Research Article

Comparative Study of Chemical Constituents of the Seeds Extract of *Acacia Seyal* and Acacia *nilotica* and its effect as antimicrobial and antioxidant

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Abstract

This study was carried out to investigate the phytochemical constituents of the seeds extract of Acacia *seyal,* and Acacia *nilotica* and to study the constituents of their oils and to test the antimicrobial and antioxidant effect of the constutionts. Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, tannins, carbohydrates, phenols, glycosides, cardiac glycosides, quinones, anthraquinone, terpenoids, coumarins and steroids in all extracts. Quantitative analysis of some important bioactive constituents was carried out. The seeds oil were analyzed by GC-MS. The GC-MS revealed that the dominant constituents in *Acacia seyal* are: 9,12-octadecadienoic acid (Z,Z)-, methyl ester (30.19%), methyl stearate (15.77%), 9-octadecenoic acid (Z)-, methyl ester (15.57%), eicosanoic acid, methyl ester (6.89%), docosanoic acid, methyl ester (6.67%), 11,14-eicosadienoic acid, methyl ester (4.16%), tetracosanoic acid, methyl ester (3.73%), cis-11-Eicosenoic acid, methyl ester (3.17). The most dominant constituents in the seeds oil of *Acacia nilotica* are: 9,12-octadecadienoic acid (Z,Z), methyl ester (48.77%), 9-octadecenoic acid (Z)-, methyl ester (28.50%), hexadecanoic acid, methyl ester (9.44%), methyl stearate (8.75%). The oils were evaluated for their antimicrobial potential against standard pathogenic microbes. In the disc diffustion bioassay, *Acacia seyal* oil showed good activity against *Bacillus subtilis*, *Staphylococcus aureu* and *Pseudomonas aeruginosa* and good anticandidal potency. However, it showed weak activity against *Escherichia coli*. The oil of *Acacia nilotca* showed good activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* with good anticandidal potency. It also showed weak activity against *Pseudomonas aeruginosa*. The antioxidant activities of the oils were evaluated using 1,1-diphenyl-2-picrylhydraxyl (DPPH). The oils of *Acacia seyal* and *Acacia nilotica* showed very high DPPH radical scavenging activity. It seems that the oil of two spices is a lead for further optimization.

Keywords: ***Acacia Seyal*, *Acacia Nilotica*, Fixed oil, GC-MS, Antimicrobial Activity.**

Introduction

Plants oils and extracts have been used for a wide variety of purposes for many thousands of years. Recently, the essential oils and various extracts of plants have provoked interest as a source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the prevention of food from the toxic effects of oxidants [1]. In this study two medicinal plant species, *Acacia Seyal and Acacia Nilotica,* were considered.

Acacia seyal is phanerophyte**,** a thorny tree 3-17m high. Bark powdery, smooth or sparsely flaking, whitish to greenish yellow or orange-red. Stipules spinescent. Leaves 1-12 cm long, leaflets 7-20 paired 3-7 x 0.5-1.3mm, oblong to linear, flower bracts pubescent, medium line conspicuous, and 2.5mm long. Calyx pubescent, 4-6 lobed, white to yellow. Ovaries sessile brown 0.5mm long, style 3-4mm long. Pods falcate, dehiscent, constricted between seeds, venation longitudinal, 7-22cm long, 0.5-0.9cm wide. Seeds are olive-to-olive-brown, glabrous, compressed, elliptic lying longitudinally in the body, funicle very long and coiled, 1.5cm long. Flowering November–April, fruiting January–May [2]. *Acacia seyal* is native to Sudan, Egypt, Eritrea, Ethiopia, Ghana, Iran, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Saudi Arabia, Senegal, Syrian Arab Republic, Tanzania, Uganda, Yemen, Republic of Zambia, and Zimbabwe. Moreover, it is exotic to Afghanistan, Bangladesh, Bhutan, India, Nepal, Portugal, Sri Lanka and the US [3].

Acacia nilotica (L.) Del. is a tree 5–20 m high with a dense spherical crown, stems and branches usually dark to black coloured, fissured bark, greypinkish slash, exuding a reddish low-quality gum. The tree has thin, straight, light, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm (3 in) long in young trees, mature trees commonly without thorns. Flowers with globulous heads 1.2–1.5 cm in diameter of a bright golden-yellow color set up either axillary or whorly on peduncles 2–3 cm long located at the end of the branches. Pods are strongly constricted, white-grey, hairy and thick [4]. The species can withstand extremely hot temperature

(>50ºC) and can also endure floods. The species is sensitive to frost when it is young [5]. Trees can flower and fruit two to three years after germination, but after high rainfall it is more quickly, usually between March and June [6]. The bark has a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns [7]. *Acacia nilotica* is a tropical and subtropical genus with species abundant throughout Asia, Australia, Africa and America. *Acacia nilotica* occurs naturally and is imperative in traditional rural and agro-pastoral systems. The largest tracts are found in Sind. It is distributed throughout the greater part of India in forest areas, roadsides, farmlands, tank foreshores, agricultural fields, village grazing lands, wastelands, bunds, along the national highways and railway lines [8].

This study was aimed to:

- Screening of active phytochemical compounds from extracts of *Acacia seyal and Acacia nilotica.*
- Quantify the total flavonoid, Alkaloids and Saponin in the seeds extract.
- Characterize the chemical profile presents in *Acacia seyal and Acacia nilotica* seeds oil by using GC-MS analysis.
- Evaluate the seeds oil for their antimicrobial activity.
- Determination of antioxidant activity.

Materials

Plant material

Acacia seyal and Acacia nilotica: were collected from the Ministry of Agriculture and Forestry farm Shendi - Sudan. The seeds of plants were blended to powder using an electric blender and stored in air-tight bottles.

Bacterial microorganisms

- *Bacillus subtilis* NCTC 8236 (Gram + ve bacteria)
- *Staphylococcus aureus* ATCC 25923(Gram +ve Bacteria)
- *Escherichia coli* ATCC 25922(Gram -ve bacteria)
- *Pseudomonas aeruginosa* ATCC27853 (Gram -ve bacteria)

Fungal microorganisms

- *Candida albicans* ATCC7596

Methods

Preparation of extracts

The extraction of the different plant extracts

and preservation of the extracts was done according to the method of Obomanu, *et al*. precisely [9], 50 g of each powdered dried seeds were extracted for 72 hours using 95% ethanol in a 200 ml beaker and later transferred to an air-tight bottle to avoid escape of the solvent. The sample was filtered using muslin cloth and later filter paper. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator to obtain crude extracts. Extract obtained was kept in an air-tight bottle until it was used for phytochemical screening test.

