion m/z at270 $[M]^+$ corresponded to an elemental composition $C_{17}H_{34}O_2$ in it masss spectra Figure 2 as well as the following fragment ions: 227, 143, 87 and 74 (base peak) which are in agreement with literature [12]. This compound had been reported to cause autolysis of membranous structures, induce significant aortic dilation, and inhibit phagocytic activity and nitric oxide production of certain cells [16].

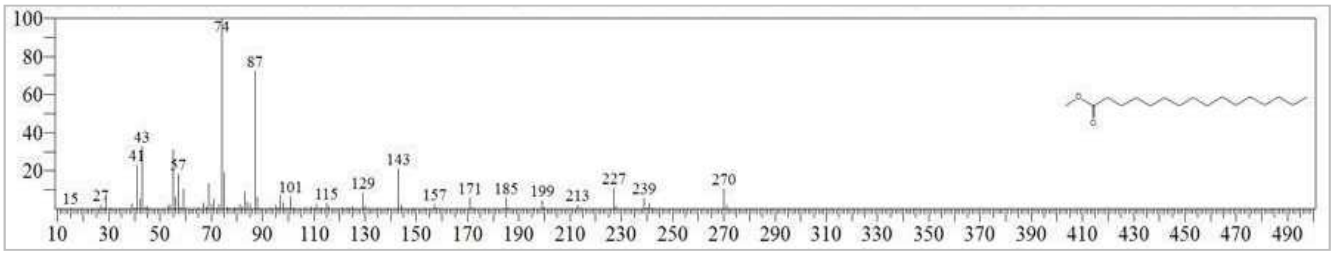

Figure 2. *The Mass spectrum of Hexadecanioc acid, methyl ester.*

The peak at 17.621min with area 1.32%, and has Ms ions *m/z* 284 $[M]^+$ correspond to formula $C_{18}H_{36}O_2$ Figure 3 as well as the following fragment ions: 253, 241, 143and 74 (base peak) which are in agreement with heptadecanioc acid, methyl ester.

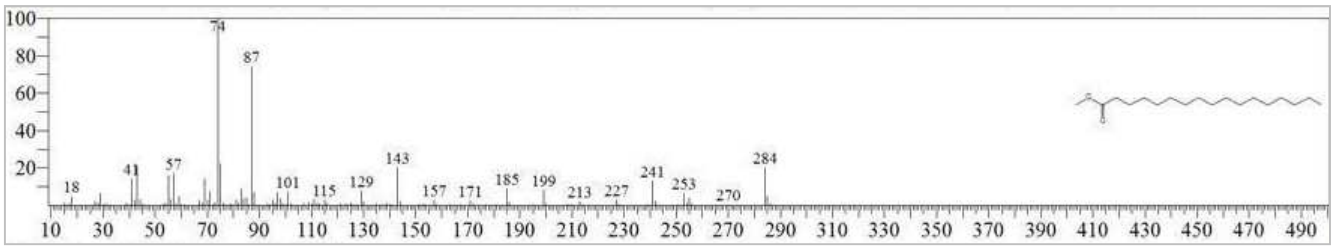

Figure 3. *The Mass spectrum of Heptadecanioc acid.*

The peak at 18.38 min with Area 19.29% on GC chromatogram, produced molecular ion peaks *m/z* at 294 $[M]^+$ corresponded to formula $C_{19}H_{34}O_2$ in it MS spectra Figure 4, in addition of fragment ions: 263, 150, 81 and 67 (base peak) by direct comparison with the Standard Mass Library spectral data and those reported in [17], this compound identified to be 9, 12 octadecadienoic acid (Z, Z), methyl ester which is known to has antifungal potential [18, 19].

