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GC-MS Analysis and Antimicrobial Activity of Sudanese *Psidium guajava* var. *pomifera* Fixed Oil

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Abstract
Fruits, like guava, contain bioactive components and regular consumption of fruits has many health promoting properties. Guava leaves are used in Sudanese traditional medicine against cough, while fruit pulp is a treatment for diarrhea. Seeds are reported to possess antimicrobial, antiallergic and anticancer activities.
In this study *Psidium guajava* fixed oil was extracted with n-hexane. The oil was screened for antimicrobial activity against six standard microorganisms. The oil showed activity against all test organisms except for *Escherichia coli*. It was partially active against the fungus *Candida albicans* but significant activity against *Staphylococcus aureus* was observed.

Keywords: *Psidium guajava,* fixed oil, GC-MS analysis, antimicrobial activity

Introduction
Psidium guajava L. is native to South America. It is a small tree with two common varieties: red guava (*Psidium guajava* var. *pomifera*) and white guava (*Psidium guajava* var. *pyrifera*) [1, 2]. Fruits, like guava, contain bioactive components and regular consumption of fruits has many health promoting properties [3-6].
Guava leaves are used in Sudanese traditional medicine against cough, while fruit pulp is a treatment for diarrhea. Seeds are reported to possess antimicrobial, antiallergic and anticancer activities [7-11].
The plant contains: vitamins, flavonoids, tannins, terpenes and essential oils [2]. The fruit pulp is rich in carotenoids, polyphenols and vitamins [12-14]. It has been demonstrated that the fruit has antioxidant properties [15-17].
Clinical studies testified that consumption of guava for 12 weeks reduced blood cholesterol, blood pressure and level of triglycerides [18, 19]. *In vivo* studies showed that feeding model animals with guava pulp resulted in significant decrease in weight and increased levels of HDL-c. Fruit pulp also showed significant hypoglycemic effect in diabetic models [10].

Materials and Methods
Materials
Plant Material
Psidium guajava was purchased from the local market-Khartoum .The plant was authenticated by The Institute of Medicinal and Aromatic Plants, Khartoum, Sudan.

Materials for antimicrobial screening
Bacterial strains
Gram positive bacteria
Bacillus subtilis and Staphylococcus aureus.

Gram negative bacteria
Escherichia coli, Pseudomonas aeruginosa.

Fungal strains
Candida albicans, Aspergillus niger

Standards
i. Gentamycin, Ampicillin: antibacterial standards.
ii. Clotrimazole: antifungal standard.

Media for G+ve bacteria
Muller Hinton agar was used as media for G +ve bacterial growth, peptone from casein 17.0g , peptone from meat 3.0g , sodium chloride 5.0g , lactose 10.0g , bile salt mixture 1.5g, neutral red 0.03g , crystal violet 0.001g , agar 13.5g

Media for G-ve bacteria
Nutrient agar (Oxoid, England) was used as media for G-ve bacterial growth : Lab. Lemco powder 1.0g , yeast extract 2.0g, peptone water 5.0g , agar No.3 15.0g , distilled water 1000ml

Media for fungi
Sabouraud agar (Oxoid, England) was used as media for fungal growth: meat peptone 5.0g, casein peptone 5.0g, dextrose 40.0g, agar 15.0g, distilled water to 1000m.

Methods
Extraction and esterification of oil
Dry powdered *Psidium guajava* seeds (300g) were exhaustively extracted with n-hexane at room temperature for 72h.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4ºC for further manipulation.
The oil (2ml) was placed in a test tube and 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. (2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously

shaken for five minutes .The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate (1µl) was injected in the GC-MS vial.

GC-MS analysis

The target oil of was analyzed by gas chromatography – mass spectrometry. A Shimadzu GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given below:
Rate: - ; Temperature (ºC) 150.0; Hold Time (min^{-1}) 1.00
Rate: 4.00; Temperature (ºC) 300.0; Hold Time (min^{-1}) 0.00
Other chromatographic conditions are displayed in Table 1.

