



Trade Science Inc.

ISSN : 0974 - 7508

Volume 9 Issue 4

Natural Products

An Indian Journal

Full Paper

NPAIJ, 9(4), 2013 [143-147]

Fatty acid constituents and antimicrobial activities of *Strawberry* and *Carica- papaya* leaves

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ABSTRACT

In this study, the fatty acids constituents of both *Strawberry* and *Carica papaya* Leaves were identified by using gas chromatography-mass spectroscopy (GC-MS). The results showed that fatty acid of *Strawberry* mainly contained about 30.28 % of,12,15- Octadecatrienoic acid methyl ester and 23.37 % Palmitic acid methyl ester, but for *Carica papaya* mainly contained about 35.6% Palmitic acid methyl ester and 14.527 % Eicosanoic acid methyl ester. Antimicrobial activities were studied against five bacterial strains and five fungal species. 0.3 ml of plant extract (10 mg / 1 ml) had inhibitory effect for all bacterial spp. and fungal spp., but 0.1 ml of two fatty acid extracts (10 mg / 1 ml) showed inhibitory effect against some bacterial spp. and fungal spp. indicating that the inhibitory effect increase with increasing the concentration of the extract.

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KEYWORDS

Strawberry;
Carica papaya;
Fatty acid;
Antimicrobial activities and
gas chromatography-mass
spectroscopy (GC-MS).

INTRODUCTION

It was reported that *strawberry* cv. Elsanta fruit and flowers contain preformed antifungal compounds which differ markedly in number and activity during flower and fruit development^[10].

The leaves and fruit of 'Earliglow' contained higher amounts of phospholipids compared to those of 'Kent', whereas 'Kent' *strawberry* roots had higher phospholipids. Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids were major fatty acids in galacto- and phospholipids of the 'Earliglow' and 'Kent' *strawberry*. PC is very rich in linolenic acid in leaves compared to the fruit and root tissues^[14]. Papaya (*Carica papaya* L.) is produced commercially in many tropical and subtropical areas of the world for domestic consumption and for export. Glo-

bal papaya production increased about 40% in a single decade (1998–2008), with an estimated 9.1 million tons produced in 2008. The top papaya producing countries are India, Brazil, Nigeria, Indonesia and Mexico^[6].

C. papaya seeds, the fruit, leave, and latex are used medicinally. The main medicinal use of *C. papaya* seeds is as a digestive agent. It is prescribed for people who have difficulty digesting protein and is used to break up blood clots after surgery; this is due to the presence of enzyme papain in the plants latex^[9]. Growth temperature has a profound influence on membrane fatty acid composition and degree of unsaturation^[7]. Changes in the composition of fatty acid components of membrane lipids are important in the acclimation of most types of plants^[8]. The physical state of the membrane lipids and the ratio of unsaturated to saturated fatty acids play an important role in determining the physiological function

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of the plant tissue^[3]. Lipid molecules are essential building blocks for every membrane of a living cell, and membranes are sites for many specific enzymatic activities, transport ions and metabolites, and hormonal receptors. The composition of membrane lipids may also be a factor in determining major biological properties of membranes that in turn may influence biological changes, such as the growth of plants^[3]. 25/12 °C (day/night) was the optimum temperature to grow strawberry^[13]. The aim of the present study was to identify the fatty acid constituents of the leaves of the Egyptian *strawberry* (*Fragaria-ananassa*) and *carica papaya*. Also to investigate the antimicrobial activities of the two extracts, for which a limited data have been previously published.

MATERIAL AND METHODS

Materials

Plant materials

Fresh leaves of *strawberry* and *carica papaya* were collected from Sharkia, Egypt, and identified by Botany Department, Faculty of Science, Zagazig University.

Test micro-organisms

The bacterial and fungal strains were personally obtained from the microbiology Lab., Botany Department, Faculty of Science, Zagazig University. Bacterial species tested were *Pseudomonas areuginosa*, *Kelbseilla sp.*, *Salmonella typhi*, *Staphylococcus aureus* and *E coli*. also fungal species were *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium sp.* and *Penicillium sp.*

Methods

Extraction

500 grams of air dried leaves thoroughly crushed and exhaustively extracted with 2 liters of petroleum ether (60-80) for 24 hours. The solvent was removed under vacuum, then hydrolysis with 10% alc. KOH for 6 hrs. over a water bath under reflux, dilution with water and extract with ether.

