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RESEARCH ARTICLE

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *PENNISETUM GLAUCUM* FIXED OIL.

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Abstract

Millets which were cultivated thousands of years ago are one of the most important cereal crops. This study was carried out to investigate the constituents of *Pennisetum glaucum* fixed oil **.** GC-MS analysis revealed the presence of 15 components dominated by: 9,12-octadecadienoic acid methyl ester (49.66%) **;** 9-octadecenoic acid methyl ester(33.16%) ; hexadecanoic acid methyl ester (6.87%) ; methyl stereate(4.28%).The disc diffusion bioassay was used to assess the antimicrobial activity of the oil. *Pennisetum glaucum* oil showed activity against all test organisms. It showed excellent activity against *Bacillus subtilis* and other bacteria except *Staphylococcus aureus* where a moderate activity was observed . It also showed good activity against the fungal species *Candida albicans* and moderate against *Aspergillus niger.*

Introduction:-

Millets represent a collective term referring to a number of small-seeded annual grasses reaching 0.5-4m in height.Millet is a cereal crop plant belonging to the grass family, Gramineae (FAO, 1972). Most of millet genera are widely distributed throughout the tropics and subtropics of the world (De Wet, 2006).

Millets which were cultivated thousands of years ago are one of the most important cereal crops(Lu et.al.,2005).In Africa it extends as belt from Sudan to Senegal.The plant can survive in arid regions with soils of low fertility (Girish et.al.,2015).In western Africa considerable morphological diversity of millet is observed. India is considered as a secondary center of diversity (Shweta, 2015).

The plant has several health promoting abilities .Millets provide minerals, significant amount of essential amino acids, fatty acids, vitamins , proteins and dietary fiber(Obilane and Manyasa, 2002; Devi et.al.,2011) . Regular consumption of whole grain, like millets, has been shown to reduce risk of diabetes, gastrointestinal disorders and cardiovascular diseases as demonstrated by some epidemiological studies(Shweta,2015)). Millets have low glycemic index and their fiber, magnesium, phenolics and tannins content reduce the risk of diabetes since they slow the sudden increase in blood glucose and insulin levels(Montonen et.al.,2003). Due to its high content of the micronutrients iron and zinc , millet , is considered useful in case of anaemia(Shweta,2015 ; Hoseney et.al. , 1987).Millets also have the potential to attenuate many degenerative diseases like hypertension . This is attributed to their antioxidant properties . Since millets do not contain gluten, they are usefull in celiac disease (Amadou

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et.al.,2011; Taylor and Emmambux,2008).Millets possess anticancer properties and this is due to their phenolic and tannin content(Grimmer et.al.,1992).

Pennisetum glaucum (Pearl millet) has about 400 genera. It is the major millet grown in Africa and it is the fourth most important cereal food crop grown in India (FAO, 1972).Pearl millet possesses high phenolic content and high free radical scavenging activity and therefore can serve as a source of antioxidants in diets (Odusola et.al.,2013).

Materials and Methods:-

Materials:-

Plant material:-

Seeds of *Pennisetum glaucum* were purchased from the local market-Omdurman, Sudan. The plant was identified and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

Test organisms:-

Microorganisms used for antimicrobial assay are shown in Table 1.

Table 1:- Test organisms

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Aspergillus niger*	fungus
6	*Candida albicans*	fungus

Methods:-

Extraction of oil from *Pennisetum glaucum* seeds:-

Powdered seeds of *Pennisetum glaucum* (400g) were extracted with n-hexane by maceration .The solvent was removed *in vacuo* to give the fixed oil.

Oil sample was esterified at room temperature via a methanolic solution of sodium hydroxide (2g of sodium hydroxide in 100ml methanol) and methanolic sulphuric acid (1ml of concentrated sulphuric acid in 99ml methanol).Following esterification , the oil was studied by gas chromatography – mass spectrometry using a Shimadzo instrument equipped with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness). Carrier gas was analytical grade Helium (purity 99.99 %) . Oven temperature program is shown below:

Rate: - ; **Temperature** :150^0 ; **Hold Time** (min^{-1}) : 1.00

Rate : 4.00 ; **Temperature** :3 00^0 ; **Hold Time** (min^{-1}) : 0.00

Other chromatographic conditions are tabulated below:

Table 2:- Chromatographic conditions.

Column oven temperature	150.0°C
Injection temperature	300.0°C
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

Antimirobial sensitivity test:-

Powdered agar (approximately 38g) was dispersed in 1 liter of distilled water for ten minutes. Then it was heated in a water bath to dissolve, swirled to mix and sterilized (autoclave) at 121^{o}C for 15 minutes .It was then cooled at 47^{o} , mixed well and poured into sterile Petri dishes.

