Constituents and Biological Activity of Sudanese *Tephrosia apollina* (Fabaceae) Seed Oil

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Abstract

Tephrosia is a genus in the family Fabaceae. This genus is distributed in tropical and sub-tropical regions.*Tephrosia* genus manifested a range of biological activities including: antifungal, antiplasmodial, antiinflammatory, antiulcer , antidiabetic and anticancer activities. In this study GC-MS analysis of *Tephrosia apollina* oil revealed the presence of the following major constituents: i)9-octadecenoic acid methyl ester(31.79%); (ii)9,12-octadecadienoic acid methyl ester(21.34%). ;(iii) hexadecanoic acid methyl ester(17.86%) and (iv)methyl stearate(16.07%).The oil was evaluated for antimicrobial potential. The studied oil showed significant activity against *Bacillus subtilis* , *Staphylococcus aureus* and *Escherichia coli* .It exhibited moderate activity against other test organisms.

Keywords: *Tephrosia apollina*, Oil, GC-MS Analysis, Antimicrobial Activity.

Introduction

Since antiquity ,herbal remedy has been used by humans to fight various diseases.Most modern medicines are associated with undesirable effects and unpredictable pharmacological actions.Thus the need for new drugs with less adverse effects is a global concern[1,2].Medicinal plants are " the sleeping giant of pharmaceutical industries " and could ,undoubtedly, provide leads for drug development and drug discovery, and Tephrosia species are no exception.

Tephrosia is a genus in the family Fabaceae. This genus is distributed in tropical and sub-tropical regions[3].*Tephrosia* genus manifested a range of biological activities including: antifungal, antiplasmodial, antiinflammatory, antiulcer , antidiabetic and anticancer activities.This genus is also used for wounds and diarrhea[4]. Many biologically active molecules have been reported from various species of this genus including flavonoids[5-11]; terpenes , sesquiterpenes , steroids[4,11-13] and retenoids[14].

The hepatoprotective properties of *Tephrosia purpurea* has been documented[15,16].The *in vivo*

antiinflammatory activity of *Tephrosia purpurea* has been reported[17]. The hypoglycemic effect of *Tephrosia calophylla* has been studied in animal models[18].The larvicidal activity of *Tephrosia egregia* has been demonstrated[19].The antimicrobial potential of *Tephrosia purpurea* has been reported[7,20].This plant also showed anticancer activity[21].

Materials and Methods

Plant material

Tephrosia apollina seeds were collected from a forest reserve around Damazin-Sudan .The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute.

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 **.**

Methods

Extraction of oil

Powdered seeds of *Tephrosia apollina* (300g) were macerated with n-hexane for 72hr.The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

Tephrosia apollina seed oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument under the following chromatographic conditions:

Table 1: Chromatographic conditions

Column oven temperature	15.0oC
Injection temperature	300.0oC
Injection mode	Split
Flow control mode	Linear velocity
Total flow	50.0ml/min.
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml.min.
Split ratio	-1.0

Antimicrobial assay

Microbial strains

The antimicrobial activity was screened using two G+ve strains: *Staphylococcus aureus* , *Bacillus subtilis;* two G-ve stains : *Escherichia coli, Pseudomonas aeruginosa* and the fungal species : *Candida albicans*.

Inoculum preparation

For bacteria, each of the bacterial strain was cultured in Mueller Hinton agar slants at 35ºC . Fungal strain was cultured in Sabouraud dextrose agar at 37ºC .The microbial growth was harvested using sterile saline solution(5ml) and diluted to a viable cell count of 10^7CFU/ml.

Antibacterial activity

The disc diffusion assay was used to screen the antibacterial activity. As basal

layer, ten ml of Mueller Hinton agar was poured into sterile Petri dishes followed by 15 ml of seeded medium previously inoculated with bacterial suspension. Sterile filter paper discs(6mm) loaded with the test oil(100mg/ml) were placed onto the top of the Mueller Hinton agar plates.The plates were incubated at 35°C for 24h. After incubation inhibition zones were measured as indicator of antibacterial activity.

For antifungal activity, the same procedure was adopted but instead of Mueller Hinton agar Sabouraud dextrose agar was used and incubation continued here for 72h at 37°C.