The ether part (the non-saponifiable part) was extracted with diethyl ether, which gives residue not used. After then aqueous part was then acidified with dil. HCL

till acidic medium then extracted with diethyl ether, the extract was analysed by GC-MS^[12].

GC-MS analysis conditions

Qualification of the fatty acids were analyzed on analyzed on Gas Chromatography Mass Spectrometry HP 6890 Series A (Agilent) by using A Thermo Scientific (TR-5MS), (5% Phenyl Polysil Phenylene Siloxane) capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness). Helium (He), having a flow rate of 1.00 ml/min, was used as carrier gas. The GC oven temperature was kept at 140°C for 5 minutes and programmed to 200°C by (5 °C" 3 min). The injector temperature was 200 °C. The amount of injection was 1µL. Also detector temperature was 220 °C.

MS spectra were taken by Mass Spectrometer HP 5973A (Agilent). Retention indices for all the components were determined according to the Van Den Dool method^[4]. Identification of the components was based on comparison of their mass spectra with those of internal (computer) library, Wiley7n.1 and PMW_Tox3.1 libraries and some reference compounds.

Preparation of diazomethane

Diazomethane was prepared from methyl amine hydrochloride as reported by Vogel^[12].

Methylation of fatty acid with diazomethane

Fatty acids were dissolved in a little anhydrous methanol and the ethereal solution of diazomethane was added in a small portion until gas evolution ceased. The mixture acquired a pale yellow color indicated the addition of excess of diazomethane, the reaction mixture was left for 10 min and ether was evaporated under nitrogen stream at room temperature. Two drops of redistilled chloroform solution was added to dissolve the fatty acids methyl esters and 10 ml of this solution were injected into the gas chromatography.

Sources of standard fatty acids:

A set of standard fatty acids of 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1 and 22:0 with a stated purity of 99% by GLC was purchased from Nu-check prop.

The purity of each fatty acid methyl ester was checked by GLC and gave one peak.

Identification and determination of fatty acids by gas liquid chromatography:

The method described by Farag *et al*^[5], was applied for determination of fatty acids by gas liquid chromatography. The methyl esters of fatty acids obtained were analyzed with a Pye Unieam Series 304 gas chromatograph equipped with dual flame ionization detector and dual channel recorder. The separation of fatty acid methyl esters was conducted using a coiled glass column (1.5m x 4 mm) Packed with Diatomite (100x 120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8 °C /min from 70 °C to 190°C, then isothermally at 190°C for 25 min with nitrogen at 30 ml/min.

Antimicrobial activities

The extract was dissolved in dimethylformamide (DMF) for antimicrobial investigation at the final concentration of (10 mg / 1 ml).

Antibacterial activity

Antibacterial activities of extract were tested using pour plate technique on nutrient agar medium. Culturing and incubated of different bacterial species were carried out at 27 °C for 24 hours. Extract was tested at two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml). After the elapse of incubation periods, the diameter of inhibition zones was measured (mm). Mean of 3 replicated was calculated. The inhibition zone formed by the extracts against the particular test bacterial strain

determined as the antibacterial activities of the extract^[11].

Antifungal activity

Czepak Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup porer (15 mm) diameter. Two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml) of the extract were transferred into the well. Dimethyl foramide (DMF) was used only as a control. The plates were incubated for 7 days at 30 °C. The inhibition zone (mm) formed by the extract against the particular test fungal strain determined as the antifungal activities of the extract.

RESULTS AND DISCUSSION

Chemical constituents of fatty acids

TABLE (1 and 2) represents the chemical composition of the fatty acid constituents of *strawberry* and *carica papaya* leaves. As can be seen from these TABLEs, 9 compounds were identified for *strawberry* leaves with major components are 30.28 % 9,12,15-Octadecatrienoic acid methyl ester and 23.37 % Palmitic acid methyl ester. Also 8 compounds were identified for *carica papaya* leaves with major components are 35.6% Palmitic acid methyl ester and 14.527 % Eicosanoic acid methyl ester.

