International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 06-11-2018; Accepted: 10-12-2018
www.pharmacyjournal.in
Volume 4; Issue 1; January 2019; Page No. 06-09

GC-MS analysis and antimicrobial activity of Sudanese *Azadirachta indica* A. juss (Meliaceae) Seed Oil

Abdel Karim M[1], Abdel AzimI[2], Maab A[3*]
[1] Sudan University of Science and Technology, Faculty of Science, Sudan
[2, 3] Alneelin University, Faculty of Pharmacy, Sudan

Abstract
Azadirachta indica (Neem) is an ever green tree in the family Meliaceae. The leaves are used by some Sudanese villagers as antimalarial. In this study the seed oil was analyzed by GC-MS. The oil was also evaluated for antimicrobial activity. The GC-MS analysis revealed the presence of 29 components. Major constituents are: 9-octadecenoic acid methyl ester (37.20%); methyl stearate (20.42%); hexadecanoic acid methyl ester (19.13%) and 9,12-octadecadienoic acid methyl ester (12.60%).
The oil was evaluated for antimicrobial activity via the agar diffusion bioassay against: Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonas aeruginosa* and the fungal species *Candida albicans.* At a concentration of 100mg/ml the oil showed good activity against *Escherichia coli.* However, at the same concentration, it exhibited partial activity against *Staphylococcus aureus* and *Bacillus subtilis.* The oil failed to give any anticandidal activity.

Keywords: *Azadirachta indica*, seed oil, GC-MS analysis, antimicrobial activity

Introduction
Azadirachta indica A. juss (Neem) is an ever green tree in the family Meliaceae. The plant may grow up to 60ft high. Flowers are used in pharmaceutical, food and cosmetic industries [1]. The fat content of the kernels is about 33-45% [2]. Neem oil (about 40% yield) can be processed into non bitter edible oil containing: oleic acid (42-50%) and linoleic acid (15%). Some pesticides have been derived from Neem [3]. Neem seed has high nutritional potential for livestock [4]. The constituent which is responsible for the distinctive odour of Neem istignic acid [5]. Some sulphur containing compounds like nimbin, nimbidin and nimbosterol have been isolated from Neem [6].
In African ethnomedicine several parts of this tree have been used to treat a wide spectrum of diseases [7-9]. Branches of *Azadirachta indica* are used in traditional medicine for dental care [10] while the leaves are used as treatment for acne [11]. Leaves are also used popularly for treatment of infected eyes. An infusion of leaves is used traditionally against sore throat [12].
All parts of *Azadirachta indica* are traditionally claimed to treat a wide array of diseases including jaundice, stomach ulcers, leprosy, chicken pox, and malaria. Leave tea is said to treat intestinal complaints, dental headache and heartburn. It also stimulates the appetite and acts as insect- repellent. In addition this tea has been used against some skin diseases and as a diuretic [13]. Neem oil is applied in the treatment of eczema as well as intestinal worms [14]. Neem-based products are popularly used for pest control in agricultural field [15].

Materials and Methods
Plant material
Seeds of *Azadirachta indica* were collected from Khartoum, Sudan. The plant was identified by direct comparison with reference herbarium sample.

Test organisms
Azadirachta indica oil was screened for antimicrobial activity using the standard microorganisms shown in Table (1).

Table 1: Test microorganisms

Sr. No.	Micro-organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeruginosa*	G-ve
4	*Escherichia coli*	G-ve
6	*Candida albicans*	fungus

Methods
Extraction of oil
Powdered shade-dried seeds of *Azadirachta indica* (300g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure to give the oil.

GC-MS analysis
Azadirachta indica fixed oil was studied by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are displayed below.

Table 2: Oven temperature program

Rate	Temperature (ºC)	Hold Time (min.$^{-1}$)
-4.00	150.0	1.00
	300.0	0.00

Table 3: 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	4.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay

Microbial suspensions

Aliquots of 24 hours broth culture of the test organisms (1 ml) were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dry nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Antimicrobial assay

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antimicrobial activity. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard drugs.

