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Phytochemical constituents and Antimicrobial Efficacy of Sudanese *Hibiscus cannabinus L.* Seeds Oil

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Abstract

Essential oils (EOs) are used in a wide variety of consumer goods such as soaps, detergents, toilet products, pharmaceuticals, cosmetics, perfumes, food products, soft drinks, alcoholic beverages and insecticides. Essential oils are generally derived from one or more plant parts, such as flowers, leaves, leaves and stems, bark, wood, roots, seeds, fruits, rhizomes and gums. Therefore, three methods of producing EOs in industry has developed, such as water distillation; water and steam distillation; and direct steam distillation. Often, the oil content is so small that oil removal is not commercially feasible by the aforementioned methods. In industry, most modern essential oil production is accomplished by solvent extraction, using volatile solvents such as petroleum ether and n-hexane. The main advantages of extraction over distillation is that uniform room temperature (usually 25 ± 5° C) can be maintained during the process. This feature is of considerable importance in essential oil extraction from different medicinal plant parts. The scope of this study included extraction of *Hibiscus cannabinus L.* seed oil using solvent extraction with characterize its phyto-chemical constituents by GC - MS technique and identify its anti-bacterial activity against four standard bacterial species and one standard fungus specie.

Hibiscus cannabinus L. is herbaceous annual plant growing under tropical climates. It has multi industrial applications including: textile, paper, fabrics, insulation and absorption materials. This

herb possesses anti-tumor, anti-oxidant, anti-hypertensive, anti-inflammatory and anti-proliferative properties. In this study, the seed oil was extracted and its components were identified by GC-MS Technique. The oil was evaluated for its antimicrobial activity against five standard human pathogens, which are *Bacillus subtilis* (*Bs.*), *Staphylococcus aureus* (*Sa.*), *Pseudomonas aeroginosa* (*Pa.*), *Escherichia coli* (*Ec.*) and *Candida albicans*(*Ca.*).Analysis results revealed the presence of 28 components dominated by 9, 12-octadecadienoic acid methyl ester (51.41%). Results showed weak activity against *Staphylococcus aureus* and moderate for other test organisms.

Keywords: *Hibiscus cannabinus L.,* Oil, GC/MS Analysis, Antimicrobial

1. Introduction

Hibiscus cannabinus L. is an herbaceous annual plant in the family Malvaceae (Paul and Smith, 2003).This plant grows under tropical climates and under good climate may reach a height of 5m within 6-8 months (Coetzee *et al*. 2008; Wood, 2003). It is a multipurpose plant with diverse industrial applications including: textile, paper, fabrics, insulation and absorption materials (Cheng, 2001; Alexopoulou *et al.* 2004; Alexopoulou and Monti, 2013; Baldwin and Graham, 2006). The inner part of the fiber of this plant is very rich in cellulose and could be used for the production of bio-ethanol(Lips *et al*. 2009). Also, it is a visible and ecologically – friendly source of cellulose (Karimi *et al*. 2014). Major producers of this plant are: China, Thailand and India (Alexopoulou *et al.* 2004). The center of origin of is probably the sub-Saharan Africa (Cheng *et al*. 2004; Dempsey, 1975).

Furthermore, it is an important medicinal plant with diverse array of biologically active molecules(Wong *et al*. 2014). This herb possesses anti-tumor, antioxidant, anti-hypertensive, anti-inflammatory and anti- proliferation properties (Maganha *et al*. 2010). This plant is rich in flavonoids and polyphenols which are associated with a reduced risk of certain types of cancer (Durgo *et al*. 2012). Its leaves are used traditionally against jaundice and scurvy (Bindhu and Umadevi, 2013).

2. Materials and Methods

2.1 Collection of plant materials and conservation

Hibiscus cannabinus L. seeds were collected based on the traditional medicinal uses and ethnomedicinal knowledge of ethnic people from a forest reserve around Damazin, Sudan. Seeds were identified by professional taxonomists at Medicinal and Aromatic Plants Research Institute,

Khartoum, Sudan. The collected sample was shade dried at room temperature in a shadow place for seven days before crunching. The specimen of seeds was kept in clean dry glass container.