Aliquots (20 ml) of molten agar were distributed into sterile Petri dishes. About (0.1ml) of the standardized bacterial stock suspension(10^{8}-10^{9} colony-forming units/ml) were soaked on agar medium plates using sterile cotton . Sterilized filter paper discs (6mm diameter) were soaked in test sample solution and then placed on the surface of the test bacteria plates. The plates were then incubated for 24h. Following incubation the, diameter of inhibition zones were measured in triplicates and averaged. The same procedure was adopted for the standard antimicrobial chemotherapeutics (positive control) . DMSO was used as negative control.

For antifungal activity, potato dextrose agar was used instead of nutrient agar . Incubation period was continued for 72h.

Results and discussion:-

The total ion chromatogram is shown in Fig. 1 , while oil constituents are shown in Table 3.

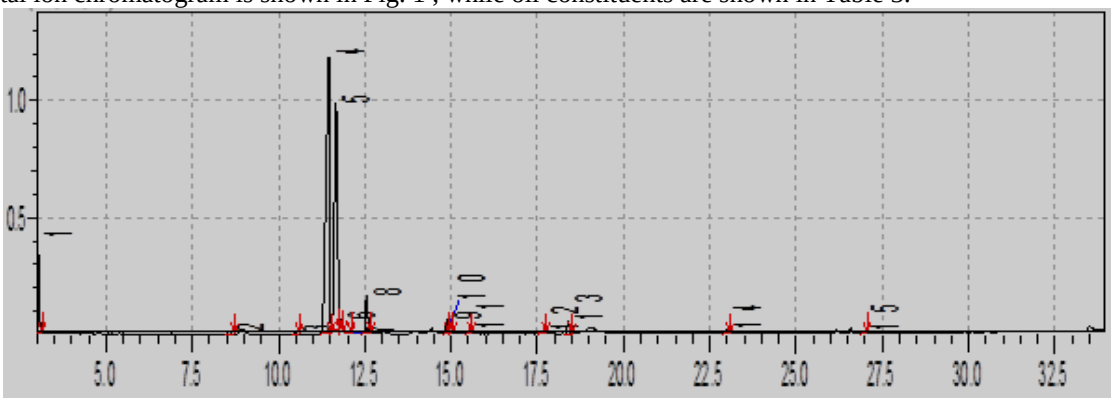

Fig.1:- Total ion Cheromatograms

Table 3:- Constituents of *Pennisetum glaucum* oil

Peak#	R.Time	Area	Area%	Name
1	3.005	12253689	6.87	Hexadecanoic acid,methyl ester
2	8.566	619208	0.35	10,13-Octadecadienoic acid, methyl ester
3	10.484	175028	0.10	Heptadecanoic acid, 16-methyl-, methyl ester
4	11.457	88599963	49.66	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
5	11.661	59158683	33.16	9-Octadecenoic acid (Z)-, methyl ester
6	11.815	2042783	1.15	11-Octadecenoic acid, methyl ester
7	11.970	317036	0.18	cis-13-Octadecenoic acid, methyl ester
8	12.548	7632582	4.28	Methyl stearate
9	14.873	1784816	1.00	Linoleic acid ethyl ester
10	14.989	1480288	0.83	8-Octadecenoic acid, methyl ester
11	15.520	624751	0.35	Nonadecanoic acid, methyl ester
12	17.680	487031	0.27	cis-11-Eicosenoic acid, methyl ester
13	18.395	2159375	1.21	Methyl 18-methylnonadecanoate
14	22.988	620021	0.35	Docosanoic acid, methyl ester
15	26.959	443431	0.25	Tetracosanoic acid, methyl ester
		178398685	100.00	

Major constituents of oil are discussed below:

i) 9,12-Octadecadienoic acid methyl ester (49.66%):-

The mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig 2 . The peak at m/z 294(RT 11.457) corresponds M^{+} $(C_{19}H_{34}O_{2})^{+}$.The peak at m/z 263 correspond to loss of a methoxyl .

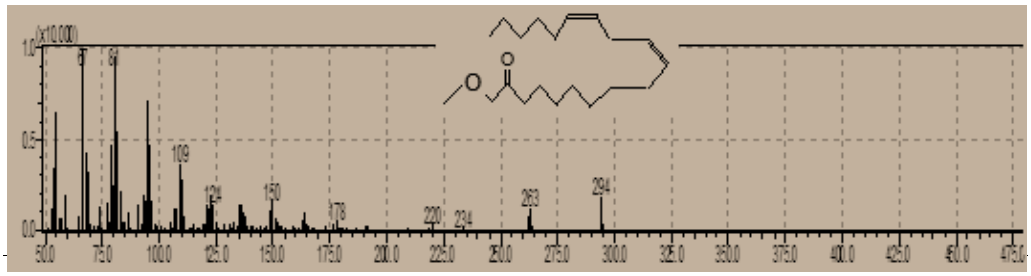

Fig.2:- Mass spectrum of 9,12-octadecadienoic acid methyl ester

ii) 9-Octadecenoic acid methyl ester(33.16%):-

The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig.3. The signal at m/z 296(RT 11.661) corresponds M^+ $(C_{19}H_{36}O_2)^+$.The peak at m/z 264 is due to loss of methoxyl function.

