Research Article

Phytochemical Screening and Investigation of Oil of *Acacia tortilis* seeds.

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Abstract

This study was carried out to assess the quality and quantity of some phytochemicals present in the seeds of *Acacia tortilis* as well as to study the constituents of its oil and to evaluate the antimicrobial and antioxidant effect of it.

Phytochemical screening revealed the presence of Flavonoids, Alkaloids, Saponins, Tannins, Carbohydrates, Phenols, Glycosides, Cardiac Glycosides, Quinones, Anthraquinone, Terpenoids, Coumarins and Steroids are present in *Acacia tortilis* extract. Quantitative analysis of some important bioactive constituents was carried out.

The oil was analyzed by GC-MS. GC-MS revealed that the dominant constituents in *Acacia tortilis* oil are; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (46.50%), 9-Octadecenoic acid (Z)-, methyl ester (23.48%), Hexadecanoic acid, methyl ester (12.64%), Methyl stearate (8.40%), Docosanoic acid, methyl ester (2.65%), Eicosanoic acid, methyl ester (2.61%). The oil was evaluated for its antimicrobial potential against standard pathogenic microbes. In the disc diffusion bioassay, *Acacia tortilis* oil showed good activity against *Bacillus subtilis*. *Escherichia coli* and *Staphylococcus aureus*. It also showed weak anticandidal potency. The oil had no activity against *Pseudomonas aeruginosa*. The antioxidant activities of the oil were evaluated using 1,1-diphenyl-2-picrylhydraxyl (DPPH). The oil of *Acacia tortilis* showed very high DPPH radical scavenging activity.

Keywords: *Acacia tortilis,* antioxidant, antimicrobial, Phytochemical.

Introduction

Plants have been widely used since ancient times as a primary remedy for various ailments. Their folk uses have encouraged various phytochemical and pharmacological screenings and

provided an unlimited source for drug discovery endeavors due to the unmatched chemical diversity [1]. Plants belonging to *Fabaceae* family are mostly herbs and some of them are shrubs and trees found in both temperate and tropical areas. The group is widely distributed and is the third-largest land plant family in terms of number of species, behind only the *Orchidaceae* and *Asteraceae*, with 730 genera and over 19400 species [2]. This family is commonly known as the legume, pea or bean family. The family is also known for its large and economically important flowering plants. The genus *Acacia* is the second largest genus of the family Fabaceae, comprising more than 1350 species worldwide, with members found in almost all habitats, of which, approximately 800 are found in Australia, 130 in Africa and 20 in India [3]. Plants of the genus *Acacia* are widely used in traditional medicine for diarrhea, urinary tract infections, headaches, sore throat, gastritis, tuberculosis and bronchial asthma [4]. Phytochemical investigation of different Acacia species showed the presence of phenolic acids, flavonoids, tannins, alkaloids and terpenes, which were responsible for numerous pharmacological effects, showing hypoglycemic, anti-inflammatory, anti-bacterial, anti-platelet aggregation, anti-hypertensive, analgesic,anticancer and anti-atherosclerotic activities [5].

Acacia tortilis is called, Umbrella Thorn acacia; Israeli Babool (in English), Sammor and Sayyal (in Arabic). It is a thorny legume tree, usually about 4 to 8m high but can grow up to 20m [6]. The crown is dense, umbrella-like and flat-topped; hence it is called Umbrella tree in some areas. Leaves are compound and the leaflets (6- 22 pairs) are very small (1- 4 mm long x 0.6 – 1 mm broad), glabrous to pubescent. Flowers are white, cream or yellow and highly aromatic. Fruits are distinguished by twisted shape; hence the epithet “tortilis”. Bark is grey-brown-black, rough and fissured. The spines are in pairs, some short and hooked up to 5 mm long, mixed with long straight slender spines up to 10 cm long [7]. *Acacia tortilis* is native to semi-arid areas of Africa and Middle-East. It is found between 15 and 30ºN and between sea level and 1000m altitude. *Acacia tortilis* is tolerant to severe drought due to its deep taproot system.

Acacia tortilis is nitrogen fixing species and thus a soil improver [8].