2.2 Tested Microorganisms

The extracted essential oil of *H. cannabinus L.* seeds was tested against five different microorganisms; two Gram positive bacterial species: *Bacillus subtilis* (*Bs.*), *Staphylococcus aureus* (*Sa.*), two Gram negative bacterial species: *Pseudomonas aeroginosa* (*Pa.*), *Escherichia coli* (*Ec.*) and one strain of *fungal specie Candida: albicans* (*Ca.*).

2.3 Oil extraction

For the extraction, *Hibiscus cannabinus L. s*eeds were firstly ground into a fine powder. A 350g of the dried powder were exhaustively extracted with n-hexane at room temperature (usually 25 ± 5^{o}C). The solvent was removed under reduced pressure to afford the oil. The extracted oil was esterified as follows: Two milliliters of oil was placed in a test tube and then 7mL of alcoholic sodium hydroxide were added followed by 7mL of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight. A (2mL) of supersaturated sodium chloride were added, then (2mL) of n-hexane were added, and then the tube was shaken vigorously for five minutes. The n-hexane layer was then separated. A (5 µL) of the n-hexane extract were mixed with 5mL diethyl ether. The solution was filtered and the filtrate (1µL) was injected in the GC-MS vial.

2.4 GC/MS analysis conditions

The extracted essential oil composition was characterized by Shimadzo GC-MS- QP2010 Ultra instrument with RTX-5 MS column (30m, length; 0.25mm diameter; 0.25µm, thickness)**.** The analysis program and conditions were as follows: Helium at 1.7mL/min was used as carrier gas; the injection volume was 1µL; column flow was 1.54mL/sec and total flow was 50.0mL/min; purge flow was 3.0mL/min; pressure was 139.3KPa; ionization potential, 70ev; The initial temperature of the column was kept at 150^{o}C and kept constant at 300^{o}C (column cleaning step) for three minutes.

2.5 Antimicrobial assay

2.5.1 Disc diffusion method

Mueller Hinton and Sabouraud dextrose agar were used as a growth media for the bacteria and the fungus respectively. The media were prepared according to manufacture instructions. Broth cultures (5.0x10^{7} CFU/mL) were streaked on the surface of the solid medium contained in Petri dishes (Tendencia, 2004). Filter paper discs (Oxid, 6mm) were placed on the surface of the inoculated agar and then impregnated with 100mg/mL of test sample. For bacteria the plates were incubated at 37^{o}C

for 24h., while for fungi the plates were incubated at 25ºC for 3days.The assay was carried in two replicates and the diameters of inhibition zone were measured and averaged. Ampicillin, gentamicin and clotrimazole were used as positive control and DMSO as negative control.

3. Results and discussion

Characterization of extracted *Hibiscus cannabinus L.* seeds oil by GC-MS analysis showed **28** constituents as are recorded in Table (**1**). The identified components were confirmed by their retention times and mass spectra fragmentation pattern. The total ion chromatograms are shown in Fig.**1**.