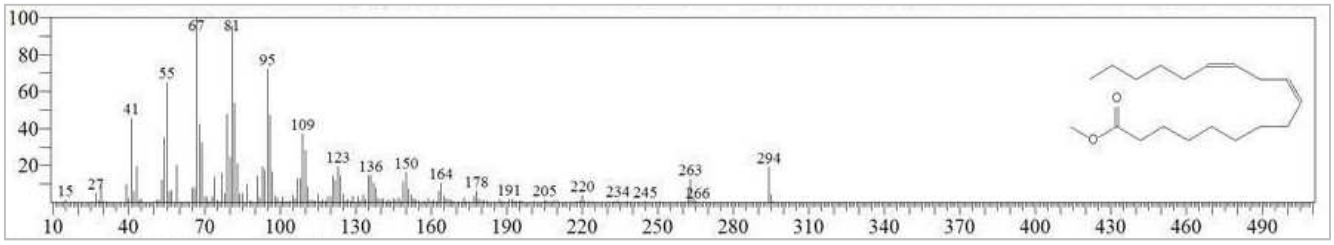

Figure 4. *The Mass spectrum of 9,12- octadecadienioc acid (Z-Z) methyl ester.*

There is another peak appeared at 18.473 min with area 34.01% it mass spectra produced molecular ion m/z at 296 $[M]^+$ corresponded to molecular formula $C_{19}H_{36}O_2$ Figure 5 as well as the following fragment ions: 222, 180, 97 and 55 (base peak) which are in agreement with 9- octadecenioc acid, methyl ester [20], which possess Antioxidant, anti-cancer [21]. The GC chromatogram showed peak at 18.629 with area 17.12%, it Mass spectrum showed ions *m/z* 298 $[M]^+$ correspond to formula $C_{19}H_{38}O_2$ Figure 6 as well as the following fragment ions: 267, 255, 199 and 74 (base peak) which are good agreement with methyl stearate. The chromatogram also showed two peaks at 20.111 and 20.315min with area 1.14% and 3.28% respectively, their mass spectrum Figure 7 and Figure 8, produced molecular ion peaks *m/z* at 324 $[M]^+$ and *m/z* at 326 $[M]^+$, these compounds identified to be Cis-11-Eicosenoic acid, methyl ester and Eicosanoic acid, methyl ester respectively.