Qualitative phytochemical analysis

The phytochemical components of the plant extracts were screened using standard procedures as described by (Harbome, 1998; Sofowora, 1993; and Trease and Evans, 1989) [10-12].

Test for Flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicated the presence of flavonoids.

Test for Alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicated the presence of alkaloids.

Test for Saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicated the presence of tannins.

Test for carbohydrates

Fehlings test, Equal volume of Fehling A (copper sulphate in distilled water) and Fehling solution B (Potassium tartarate and sodium hydroxide in distilled water) reagents were mixed with few drops of crude extract and boiled, a brick red precipitate of cuprous oxide forms, indicate the presence of reducing sugar.

Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicated the presence of phenols.

Test for Glycosides

To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution

was added. Formation of pink color indicated the presence of glycosides.

Test for Cardiac Glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated the presence of cardiac glycosides.

Test for Quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicated the presence of quinones.

Anthraquinone

To 1ml of plant extract, a few drops of 10% ammonia solution was added and the appearance of pink color precipitate indicated the presence of anthraquinones.

Test for Terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of the residue re-extracted three times with the same volume of ethanol. The whole solution of each seeds sample was filtered through Whatman filter paper No 42 (125 mm). The filtrate of each seeds sample was later transferred into a crucible and evaporated to dryness over a red brown color at the interface indicated the presence of terpenoids.

Test for Coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicated the presence of coumarins.

Steroids and Phytosteroids

To 1ml of plant, an extract equal volume of chloroform was added and subjected with a few drops of concentrated sulphuric acid where the appearance of brown ring indicated the presence of steroids and appearance of bluish brown ring indicated the presence of phytosteroids.

Quantitative phytochemical analysis

Determination of flavonoids

Each seeds powder weighing about 10 g was placed in a 250 cm^3 beaker and 50 cm^3 of 80% (v/v) aqueous methanol added, covered and allowed to stand For 24 hours at room temperature. The supernatant was discarded and water bath. The crucible and its contents were cooled in a desiccator and weighed until constant weight was obtained [13]. The percentage of flavonoids is expressed mathematically as:

$$\% \; Flavonoids \; \% = \frac{Weight\ of\ flavonoids}{Weight\ of\ sample} \times 100$$

Determination of Alkaloids

About 5 g of each seeds powder was weighed into a 250-cm^3 beaker and 200 cm^3 of 10 % (v/v) acetic acid in ethanol was added to each seeds Powder and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation was complete. The whole mixture was allowed to settle for 3 hours, the supernatant was discarded and then precipitates washed with 20cm^3 of 0.1M of ammonium hydroxide and then filtered using Gem fitter paper (12.5cm). The residue was dried in an oven and weighed using electronic weighing balance [10].

The percentage of alkaloid can be expressed mathematically as:

$$\% \; Alkaloids \; \% = \frac{Weight \; of \; Alkaloids}{Weight \; of \; sample} \times 100$$

Determination of Saponin

20 gram of each seeds powder was put in to a 250 cm^3 conical flask and 100 cm^3 of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55ºC. The mixture was filtered and the residue re-extracted with another 100 cm^3 of 20% (v/v) aqueous ethanol, heated for 4 hours at a constant temperature of 55ºC with constant stirring. The combined extract was reduced to 40 cm^3 over the water bath at a temperature of 90ºC. The concentrate was transferred in to a 250cm^3 separator funnel and 20cm^3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice. 60cm^3 of n- butanol was added and the butanol extract was washed twice with 10cm^3 of 5 % (w/v) sodium chloride. The sodium chloride layer was discarded and the remaining solution heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight [14]. The saponin content was calculated as a percentage:

$$\% \; Saponin \; \% = \frac{Weight \; of \; Saponin}{Weight \; of \; sample} \times 100$$

Oil Extraction

The oil from the seeds was extracted with the Soxhlet method according to the Methods of analysis used for chemical control of food of the National Institute

of Health, 1996. A sample of 500 g of mature seeds was extracted using n-hexane, for 6 hrs. The solvent was removed with a rotary evaporator and the oil was kept in the fridge at 4°C for further manipulation.

GC-MS analysis

The qualitative and quantitative analysis of the sample was done by using GM/MS technique model (GC/MS-QP2010-Ultra) from japans 'Simadzu Company, with capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61ml/min, the temperature program was started from 60^0C with rate 10^0C/min reaching 300^0C as final temperature degree, the injection port temperature was 300^0C, the ion source temperature was 200^0C and the interface temperature was 250^0C. The sample was analyzed by using scan mode in the range of m/z 50-550 charges to ratio. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library, the National Institute of Standards and Technology (NIST).

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Table (1): Chromatographic conditions

Column oven temperature	150.0ºC
Injection temperature	300.0ºC
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial test

Bacterial suspensions

One ml aliquots of a 24 hours broth cultureof the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterialgrowth was harvested and washed off with 100 ml sterile normal saline, to produce a suspensionsuspension containing about 10^8- 10^9 C.F.U/ ml. Thesuspension was stored in the refrigerator at 4° Cconditions were maintained constantly so that till used. The average number of viableorganisms per ml of the stock suspension wasdetermined by means of the surface viable counting technique (Miles and Misra, 1938) [15]. Serial dilutions of the stock suspension weremade in sterile normal saline solution and ml volumes of the appropriate dilution weretransferred by micro pipette onto the surface ofdried nutrient agar plates. The plates werallowed to stand for two hours at roomtemperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constantly so that suspensions with very close viable counts would be obtained.

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 0.024 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspended was stored in the refrigerator until used.

Testing of antimicrobial susceptibility

The paper disc diffusion method was used to screen the antimicrobial 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 suspensions 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.

Antioxidant activity of seeds oil

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method by (Shimada, *et al*., 1992) [16] with some modifications. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 µm. The extract was dissolved in DMSO (500µg/ml. concentration), while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. The percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and propyl gallate (PG). All tests and analysis were run in triplicate.

