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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *CARICA PAPYA* GROWN IN SUDAN

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ABSTRACT

Carica papya is a large perennial plant in the family Caricaceae. This plant is cultivated in tropical and subtropical regions for its economic and medicinal importance. All parts of the plant are used as natural remedy against a wide array of ailments.In this study, the oil from *Carica papya* seeds has been analyzed by GC-MS. The GC-MS analysis showed 20 components. Major constituents are : i) 9-octadecenoic acid methyl ester (28.06 %) ii)oleic acid (12.86%) iii) ethyl oleate(11.52 %) iv)hexadecanoic acid methyl ester(7.78%) v)stigmasterol (7.57%) vi)2-dodecen-1-yl(-) succenic acid anhydride (5.85%) and vii)gamma-sitosterol(5.40%). The antmicrobial activity of the oil has been assessed. At a concentration of 100mg/ml, The oil showed significant activity against *Escherichia coli* and *Pseudomonas aeroginosa* .It also exhibited moderate activity against *Bacillus subtilis* and *Staphylococcus aureus* beside weak anticandidal activity.

***KEYWORDS*:** *Carica papya*, Oil, Constituents, Antimicrobial Activity.

INTRODUCTION

Medicinal herbs are gaining popularity specially in developing countries where modern medicines are beyond affordability beside being associated with side effects.[1,2] Medicinal plants are endowed with bioactive molecules which may induce changes in human physiology.[3]

Carica papya is a large perennial plant in the family Caricaceae.[4] This plant is cultivated in tropical and subtropical regions for its economic and medicinal importance.[5] All parts of the plant(leaves, roots,peel, flowers and seeds) are used as natural remedy against a wide array of ailments.[6] Fruit has a nutritional value. It contains vitamins, mineals, enzymes, proteins, polysaccharides beside sterols, flavonoids, alkaloids and saponins.[7,8] *Carica papya* has been used traditionally against many diseases including heart diseases, low sperm count, kidney failure and uterus fibroid.[9] Leaves are rich in flavonoids and alkaloids. They are traditionally used as hypoglycaemic, antiinflammatory, hepatoprotective, antihypertensive, antiviral, antimalarial and anticancer.[10-12] In vitro and in vivo studies demonstrated that the leaves possess antiinflammatory, antioxidant, antiplasmodial, antibacterial , antidenge and anti cancer effect.[13]

Flowers are emmengogue, febrifuge and are used in ethnomedicine against jaundice,hypertension, intestinal helminthiasis, malaria, diabetes and cancer.[2,14,15] Seeds have some pharmacological activities including antiinflammatory, antimicrobial, anthelmintic, contraceptive and analgesic effects.[16] Seeds are fermifuge, pain alleviator and thirst quencher. They are used by local healers for hypertension, hypercholesterolemia, diabetes and intestinal worms.[2,17] Peel extracts exhibited a range of biological activities including antibacterial, antioxidant and anticancer activites.[18-20] Root is a remedy for typhoid fever, wounds, urethritis, gastroenteritis, abdominal pain and pneumonia.[21,22]

MATERIALS AND METHODS

Plant material

Seeds of *Carica papya* were collected from Damazin-Sudan. The plant was authenticated by The Medicinal and Aromatic Plants Research Institute-Sudan.

Instruments

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

Test organisms

Carica papya oil were assessed for antimicrobial activity using the standard microorganisms shown in Table(1).

Table 1: Test organisms.

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
6	*Candida albicans*	fungi

Extraction of oil

Powdered seeds of studied plant (350g) were macerated with n-hexane.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

GC-MS analysis

The extracted oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo 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. Chromatographic conditions are as follows: 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;** split ratio**:-1.**

Antimicrobial assay

i)-Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^{8}-10^{9}colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

ii)-Preparation of fungal suspensions

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

iii)-Testing for antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the oil. (2ml) of the standardized bacterial 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 of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a test sample.

The agar discs were removed, alternate cup were filled with 0.1 ml samples of each compound using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

After incubation, the diameters of the resultant growth inhibition zones were measured as average of two replicates.