Acacia tortilis provides high quality animal fodder, fuel wood, charcoal, gums and other products as well as contributes to soil stabilization and improvement through nitrogen fixation. Their particular value in arid zones lies in their extreme resistance to heat, drought, salinity and alkalinity, drifting sand, grazing and repeated cutting [9]. Apart from this, timber is also used for fence posts, firewood, furniture and wagon wheels. The prolific pods made good fodder for desert grazers and the foliage is also palatable. Reports of folk medicine on these species revealed that stems of *A. tortilis* have been used in Somalia to treat asthma, and dried powdered bark has been used as a disinfectant in wound healing [10]. In Egypt and Yemen, Infusion of bark used as sedative, analgesic and disinfectant. Bark infusion also applied against jaundice, diarrhoea, pulmonary diseases. For stomach ache and digestive problems. Sap is taken directly for the treatment of malaria [11]. *A. tortil* is showed marked inhibition of mammalian -glucosidase significantly reducing postprandial blood glucose level [12].

This study was aimed to:

- Determine the phytochemical constituents present in the seeds extract of *Acacia tortilis*.
- Determine the yield percentage of flavonoids, alkaloids and saponins in the seeds extract.
- Analysis of oil of *Acacia tortilis* by GC-MS.
- Screening the antimicrobial activity of oil.
- Evaluate the antioxidant activities of oil.

Materials

Plant material

Acacia tortilis: was collected from the Ministry of Agriculture and Forestry farm Shendi - Sudan. The plant seeds were blended into 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 [13], 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. The extract obtained was kept in an air-tight bottle until used for the 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) [14-16].

Test for Flavonoids

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

Test for Alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. Presence of a green color or white precipitate indicates 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 indicates 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 indicates 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 a few drops of crude extract and boiled, a brick red precipitate of cuprous oxide forms, indicates the presence of reducing sugar.

Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates 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 indicates 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 a brown ring at the interface indicates 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 were added and the appearance of a pink color precipitate indicates 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 a red-brown color at the interface indicates the presence of terpenoids.

Test for Coumarins

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

Steroids and Phytosteroids

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

Quantitative phytochemical analysis

Determination of flavonoids

10 g of powder seed was placed in a 250 cm^3 beaker, then 50 cm^3 of 80% aqueous methanol added, covered and allowed to stand for 24 hours at room temperature. The supernatant was discarded and the residue re-extracted three times with the same volume of ethanol. The whole solution of the seeds sample was filtered through Whatman filter paper No 42 (125 mm). The filtrate was then transferred to a crucible and evaporated

to dryness over a water bath. The crucible and its contents were cooled in a desiccator and weighed until a constant weight was obtained [17]. The percentage of flavonoids is expressed mathematically as:

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

Determination of Alkaloids

5 g of seed powder was placed into a 250 cm^3 beaker, and 200 cm^3 of 10 % acetic acid in ethanol was added to seed powder and allowed to stand for 4 hours. Then the mixture was filtered and the extract was concentrated in a water bath to one - quarter of the original volume followed by the 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 the precipitate washed with 20cm3 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 an electronic weighing balance [14].

The percentage of alkaloid can be expressed mathematically as:

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

Determination of Saponins

20 g of seed powder was put in 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 55°C. The mixture was filtered and the residue re-extracted with another 100 cm^3 of 20% aqueous ethanol, heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was reduced to 40 cm^3 over water bath at a temperature of 90°C. 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 % 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 [18]. The saponins content was calculated as a percentage:

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

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 h. The solvent was removed with a rotary evaporator and the oil was kept in the fridge at 4ºC 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), results were recorded.

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

1.0 ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108- 109 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938) [19]. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature 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 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 ml 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 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 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on the 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) [20] 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

Phytochemical screening of *Acacia tortilis* for various compound classes was carried out using standard procedures as described by Harbome 1998, Sofowora 1993 and Trease and Evans 1989. Phytochemical screening of the seeds extracts revealed that Flavonoids, Alkaloids, Saponins, Tannins, Carbohydrates, Phenols, Glycosides, Cardiac Glycosides, Quinones, Anthraquinone, Terpenoids, Coumarins and Steroids are present (Table 2).

