The Pharmaceutical and Chemical Journal, 2020, 7(2):28-33

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Constituents of Sudanese *Cassia auriculata* Linn.(Caesalpiniaceae) Oil

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Abstract Traditional medicine is playing an important role in primary health care in developing countries. Increasing research on the impact of secondary metabolites on human physiology potentiated the applications of medicinal plants**.** *Cassia auriculata* L. is a plant of many medicinal attributes**.** The plant is used against diarrhea, leprosy, intestinal worms, female infertility, rheumatism and conjunctivitis**.** In this study, the oil from *Cassia auriculata* was analyzed by GC-MS. The GC-MS analysis showed sixteen components. Major constituents are : 9,12-octadecadienoic acid (Z,Z)- methyl ester (33.02%); 9-octadecenoic acid (Z)-methyl ester (26.77%); hexadecanoic acid methyl ester (17.59%) and methyl stereate(15.86%)**.** The antimicrobial potential of the oil was attempted via the cup plate agar diffusion bioassay against five human pathogens(*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*).However, the oil failed to exhibit any antimicrobial activity.

Keywords *Cassia auriculata*, Oil, GC-MS Analysis, Antimicrobial Activity

Introduction

Ethnomedicine is still playing an important role in primary health care in developing countries where modern medicines,with their known adverse effects , are usually beyond affordability. Increasing research on the impact of secondary metabolites on human physiology potentiated the applications of medicinal plants. Now about 50% of the marketed medicines are derived directly or indirectly from plants. Natural products are potential leads for drug development and drug design [1].

Cassia auriculata L. is a plant of many medicinal attributes in the family Caesalpiniaceae**.** Phytochemical investigation of pod husk revealed the presence of emodin, rubiadin, β-sitosterol, anthracene derivatives and flavonoids [2]. Leaves contain , among others, α-tocopherol derivative, octadecenal and hexadecanoic acid. The flowers contain sitosterol, emodin and some anthraquinones [3]. The ethanolic extract of leaves contain many secondary metabolites including alkaloids, tannins, flavonoids and saponins [4]. Cassia auriculata is used traditionally as antidiabetic [5], antipyretic [6], hepaoprotective, antiviral and antispasmodic4. The plant is also used against diarrhea, leprosy, intestinal worms, female infertility [7], rheumatism [8] and conjunctivitis [9]. Flowers are used as a natural remedy for nocturnal emissions, throat irritation, diabetes, and urinary discharge8. The bark is used in the treatment of skin diseases [10], while seeds are used against eye inflammation [11]. The antioxidant [4], anthelmintic [12], antihyperglycemic [13], antilipidemic [14], antipyretic [6], hepatoprotective and antiinflammatory properties of Cassia auriculata have been documented [15,16].

Materials and Methods

Plant material

Seeds of *Cassia auriculata* were collected from a forest reserve around Damazin, Sudan. The plant was identified and authenticated by The Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

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 *Cassia auriculata* oil was estimated by the cup plate agar diffusion assay using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli*(G-ve) and the fungal species *Candida albicans.*

Methods

Extraction of oil

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

GC-MS analysis

Cassia auriculata 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 (^{o}C)	Hold Time
1.00	150.0	-
0.00	300.0	4.00

Table 2: Chromatographic conditions

Column oven temperature	150.0^{o}C
Injection temperature	300.0^{o}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

The cup plate agar diffusion assay was adopted ,with some minor modifications ,to assess the antimicrobial activity of the studied oil. Two (ml) of standardized microbial stock suspension were mixed with 200ml of sterile molten agar which was maintained at 45^{o}C in a water bath. Twenty (ml) aliquots of the agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates a cup(6mm,diamer) was cut using sterile cork borer (No.4). The agar discs were removed and cups were filled with test sample and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37^{o}C for 24h. For fungi, the incubation continued for 72h.Experiments were conducted in duplicates and the diameters of inhibition zones were measured and averaged as indicator of activity.