RESULTS AND DISCUSSION

Gas chromatography - mass spectrometry has been used for the identification and quantification of the *Carica papaya* oil. The analysis revealed the presence of 20 components - Table (2).The total ion chromatogram is presented in Fig.1.

Fig. 1: Total ion chromatograms.

Table 2: Constituent of *Carica papaya* oil.

No.	Name	Ret.Time	Area%
1.	Hexadecanoic acid, methyl ester	16.396	7.78
2.	n-Hexadecanoic acid	16.834	1.98
3.	Hexadecanoic acid, ethyl ester	17.090	3.22
4.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.143	4.29
5.	9-Octadecenoic acid (Z)-, methyl ester	18.188	28.06
6.	Methyl stearate	18.409	2.53
7.	Oleic Acid	18.630	12.86
8.	Ethyl Oleate	18.817	11.52
9.	Octadecanoic acid, ethyl ester	19.036	0.84
10.	Hexadecanoic acid, tetradecyl ester	19.886	0.28
11.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	19.951	0.29
12.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	20.030	1.67
13.	Oleoyl chloride	21.194	1.36
14.	6-Octadecenoic acid, (Z)-	21.412	0.58
15.	Decyl oleate	21.476	0.46
16.	Octadecanoic acid, 2-propenyl ester	21.536	0.42
17.	cis-9-Hexadecenal	21.601	3.04
18.	Stigmasterol	22.847	7.57
19.	2-Dodecen-1-yl(-)succinic anhydride	23.051	5.85
20.	.gamma.-Sitosterol	23.979	5.40

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

1. 9-Octadecenoic acid methyl ester (28.06 %)
2. Oleic acid (12.86 %)
3. Ethyl oleate(11.52 %)
4. Hexadecanoic acid methyl ester(7.78%)
5. Stigmasterol (7.57%)
6. 2-Dodecen-1-yl(-)succenic acid anhydride (5.85%)
7. gamma-Sitosterol(5.40%)

The GC-MS analysis of the studied oil showed a mass spectrum (Fig. 2) identical with that of 9-octadecenoic acid methyl ester where the peak at m/z 296 (RT.18.188) accounts for : M^+ $[C_{19}H_{36}O_2]$.The analysis also showed a mass spectrum(Fig.3) characteristic of oleic acid.The molecular ion: $M^+[C_{18}H_{34}O_2]^+$ appeared at m/z282(RT.18.630). A Mass spectrum(Fig. 4) characteristic of ethyl oleate was also shown: the peak at m/z310(RT.18.817) is attributed to the molecular ion :$M^+[C_{20}H_{38}O_2]^+$.Hexadecanoic acid was detected by its retention time(16.396) and mass spectrum(Fig.5) where the molecular ion $M^+[C_{17}H_{34}O_2]$ was detected at m/z270. Stigmasterol was also detected by its retention time(22.847) and mass spectrum(Fig.6) which revealed m/z412 for : $M^+[C_{29}H_{48}O]$. 2-dodecen-1-yl(-)-succenic anhydride was also detected . The peak(Fig.7) at m/z266(RT.23.051) is due to the molecular ion: $M^+[C_{16}H_{26}O_3]^+$. A mass spectrum Characteristic of ɤ-sitosterol (Fig.8) was observed.The signal at m/z414(RT.23.979)is due to $M^+[C_{29}H_{50}O]$.

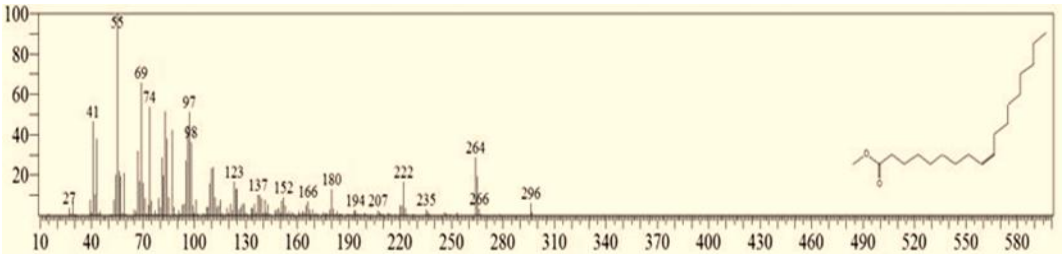

Fig. 2: Mass spectrum of 9-octadecenoic acid methyl ester.