The agar discs were removed, alternate cups were filled with 0.1 ml samples of each test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured.

Results and Discussion

GC-MS analysis of *Azadirachta indica* oil was conducted and the identification of the constituents was accomplished by retention times and MS fragmentation pattern. A 90-95% match was observed when comparing the mass spectra with the database on MS library.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 29 constituents (Table 4). The typical total ion chromatograms (TIC) is depicted in Fig.1.

The major constituents of the oil are

1. 9-Octadecenoic acid methyl ester (37.20%)
2. Methyl stearate (20.42%)
3. Hexadecanoic acid methyl ester (19.13%)
4. 9, 12-Octadecadienoic acid methyl ester (12.60%)

Table 4: Constituents of *Azadirachta indica* oil

Peak#	R.Time	Area	Area%	Name
1	6.020	199124	0.05	Cyclopropane, 1,2-dimethyl-1-pentyl-
2	6.215	197921	0.05	(S)-(+)-6-Methyl-1-octanol
3	6.978	406076	0.11	L-.alpha.-Terpineol
4	10.250	53973	0.01	trans-.alpha.-Bergamotene
5	10.316	14719	0.00	.alpha.-ylangene
6	10.435	35005	0.01	1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)
7	11.253	141578	0.04	Dodecanoic acid, methyl ester
8	13.488	81162	0.02	6,10-Dodecadienoic acid, 3,7,11-trimethyl-,
9	13.563	1440425	0.37	Methyl tetradecanoate
10	14.639	206952	0.05	Pentadecanoic acid, methyl ester
11	15.429	209999	0.05	7-Hexadecenoic acid, methyl ester, (Z)-
12	15.470	865688	0.22	9-Hexadecenoic acid, methyl ester, (Z)-
13	15.698	73645291	19.13	Hexadecanoic acid, methyl ester
14	16.371	125446	0.03	Hexadecanoic acid, 14-methyl-, methyl este
15	16.433	248930	0.06	cis-10-Heptadecenoic acid, methyl ester
16	16.641	1060845	0.28	Heptadecanoic acid, methyl ester
17	17.341	48484713	12.60	9,12-Octadecadienoic acid (Z,Z)-, methyl e
18	17.439	143177835	37.20	9-Octadecenoic acid (Z)-, methyl ester
19	17.621	78612946	20.42	Methyl stearate
20	19.099	2364052	0.61	Oxiraneoctanoic acid, 3-octyl-, methyl este
21	19.133	963302	0.25	cis-11-Eicosenoic acid, methyl ester
22	19.334	20218704	5.25	Eicosanoic acid, methyl ester
23	20.591	735650	0.19	9-Octadecenoic acid, 1,2,3-propanetriyl est
24	20.950	4131698	1.07	Docosanoic acid, methyl ester
25	21.715	478746	0.12	Tricosanoic acid, methyl ester
26	22.192	410963	0.11	Hexatriacontane
27	22.454	3077201	0.80	Tetracosanoic acid, methyl ester
28	23.194	1073341	0.28	Squalene
29	23.599	2255649	0.59	Tetratriacontane
		384917934	100.00	

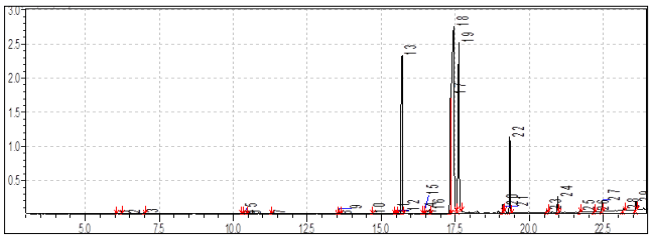

Fig 1: Total ions chromatograms

Major constituents are discussed below

9-Octadecenoic acid methyl ester (37.20%)