Results and Discussion

Constituents of the oil

The oil was analyzed by GC-MS . The analysis showed 22 components. Fatty acids constituted 97.96% , the rest is ϑ-sitosterol (2.04%). The oil was dominated by : (i)9-octadecenoic acid methyl ester(31.79%) ; 9,12-octadecadienoic acid methyl ester(21.34%); hexadecanoic acid methyl ester(17.86%) and methyl stearate(16.07%). The total ions chromatogram is shown in Fig. 1, while the constituents of the oil are displayed in Table 1.

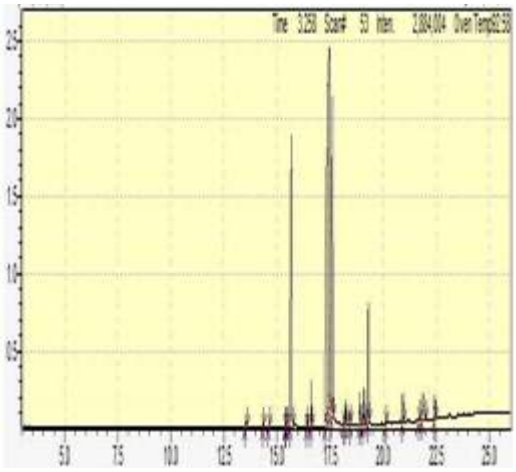

Fig.1 : Total ions chromatograms

(i)9-octadecenoic acid methyl ester(31.79%)

The mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 2.The peak at m/z 296, which appeared at R.T. 17.460 , in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$ .The peak at m/z265 corresponds to loss of a methoxyl function.

(ii)9,12-octadecadienoic acid methyl ester(21.34%)

Fig. 3 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester .The signal at m/z 294, which appeared at R.T. 17.370 corresponds : $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 is due to loss of a methoxyl .

(iii) hexadecanoic acid methyl ester(17.86%)

The mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 4.The peak at m/z 270 (R.T. 15.681) accounts for the

molecular ion: $M^+[C_{17}H_{34}O_2]^+$ .The signal at m/z239 is attributed to loss of a methoxyl .

(iv)methyl stearate(16.07%)

Fig. 5 illustrates the mass spectrum of methyl stearate .The signal at m/z 298, which appeared at R.T. 15.783 corresponds : $M^+[C_{19}H_{38}O_2]^+$ .The peak at m/z267 is due to loss of a methoxyl function.