Results and Discussion

Phytochemical analysis

The seeds extract of *Acacia seyal and Acacia nilotica* were subjected to phytochemical screening, which indicated the presence of Flavonoids, Alkaloids, Saponins, Tannins, Carbohydrates, Phenols, Glycosides, Cardiac Glycosides, Quinones, Anthraquinone, Terpenoids, Coumarins and Steroids in both extract (Table 2).

Table (2): phytochemical screening analysis of *Acacia seyal and Acacia nilotica* seeds extracts

Phytochemical	Plant extract		
compounds	***Acacia seyal***	***Acacia nilotica***
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+

Tannins	+	+
carbohydrates	+	+
Phenols	+	+
Glycosides	+	+
Cardiac Glycosides	+	+

+ (contain this phytochemical compound)

Quantitative phytochemical analysis

Quantitative determination of the total active constituents (Flavonoids, Alkaloids and Saponins) of the plants under investigation is as summarized in Table (3). The results revealed that maximum Flavonoids content (8.95 ± 0.04%) was recorded in *Acacia nilotica* followed by *Acacia seyal* (8.05 ± 0.03%). Alkaloids were observed to contain the highest percentage in *Acacia nilotica* (7.1 ± 0.01%) followed by *Acacia seyal* (6.3 ± 0.02%). Maximum quantity of Saponins were observed in *Acacia nilotica* (2.15 ± 0.03%) followed by *Acacia seyal* (2.1 ± 0.00%).

Table (3): percentage of Flavonoids, Alkaloids and Saponins

Quinones	+	+
Anthraquinone	+	+
Terpenoids	+	+
Coumarins	+	+
Steroids	+	+

Plant species	Flavonoids (%)	Alkaloids (%)	Saponins (%)
Acacia seyal	8.05 ± 0.03	6.3 ± 0.02	2.1 ± 0.00
Acacia nilotica	8.95 ± 0.04	7.1 ± 0.01	2.15 ± 0.03

GC-MS analysis of *Acacia seyal* seeds oil

The seeds oil of *Acacia seyal* was analyzed by GC-MS. Twenty-eight components appeared in total ion chromatogram (Fig1). Different components were quantified and identified by their retention time and mass spectra and a tabulation of constituents is presented in Table (4).

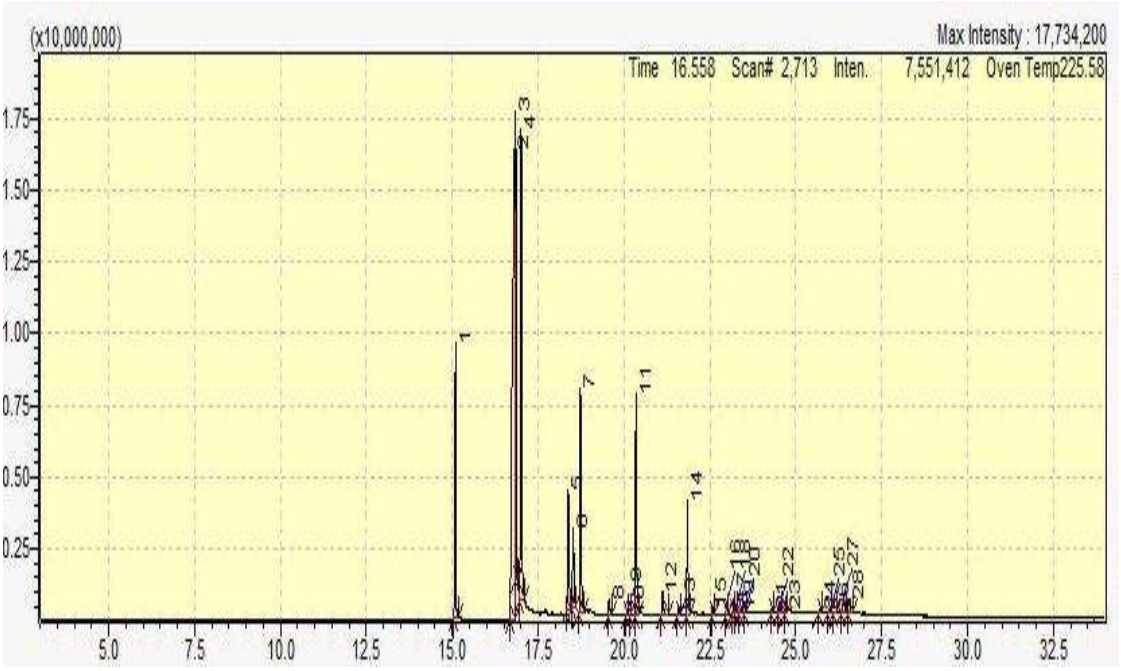


Fig.1: Total ion Cromatograms of *Acacia seyal* seeds oil

Table (4): Constituents of *Acacia seyal* seeds oil

ID#	Name	Ret.Time	Area	Area%
1.	Hexadecanoic acid, methyl ester	15.094	21847889	8.46
2.	9,12-octadecadienoic acid (Z,Z)-, methyl ester	16.806	77962744	30.19
3.	9-octadecenoic acid (Z)-, methyl ester	16.846	40216972	15.57
4.	Methyl stearate	17.005	40734668	15.77
5.	11,14-eicosadienoic acid, methyl ester	18.376	10758513	4.16
6.	cis-11-eicosenoic acid, methyl ester	18.525	8182598	3.17
7.	Eicosanoic acid, methyl ester	18.730	17810999	6.89
8.	Heneicosanoic acid, methyl ester	19.566	1307215	0.51
9.	Eicosane	20.074	239445	0.09
10.	13-docosenoic acid, methyl ester, (Z)-	20.181	88499	0.03
11.	Docosanoic acid, methyl ester	20.345	17752490	6.87
12.	Tricosanoic acid, methyl ester	21.120	2632596	1.02
13.	Docosane	21.579	654467	0.25
14.	Tetracosanoic acid, methyl ester	21.845	9633368	3.73
15.	Pentacosanoic acid, methyl ester	22.570	509618	0.20
16.	Tetracosane	22.979	805176	0.31
17.	Pentadecane, 8-hexyl-	23.193	1092775	0.42
18.	Hexacosanoic acid, methyl ester	23.252	353422	0.14
19.	Tetratriacontane	23.389	231913	0.09
20.	Nonacosane	23.516	89761	0.03
21.	Tetratetracontane	24.303	193237	0.07
22.	Pentatriacontane	24.511	590110	0.23
23.	Hexatriacontane	24.694	184256	0.07
24.	Campesterol	25.718	382688	0.15
25.	Stigmasterol	25.991	395792	0.15
26.	Dotetracontane	26.130	116294	0.05
27.	Fucosterol	26.391	1982088	0.77
28.	.gamma.-Sitosterol	26.542	1584104	0.61