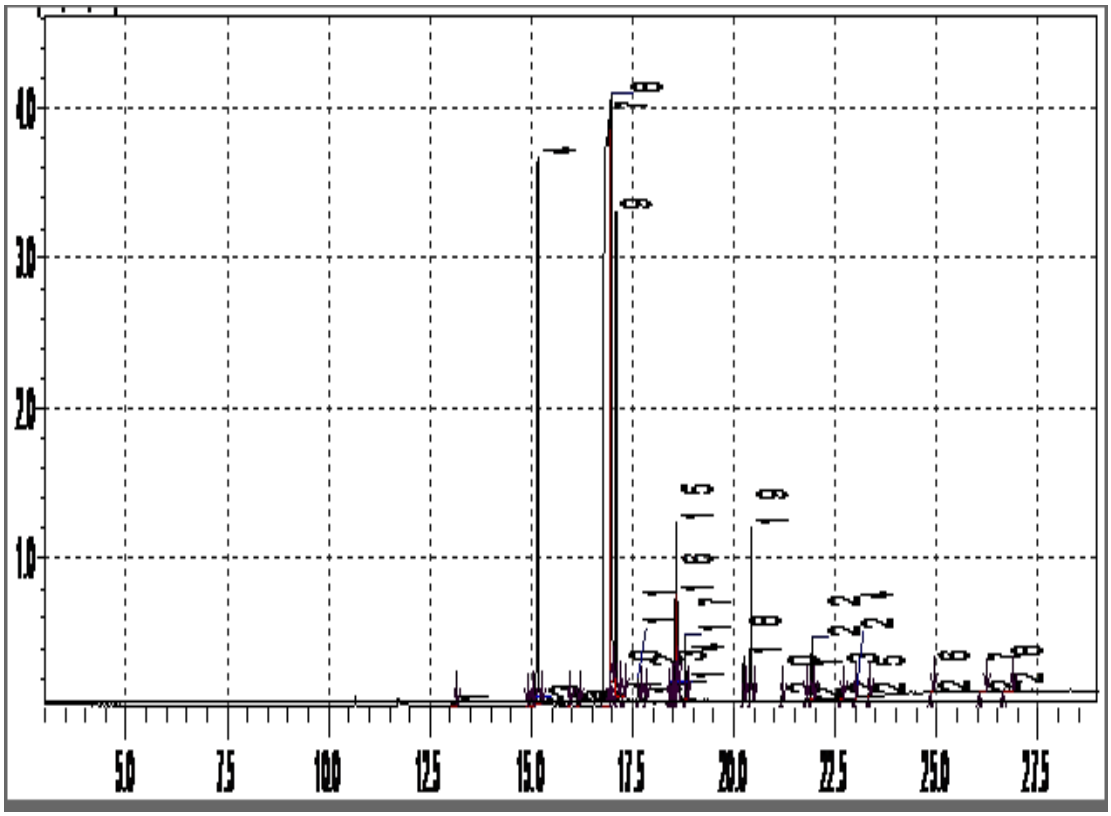

Fig.1: Total ion chromatograms

Table 1: Phytochemical components of *Hibiscus cannabinus L.* seeds oil

No.	Component name	RT(mins)	Area%

1.	Methyl tetradecanoate	13.044	0.10
2.	7-Hexadecenoic acid, methyl ester, (Z)-	14.896	0.02
3.	9-Hexadecenoic acid, methyl ester, (Z)-	14.940	0.20
4.	**Hexadecanoic acid, methyl este**r	**15.158**	**13.19**
5.	cis-10-Heptadecenoic acid, methy l ester	15.914	0.07
6.	Heptadecanoic acid, methyl ester	16.123	0.09
7.	**9,12-Octadecadienoic acid (Z,Z)-, methyl este**r	**16.926**	**51.41**
8.	**9-Octadecenoic acid (Z)-, methyl ester**	**16.969**	**14.34**
9.	Methyl stearate	17.092	9.18
10.	Methyl 10-trans,12-cis-octadecadienoate	17.284	0.16
11.	Methyl 9-cis,11-trans-octadecadienoate	17.630	0.42
12.	10-Nonadecenoic acid, methyl ester	17.822	0.08
13.	8,11-Eicosadienoic acid, methy l ester	18.409	0.21
14.	11,14-Eicosadienoic acid, methyl ester	18.447	0.23
15.	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	18.568	2.82
16.	cis-11-Eicosenoic acid, methyl ester	18.590	1.05
17.	Eicosanoic acid, methyl ester	18.800	1.02
18.	13-Docosenoic acid, methyl ester, (Z)-	20.238	0.76
19.	Docosanoic acid, methyl ester	20.421	2.84
20.	Tricosanoic acid, methyl ester	21.187	0.10
21.	15-Tetracosenoic acid, methyl ester, (Z)-	21.765	0.04
22.	Tetracosanoic acid, methyl ester	21.921	1.09
23.	Squalene	22.640	0.09
24.	Cholestane	22.997	0.02
25.	Hexacosanoic acid, methyl ester	23.322	0.03
26.	Vitamin E	24.887	0.04
27.	Stigmasterol	26.144	0.06
28.	.gamma.-Sitosterol	26.712	0.34

