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CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *DICOMA TOMENTOZA* GROWN IN SUDAN

Abdel Karim M.[1*], Amin-Eldin E.[1] and M. Alla[2]

[1]Sudan University of Science and Technology, Faculty of Science (Sudan).
[2]Delta College of Science and Technology (Sudan).

Corresponding Author: Abdel Karim M.
Sudan University of Science and Technology, Faculty of Science (Sudan).

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ABSTRACT

Intensive pharmacological reports on the impact of bioactive phytochemicals on human physiology potentiated the applications of medicinal plants**.** Dicoma is a genus of approximately 35 species in the family Compositeae. Most of these species are small shrubs or even trees and they may grow in diverse habitats including deserts. *Dicoma tomentosa* is distributed in tropical Africa and Asia.In African system of medicine the plant is used against malaria.In this study, the oil from *Dicoma tomentosa* seeds has been analyzed by GC-MS. The GC-MS analysis showed 18 components. Major constituents are : 9-octadecenoic acid methyl ester (39.78 %) ; methyl 5;6-octadecadienoate(24.57%) ; hexdecanoic acid methyl ester(16.57 %) and 9;12-octadecadienoic acid methyl ester(14.50%) .The antmicrobial activity of the oil has been assessed. At a concentration of 100mg/ml, the oil showed significant activity against *Escherichia coli*. It also exhibited moderate activity against *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Candida albicans*.

KEYWORDS: *Dicoma tomentosa*, Oil, Constituents, Antimicrobial Activity.

INTRODUCTION

Dicoma is a genus of approximately 35 species in the family Compositeae.[1] Most of these species are small shrubs or even trees and they may grow in diverse habitats including deserts.[2] *Dicoma tomentosa* is distributed in tropical Africa and Asia. In African system of medicine the plant is used against malaria.[3] *Dicoma tomentosa* contains sesquiterpenes,[4-6] sterols and triterpenes[7,8] beside some flavonoids[9-12]

Dicoma tomentosa is used by some communities as tooth cleaner.[13] It is used traditionally against wounds and as febrifuge.[14] The antiplasmodial activity of *Dicoma tomentosa* has been reported.[15-17]

MATERIALS AND METHODS

Plant material

Seeds of *Dicoma tomentoza* were collected from Damazin-Sudan. The plant was authenticated by the Medicinal and Aromatic Plants Research Institute-Khartoum-Sudan.

Instruments

A shimadzo GC-MS-QP2010 ultra instruments with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25µm, thickness) was used for GC-MS analysis.

Test organisms

Dicoma tomentoza oil was screened for antibacterial and antifungal activity using the standard microorganisms shown in Table (1)

Table 1: Test organisms.

Ser.No	Microorganism	Type
1	*Bacillus subtilis*	G+ve
2	Staphylcococcus aureus	G+ve
3	Pseudomonas aeroginesa	G-ve
4	Escherichia coli	G-ve
5	Candida albicans	fungus

METHODS

Extraction of oil

Dicoma tomentoza seeds(350g) were macerated with hexane at room temperature. The solvent was removed under reduced pressure to give the oil.

The oil was esterified as follows: A methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol. The oil (2ml) was placed in a test tube and (7ml) of methanolic sodium hydroxide were added followed by (7ml) of an alcoholic sulphuric acid. The

tube was shaken vigorously for 3 minutes and then left over night. Then (2ml) of n-hexane were added and the tube was vigorously shaken for 3 minutes .The hexane layer was diluted with 5ml diethyl ether. One gram of sodium sulphate was added as drying agent. The solution was filtered. The filtrate (1µl) was injected in GC-MS vial.

GC-MS analysis

The studied oil was analyzed by gas chromatography - mass spectrometry. A Shimadzo GC-MS-QP2010 ultra instrument was used. Helium (purity; 99.99%) was used as carrier gas. Oven temperature program is given in table 2.

Table 2: Oven temperature program.

Rate	Temperature (c)	hold time $(min.^{-1})$
-	50.0	0.00
7	180.0	0.00
10	300.0	0.00

Chromatographic conditions are as follows: 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.**

Testing of antibacterial susceptibility

The paper disc diffusion method was used to screen the antimicrobial activity of the oil and performed by 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 10^8 cfu/ml (Turbidity=McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1. ; 6mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of test sample. The inoculated plates were incubated at 37^0C for 24 hours in the invested position. The diameters (mm) of the inhibition zones were measured as average of two replicates.