The following major constituents were detected by GC-MS:

9,12-octadecadienoic acid (Z,Z)-, methyl ester (30.19%)

Synonyms: Linoleic acid, methyl ester. This compound was reported with Anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge, anti-histaminic, anti-eczemic, anti-acne, 5-Alpha reductase inhibitor anti-androgenic, anti-arthritic, anti-coronary, and insectifuge [17].

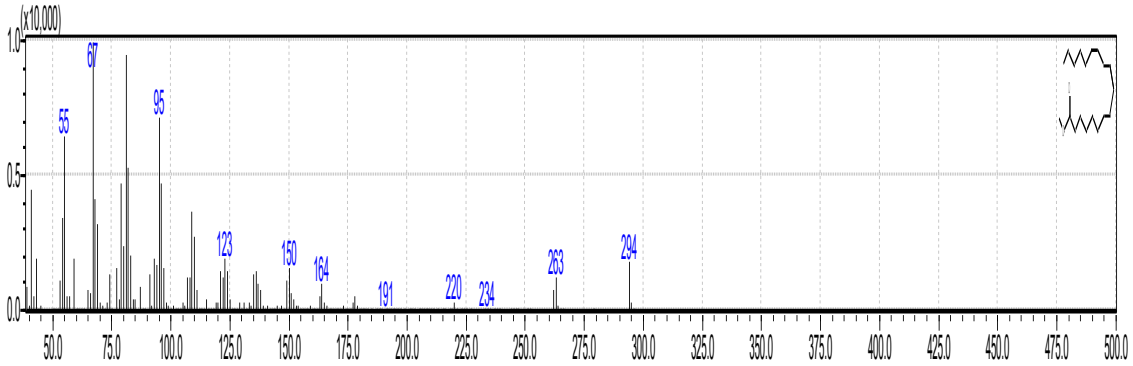


Fig.2: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

The EI mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is shown in Fig. 2. The peak at m/z 294, which appeared at R.T. 16.806 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxy function.

Methyl stearate (15.77%)

Synonyms: Octadecanoic acid, methyl ester and Stearic acid, methyl ester. This compound was reported with anti-foaming agent, fermentation nutrient, and flavoring agent [18, 19].

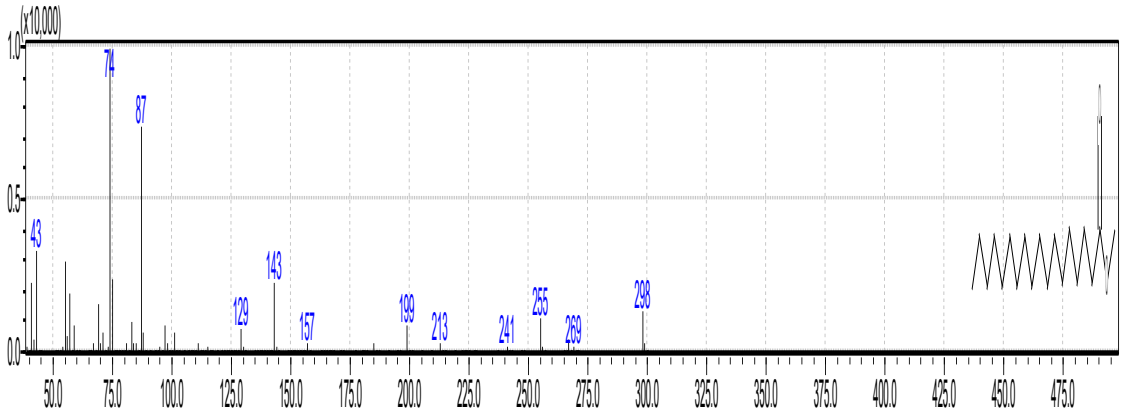


Fig.3: Mass spectrum of Methyl stearate

The EI mass spectrum of Methyl stearate is shown in Fig. 3. The peak at m/z 298, which appeared at R.T. 17.005 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{38}O_2]^{+}$. The peak at m/z 269 corresponds to loss of a methoxy function.

9-octadecenoic acid (Z)-, methyl ester (15.57%)

Synonyms: Oleic acid, methyl ester. This compound was reported with following potential properties like Flavour [20], cancer preventive [21], anti-inflammatory [22] and anti-microbial activity [23].

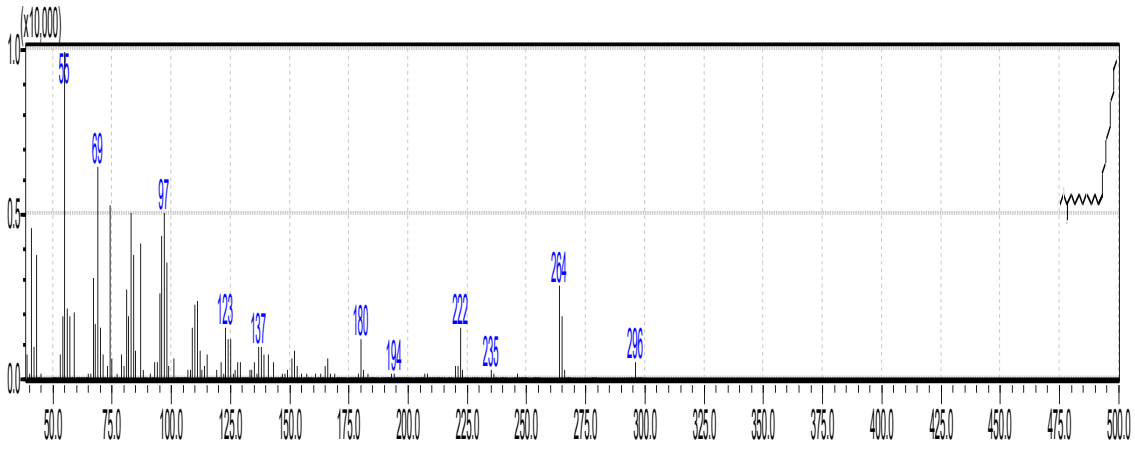


Fig.4: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

The EI mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig.4. The peak at m/z 296, which appeared at R.T. 16.846 in total ion chromatogram,

corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 264 corresponds to loss of a methoxy function.