Results and Discussion

The oil was analyzed by GC-MS. This analysis revealed the presence of 16 components(Table 1). Characteriation of constituents was based on retention times and the mass spectrum fragmentation pattern. Major constituents are discussed below:

Table 3: Consitituents of *Cassia auriculata* oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.200	0.06
2.	Pentadecanoic acid, methyl ester	15.320	0.04
3.	7,10-Hexadecadienoic acid, methyl ester	16.092	0.03
4.	7-Hexadecenoic acid, methyl ester, (Z)-	16.150	0.08
5.	9-Hexadecenoic acid, methyl ester, (Z)-	16.196	0.13
6.	Hexadecanoic acid, methyl ester	16.399	17.59
7.	cis-10-Heptadecenoic acid. methyl ester	17.212	0.05
8.	Heptadecanoic acid, methyl ester	17.424	0.12
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.164	33.02
10.	9-Octadecenoic acid (Z)-. methyl ester	18.209	26.77
11.	Methyl stearate	18.417	15.86
12.	cis-11-Eicosenoic acid, methyl ester	20.063	0.50
13.	Eicosanoic acid, methyl ester	20.266	3.21
14.	Docosanoic acid. methyl ester	21.976	1.84
15.	Tricosanoic acid, methyl ester	22.781	0.20
16.	Tetracosanoic acid, methyl ester	23.555	0.50

Major constituents are:

i) 9,12-octadecadienoic acid (Z,Z)- methyl ester (33.02)

ii) 9-Octadecenoic acid (Z)- methyl ester (26.77%)

iii) Hexadecanoic acid methyl ester (17.59%)

iv) Methyl stereate (15.86%)

9,12-octadecadienoic acid-(ZZ)-, methyl ester (33.02%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 1. The peak at m/z294 (R.T. 18.164) 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. Linoleic acid is used in the biosynthesis of arachidonic acid. It exists in lipids of cell membrane [17]. It has been reported that linoleic acid possesses antiinflammatory, skin-lightenting and anti-acne properties [18,19,20].

9-octadecenoic acid –(Z)-, methyl ester (26.77%)

The mass spectrum of 9-octadecenoic acid(oleic acid) methyl ester is displayed in Fig. 2. The peak at m/z 296 (R.T. 18.209) 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. With the exception of palmitic acid , oleic acid is the most abundant fatty acid in human adipose tissue. It has been shown that oleate, as main constituent of olive oil, can protect against cardiovascular insulin resistance, improve endothelial dysfunction in response to proinflammatory signals and reduce proliferation and apoptosis in VSMC' s [21].

hexadecanoic acid , methyl ester(17.59%)

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 with R.T. 16.399 in total ion chromatogram. The fragment at m/z239 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. There is convincing evidence that increased intake of this acid may increase the risk of developing cardiovascular diseases [22].

Methyl stearate (15.86%)

Fig. 4 shows the mass spectrum of methyl stereate. The signal at m/z 298(R.T. 18.417) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z267 accounts for loss of a methoxyl .