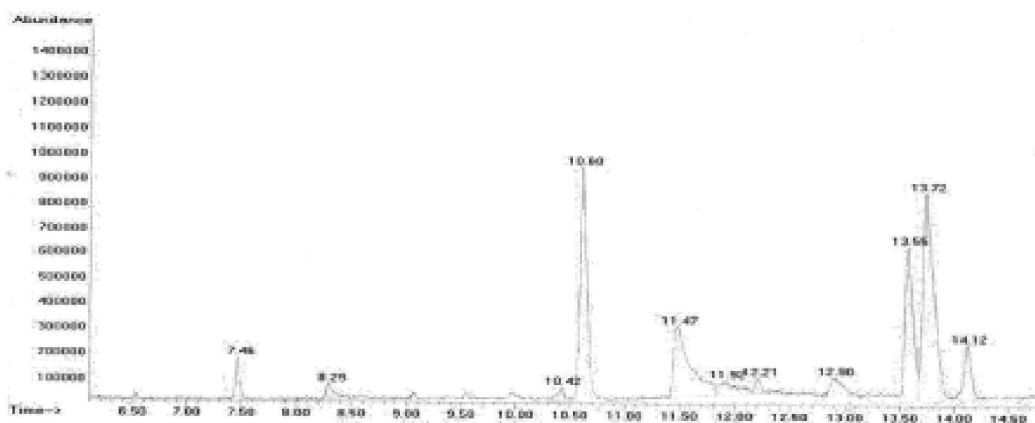


Figure 1 : GC/MS chromatogram of fatty acid constituents of *strawberry* leaves.

Results of antimicrobial activity

Antibacterial activity

Data in figure (3); evaluate that extract of *strawberry* has resistance against all species at 1 and 3 mg

concentrations under investigation. But for *papaya* extract it has resistance against *Salmonella typhi* and *Escherichia coli* at 1 mg concentration and it has resistance against all species at 3 mg concentration. These result agreement with that obtained by Ayoola^[2], and

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ayad^[1]. Ayoola reported that, the alcoholic extracts of clove, ginger, peppermint spearmint and thyme were

the most effective than aqueous extracts against *E.coli*. isolated.

TABLE 1 : Fatty acid constituents of *strawberry* leaves.

Compound name	Rt	Peak area	Molecular weight
Myristic acid methyl ester	7.46	2.49	242
Pentadecanoic acid methyl ester	8.28	1.804	256
Palmitic acid methyl ester	10.58	23.37	270
Pentadecanoic acid methyl ester	11.47	16.199	256
14-methyl Hexadecanoic acid methyl ester	12.21	1.248	284
Eicosanoic acid methyl ester	12.89	3.29	326
8,11 - Octadecadienoic acid methyl ester	13.55	15.905	294
9,12,15- Octadecatrienoic acid methyl ster	13.71	30.28	292
Stearic acid methyl ester	14.12	5.39	298

TABLE 2 : Fatty acid constituents of *carica papaya* leaves.

Compound name	Rt	Peak area	Molecular weight
Myristic acid methyl ester	7.44	9.23	242
Pentadecanoic acid methyl ester	8.63	2.306	256
Palmitic acid methyl ester	10.62	35.6	270
14-methyl Hexadecanoic acid methyl ester	11.53	7.723	284
Eicosanoic acid methyl ester	12.29	14.527	326
10,13 - Octadecadienoic acid methyl ester	13.67	13.769	294
9,12,15- Octadecatrienoic acid methyl ster	13.89	10.33	292
12-methyl Tetradecanoic acid methyl ester	14.14	6.6	256

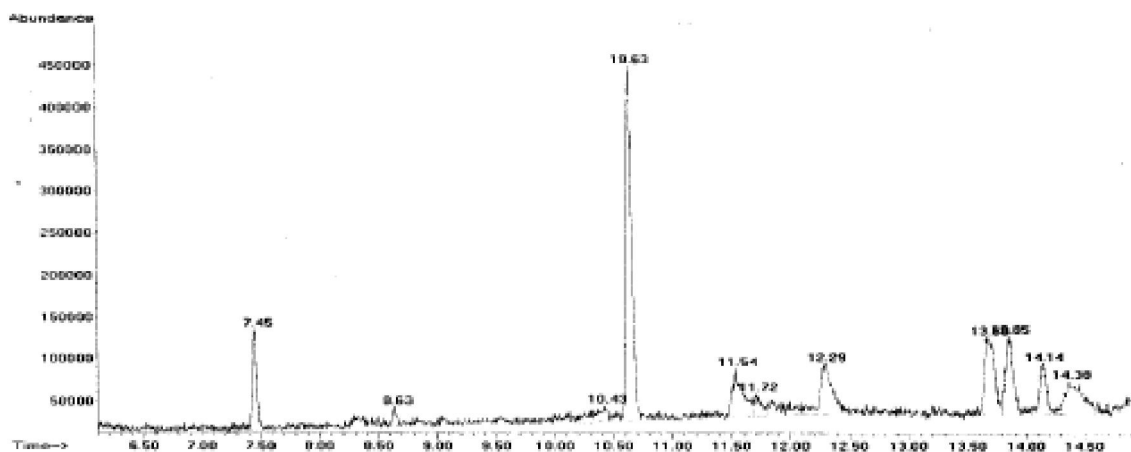


Figure 2 : GC/MS chromatogram of fatty acid constituents of *carica papaya* leaves.

