Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/4645
DOI URL: http://dx.doi.org/10.21474/IJAR01/4645
ISSN NO. 2320-5407

RESEARCH ARTICLE

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *SORGHUM SUDANESE* (PIPER) STAPF. FIXED OIL.

***Abdel Karim. M[1], Asma M. H[2], Mustafa. S. K.[2] and Khalid, M. S.[3].**

1. Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.
2. University of Nyala , Faculty of Education, Dept. of Chemistry..
3. International University of Africa, Faculty of Pharmacy.

Manuscript Info

Manuscript History

Received: 28 April 2017
Final Accepted: 30 May 2017
Published: June 2017

Key words:-
Sorghum sudanese, Fixed Oil, GC-MS, Antimicrobial Activity

Abstract

The present study was undertaken to investigate the chemical constituents of *Sorghum sudanese* seed oil and to assess its antimicrobial activity. 25 components were detected by GC-MS analysis. Major constituents are: 9,12-octadecadienoic acid methyl ester(40.52%) ; 9-octadecenoic acid methyl ester (33.60%), hexadecanoic acid methyl ester(17.99%) . The oil was evaluated for antimicrobial activity against : (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative : *Escherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*) . The oil showed excellent activity against all test organisms and specially against *Bacillus subtilis* at a concentration of 100mg/ml. At all test concentrations the oil gave significant activity against *Bacillus subtilis* . Excellent antifungal activity against *Candida albicans* was demonstrated at concentrations of 100,50 and 25 mg/ml. It seems that the oil is a lead for a potent antimicrobial agent.

Introduction:-

Sorghum is a genus of flowering plants in the Poaceae(grass family). Seventeen of the 25 species are native to Australia. Sorghum is divided into five basic races: bicolor, guinea, caudatum, kafir and durra[l](Harlan and de Wet,1975 ; Hitchcock ,1971). The genus Sorghum is characterized by spikelets borne in pairs (FAO,1995) . Many Sorghum species are used as fodder plants, while one species is grown for grain (Mutegi et.al.,2000). The species *Sorghum tricolor*(Watson,1983) is native to Africa and has many cultivated varieties(URL-1).This species is an important crop worldwide and is used as food. The variety known as sweet sorghum is used in ethanol production and bio-oil (URL-2) .

Singh and Axtell(1973) evaluated the chemical composition of different Sorghum varieties ; protein(10.6-18.5%); starch (55.6-75.2%), crude fiber(1/0-3.4%), fat (2.1-7.6%) and ash (1.6-3.3%). Sankara and Deosthale(1980) assessed the mineral composition of sorghum kernels (mg/100g) : phosphorus(352) ; magnesium(171); calcium (15); iron(4.2); zinc(2.5), copper(0.44), manganese(1.15) beside small amount of molybdenum and chromium.

The cultivated variety – *Sorghum sudanese* - is used extensively for feeding livestock .It requires a large amount of water to achieve maximum yield (Husain et.al.,1991; Merrill et.al.,2007 ; Marsalisa et.al.,2010; Sowinski and Szydelko,2011).

Corresponding Author:- Abdel Karim. M.
Address:- Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

Materials and Methods:-

Plant material:-

Sorghum sudanese seeds were purchased from the local market – Khartoum, Sudan. The plant was authenticated by Institute of Aromatic and Medicinal Plants- Khartoum ,Sudan.

Test organisms:-

Sorghum sudanese oil was screened for antimicrobial activity using the standard microorganisms: *Bacillus subtilis* (Gram +ve) , *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve) , *Escherichia coli* (Gram – ve) and the fungal species *Candida albicans*.

Methods:-

Extraction of oil from *Sorghum sudanese* seeds:-

Dry-powdered seeds of *Sorghum sudanese* (300g) were macerated with n-hexane at room temperature for 48h..The solvent was removed under reduced pressure to give the oil. A methanolic solution of sodium hydroxide and a methanolic sulphuric acid were used to esterify the oil .

GC-MS Analysis:-

Sorghum sudanese fixed oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo Ultra instrument with RTX-5MS column (30m,length ; 0.25mmdiameter ; 0.25 µm, thickness). Helium (purity; 99.99 %) was the carrier gas. Oven temperature program and other chromatographic conditions are depicted in Tables 1 and 2 respectively.

Table 1:- Oven temperature program.

