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RESEARCH ARTICLE

BIOLOGICAL ACTIVITY OF FATTY ACID CONSTITUENTS OF THE EGYPTIAN *STRAWBERRY*AND *CARICA- PAPAYA* LEAVES.

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Abstract

In our study we aimed to identify the fatty acid constituents of both *Strawberry* and *Carica papaya* leaves by using gas liquid chromatography (GLC). The results showed that we have nine fatty acids for *Strawberry* leaves, with main constituents 30.28 % of 9,12,15- Octadecatrienoic acid methyl ester and 23.37 % Palmitic acid methyl ester, but for *Carica papaya* we obtained eight fatty acid compounds with main constituents 35.6% Palmitic acid methyl ester and 14.527 % Eicosanoic acid methyl ester. Antimicrobial activities of fatty acids of both two plants were studied against five bacterial strains and five fungal species. Results founded that; 0.3 ml concentration of the two plant extracts (10 mg / 1 ml) had inhibitory effect for all bacterial spp. and fungal spp.,but 0.1 ml concentration 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.

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 (Terry et al., 2004). The leaves and fruit of 'Earliglow' contained higher amounts of phospholipids compared to those of 'Kent', whereas 'Kent' *strawberry* roots had higher phospholipids. Palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$) 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 (Wang and Lin, 2006). Papaya (*Carica papaya* L.) is produced commercially in many tropical and subtropical areas of the world for domestic consumption and for export. Global 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 (FAO, (2010). *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 (Oduola et al., 2007). Growth temperature has a profound influence on membrane fatty acid composition and degree of unsaturation (Lyons, 1973). Changes in the composition of fatty acid components of membrane lipids are important in the acclimation of most types of plants (Martin et al., 1976). 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 of the plant tissue (Brenner, 1984). 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 (Brenner, 1984). 25/12 ^{0}C (day/night) was the optimum temperature to grow *strawberry* (Wang and Camp, 2000). Due to the lack of the previous studies and in continuation of our studies (El-Mesallamy et al., 2012 and Abd El Azim et al., 2014); we aimed to study the

fatty acids constituents and antimicrobial activities of the leaves of the Egyptian *strawberry* (*Fragaria-ananassa*) and *carica papaya*.

Material and Methods:-

Materials:-

Plant materials:-

Fresh leaves of *strawberry* and *carica papaya* were collected from Sharkiaa, 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 *Psudomonas areuginosa*, *Kelbseilla* sp., *Salmonella typhi*, *Staphyllococcus 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 two liters of petroleum ether (60-80) for 24 hours. The solvent was removed under vacuum, then hydrolysis with 10% alc. KOH for 6 hours 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, leave the ether to evaporate to give a residue which is the saponifiable fraction (fatty acid)(Vogel, 1975).

Preparation of diazomethane:-

Diazomethane was prepared from methyl amine hydrochloride as reported by (Vogel, 1975).

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., 1986), was applied for determination of fatty acids by gas liquid chromatography. The methyl esters of fatty acids obtained were analyzed with a Pye Unicam Series 304 gas chromatograph equipped with dual flam 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 inch) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8 ^{0}C /min from 70 ^{0}C to 190 ^{0}C, then isothermally at 190 ^{0}C for 25 min with nitrogen at 30 ml/min.

Antimicrobial activities:-

The extract was dissolved in dimethyl formamide (DMF) for the 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 (Vaghasiya et al., 2004).

Antifungal activity:-

Czapek 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. Dimethylformamide (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.

Result and Discussion:-

Chemical constituents of fatty acids:-

Chemical constituents of fatty acid of *strawberry* leaves; were characterized and identified by using GLC as shown in figure 1 and table 1.

Table 1: Fatty acids constituent of *strawberry* leaves.

Compound name	Retention time	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 ester	13.71	30.28	292
Stearic acid methyl ester	14.12	5.39	298