Table 1: Chromatographic conditions

Column oven temperature	150.0ºC
Injection temperature	300.0ºC
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 activity

The oil from *Psidium guajava* seeds was screened for its antimicrobial activity against six standard human pathogens (*Bacilus subtilis* (Bs), *Staphylococcus aureus* (Sa), *Escherichia colli* (Ec), *Pseudomonas aeruginosa* (Pa), *Aspergillus niger* (An) and *Candida albacans* (Ca).

One ml aliquot of 24 hours broth culture of the test organisms were aseptically distributed onto Mueller Hinton agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in (100 ml) of normal saline producing a suspension containing about 108 - 104 colony forming units per ml. The suspension was stored in the refrigerator at 4ºC until used. The average of viable organism per ml of the saline suspension was determined by means of the surface viable counting technique. Serial dilution of the stock suspension were made in sterile saline in tubes and one drop volumes (0-20ml) of the appropriate dilution were transferred by adjustable volume micropipette onto the surface of dried agar plates. The plates were allowed to stand for two hours at room temperature for drop to dry, and then incubated at 37ºC for 24 hours.

Fungal cultures were maintained on Sabouraud dextrose agar and incubated at 25ºC for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

To determine the antibacterial activity of the oil, the cup- plate agar diffusion method was adopted with some minor modification. (2ml) of the standard bacteria stock suspension were mixed with (200ml) of sterile molten nutrient agar which was maintained at 45ºC. (20ml) aliquot of incubated agar were distributed into sterile Petri dishes. The agar was left to settle and each plate was cut using sterile cork-borer (No.4) and agar discs were removed. Alternates cups were filled with (0.1ml) of test sample using adjustable pipette and allowed to diffuse at room temperature. The Petri dishes were then incubated in the upright position at 37ºC for 18hr. Two replicates were carried out for the test sample .After incubation the diameters of growth inhibition zones were measured and averaged.

The above method was applied for the antifungal activity but Sabouraud dextrose agar was used this time. The inoculated medium was incubated at 25ºC for two days for *candida albicans* and for three days for *Aspergillus niger*.

Results and discussion

GC-MS analysis of *Psidium guajava* oil

Psidium guajava fixed oil was obtained via maceration of seeds. The oil was studied by GC-MS which revealed the presence of 30 constituents. The total ion chromatograms is displayed in Fig. 1, while the different constituents of the oil are depicted in Table 2.