Table 2: phytochemical screening analysis of *Acacia tortilis*

Phytochemical compounds	Plant extract *Acacia tortilis*
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
carbohydrates	+
Phenols	+
Glycosides	+
Cardiac Glycosides	+

Quinones	+
Anthraquinone	+
Terpenoids	+
Coumarins	+
Steroids	+

+ (contain this phytochemical compound)

Quantitative phytochemical analysis

Quantitative evaluation of the amount of individual bioactive constituents of the plants under investigation is as summarized in Table (3).

Table 3: percentage of Flavonoids, Alkaloids and Saponins

Plant species	Flavonoids (%)	Alkaloids (%)	Saponins (%)
Acacia tortilis	8.7 ± 0.00	5.4 ± 0.00	2.05 ± 0.02

GC-MS analysis of *Acacia tortilis* oil

The oil of *Acacia tortilis* was analyzed by GC-MS. Sixteen components appeared in total ion chromatogram (Fig.1). Different components were quantified and identified by their retention time and mass spectra and a tabulation of constituents is presented in Table (4).

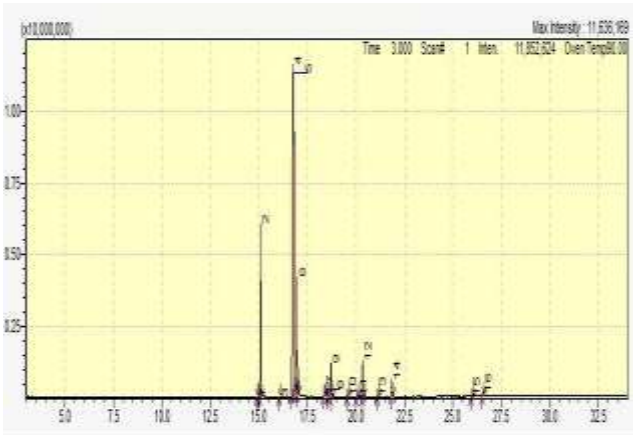


Fig. 1: Total ion Cromatograms of *Acacia tortilis* oil

Table 4: Constituents of *Acacia tortilis* oil

ID#	Name	Ret.Time	Area	Area%
	9-Hexadecenoic acid, methyl ester, (Z)-	14.902	33422	0.04
	Hexadecanoic acid, methyl ester	15.078	11894802	12.64
	Heptacosanoic acid, methyl ester	16.081	22204	0.02
	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.768	43764096	46.50
	9-Octadecenoic acid (Z)-, methyl ester	16.804	22104943	23.48
	Methyl stearate	16.985	7903823	8.40
	11,14-Eicosadienoic acid, methyl ester	18.383	991391	1.05
	11-Octadecenoic acid, methyl ester	18.527	638984	0.68
	Eicosanoic acid, methyl ester	18.731	2457494	2.61
	Heneicosanoic acid, methyl ester	19.567	58995	0.06
	13-Docosenoic acid, methyl ester	20.061	57136	0.06
	Docosanoic acid, methyl ester	20.343	2493512	2.65

	Tricosanoic acid, methyl ester	21.122	110121	0.12
	Tetracosanoic acid, methyl ester	21.844	1076794	1.14
	Stigmasterol	25.980	190731	0.20
	.gamma.-Sitosterol	26.541	330466	0.35

The following major constituents were detected by GC-MS:

9,12-Octadecadienoic acid (Z,Z)-, methyl ester (46.50%)

Synonyms: Linoleic acid, methyl ester. This compound was reported with Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, and Insectifuge [21].

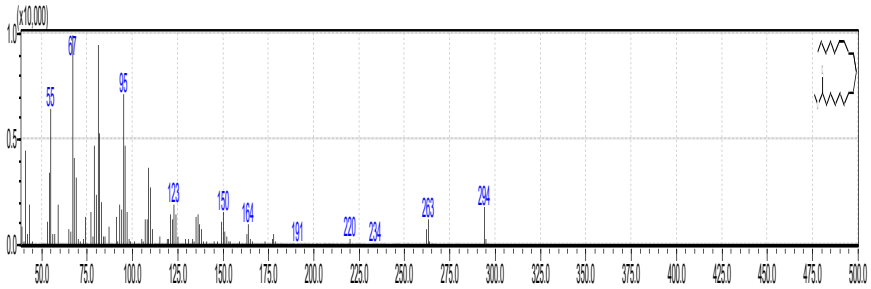


Fig. 2: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

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. 16.768 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 (23.48%)

Synonyms: Oleic acid, methyl ester. This compound was reported with following potential properties like Flavour [22], cancer preventive [23], anti-inflammatory [24] and antimicrobial activity [25].