Rate	Temperature(C)	Hold time ($mim.^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial Assay:-

One gram of the oil was weighed and dissolved in 10ml of DMSO to obtain a concentration of 100mg/ml. This was the initial concentration of the oil used to check the antimicrobial activities. Diffusion method was the method used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to the manufacturer's instructions, sterilized at 121^{o}C for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify. The sterilized media were sealed with 0.1ml of the standard inoculums of the test microbe (Mueller Hinton agar was sealed with the bacteria and Sabouraud dextrose agar sealed with the fungus). The inoculums were spread over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters, a well was cut at the centre of each inoculated medium. Serial dilutions of the oil (0.1ml) were then introduced into the well on the inoculated medium. Incubation of the inoculated medium was made at 37^{o}C for 24 hours for the bacteria and at 30^{o}C and for 4 days for the fungus. After incubation each plate of the medium was observed for the growth inhibition zone. The zone was measured with a transparent ruler and the results were recorded in millimeters.

Results and Discussion:-

GC-MS analysis of *Sorghum sudanese* fixed oil:-

GC-MS analysis of *Sorghum sudanese* oil was conducted and the identification of the constituents was based on the MS library (NIST) and further confirmed by interpreting the fragmentation pattern. MS library revealed about 90-95% match. The GC-MS analysis of the studied oil revealed the presence of 25 components(Table 3).The typical total ion chromatograms (TIC) is depicted in Fig.1.

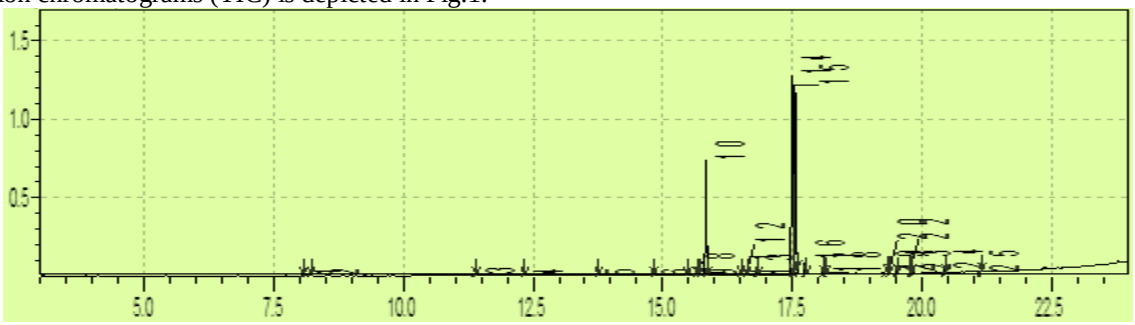

Fig. 1:- Total ion chromatograms

Table 3:- Contituents of *Sorghum sudanese* oil

Peak#	R.Time	Area	Area%	Name
1	8.048	82655	0.12	Octadecane, 1,1-dimethoxy-
2	8.188	148210	0.21	Nonanal dimethyl acetal
3	11.375	214379	0.31	Butylated Hydroxytoluene
4	12.308	121335	0.18	2-Octanol, 8,8-dimethoxy-
5	13.726	48747	0.07	Methyl tetradecanoate
6	14.801	33473	0.05	Methyl 13-methyltetradecanoate
7	15.436	38057	0.05	2H-Oxireno[3,4]cyclopenta[1,2-c]furan-2-one,
8	15.638	463598	0.67	9-Hexadecenoic acid, methyl ester, (Z)-
9	15.680	97685	0.14	9,12-Hexadecadienoic acid, methyl ester
10	15.833	12469450	17.99	Hexadecanoic acid, methyl ester
11	16.492	97225	0.14	Hexadecanoic acid, ethyl ester
12	16.602	86785	0.13	cis-10-Heptadecenoic acid, methyl ester
13	16.810	108646	0.16	Heptadecanoic acid, methyl ester
14	17.501	28084416	40.52	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
15	17.545	23287293	33.60	9-Octadecenoic acid (Z)-, methyl ester
16	17.747	1429673	2.06	Methyl stearate
17	18.091	265724	0.38	9,12-Octadecadienoic acid, ethyl ester
18	18.132	203382	0.29	Ethyl Oleate
19	19.301	543579	0.78	11-Eicosenoic acid, methyl ester
20	19.356	166451	0.24	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrie
21	19.505	265076	0.38	Eicosanoic acid, methyl ester
22	19.765	313263	0.45	n-Propyl 9,12-octadecadienoate
23	19.790	253336	0.37	Butyl 9-octadecenoate or 9-18:1
24	20.425	376743	0.54	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethy
25	21.126	107392	0.15	Docosanoic acid, methyl ester
		69306573	100.00	

Major constituents of the oil are discussed below:-

9,12-Octadecadienoic acid methyl ester (40.52%)

Fig. 2 shows the EI mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z294, which appeared at R.T. 17.501 in total ion chromatogram, corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$.The peak at m/z263 corresponds to loss of a methoxyl function.