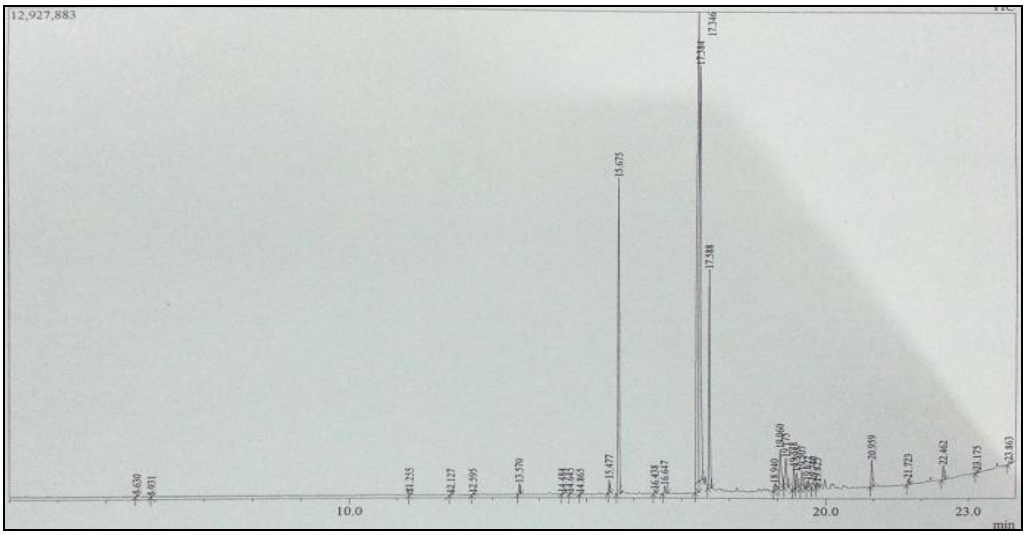

Fig 1: Total ions chromatograms

Table 2: Costituents of Guava oil

No.	Area %	RT	Name
1	5.630	0.09	Benzoic acid methy ester
2	5.031	0.02	Octanoic acid methyl ester
3	11.255	0.08	Dodecanoic acid methyl ester
4	12.127	0.06	(-)-Spathulenol
5	12.598	0.08	Apiol
6	13.570	0.45	Methyl tetradecanoate
7	14.484	0.06	5-Octadecenoic acid methyl ester
8	14.645	0.06	Pentadecanoic acid methyl ester
9	14.865	0.04	2- Pentadecanone ,6,10,14-trimethyl
10	15.477	0.53	9-Hexadecenoic acid methyl ester
11	15.675	15.96	Hexadecanoic acid methyl ester
12	16.438	0.16	9,12-Octadecadienoyl chloride
13	16.647	0.33	Heptadecanoic acid methyl ester
14	17.340	36.39	9,12-Octadecadienoic acid(z, z-) methyl ester
15	17.384	23.44	9-Octadecadienoic acid(z-) methyl ester
16	17.588	10.53	Methyl stearate
17	18.940	0.31	9,12-Octadecadienoic acid methyl ester
18	19.060	2.58	9-1-Butyltricyclo[4.2.1.1]decane-1,10-
19	19.175	2.17	E,E,Z-1,3,12-Nonadecatriene=5,14-diol
20	19.338	1.02	Eicosanoic acid methyl ester
21	19.395	0.77	PGHI methyl ester
22	19.507	1.08	1-Naphthalenol decahydro-4α-methyl
23	19.622	0.30	2-Butyl-3-methyl-5-(2-methylpropyl-enyl)
24	19.740	0.49	α- d-Xylopyranoside, methyl-2,3,4-triol
25	19.825	0.33	Methyl-2-octylcyclopropene-1-octanoate
26	20.959	1.20	Docosanoic acid methyl ester
27	21.723	0.31	Tricosanoic acid methyl ester
28	22.462	0.78	Tetracosanoic acid methyl ester
29	23.175	0.14	Pentacosanoic acid methyl ester
30	23.865	0.20	Hexacosanoic acid methyl ester
		100.00	

The following constituents were detected as major components:

i. 9, 12-Octadecadienoic acid (36.39%)

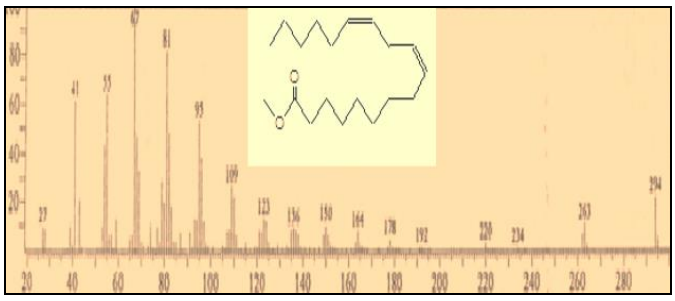

Fig 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 2.The peak at m/z 294, which appeared at R.T. 17.346 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{34}O_2]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.

ii. 9-Octadecenoic acid methyl ester (20.37%)