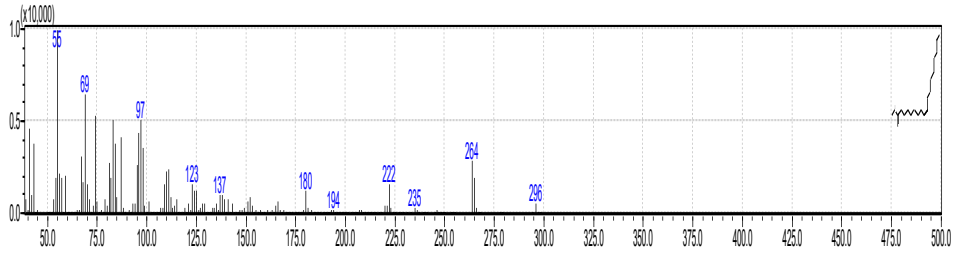


Fig.3: Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester

The EI mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester is shown in Fig. 3 ~~3.3~~. The peak at m/z 296, which appeared at R.T. 16.804 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 (12.64%)

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

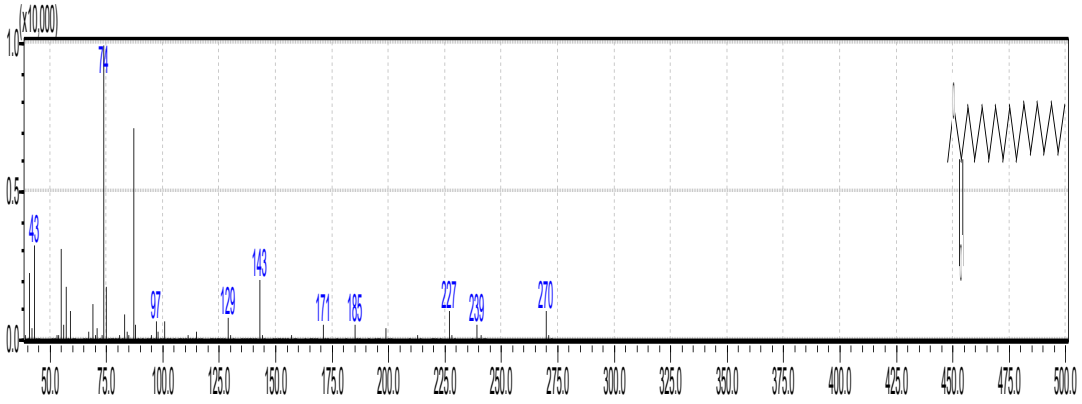


Fig.4: Mass spectrum of Hexadecanoic acid, methyl ester

The EI mass spectrum of Hexadecanoic acid, methyl ester is shown in Fig. 4 ~~3.4~~. The peak at m/z 270, which appeared at R.T. 15.078 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 (8.40%)

Synonyms: Octadecanoic acid, methyl ester and Stearic acid, methyl ester. This compound was reported with Antifoaming

agent, fermentation nutrient, flavoring agent [28, 29].