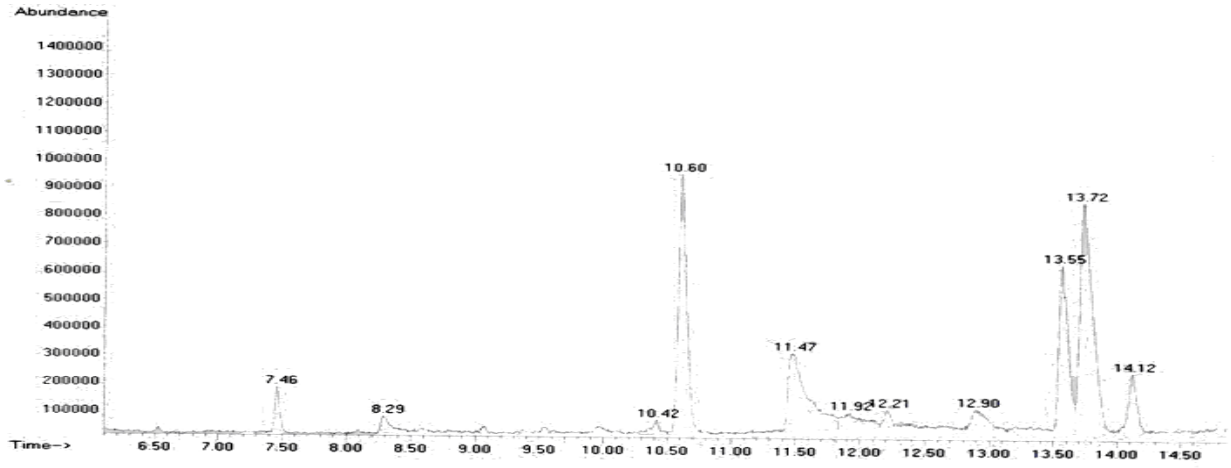

Figure 1: GLC chromatogram of fatty acid constituents of *strawberry* leaves.

As was seen from the above data in figure 1 and table 1, nine compounds were characterized and identified by using GLC, and the main constituents of fatty acid of *strawberry* leaves were found to be 30.28 % 9,12,15-Octadecatrienoic acid methyl ester and 23.37 % Palmitic acid methyl ester.

Also the same, Chemical constituents of fatty acid of *carica papaya* leaves; were characterized and identified by using GLC as shown in figure 2 and table 2.

Table (2): Fatty acids constituent of *carica papaya* leaves.

Compound name	Retention time	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 ester	13.89	10.33	292
12-methyl Tetradecanoic acid methyl ester	14.14	6.6	256

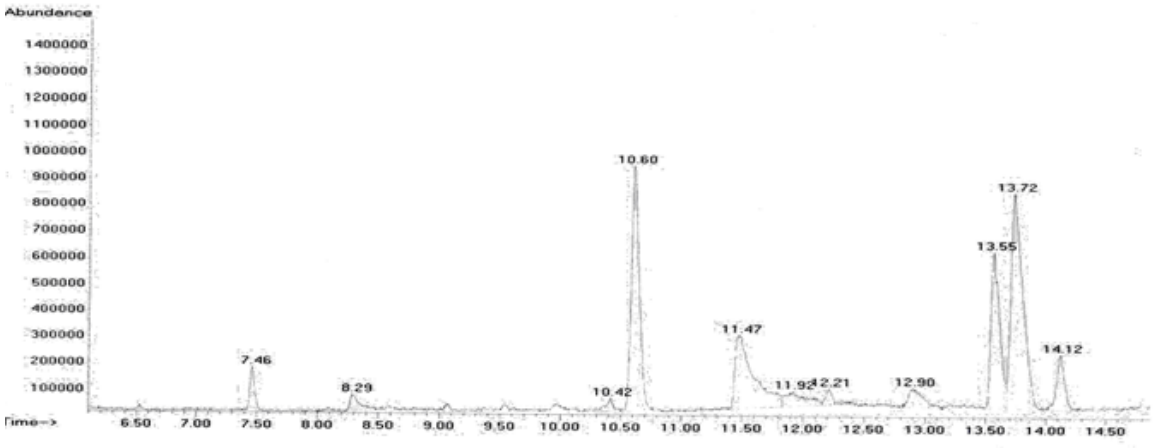

Figure 2: GLC chromatogram of fatty acid constituents of *carica papaya* leaves.

As was seen from the above data in figure 2 and table 2, eight compounds were characterized and identified by using GLC, among them, two compounds were found to be represents the major components of fatty acid of *carica papaya* leaves; 35.6% Palmitic acid methyl ester and 14.527 % Eicosanoic acid methyl ester.

Results of antimicrobial activity:-

Antibacterial activity:-

Data found in figure (3); evaluate that extract of *strawberry* has resistance against all species at 1 and 3 mg concentrations under investigation. But for *carica 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 results agreement with these obtained by (Ayad, 2009 and Ayoola et al., 2008). Ayoola reported that, the alcoholic extracts of clove, ginger, peppermint spearmint and thyme were the most effective than aqueous extracts against *E. coli* .isolated.