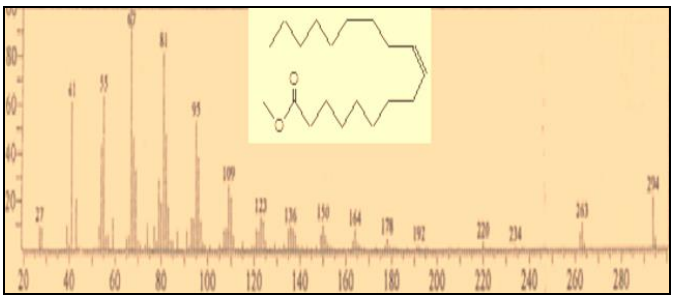

Fig 3: Mass spectrum of 9-octadecenoic acid methyl ester

Fig. 3 displays the mass spectrum of 9-octadecenoic acid methyl ester**.** The signal at m/z 294(R.T. 17.384) corresponds: M^{+} $[C_{19}H_{34}O_2]^{+,}$ while the peak at m/z263 accounts for loss of a methyl.

iii. Hexadecanoic acid methyl ester (15.96%)

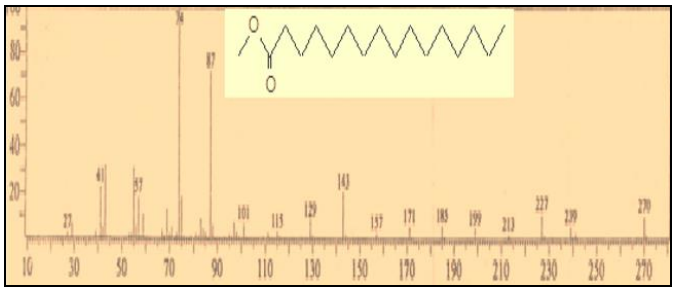

Fig 4: Mass spectrum of hexadecanoic methyl ester

The mass spectrum of hexadecanoic acid methyl ester is given in Fig.4.The peak at m/z 270, which appeared at R.T. 15.675 corresponds to the molecular ion: M^+ $[C_{17}H_{34}O_2]^+$.The signal at m/z239 corresponds to loss of a methoxyl group.

iv. Methyl stearate (10.53%)

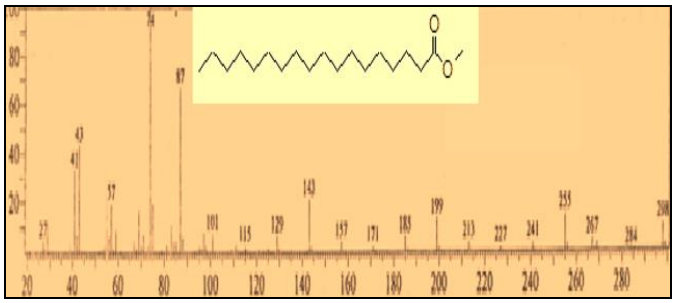

Fig 5: Mass spectrum of methyl stearate

The mass spectrum of methyl stearate is displayed in Fig. 5. The spectrum showed m/z 298 (R.T. 17.588 in total ion chromatogram) .Appearantly, m/z 298 corresponds to M^+ $[C_{19}H_{38}O_2]^+$.The fragment at m/z267 is attributed to loss of a methoxyl function.

Antimicrobial activity

Guava oil was screened for antimicrobial activity against six standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table (3) .The results were interpreted in terms of the commonly used terms (<9mm: inative; 9-12mm: partially active; 13-18mm: active ;> 18mm: very active). Tables (4 and 5) show the antibacterial and antifungal activities of standard drugs respectively.

Table 3: Antimicrobial activity of *Oil: M.D.I.Z* (mm)

Drug	Conc .(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
A.digitata oil	100	-	15	24	15	9	13

Table 4: Antibacterial activity of standard chemotherapeutic

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 5: Antifungal activity of standard chemotherapeutic agent

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

- Ec.: *Escherichia coli*
- Ps.: *Pseudosssmonas aeruginosa*
- *Bs.: Bacillus subtilis*
- *Staphylococcus aureus*
- An.: *Aspergillus niger*
- Ca.: *Candida albicans*

The oil showed activity against all test organisms except for *Escherichia coli*. It was partially active against the fungus *Candida albicans* but significant activity against *Staphylococcus aureus* was observed.

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