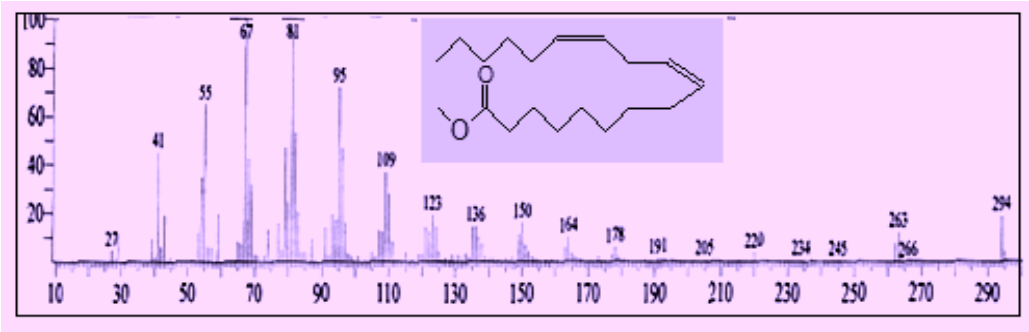

Figure 1: Mass spectrum of 9,12-octadecadienoic acid methyl ester

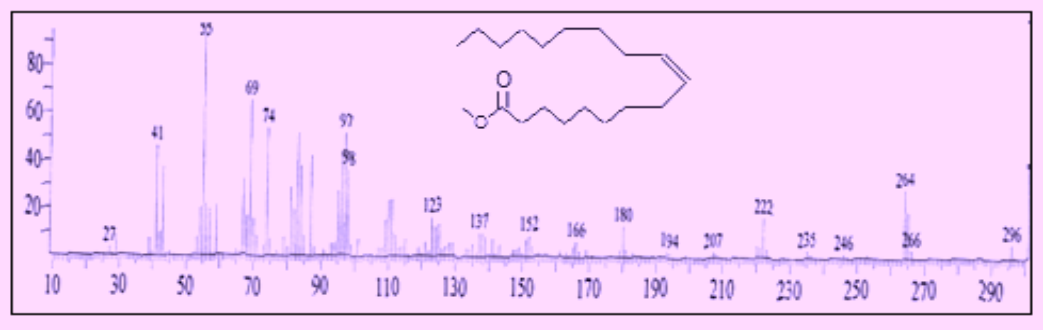

Figure 2: Mass spectrum of 9-octadecenoic acid methyl ester

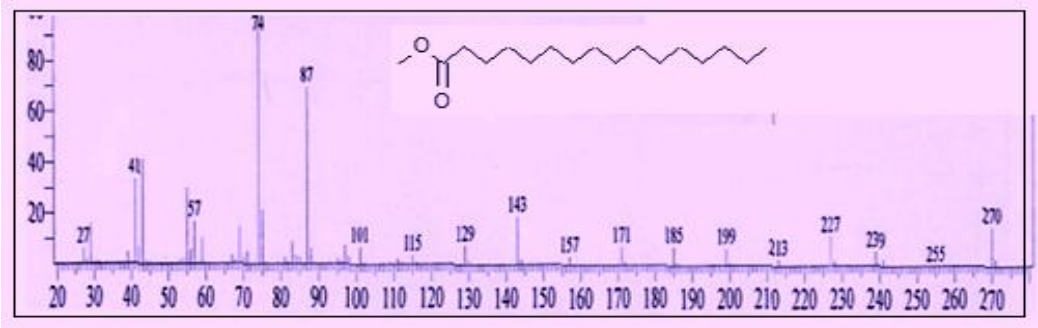

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

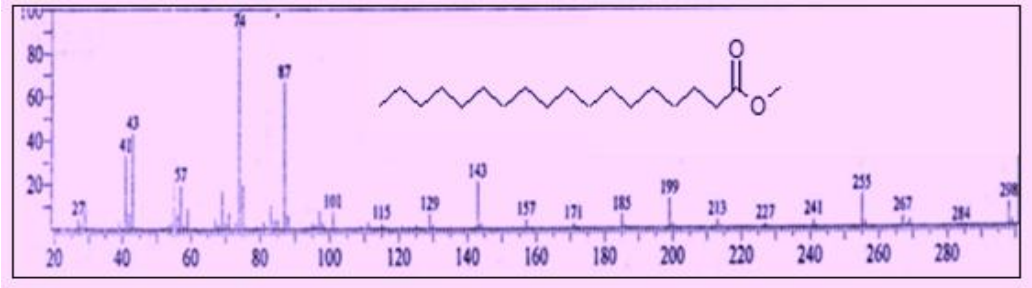

Figure 4: Mass spectrum of methyl stereate

Antimicrobial activity

In cup plate agar diffusion assay,the antimicrobial activity of *Cassia auriculata* oil was attempted. However, the oil failed to show any activity against all test organisms (Table 4).

Table 4: Antibacterial activity of oil

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	--	-	--	--	--
Ampicilin (40mg/ml)	30	15	--	--	--
Gentacycin (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*

Ca.: *Candida albicans*

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