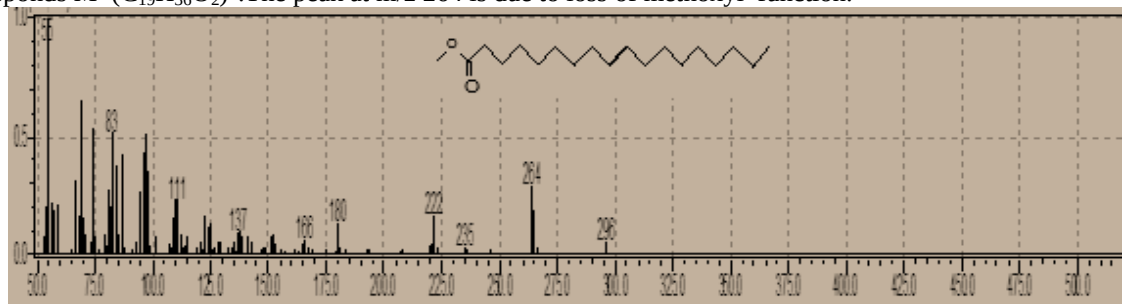

Fig. 3:- Mass spectrum of 9-octadecenoic acid methyl ester

iii)Hexadecanoic acid methyl ester (6.87%):-

The molecular ion M^+ $(C_{17}H_{34}O_2)^+$ for hexadecanoic acid appeared at m/z270(RT, 3.005). The peak at m/z 239 is due to loss of a methoxyl (Fig.4).

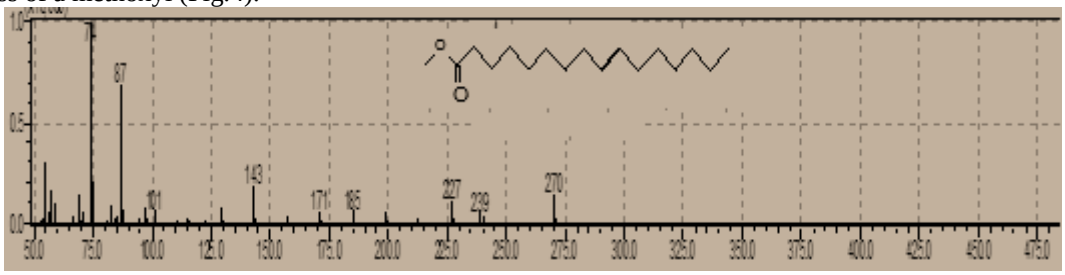

Fig.4:- mass spectrum of hexadecanoic acid methyl ester

iv) Methyl stearate(4.28%)

The mass spectrum of methyl stearate is displayed in Fig.5 . The peak at m/z298(RT 12.548) corresponds M^+ $(C_{19}H_{38}O_2)^+$, while the signal at m/z267 accounts for loss of a methoxyl.

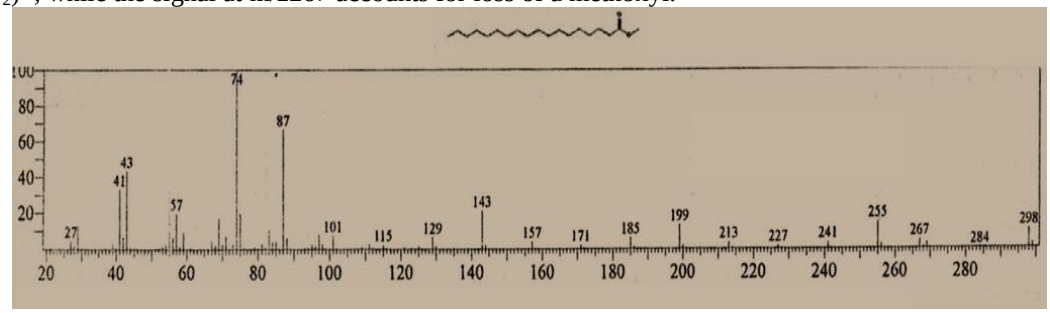

Fig.5:- Mass spectrum of methyl stereate

Antimicrobial assay:-

Fixed oil from *Pennisetum glaucum* was screened for antimicrobial activity against six standard human pathogens. Diameters of the growth inhibition zones are displayed 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) show the antimicrobial activity of standard drugs.

Table 4 : Antibacterial activity of *Pennisetum glaucum* oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Pennisetum glaucum oil	100	16	17	14	19	15	14

Table 5 : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	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*

Pennisetum glaucum oil showed activity against all test organisms. It showed excellent activity against *Bacillus subtilis* and other bacteria except *Staphylococcus aureus* where a moderate activity was observed . It also showed good activity against the fungal species *Candida albicans* and moderate against *Aspergillus niger*

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