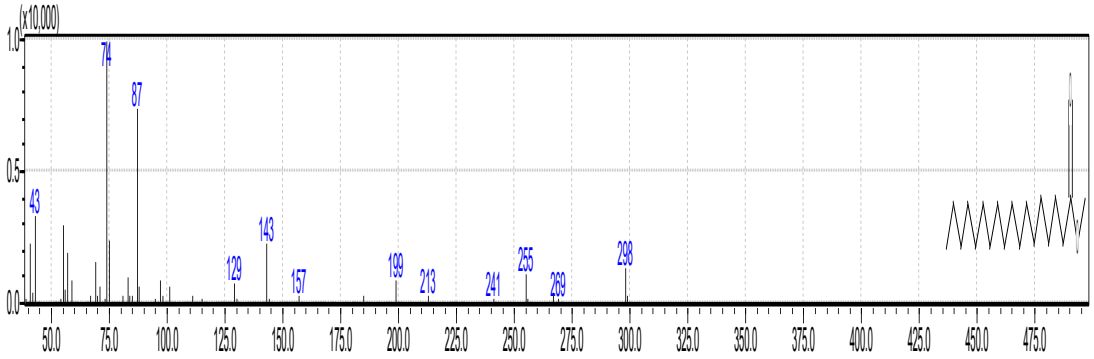


Fig. 5: Mass spectrum of Methyl stearate

The EI mass spectrum of Methyl stearate is shown in Fig. 5. The peak at m/z 298, which appeared at R.T. 16.985 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.

Docosanoic acid, methyl ester (2.65%)

Synonyms: Behenic acid, methyl ester.

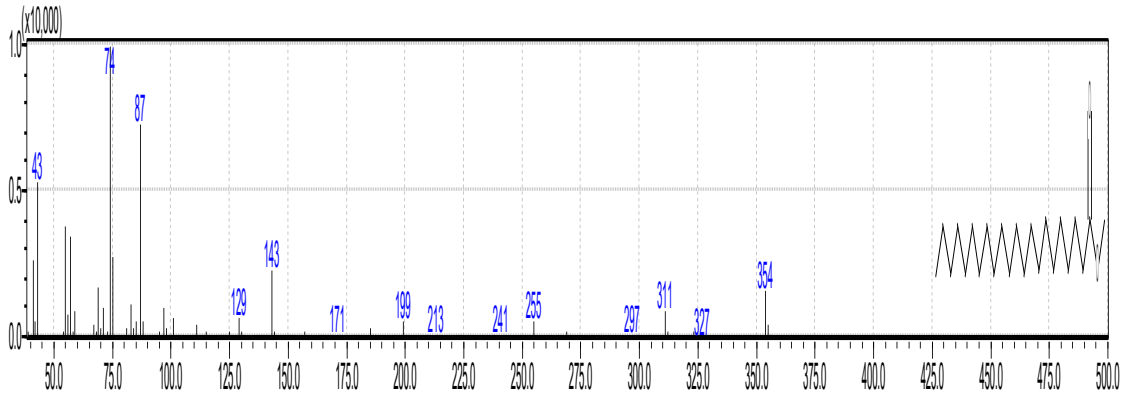


Fig.6: Mass spectrum of Docosanoic acid, methyl ester

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. 20.343 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.

Eicosanoic acid, methyl ester (2.61%)

Synonyms: Methyl arachisate, Methyl eicosanoate and Arachidic acid methyl ester. The compound was reported to be Alpha-glucosidase inhibitors activity [30].

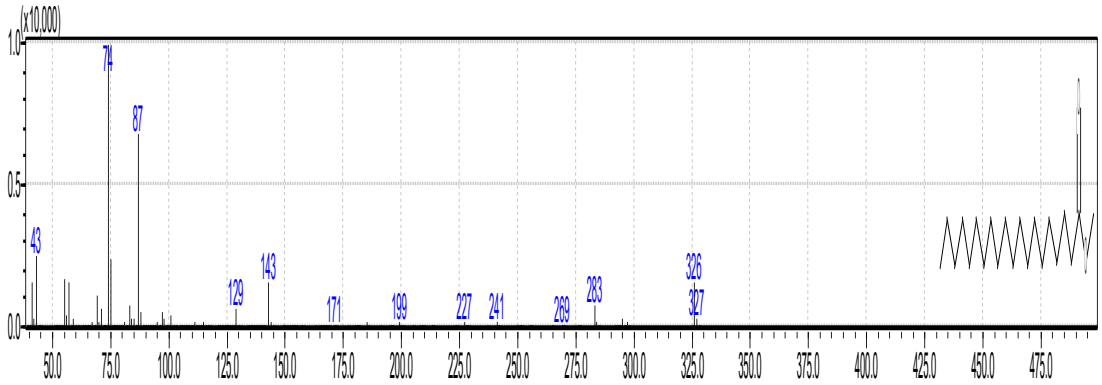


Fig.7: Mass spectrum of Eicosanoic acid, methyl ester

The EI mass spectrum of Eicosanoic acid, methyl ester is shown in Fig. 7. The peak at m/z 326, which appeared at R.T. 18.731 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.