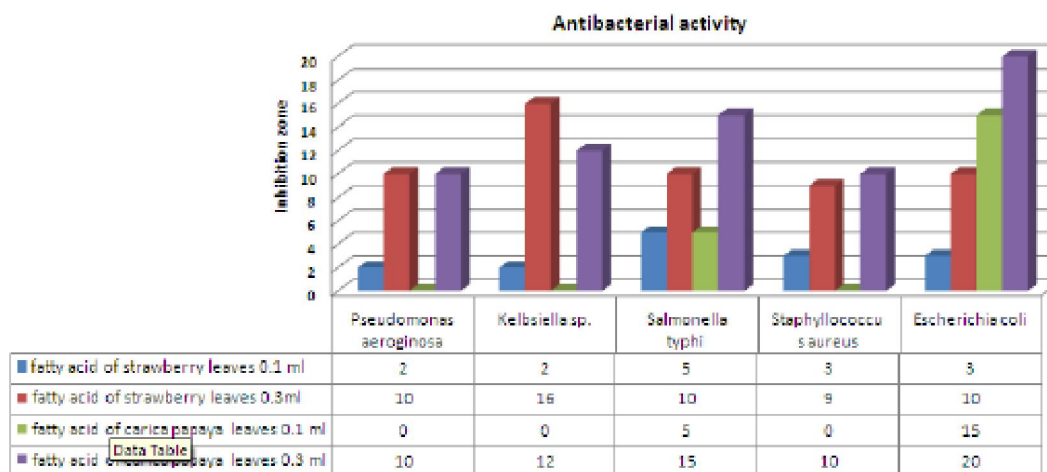


Figure 3 : Antibacterial activity statically representation of the fatty acids of *strawberry* and *carica-papaya*.

Antifungal activity

The two extracts had different antifungal activities against the tested fungal strains. Fatty acid of *strawberry* at 0.1 ml concentration showed inhibitory activity against

only *penicillium* sp, but fatty acid of *carica papaya* leaves showed inhibitory activity against *penicillium* sp. and *Fusarium oxysporum* at 0.1 ml concentration. The two extracts at 0.3 ml concentration showed inhibitory activity against all species (Figure 4).

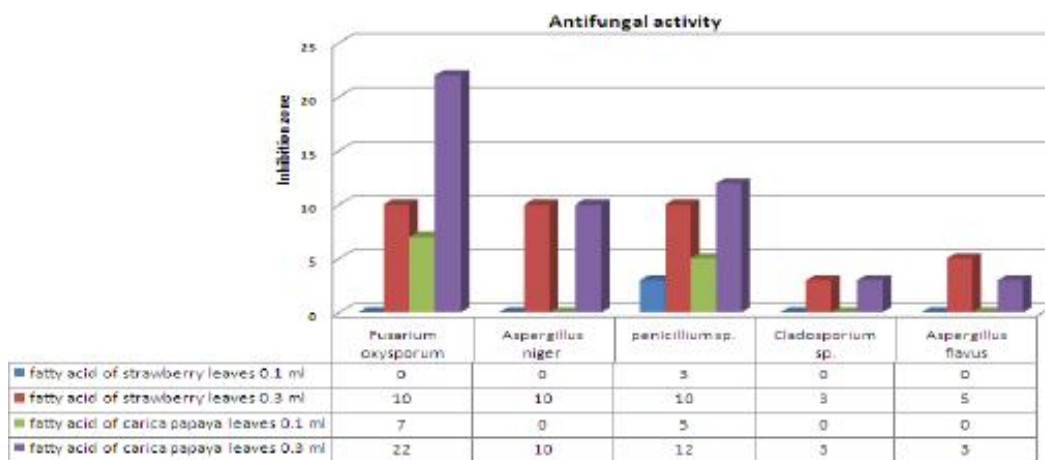


Figure 4 : Antifungal activity statically representation of the fatty acids of *strawberry* and *carica papaya*

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