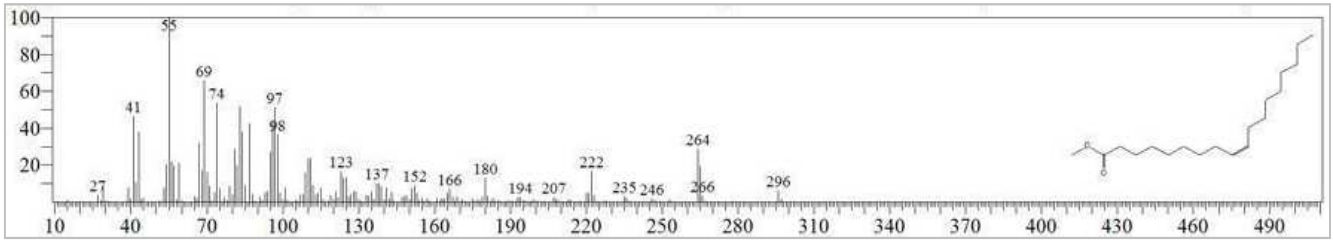

Figure 5. *The Mass spectrum of 9-octadecenioc acid, methyl ester.*

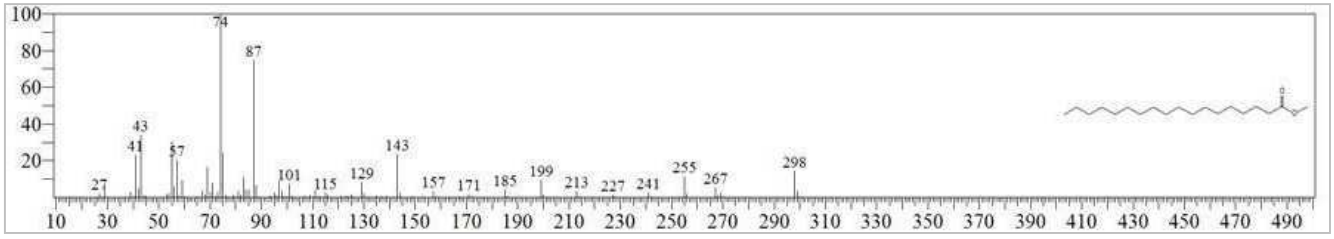

Figure 6. *The Mass spectrum of Methyl stearate.*

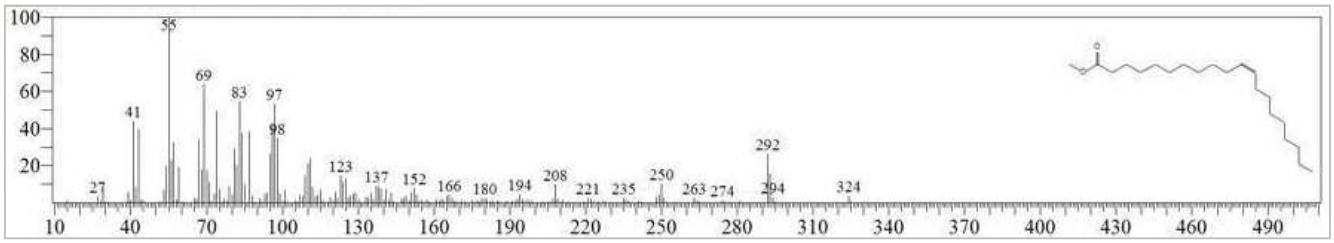

Figure 7. *The Mass spectrum of cis-11-nEicosenoic acid, methyl ester.*

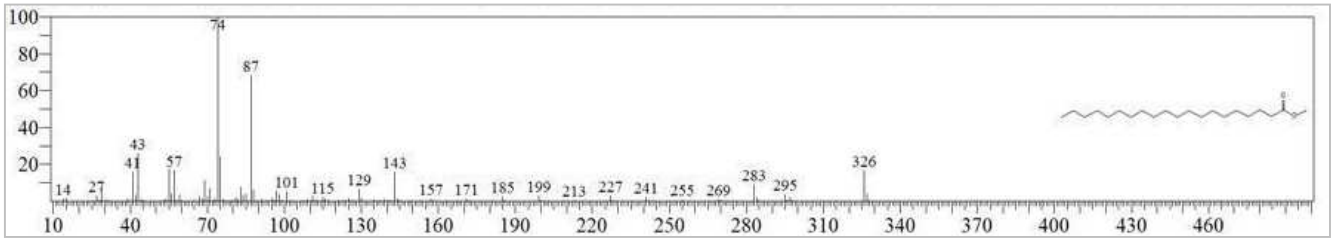

Figure 8. *The Mass spectrum of Eicosenoic acid, methyl ester.*

The seeds oil of *A. squmosa* showed an excellent antibacterial activity against prepared antibacterial comparison to standard, Ampicillin. The oil showed high inhibitory effect against against Pseudomonus aeregnosia (16mm), Bacillus subitus, aeregnosia (15mm), Candida albicans (15mm) moderate against *Escherichia coli* (13mm) and *Candida albicans* the observations results were recorded in Table 3.

Table 3. *Antibacterial activity of the A. squmosa seeds oil.*

Sample conc. 100 µl/ml	Zone of inhibition (mm)				
	Ec	Ps	Sa	Bs	Ca
A. squmosa seeds oil	13	16	10	15	15
Ampicillin	16	-	-	17	14

Ec=Escherichia coli, ps = Pseudomonus aureus; Sa=Staphylococcus Bs =Bacillus subitus, aeregnosia; Ca=Candida albicans.

According to DPPH scavenging activity was calculated Table 4. The seeds oil showed moderately antioxidant potential 50± 0.09 μg/mL activity comparable to that of Propyl Gallate (Standard) 93± 0.01 μg/mL against DPPH. The antioxidant activity may be attributed to the presence of unsaturated fatty acids. The lipid content may change due to oxidation of the oil which in turn affects the antioxidant potential of the oil.

Table 4. *Antioxidant activity of the A. squmosa seeds oil.*

Sample Code	%RSA±SD (DPPH) μg/mL
A. squmosa seeds oil	50± 0.09
Propyl Gallate (Standard)	93± 0.01

4. Conclusion

Phytochemical screening of ethanolic extacts of the *A. squmosa* seeds showed to contain alkaloids, flavonoids, tannins, triterpenes and carbohydrate, antibacterial activity of the seed oil was gave normal results ranged between weak to moderate activities against all bacterial organisms, GC-Ms analysis of the seeds oil reveals twenty one chemical constituents have been identified moreover the anti-oxidant activity and gave a moderately activity than DPPH radical scavenging assay

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