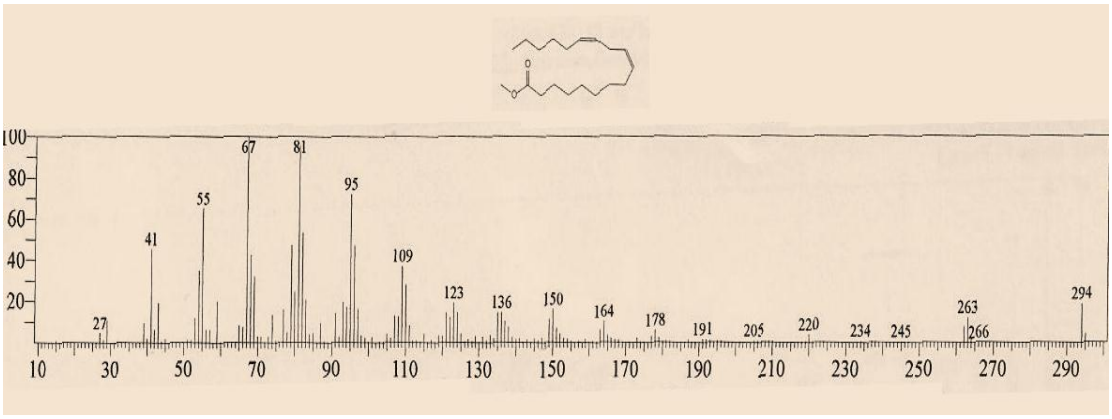

Fig. 2:- Mass spectrum of 9,12-octadecadienoic acid methyl ester

9-Octadecenoic acid methyl ester(33.60%):-

Fig. 3 shows the mass spectrum of 9-octadecenoic acid methyl ester. The signal at m/z 296 (R.T. 17.545) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl group.

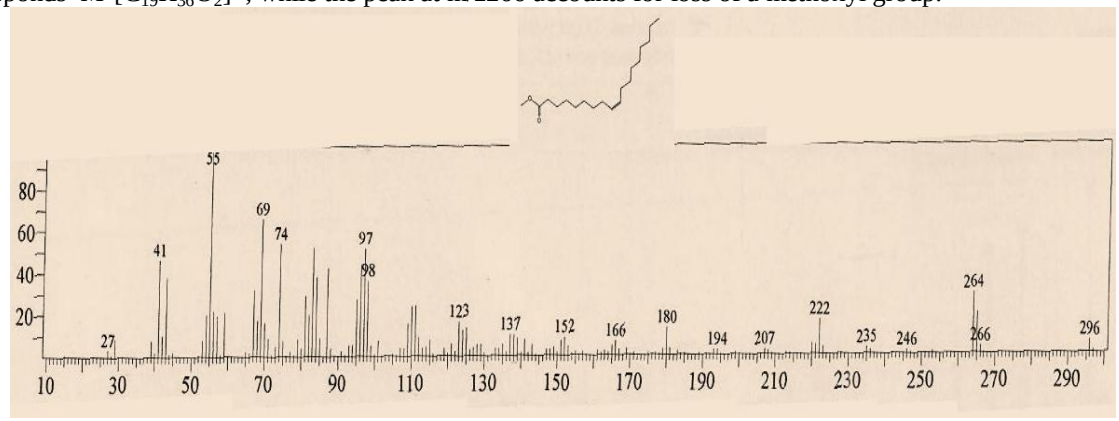

Fig. 3:- Mass spectrum of 9-octadecenoic acid methyl ester

Hexadecanoic acid methyl ester(17.99%):-

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 4.The peak at m/z 270, which appeared at R.T. 15.833 corresponds $M^+[C_{17}H_{34}O_2]^+$, while the peak at m/z239 is attributed to loss of a methoxyl function.

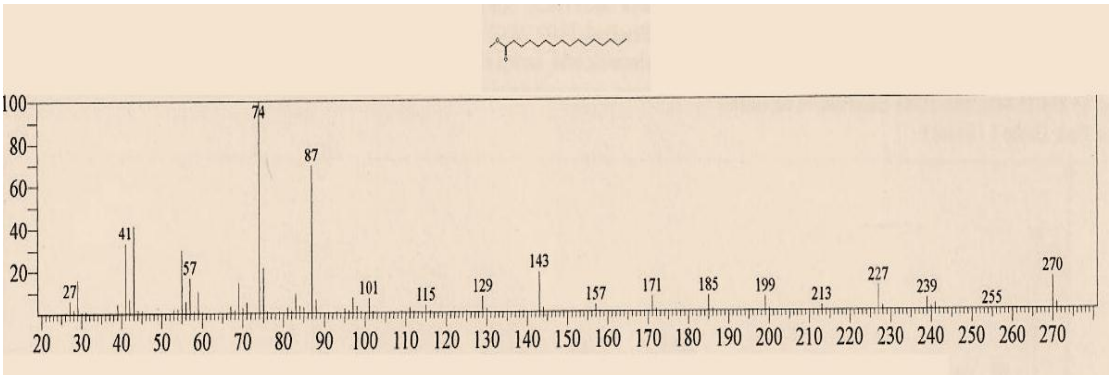

Fig. 4:- Mass spectrum of hexadecanoic acid methyl ester

.

