

## GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *TRICHYSPERMUM AMMI* MARKETED IN KHARTOUM (SUDAN)

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### ABSTRACT

*Trichyspermum ammi* is a herb of diverse uses in Sudanese ethnomedicine. It is an annual herbaceous plant reaching 30-70cm in height. Fruits are claimed to have antimicrobial, antiseptic and antihelminthic properties. The oil from *Trichyspermum ammi* was studied by GC-MS. It was also evaluated for antimicrobial activity. The GC-MS analysis showed eleven components. Major constituents are: 9-octadecenoic acid –(Z)- methyl ester(61.11%), 9,12-octadecadienoic acid-(ZZ)-, methyl ester(29.57%) and hexadecanoic acid methyl ester(6.53%). The oil showed significant antibacterial activity against *Staphylococcus aureus*. It also showed significant anticandidal activity. It exhibited partial activity against *Pseudomonas aeruginosa*.

**KEYWORDS:** *Trichyspermum ammi*, Oil, GC-MS analysis, Antimicrobial Activity.

### INTRODUCTION

*Trichyspermum ammi* is a herb of multidimensional uses in Sudanese ethnomedicine.

It is an annual herbaceous plant reaching 30-70cm in height. *Trichyspermum ammi* belongs to family Apiaceae which comprises around 2700 species. The plant is indigenous to the middle east.<sup>[1,2]</sup> Fruits are claimed to have antimicrobial, antiseptic and antihelminthic properties.<sup>[3]</sup> A major constituent of this herb – thymol – is known to exhibit antifungal, germicidal and spasmolytic activities.<sup>[4]</sup> Another bioactive constituent – carvacrol is associated with expectorant and antifungal properties.<sup>[5]</sup> The fruits are used traditionally against nausea, vomiting and abdominal anorexia.<sup>[6]</sup> Powdered fruits are inhaled to relief cold.<sup>[7,8]</sup> It has been reported that the fruits exhibited antiulcer activity.<sup>[7]</sup> *In vivo* studies demonstrated that supplementation of *Trichyspermum ammi* enhanced the digestive enzymes and promoted secretion of bile acids.<sup>[7]</sup> The antifungal activity of this plant against a panel of fungi has been reported.<sup>[7]</sup> The activity is probably due to the phenolics of *Trichyspermum ammi*. The antiinflammatory effect of seed extracts has been demonstrated in model animals.<sup>[9]</sup> It has been shown that the plant extracts exhibited *in vivo* hepatoprotective properties.<sup>[10]</sup> The hypotensive and broncho-dilating effect of *Trichyspermum ammi* has been

reported.<sup>[11]</sup> Also the hypolipidemic effect of *Trichyspermum ammi* seeds has been testified.<sup>[12]</sup>

### MATERIALS AND METHODS

#### Plant material

Fruits of *Trichyspermum ammi* were purchased from the local market, Khartoum, Sudan. The plant was identified and authenticated by direct comparison with reference herbarium sample.

#### Instruments

For GC-MS analysis a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used.

#### Test organisms

The antimicrobial potential of *Trichyspermum ammi* oil was estimated by the cup plate agar diffusion assay using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Escherichia coli* (G-ve) and the fungal species *Candida albicans*.

#### Methods

##### Extraction of oil

Powdered fruits of *Trichyspermum ammi* (250g) were macerated with n-hexane for 48hr. The solvent was removed under reduced pressure giving the oil.

**GC-MS analysis**

*Trichyspermum ammi* fruit oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. chromatographic conditions are shown below .

**Table 1: Oven temperature program.**

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

**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

**Antimicrobial assay****RESULTS AND DISCUSSION****Table 3: Consituents of *Trichyspermum ammi* oil.**

No.	R.Time	Area%	Name
1	13.532	0.03	Methyl tetradecanoate
2	14.602	0.05	1-Pentadecanoic acid, methyl ester
3	15.431	0.35	9-Hexadecenoic acid, methyl ester
4	15.627	6.53	Hexadecanoic acid, methyl ester
5	16.605	0.04	Heptadecenoic acid, methyl ester
6	17.298	29.57	9,12-Octadecadienoic acid-(ZZ)-, methyl ester
7	17.378	61.11	9-Octadecenoic acid -(Z)-, methyl ester
8	17.542	1.75	Methyl stearate
9	19.074	0.39	6-Octadecenoic acid -(Z)-, methyl ester
10	19.297	0.14	Eicosanoic acid, methyl ester
11	20.920	0.04	Docosanoic acid, methyl ester
		100.00	

The constituents of *Trichyspermum ammi* fruit oil were characterized by GC-MS analysis. Identification of the constituents was established by comparison of the retention times and also by consulting the MS library (NIST). The GC-MS analysis of the *Trichyspermum ammi* oil gave 11 components (Table 3). Major constituents are briefly discussed below:

**9-octadecenoic acid -(Z)-, methyl ester(61.11%)**

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 1. The peak at m/z 296 (R.T. 17.378) corresponds  $M^+[C_{19}H_{36}O_2]^+$ , while the signal at m/z266 is attributed to loss of a methoxyl.

Oleic acid occurs naturally in many animal and vegetable oils and fats. It is a monounsaturated omega-9 fatty acid. Oleic acid is used in food industries. It is also used in soap industries and as emollient.<sup>[13,14]</sup> With the exception

The cup-plate agar diffusion bioassay was adopted, with some minor modifications, to assess the antimicrobial activity of the studied oil. (2ml) of the standardized microbial 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 the nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates a cup (6 mm in diameter) was cut using sterile cork borer (No 4). The agar discs were removed and cups were filled with samples of test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37°C for 24 hours (for bacteria) and at 25° for 3 days for fungi. Experiments were carried out in duplicates. After incubation, the diameters of the resultant growth inhibition zones were measured and averaged.

of palmitic acid , oleic acid is the most abundant fatty acid in human adipose tissue.