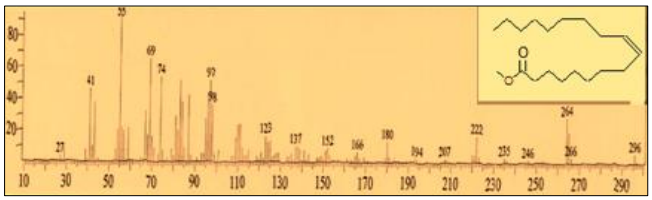

Fig 2: Mass spectrum of 9-octadecenoic acid methyl ester

The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 2. The peak at m/z 296, which appeared at R.T. 17.439 in total ion chromatogram, corresponds M+ [C19H36O2]+.The signal at m/z266 is due to loss of a methoxyl function.

1. Methyl stearate (20.42%)

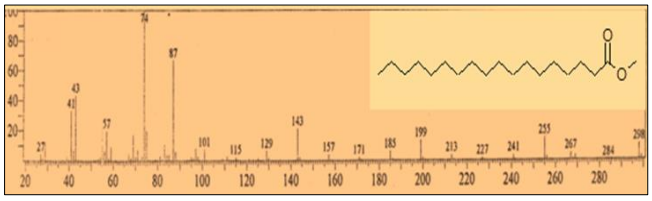

Fig 3: Mass spectrum of methyl stearate

The EI mass spectrum of methyl stearate is displayed in Fig.3.The peak at m/z 298 with R.T. 17.621 is due to $M^{+}[C_{19}H_{38}O_2]^{+\cdot}$ while the signal at m/z267 corresponds to loss of a methoxyl group.

2. Hexadecanoic acid methyl ester (19.13%)

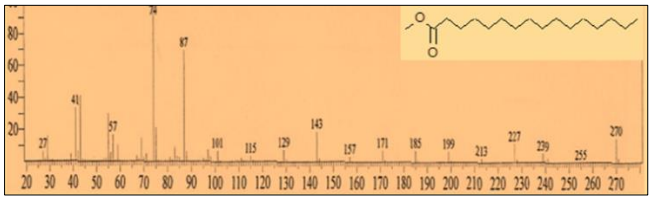

Fig 4: Mass spectrum of hexadecanoic acid methyl ester

Fig. 4 shows the mass spectrum of hexadecanoic acid methyl. The peak m/z 270 (R.T. 15. 698) was detected in the spectrum. It corresponds $M^{+}[C_{17}H_{34}O_2]^{+\cdot}$ The peak at m/z239 is due to loss of a methoxyl.

3. 9, 12-Octadecadienoic acid methyl ester (12.60%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is depicted in Fig.5. The signal which was observed at m/z294(R.T. 17.341) is due to M+[C19H34O2]+, while the signal at m/z263 corresponds to loss of a methoxyl.

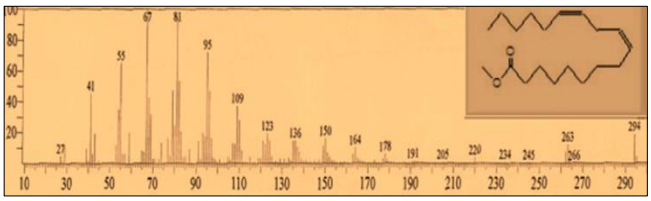

Fig 5: Mass spectrum of 9, 12-octadecadienoic acid methyl ester

Antimicrobial activity

Azadirachta indica seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 5.The results were interpreted in commonly used terms (<9mm: inative;9-12mm:partially active; 13-18mm: active; >18mm:very active).Tables 6 and 7 represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents respectively.

At a concentration of 100mg/ml the oil showed good activity against *Escherichia coli*. However, at the same concentration, it exhibited partial activity against *Staphylococcus aureus* and *Bacillus subtilis*.