Eicosanoic acid, methyl ester (6.89%)

Synonyms: Methyl arachisate, methyl eicosanoate and arachidic acid methyl ester. The compound was reported to be alpha-glucosidase inhibitors activity [24].

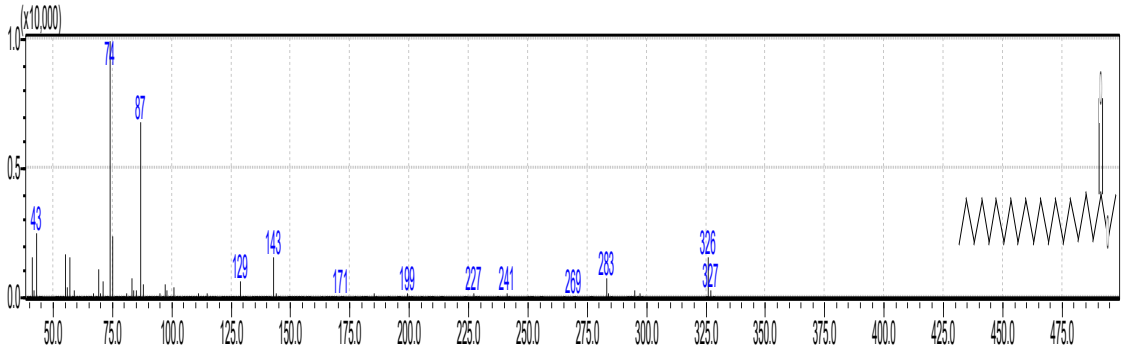


Fig.5: Mass spectrum of Eicosanoic acid, methyl ester

The EI mass spectrum of Eicosanoic acid, methyl ester is shown in Fig. 5. The peak at m/z 326, which appeared at R.T. 18.730 in total ion chromatogram, corresponds to $M^+[C_{21}H_{42}O_2]^+$. The peak at m/z 283 corresponds to loss of an allyl Cation function.

Docosanoic acid, methyl ester (6.67%)

Synonyms: Behenic acid, methyl ester.

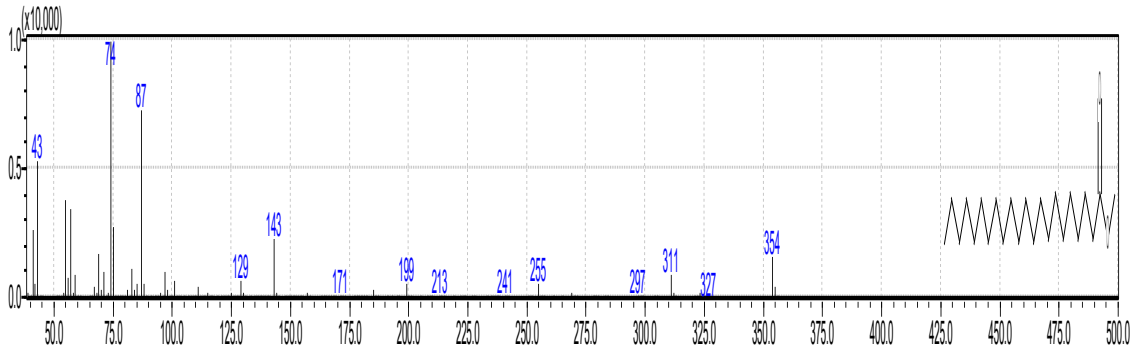


Fig.6: Mass spectrum of Docosanoic acid, methyl ester

The EI mass spectrum of Docosanoic acid, methyl ester is shown in Fig. 6. The peak at m/z 354, which appeared at R.T. 20.345 in total ion chromatogram, corresponds to $M^+[C_{23}H_{46}O_2]^+$. The peak

at m/z 311 corresponds to loss of an allyl Cation function.

11,14-eicosadienoic acid, methyl ester (4.16%)

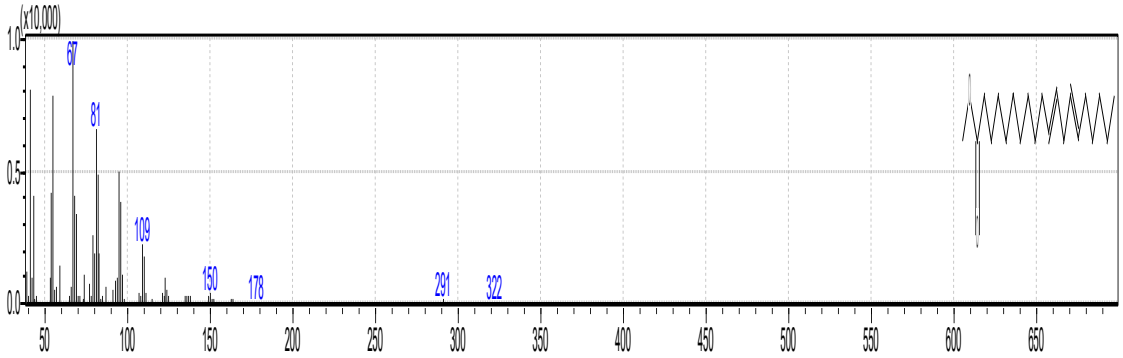


Fig.7: Mass spectrum of 11,14-eicosadienoic acid, methyl ester

The EI mass spectrum of 11,14-eicosadienoic acid, methyl ester is shown in Fig. 7. The peak at m/z 322, which appeared at R.T. 18.376 in total ion chromatogram, corresponds to $M^+[C_{21}H_{38}O_2]^+$. The peak at m/z 291 corresponds to loss of a methoxy function.