Results in Table (**1**) and Figure (**1**) reveal that the major components of the extracted oil are: 9, 12-Octadecadienoic acid methyl ester (51.41%), 9-Octadecenoic acid methyl ester (14.34%) and hexadecanoic acid methyl ester (13.19%). Mass spectra of these major components are shown in Figures (**2**, **3**, **4**).

The electron ionization (EI) mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Figure (**2**). The peak at m/z 294, which appeared at retention time (RT): 16.962 in total ion chromatogram, corresponds: $M^+[C_{19}H_{34}O_2]^+$ .The signal which appeared at m/z263 attributed to the loss of methoxyl group. Figure (**3**) shows the mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296 (RT: 16.969) accounts for: $M^+ [C_{18}H_{34} O_2]^+$. Figure (**4**) illustrates the mass spectrum of hexadecanoic acid methyl ester. The peak at m/z 270 (RT. 15.158) was detected in the spectrum corresponds: $M^+[C_{17}H_{34}O_2]^+$ .The signal at m/z 239 is due to loss of a methoxyl group.

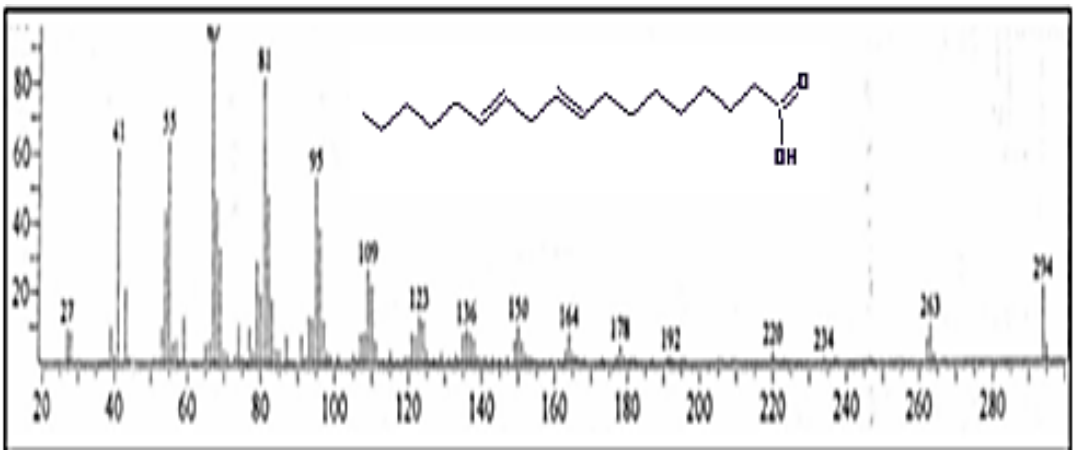

Fig. 2: Mass spectrum of 9, 12-octadecanoic acid methyl ester

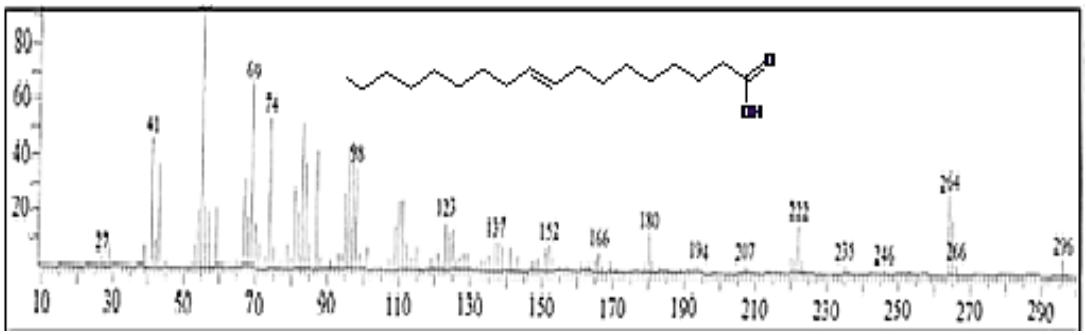

Fig.3: Mass spectrum of 9-octadecenoic acid methyl ester

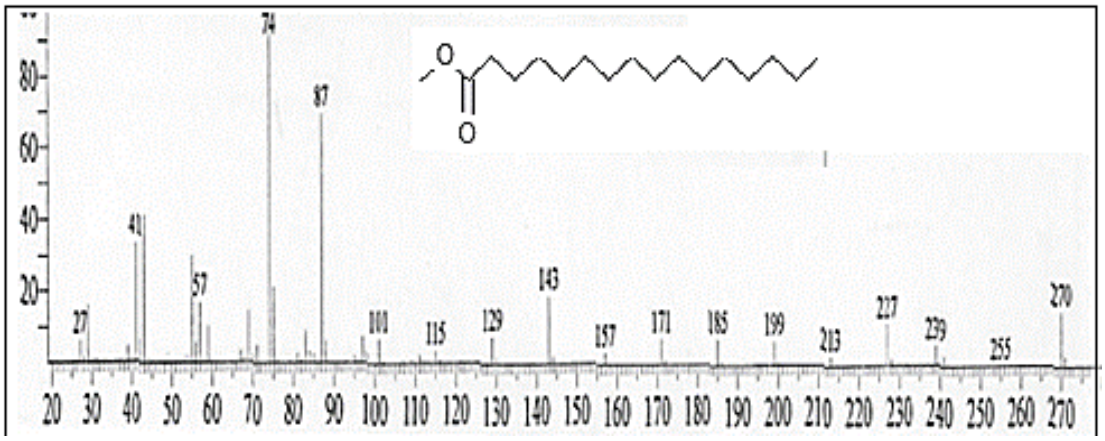

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester

Extracted *H. cannabinus L. seeds* oil was evaluated for its antimicrobial activity against the four standard bacterial strains and candida fungus using disc diffusion assay. The average of the diameters of the growth inhibition zones are recorded in Table (**2**). Results were interpreted in