RESULTS AND DISCUSSION

GC/MS analysis

Gas chromatography - mass spectrometry has been used for the identification and quantification of the *Dicoma tomentoza* oil. The analysis revealed the presence of 18 components - Table (3).The total ion chromatogram is presented in Fig.1

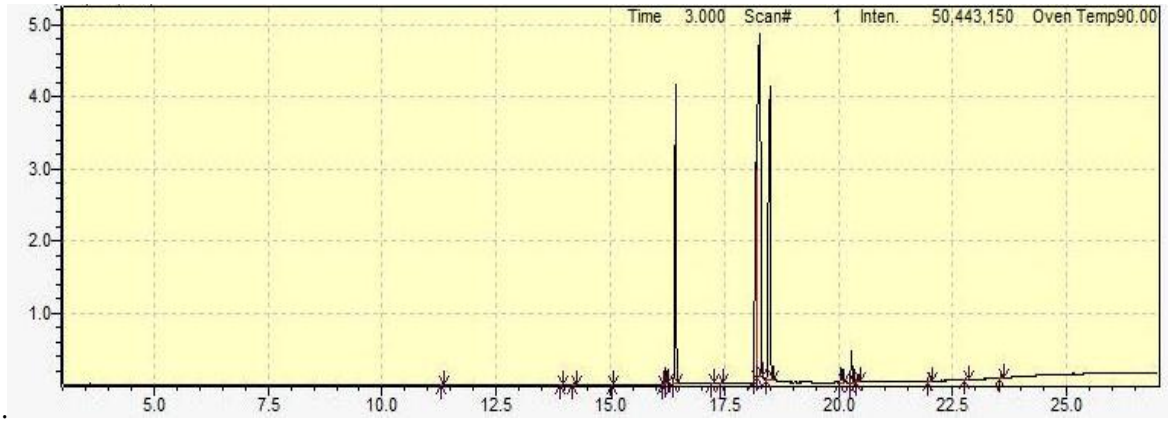

Fig. 1: Total ion chromatograms.

The following compounds were detected in the chromatogram as major constituents:

1. 9-Octadecenoic acid methyl ester (39.78 %)
2. Methyl 5; 6-octadecadienoate(24.57%)
3. Hexdecanoic acid methyl ester(16.57 %)
4. 9;12-Octadecadienoic acid methyl ester(14.50%)

The GC/MS analysis revealed a mass spectrum(Fig.2) characteristic of 9-octadecenoic acid methyl ester where

the peak at m/z 296 (RT.18.274) is due to M^+ $[C_{19}H_{36}O_2]^+$. It also showed a spectrum(Fig.3) matching that of methyl 5;6-octadecadienoate: the signal at m/z294(RT.18.496) is due to the molecular ion: $M^+[C_{19}H_{34}O_2]^+$.

The GC/MS analysis also revealed the presence of hexdecanoic acid methyl ester.The peak at m/z 270 (Fig.4) which appeared at (RT.16.430) is due to M^+ $[C_{17}H_{34}O_2]^+$. The analysis gave a mass spectrum(Fig.5) characteristic of 9;12-octadecadienoic acid methyl ester. The molecular ion: M^+ $[C_{19}H_{34}O_2]^+$ appeared at m/z 294(RT. 18.186).

Table 3:Constituent of the oil.

No.	Name	Ret.Time	Area%
1.	.gamma.-Muurolene	11.305	0.03
2.	Methyl myristoleate	13.914	0.01
3.	Methyl tetradecanoate	14.195	0.11
4.	cis-5-Dodecenoic acid, methyl ester	15.055	0.01
5.	7-Hexadecenoic acid, methyl ester, (Z)-	16.163	0.15
6.	9-Hexadecenoic acid, methyl ester, (Z)-	16.211	0.69
7.	Hexadecanoic acid, methyl ester	16.430	16.57
8.	cis-10-Heptadecenoic acid, methyl ester	17.229	0.05
9.	Heptadecanoic acid, methyl ester	17.441	0.08
10.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.186	14.50
11.	9-Octadecenoic acid (Z)-, methyl ester	18.274	39.78
12.	Methyl 5,6-octadecadienoate	18.496	24.57
13.	cis-11-Eicosenoic acid, methyl ester	20.055	0.95
14.	Eicosanoic acid, methyl ester	20.280	1.47
15.	6,9-Octadecadienoic acid, methyl ester	20.430	0.27
16.	Docosanoic acid, methyl ester	21.983	0.48
17.	Tricosanoic acid, methyl ester	22.789	0.08
18.	Tetracosanoic acid, methyl ester	23.563	0.20

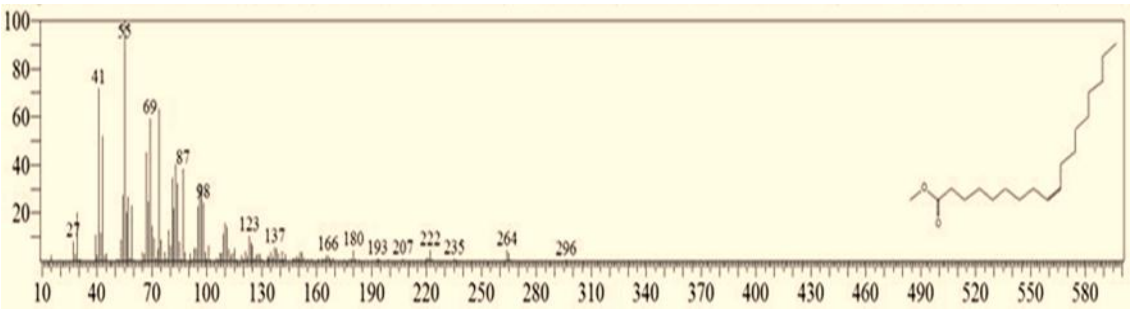

Fig.2: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester.