Tetracosanoic acid, methyl ester (3.73%)

This compound was reported with anti-oxidant, anti-microbial, Lubricant, anti-cancerous, and cosmetics [25].

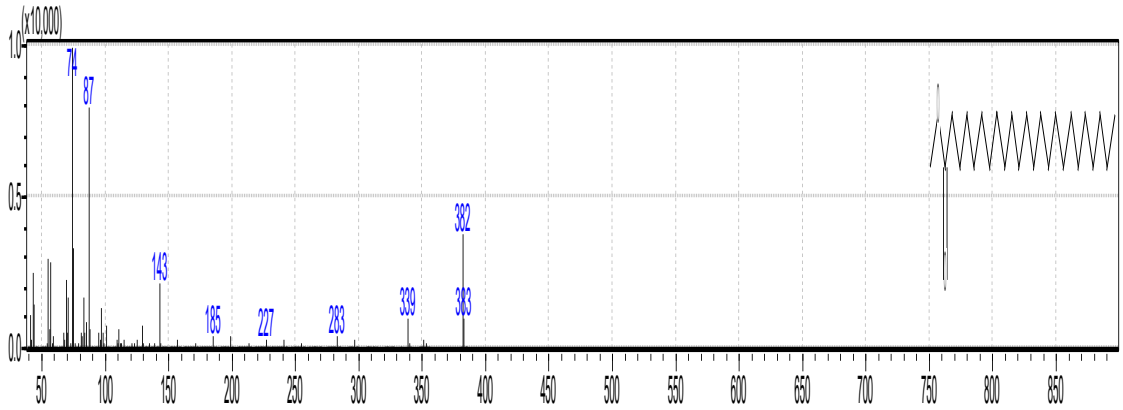


Fig.8: Mass spectrum of Tetracosanoic acid, methyl ester

The EI mass spectrum of Tetracosanoic acid, methyl ester is shown in Fig. 8. The peak at m/z 382, which appeared at R.T. 21.845 in total ion chromatogram, corresponds to $M^+[C_{25}H_{50}O_2]^+$. The peak at m/z 339 corresponds to loss of an Allyl Cation function.

Cis-11-eicosenoic acid, methyl ester (3.17)

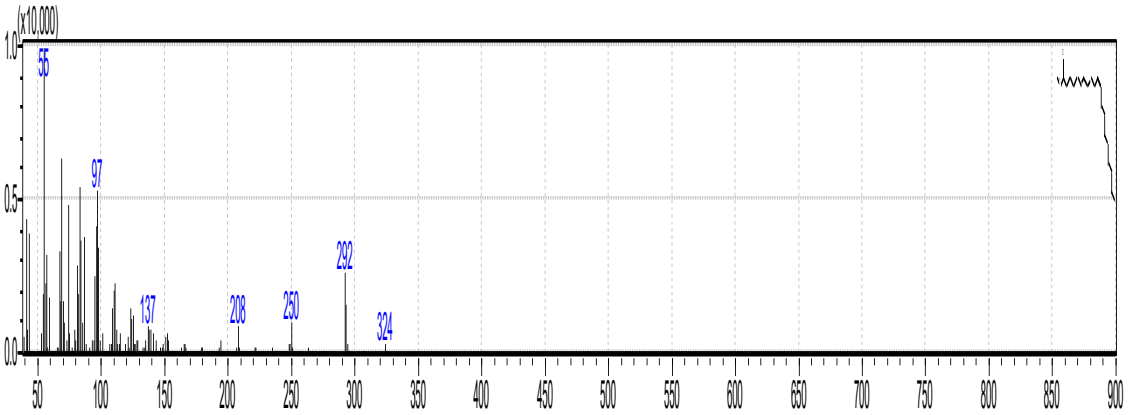


Fig.9: Mass spectrum of cis-11-eicosenoic acid, methyl ester

The EI mass spectrum of cis-11-eicosenoic acid, methyl ester is shown in Fig. 9. The peak at m/z 324, which appeared at R.T. 18.525 in total ion chromatogram, corresponds to $M^+[C_{21}H_{40}O_2]^+$. The peak at m/z 292 corresponds to loss of a methoxy function.

GC-MS analysis of *Acacia nilotica* seeds oil

The seeds oil of *Acacia nilotica* was analyzed by GC-MS. Nine components appeared in total ion chromatogram (Fig.10). Different components were quantified and identified by their retention time and mass spectra and a tabulation of constituents is presented in Table (5).