Methyl stearate(2.06%):-

Mass spectrum of methyl stearate is displayed in Fig. 5. The peak at m/z 298, which appeared at R.T. 17.747 corresponds $M^+[C_{19}H_{38}O_2]^+$.The peak at m/z267 accounts for loss of a methoxyl .

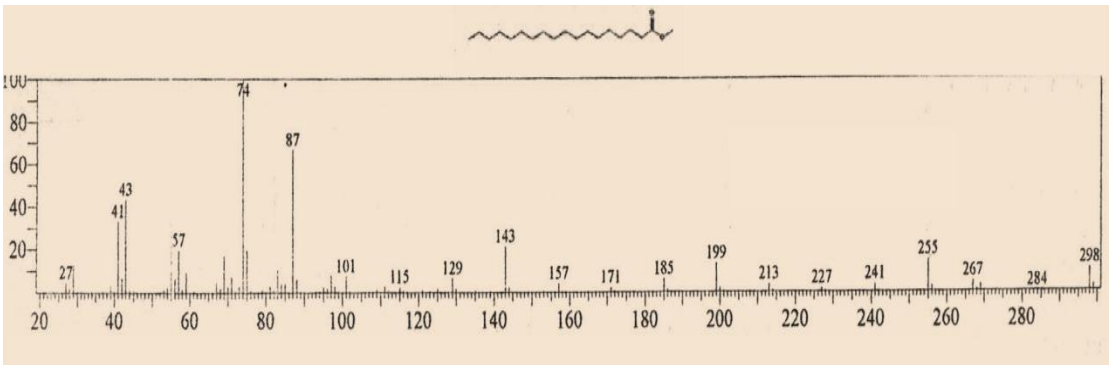

Fig. 5:- Mass spectrum of methyl stearate

Antibacterial Activity:-

The antimicrobial activity of the oil was assessed against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (4) .The results were interpreted as follows : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (5) and (6) represent the antimicrobial activity of standard chemotherapeutic agents.

Table 4:- Antimicrobial activity of *Sorghum sudanese* oil

Type	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Oil	100	18	25	20	20	20
	50	14	21	17	18	18
	25	14	18	15	18	18
	12.5	14	18	14	14	-
	6.25	12	18	12	13	-

Table 5:- Antibacterial activity of standard chemotherapeutic agents

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

Table 6:- Antifungal activity of standard chemotherapeutic agent

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

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

The oil showed excellent activity against all test organisms and specially against *Bacillus subtilis* at a concentration of 100mg/ml. At all test concentrations, the oil gave significant activity against *Bacillus subtilis* . Excellent antifungal activity against *Candida albicans* was demonstrated at concentrations 100,50 and 25 mg/ml.

References:-

1. **FAO,** "Sorghum and Millet in Human nutrition" , Food and Agriculture Organization, Rome ,1995.
2. **Harlan,** J.R. and de Wet, J.M.J. , *Crop Sci.*,1975, **12** , 172.
3. **Hitchcock**, A.S. : In "Manual of the Grasses of the United States". U.S.D.A. Mscellaneous Publication No. 200 , New York. 2nd ed. , 1971.
4. **Hussain**,A., Mohamed,D.,Bhatti,M., Zahid, M.,*Pak. J. Agrid. Res*.,1991, **12**,58.
5. **Merrill**,S.D., Tanaka,D.L., Krupinsky,J.M., Liebig, M.A., Hanson,J.D., *Agro. J.* ,2007, ,**99**,931.
6. **Mutegi**,E.,Sagnard,F.,Muraya,M.,Kanyenji,B.,Rono,B., Mwongera,C.,Kamau,J., *Genetic Resources and Crop Evolution*,2000 **, 57**(2),243.
7. **Marsalisa**,M.A.,Angadi,S.V.,Contreras- Govea,F.E., *Field Crop Res*.,2010,**116**, 52.
8. **Singh,**R. and Axtell,J.D., *Crop Science* ,1973,**13** , 535.
9. **Sankara**,R.D. and Deosthale,Y.G., *Nutr. Rep. Int.*, 1980,**22**,723.
10. **Sawinski**,J., Szydelko,E.,*Polish J. Agron*., 2011, **4,** 23.
11. **(URL-1)** : www.Articles.extension,org./Sweet Sorghum for Biofuel Productin
12. **(URL-2)** : www. The plant List . Org./Sorghum.
13. **Watson,**AM., "Agricultural Innovations in the Early Islamic World", Cambridge University Press, Cambridge,1983