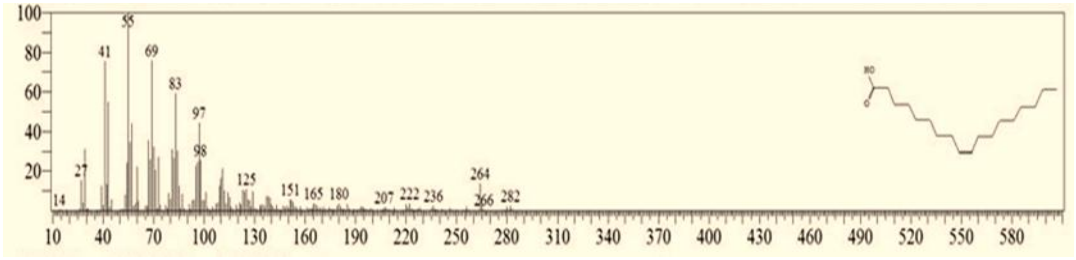

Fig. 3: Mass spectrum of oleic acid.

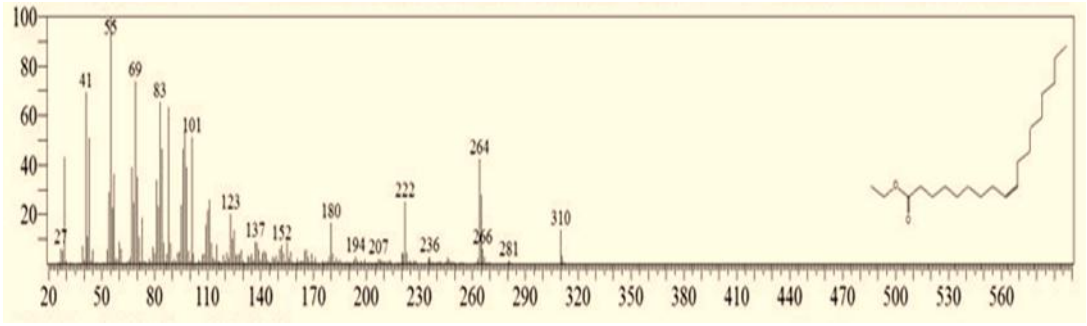

Fig. 4: Mass spectrum of ethyl oleate.

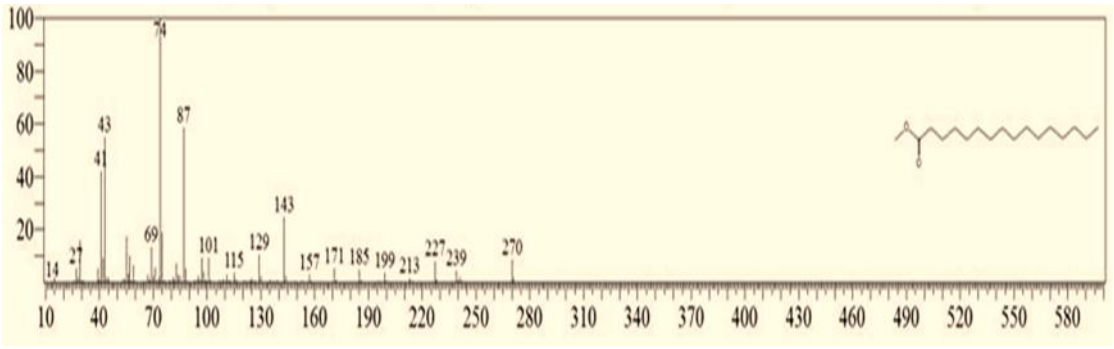

Fig. 5: Mass spectrum of hexadecanoic acid, methyl ester.