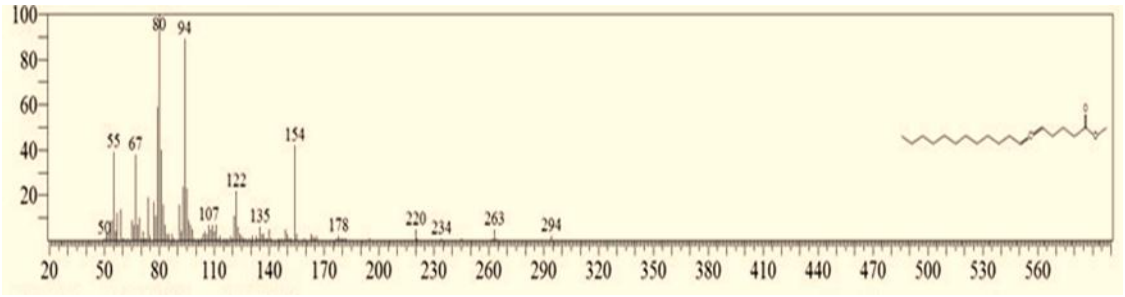

Fig.3: Mass spectrum of methyl 5;6-octadecanoate.

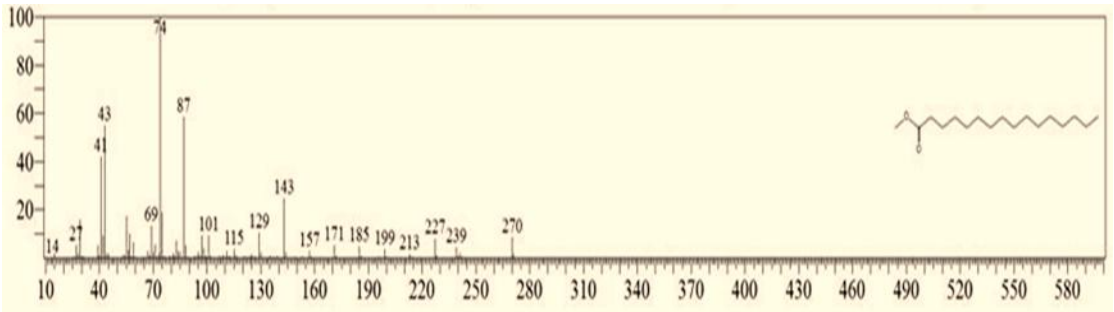

Fig. 4: Mass spectrum of hexadecanoic acid, methyl ester.

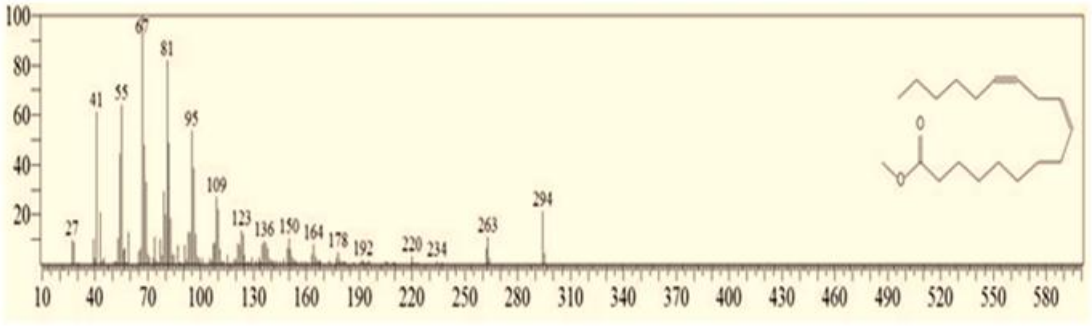

Fig. 5: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester.

Antimicrobial activity

Dicoma tomentoza oil was assessed for antimicrobial activity against five standard organisms. The inhibition zones are presented in Table 4. The oil showed significant activity against *Escherichia coli*. It also showed moderate activity against *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Candida albicans*. Tables 5 and 6 illustrate the antimicrobial activity of standard drugs.

Table 4: Inhibtion zones(mm) of *Dicoma tomentoza* oil.

Sample	Sa	Bs	Ec	Pa	Ca
Oil(100mg/ml)	14	---	20	13	15

Sa.: Staphylococcus aureus.
Bs.: Bacillus subtilis.
Ec.: Escherichia coli.
Pa.: Pseudomonas aeroginosa.
Ca.: Candida albicans.

Table 5: Inhibition zones of standard antibacterial agents.

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

Table 6: Inhibition zone (mm)s of standard antifungal agent.

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

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