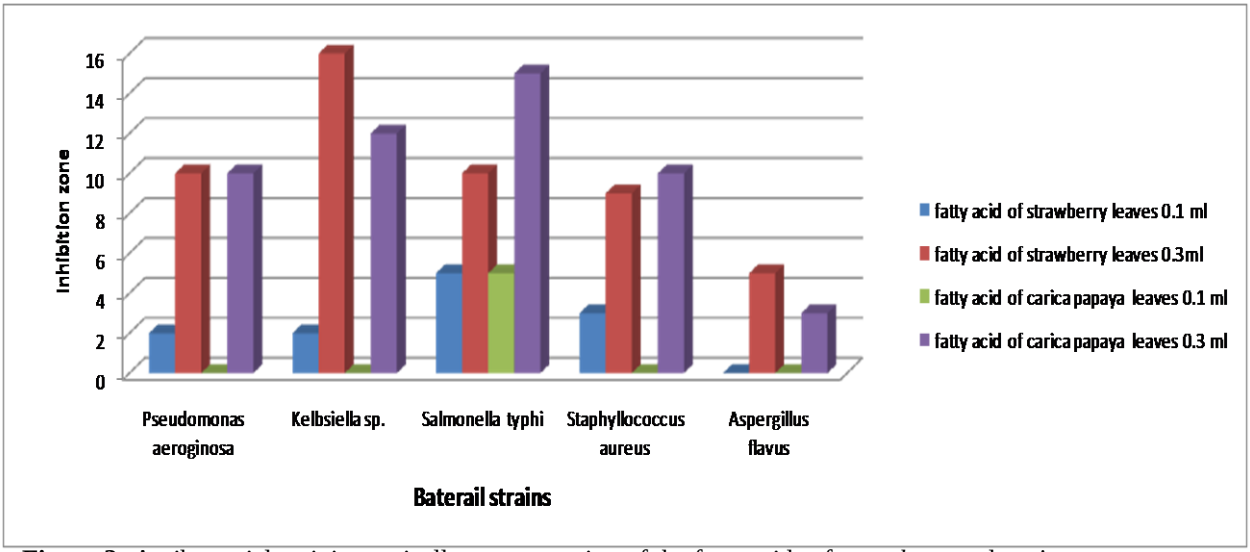

Figure 3: Antibacterial activity statically representation of the fatty acids of *strawberry*and *carica-papaya*.

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).

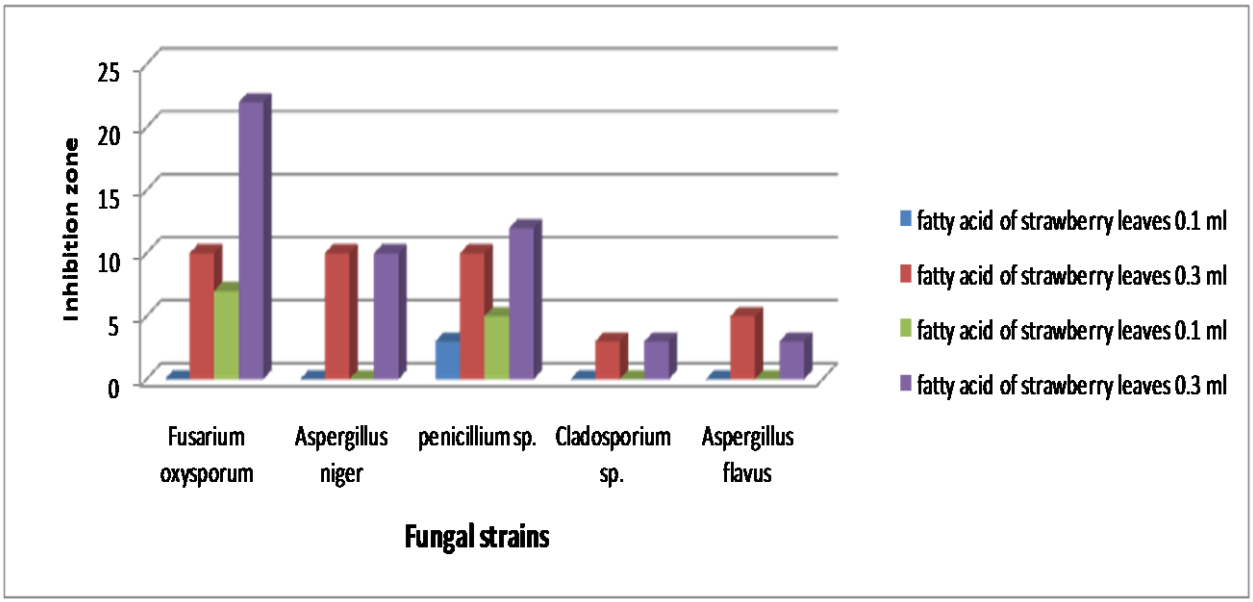

Figure 4: Antifungal activity statically representation of the fatty acids of *strawberry* and *carica papaya*

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