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 Table (5). 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 tortilis* oil showed good activity against *Bacillus subtilis*. *Escherichia coli* and *Staphylococcus aureus*. It also showed weak anticandidal potency. The oil had no activity against *Pseudomonas aeruginosa*.

Table 5: The antimicrobial activity of *Acacia tortilis* oil

Microorganism	**species**	**type**	Inhibition zone diameter (mm) Con (100mg/ml)
Bacteria	*Bacillus subtilis*	+ ve	15
	Staphylococcus aureus	+ ve	13
	Escherichia coli	+ ve	15
	Pseudomonas aeruginosa	- ve	-
Fungi	*Candida albicans*	- ve	12

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 (DPPHassay), while propyl galate was used as standard antioxidant.

Based on findings of Dudonne, *et al*. (2009) [31], 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 are shown in Table (6). The oil of *Acacia tortilis* showed very high DPPH radical scavenging activity.

Table 6: the radical scavenging assay (DPPH) of *Acacia tortilis* oil

Sample	RSA%
Acacia tortilis	89 ± 0.06
propyl gallate (standard)	92 ± 0.01

RSA* = Radicals scavenging assay

References

[1] Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Yoga Latha, L., (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr. J. Tradit. Compl. Altern. Med., **8** (1):1-10.

[2] Judd, W.S., Campbell, C.S., Kellog, E.A., Stevens, P.F., Donoghue, M.J. (2002). Plant Systematics: a phylogenetic approach, Sinauer Axxoc, 287-292, ISBN 0-87893-403-0.

[3] Jelassi, A., Zardi-Bergaoui, A., Ben Nejma, A., Belaiba, M., Bouajila, J., Ben Jannet, H., (2014). Two new unusual monoterpene acid glycosides from Acacia cyclops with potential cytotoxic activity. *Bioorg. Med. Chem. Lett*., **24** (16): 3777-3781.

[4] Stohs, S.J., Bagchi, D., 2015. Antioxidant, anti-inflammatory, and chemoprotective properties of Acacia catechu heartwood extracts. Phytother Res., **29** (6): 818-824.

[5] Jelassi, A., Ayeb-Zakhama, A.E., Nejma, A.B., Chaari, A., Harzallah-Skhiri, F., Jannet, H.B., 2016. Phytochemical composition and allelopathic potential of three Tunisian Acacia species. Ind. Crop. Prod., 83: 339-345.

[6] Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Anthony, S. (2009). Agroforestry database: A tree reference and selection guide. Version 4.0. World agroforestry center, Kenya.

[7] Sunita, V. (2016). A Review Study on Acacia Tortilis. *International Journal of Advanced Research*. Volume 4,

[8] Araya, M.R., Ngungi, R.K., Musimba, N.R., Nyariki, D.M., Arbonnier, M. (2009). Arbres, arbustes et lianes des zones seches d'Afrique de l'Ouest. Versailles Ed Quae, MHNH. 574.

[9] Katende, A.B. Birnie, A. and Tengnas, B. (1995). Useful Trees and Shrubs for Uganda: Identification, Propagation and Management for Agricultural and Pastoral Communities. ISBN 9966-896-22-8.

[10] Verma, S., (2016). A review study on *Acacia tortilis*. *Int. J. Life Sci. Pharma Res*., **6** (2):24-26.

[11] Al-Fatimi, M., Wurster, M., Schroder, G. and Lindequist, U. (2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology*, **111**(3): 657-666.

[12] Bisht, S., Kant, R., Kumar, V. (2013). a-d-Glucosidase inhibitory activity of polysaccharide isolated from *Acacia tortilisgum exudate*. *Int. J. Biol*. Macromol. 59: 214-220.