**9,12-octadecadienoic acid-(ZZ)-, methyl ester (29.57%)**

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2 The peak at m/z294 (R.T. 17.298) coincides with  $M^+[C_{19}H_{34}O_2]^+$ , while the peak at m/z263 is due to loss of a methoxyl.

9,12-Octadecadienoic(linoleic acid) belongs to one of the two families of essential fatty acids. Such acids can not be synthesized by human bodies and are available through diet.<sup>[13]</sup> Linoleic acid is used in the biosynthesis of arachidonic acid. It exists in lipids of cell membrane.

**Hexadecanoic acid, methyl ester (6.53%)**

Figure 3 shows the mass spectrum of hexadecanoic acid methyl ester The molecular ion:  $M^+[C_{17}H_{34}O_2]^+$  appeared

at  $m/z$  270 at R.T. 15.627 in total ion chromatogram. The fragment at  $m/z$  239 is due to loss of a methoxyl function.

Hexadecanoic acid (palmitic acid) is a  $C_{16}$  saturated fatty acid. It is the most common fatty acid in plants and animals.

This acid is the precursor of long chain fatty acids. Pamitic acid is a lipid constituent of human breast milk.<sup>[15,16]</sup>

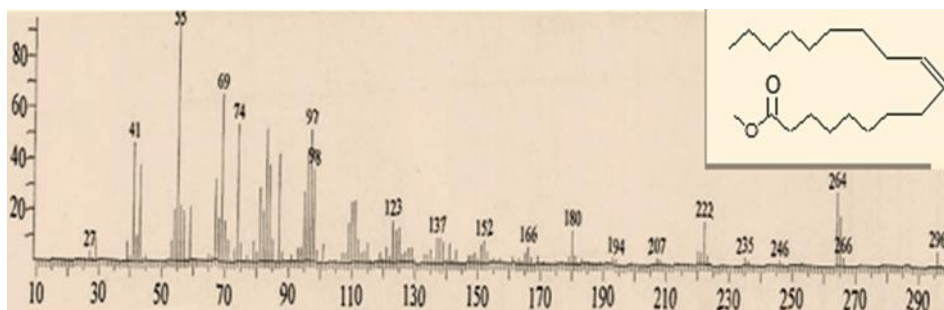


Fig. 1: Mass spectrum of 9-octadecenoic acid methyl ester.

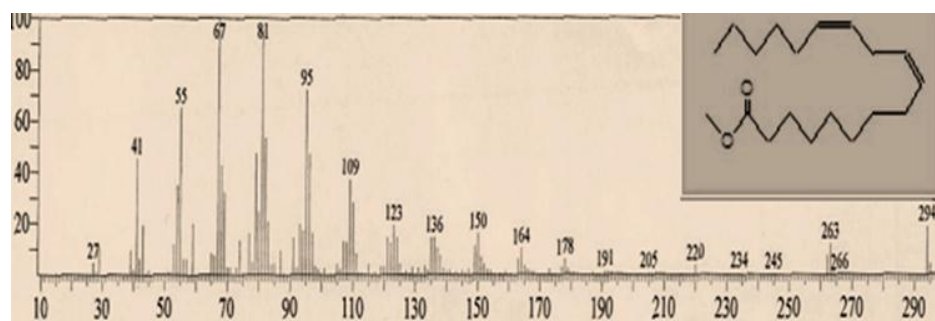


Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester.

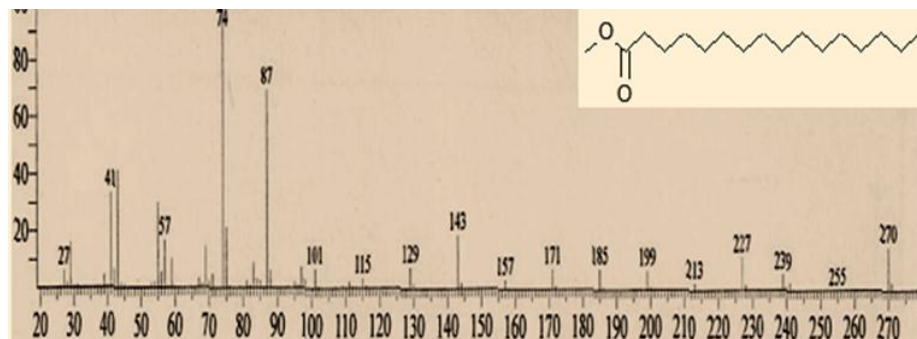


Fig. 3: Mass spectrum of hexadecanoic acid methyl ester.

#### Antimicrobial activity

In cup plate agar diffusion assay, *Trichyspermum ammi* oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (4). Ampicilin, gentamycin and clotrimazole were used as positive controls.

The oil showed significant antibacterial activity against *Staphylococcus aureus*. It also showed significant anticandidal activity. It exhibited partial activity against *Pseudomonas aeruginosa*. However, it did not show inhibitory effect against both *Escherichia coli* and *Bacillus subtilis*.

Table 4: Antibacterial activity of oil.

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	21	-	--	11	24
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamycin (40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

<9mm: Inactive; 9-12mm: partially active; 13-18mm: active; >18mm very active

Sa.: *Staphylococcus aureus*

Bs.: *Bacillus subtilis*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

An.: *Aspergillus niger*

Ca.: *Candida albicans*

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