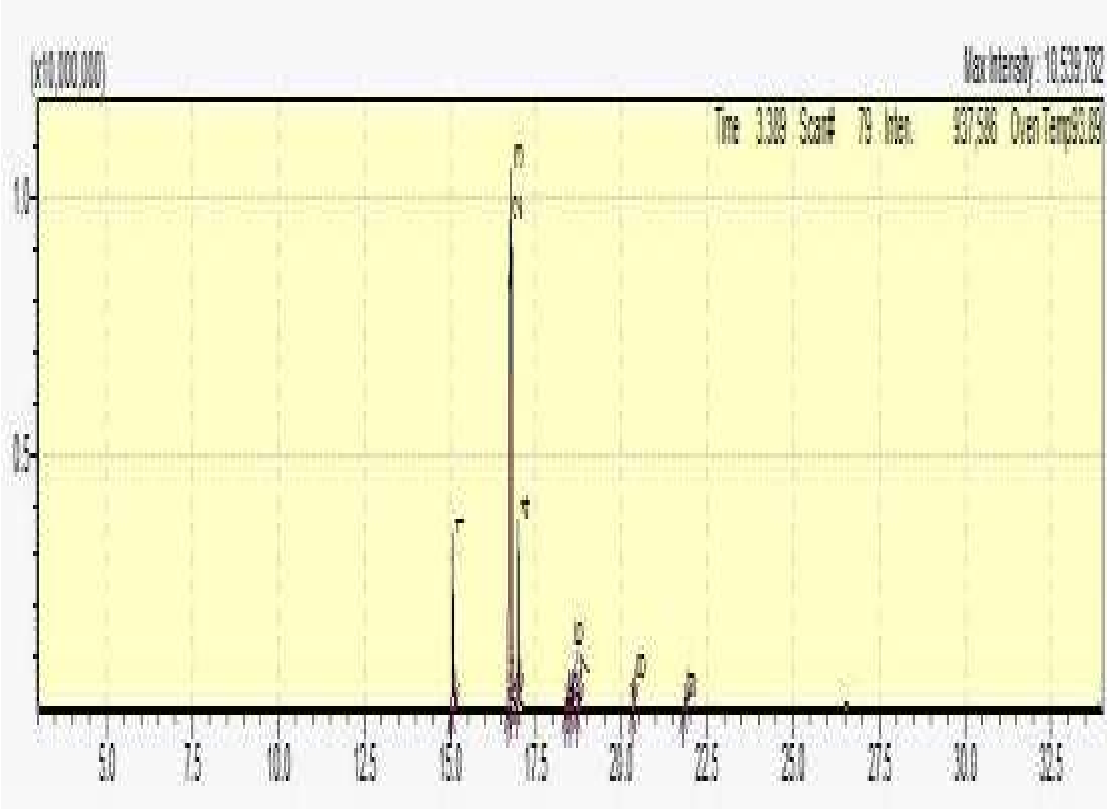


Fig.10: Total ion Cromatograms of *Acacia nilotica* seeds oil

Table (5): Constituents of *Acacia nilotica* seeds oil

ID#	Name	Ret.Time	Area	Area%
1.	Hexadecanoic acid, methyl ester	15.068	6270387	9.44
2.	9,12-octadecadienoic acid (Z,Z)-, methyl ester	16.753	32384744	48.77
3.	9-octadecenoic acid (Z)-, methyl ester	16.791	18928109	28.50
4.	Methyl stearate	16.981	5810972	8.75
5.	9,15-octadecadienoic acid, methyl ester	18.384	405439	0.61
6.	11-octadecenoic acid, methyl ester	18.527	271913	0.41
7.	Eicosanoic acid, methyl ester	18.731	1011306	1.52
8.	Docosanoic acid, methyl ester	20.345	938803	1.41
9.	Tetracosanoic acid, methyl ester	21.846	390091	0.59

The following major constituents were detected by GC-MS:

9,12-octadecadienoic acid (Z,Z)-, methyl ester (48.77%)

Synonyms: Linoleic acid, methyl ester. This compound was reported with anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge, anti-histaminic, anti-eczemic, anti-acne, 5-Alpha reductase inhibitor anti-androgenic, anti-arthritic, anti-coronary, and insectifuge [17].

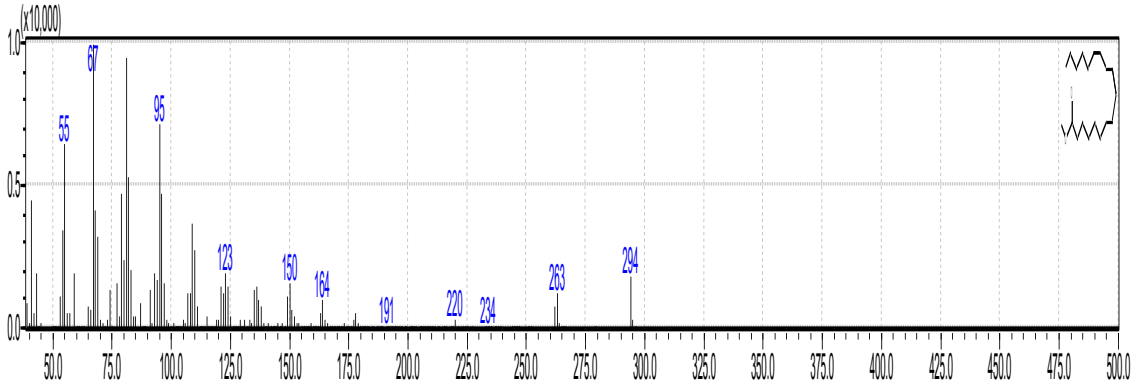


Fig.11: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

The EI mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester is shown in Fig. 11. The peak at m/z 294, which appeared at R.T. 16.753 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxy function.

9-Octadecenoic acid (Z)-, methyl ester (28.50%)

Synonyms: Oleic acid, methyl ester. This compound was reported with following potential properties like flavour [20], cancer preventive [21], anti-inflammatory [22] and anti-microbial activity [23].

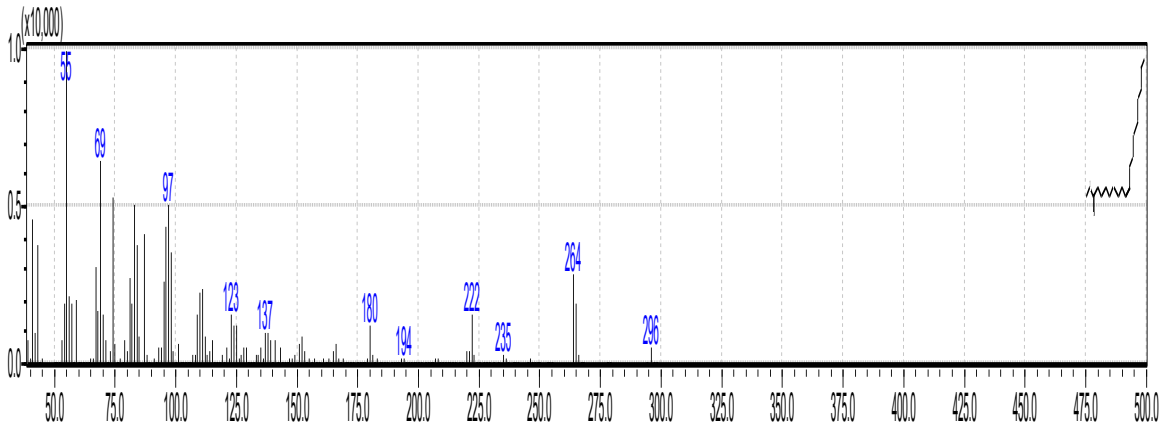


Fig.12: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

The EI mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig. 12. The peak at m/z 296, which appeared at R.T. 16.791 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 264 corresponds to loss of a methoxy function.

Hexadecanoic acid, methyl ester (9.44%)

Synonyms: Palmitic acid, methyl ester. Anti-bacterial, hypercholesterolemic, lubricant, [26], flavours, cosmetic, and perfumery [27], properties of this compound have already been reported.