[13] Obomanu, F.G., Fekarurbobo, G.K. and Howard, I.C. (2005). Antimicrobial activity of extracts of the leaves of L*epidagathis alopecuroides* (Vahl). *J. Chem. Soc. Nig*., **30**(1):33-35.

[14] Harborne, J.B. (1998). Phytochemical Methods: A Guide to Modern Techniques of plant Analysis. Chapman and Hall Ltd, London. pp. 279.

[15] Sofowora, A. (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa Edition. Spectrum Books Ltd., Nigeria, 150-156.

[16] Trease, G.E. and Evancs, WC. (1989). Pharmacognosy. 11th Edition, Bailliere Tindall, London, pp: 45-50.

[17] Boham, B.A. and Kocipai, A.R. (1994). Flavonoids and Condensed Tannins from Leaves of *Hawaiian Vaccinium vaticulatum* and *V. calycinium*. *Pacific Sci*.,48:458-463.

[18] Obdoni, B.O. and Ochukpo, P.O. (2000). Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homostatic Plants in Edo and Delta States of Nigeria. *Global J. Pure & Appl. Sci*., **8**(2):203-208.

[19] Miles, A. and Misra, S. (, 1938). The estimation of the bactericidal power of the blood. *J. Hyg.*, 38: 732-748.

[20] Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J Agric Food Chem*.,40:945-8.

[21] Parthipan, B., Suky, M.G.T. and. Mohan, V.R. (2015). GC-MS Analysis of Phytocomponents in *Pleiospermium alatum* (Wall. ex Wight & Arn.) Swingle, (Rutaceae). *Journal of Pharmacognosy and Phytochemistry*, **4**(1): 216-222.

[22] Omolosa, A. D. and Vagi, J. K. (2001). Broad-Spectrum antibacterial activity of Trigonella foenum-graecum. *Nat. Prod. Sci*., **7**(1): 13- 16.

[23] Simin, K., Ali, Z., Khalid-Uz-Zaman, S.M. and Ahmad, V.U. (2000). Structure and biological activity of a new rotenoid for *pongamia pinnata*. *Nat. Prod. Lett.*, **10**(5): 351 - 357.

[24] Yunfeng, Z., Dong, W., Siyuan, G., Xuewu, Z., Mingfu, W. and Feng, C. (2007). Chemical components and antioxidant activity of the volatile oil from *cassia tora* L. Seed prepared by

supercritical fluid extraction. *Journal of Food Lipids*, **14**(4): 411 - 423.

[25] Wagh, P., Rai, M., Deshmukh, S. K. and Durate, M.C.T. (2007). Bio-activity of oils of *Trigonella foenum-graecum* and *Pongamia pinnata*. *African Journal of Biotechnology*, **6**(13): 1592- 1596.

[26] Kujumgiev, A., Bankova, V., Ignatova, A. and Popov, S. (1993). Antibacteral activity of propolis, some of its components and their analogs. *Pharmazie*, 48: 785 - 786.

[27] Kroes, B. H., van der Berg, A. J. J., Quarles van Ufford, H. C., van Dijk, H. and Labadie, R. P. (1991). Inti-Inflammatory Activity of Gallic Acid. *Planta Med.*, 58: 499 - 504.

[28] Nascimento, A.M., Conti, R., Turatti, I.C., Cavalcanti, B.C., Costa-Lotufo, L.V., Pessoa, C., Moraes, M.O., Manfrim, V., Toledo, J.S., Cruz, A.K. and Pupo, M,T. (2012). Bioactive extracts and chemical constituents of two endophytic strains of *Fusarium oxysporum*. *Revista Brasileira de Farmacognosia*, **22**(6): 1276-81.

[29] Mulyono, N., Lay, B.W., Ocktreya, L. and Rahayu, S. (2013). Antidiarrheal activity of Apus bamboo (*Gigantochloa apus*) leaf extract and its bioactive compounds. *American Journal of Microbiology*, **4**(1): 1-8.

[30] Elaiyaraja, A. and Chandramohan, G. (2016). Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. *Journal of Pharmacognosy and Phytochemistry*, **5**(6): 158-171.

[31] Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., Merillon, J.M. (2009). Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. *J Agric Food Chem*., **57**: 1768–1774.