conventional terms: (<9 mm: inactive; 9 -12 mm: partially active; 13-18 mm: active >18 mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls.

Table 2: Inhibition zones (mm/mg sample)

Concentartion (mg/mL)	***Sa.***	***Bs.***	***Ec.***	***Pa.***	***Ca.***
Oil (100)	12	14	14	13	14
Ampicilin (40)	30	15	--	--	--
Gentamicin (40)	19	25	22	21	--
Clotrimazole (30)	--	--	--	--	38

Where: ***Sa.****: Staphylococcus aureus;* ***Bs.****: Bacillus subtilis;* ***Ec****.: Escherichia coli;* ***Pa.****: Pseudomonas aeruginos*a; Ca.*: Candida albicans.*

Basis on disc diffusion method results as shown in Table (**2**), its clear that the average minimum inhibitory concentration (MIC) values of *H. cannabinus* oil against the selected standard organisms. The results showed that the extracted oil is active against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans,* but it seems partially active against *Staphylococcus aureus*. The bacteriostatic effect of essential oil may attribute to evaporation and miss of oil original quality (Bakkali *et al*. 2008).

Conclusion

It could be concluded that *H. cannabinus L.* seeds oil has better effects against Gram negative bacteria (*Pa.*, *Ec.*), Gram positive (*Bs.*) and (*Ca.*) fungi than Gram positive (*Sa.*) bacteria.

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