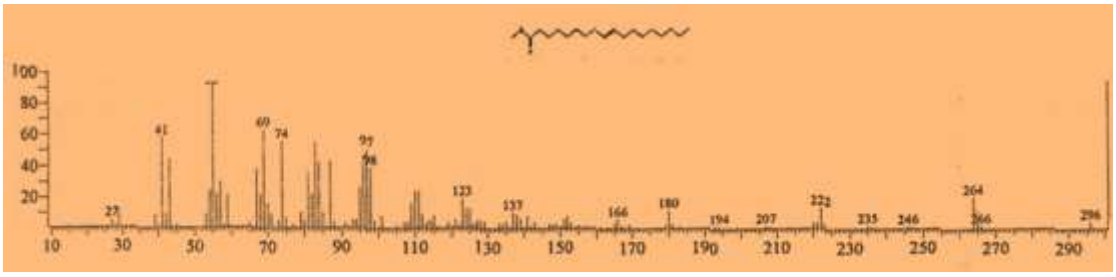

Fig. 2: Mass spectrum of 9-octadecenoic acid methyl ester

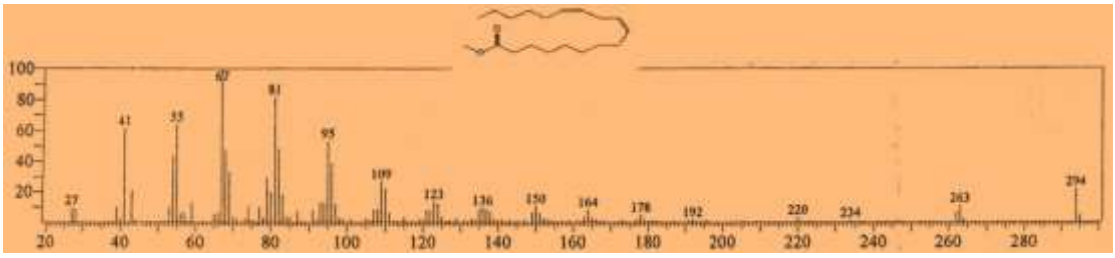

Fig. 3: Mass spectrum of 9,12-octadecadienoic acid methyl ester

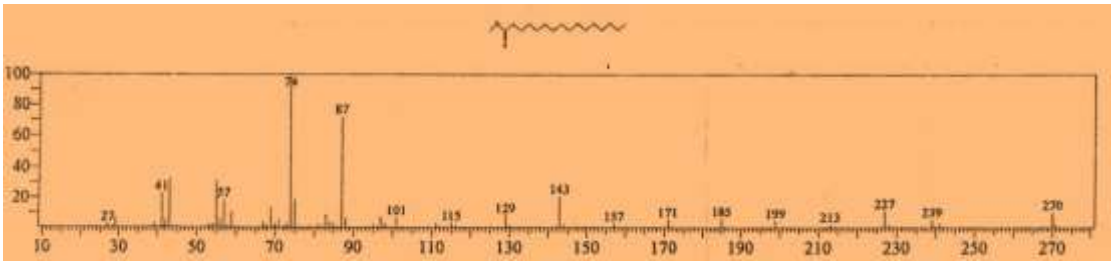

Fig. 4: Mass spectrum of hexadecanoic methyl ester

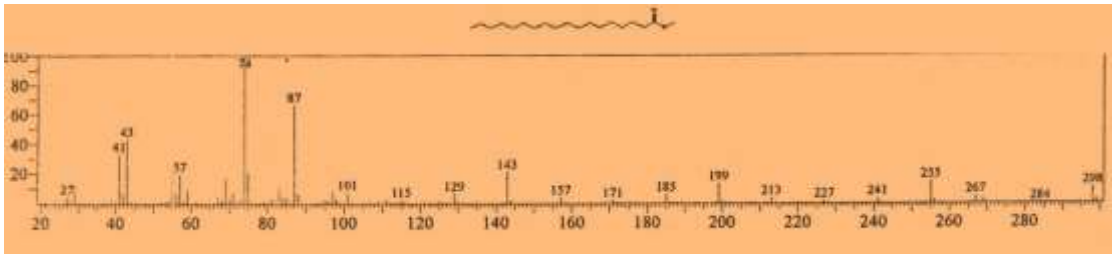

Fig. 5:Mass spectrum of methyl stearate

Table 1: Constituents of the oil

ID#	Name	Ret.Time	Area%
1	Methyl tetradecanoate	13.531	0.13
2.	cis-5-Dodecenoic acid, methyl ester	14.341	0.03
3.	Pentadecanoic acid, methyl ester	14.606	0.09
4	7-Hexadecenoic acid. methyl ester. (Z)-	15.397	0.11
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.440	0.45
6.	Hexadecanoic acid, methyl ester	15.681	17.86
7.	cis-10-Heptadecenoic acid, methyl ester	16.404	0.27
8.	Heptadecanoic acid, methyl ester	16.613	1.21
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.370	21.34
10.	9-Octadecenoic acid (Z)-, methyl ester	17.460	31.79
11.	Methyl stearate	17.615	16.07
12.	cis-11,14-Eicosadienoic acid, methyl ester	18.155	0.14
13.	cis-10-Nonadecenoic acid, methyl ester	18.226	0.60
14.	Nonadecanoic acid, methyl ester	18.445	0.39
15.	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.907	0.92
16.	cis-11-Eicosenoic acid, methyl ester	19.104	1.46
17.	Eicosanoic acid, methyl ester	19.307	3.42
18.	Heneicosanoic acid, methyl ester	20.129	0.07
19.	Docosanoic acid, methyl ester	20.921	0.75
20.	Tricosanoic acid, methyl ester	21.687	0.10
21.	.gamma.-Sitosterol	21.872	2.04
22.	Tetracosanoic acid, methyl ester	22.426	0.76

Antimicrobial assay

Tephrosia apollina oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (2).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil showed significant activity against *Bacillus subtilis* , *Staphylococcus aureus* and *Escherichia coli* .It exhibited moderate activity against other test organisms.

Table 2 : Inhibitory effect of *Beta vulgaris* oil

Sample	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	23	22	21	15	15
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

Sa.*: Staphylococcus aureus*

Ec.*: Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Bs.*: Bacillus subtilis*

Ca.: *Candida albicans*

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