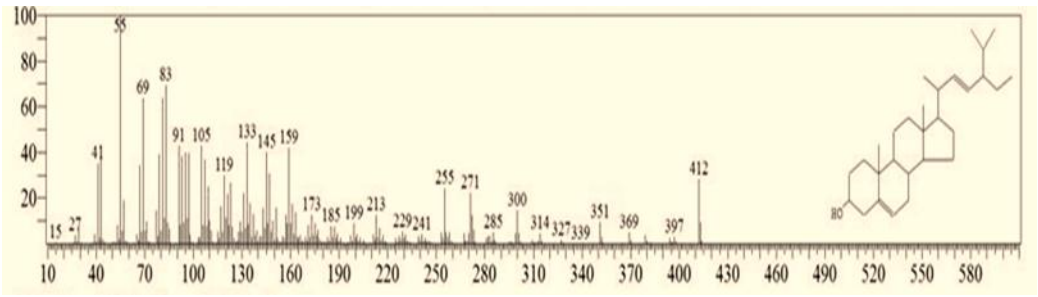

Fig. 6: Mass spectrum of stigmasterol.

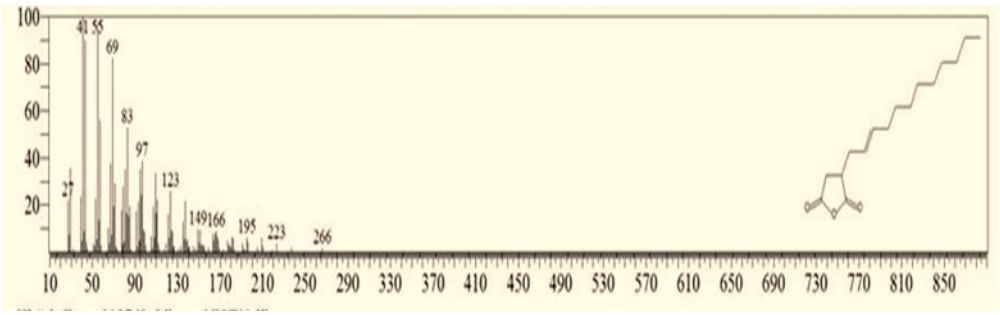

Fig. 7: Mass spectrum of 2-dodecen-1-yl(-)-succenic anhydride.

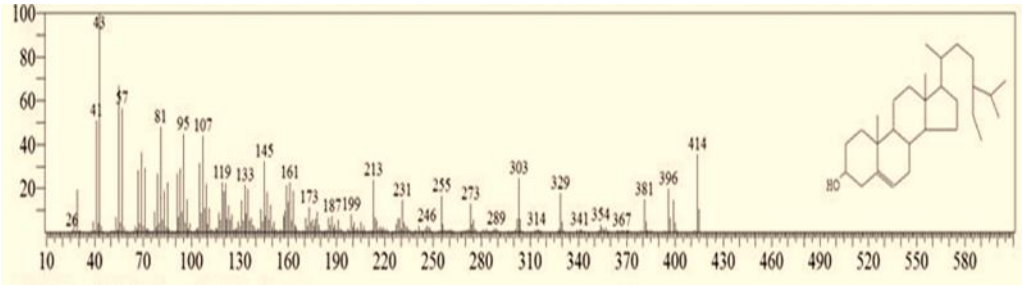

Fig. 8: Mass spectrum of gamma-sitosterol.

Antimicrobial activity

Carica papya oil was screened for antimicrobial activity against five standard organisms. The inhibition zones are presented in Table 3. The oil showed significant activity against *Escherichia coli* and *Pseudomonas aeroginosa* .It also exhibited moderate activity against *Bacillus subtilis* and *Staphylococcus aureus* beside weak anticandidal activity. Tables 4 and 5 illustrate the antimicrobial activity of standard drugs.

Table 3: Inhibtion zones(mm) of the oil.

Sample	Sa	Bs	Ec	Pa	Ca
Oil 100mg/ml	15	16	19	19	11

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

Table 4: Inhibition zones of standard antibacterial agents.

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	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: 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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