Table 5: Antimicrobial Activity of the oil

Oil	Antibacterial activity				
	Gram positive		Gram negative		
mg/ml	*Bs.*	*Sa.*	*Ec.*	*Pa.*	*Ca.*
100	10	10	14	--	--

Table 6: Antibacterial activity of standard drugs

Drug	Conc. (mg/ml)	*Bs*	*Sa*	*Ec*	*Ps*
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamicin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table7: Antifungal activity of standard drug

Drug	Conc. (mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa: Staphylococcus aureus
Ek: Escherichia coli
Pa: Pseudomonas aeruginosa
An: Aspergillus Niger
Ca: Candida albicans
Bs: Bacillus subtilis

References

1. Nursing RG, Prabhakara RPG, Satyanaraya A. Fatty acid, volatile oil composition and antioxidant activity of shade dried neem (*Azadirachta indica* L.) flower powder, International Food Research Journal. 2014; 21(2):807-813.
2. Eckey EW. Vegetable Fats and Oils, Reinhold publishing, corporation, 1954, New York, PP559.
3. Rahim M. Biological activity of azadirachtin-enriched Neem kernel extracts against *Rhyzopertha dominica* in stored wheat, Journal of Stored Product Research. 1998; 34:123-128.
4. Udayashekhara PP. Chemieal composition and biological evaluation of debitterised and defatted Neem

(*Azadirachta indica*) seed kernel cake, Journal of American Oil Chemists Society1. 1987; 98764:1348-1352.
5. IWU MM. Handbook African Medicinal Plants, CRC Press London, 1993, 124-128.
6. Kar Kar CN. Utilization of Neem (*A. indica*) and its by-products: Report of the modified Neem cake manorial project, Directorate of non-edible oils soap industry, Khadi and Village Industries Commission, Bombay, India, 1969-76.
7. WHO. General guidelines for methodologies on research and evaluation of traditional medicine, 2001.
8. Shahed S, Ahmed F, Saha SK, Das SC, Bachar SC. Evolution of Brine on some drug resistance bacteria in Bangladesh, Pharmacognosy J. 2011; 3(20):66-71.
9. Velusamy P, Das J, Pachaiappon R, Vaseeharan B, Pandian K. Greener approach for synthesis of antibacterial silver nanoparticles using aqueous solution of Neem gum (*Azadirachta indica* L). Ind Crops Prod. 2015; 66:103-9.
10. Prashant G, Chandu G, Chandu G, Murulikrishna K, Shafiulla M. The effect of mango and Neem extract on four organisms causing dental caries: *streptococcus mutans. streptococcus salivavius, streptococcus mitis*, and *Streptococcus sanguis*: An in vitro study. Indian J Dent Res. 2007; 18(4):148-51.
11. Bhowmik D, Yadav J, Tripathi K, Kumar KS. Herbal remedies of *Azadirachta indica* and its medicinal application, J Chem Pharm Res. 2010; 2(1):62-72.
12. Biswas K, Chattopadhyay I, Banerjee R, Bandyopadyay U. Biological activities and medicinal properties of Neem (*Azadirachta indica*) Curr sci. 2002; 82(11):1336-45.
13. Sujarwo W. Keim AP, Caneva G, Toniolo C, Nicoletti M. Ethnobotanical uses of Neem (*Azadirachta indica* A. juss, Meliaceae) leaves in Bali(Indonesia) and the Indian subcontinent in relation with historical background and photochemical properties J. Ethnopharmacol. 2016; 189:18693.
14. Forster P, Moser G. Status report on global Neem usage: Deutsche Gesellschaft fur Technische Zusammenarbeit (G T Z). Eschborn, Germany, 2000, 122.
15. Habluetzel A, Lucantoni L, Esposito F. *Azadirachta indica* as apublic health tool for the control of malaria and other vector-borne diseases. Indian J. Med. Res. 2009; 130(2):112-4.