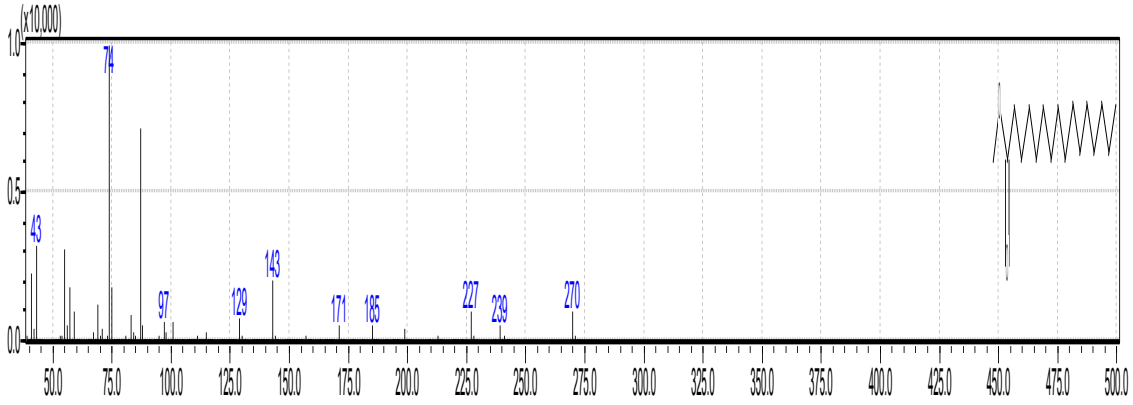


Fig.13: Mass spectrum of Hexadecanoic acid, methyl ester

The EI mass spectrum of Hexadecanoic acid, methyl ester is shown in Fig. 13. The peak at m/z 270, which appeared at R.T. 15.068 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$. The peak

at m/z 239 corresponds to loss of a methoxy function.

Methyl stearate (8.75%)

Synonyms: Octadecanoic acid, methyl ester and Stearic acid, methyl ester. This compound was reported with anti-foaming agent, fermentation nutrient and flavoring agent [18, 19].

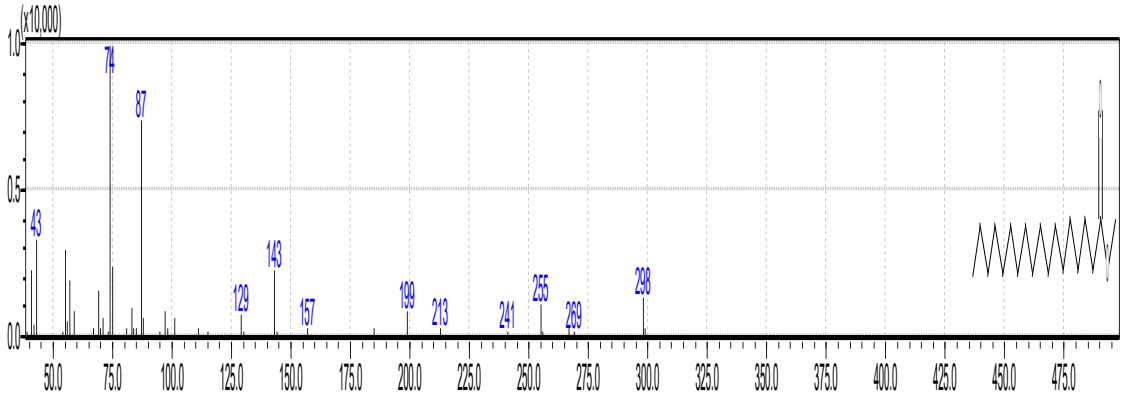


Fig.14: Mass spectrum of Methyl stearate

The EI mass spectrum of Methyl stearate is shown in Fig. 14. The peak at m/z 298, which appeared at R.T. 16.981 in total ion chromatogram, corresponds to $C_{19}H_{38}O_2$ $M^+[C_{19}H_{38}O_2]^+$. The peak at m/z 269 corresponds to loss of a methoxy function.

Antimicrobial activity

The target oils were evaluated for antimicrobial activity via the cup plate agar diffusion assay. The average of the diameters of the growth inhibition zones are shown in Tables (6-7). The results were interpreted in terms of the commonly used terms (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). In the disc diffustion bioassay, *Acacia seyal* seeds oil showed good activity against *Bacillus subtilis*, *Staphylococcus aureu* and *Pseudomonas aeruginosa* and good anticandidal potency. However, it showed weak activity against *Escherichia coli*. The seeds oil of *Acacia nilotca* showed good activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* and good anticandidal potency. It also showed weak activity against *Pseudomonas aeruginosa*.

Table (6): The antimicrobial activity of *Acacia seyal* seeds oil

Microorganism	species	type	Inhibition zone diameter (mm)
			Con (100mg/ml)
Bacteria	*Bacillus subtilis*	+ ve	14
	Staphylococcus aureus	+ ve	14
	Escherichia coli	+ ve	10
	Pseudomonas aeruginosa	- ve	13
Fungi	*Candida albicans*	- ve	15

Table (7): The antimicrobial activity of *Acacia nilotca* seeds oil

Microorganism	species	type	Inhibition zone diameter (mm)
			Con (100mg/ml)
Bacteria	*Bacillus subtilis*	+ ve	15
	Staphylococcus aureus	+ ve	14
	Escherichia coli	+ ve	14
	Pseudomonas aeruginosa	- ve	12
Fungi	*Candida albicans*	- ve	14

Antioxidant activity

The target oils were tested for antioxidant screening for their free radical scavenging properties using 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH assay), while propyl galate was used as standard antioxidant. Based on findings of Dudonne, *et al*. (2009) [28], DPPH inhibition was categorized as very low (1-10%), low/week (10 - 30%), moderate (30-60%), high (61-69%) and very high (70-100%). The Radicals scavenging assay is shown in Table (8). The oils of *Acacia seyal* and *Acacia nilotica*

showed very high DPPH radical scavenging activity.

Table (8): the radical scavenging assay (DPPH) of *Acacia seyal* and *Acacia nilotica* seeds oil

Sample	**RSA%**
Acacia seyal	91 ± 0.04
Acacia nilotica	92 ± 0.03
propyl gallate (standard)	92 ± 0.01

RSA* = Radicals scavenging assay

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