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Chemical Constituents and Cytotoxic Evaluation of *Abelmoschus esculentus* L. Leaves Grown in Egypt

Ali Mohamed El-Hagrassi[1*], Abeer Fouad Osman[2], Dina Mahfouz Eskander[2] and Mahmoud Ibrahim Nassar[2]

[1]*Phytochemistry and Plant Systematics Department, Pharmaceutical Industries Division, National Research Centre, 33 El Bohouth St. (Former El Tahrir St.), 12622-Dokki, Giza, Egypt*

[2]*Chemistry of Natural Compounds Department, Pharmaceutical Industries Division, National Research Centre, 33 El Bohouth St. (Former El Tahrir St.), 12622-Dokki, Giza, Egypt*

ABSTRACT

The aim of the present work is to investigate the phenolic profile and the non-polar contents of Abelmoschus esculentus L. leaves as well as, studying the cytotoxic activity of three tested extracts. The chemical compounds of the defatted aqueous methanolic extract of Abelmoschus esculentus L. leaves were analyzed using liquid chromatography-electrospray ionization mass spectrometry technique (LC-ESI-MS) by which twenty two compounds can be identified and classified as seven phenolic acids and their derivatives, with fifteen flavanol derivatives.

Petroleum ether fraction of Abelmoschus esculentus L. leaves was analyzed by gas chromatography-mass spectrometry (GC/MS), characterized 31 compounds. Cytotoxic activity was evaluated by using MTT assay against four carcinoma cell lines: HELA, MCF, PC3 and HepG2 (cervix, breast, prostate and liver carcinoma cell lines, respectively).

Keywords: Phenolic; Flavonoid compounds; LC-ESI-MS; GC/MS; *Abelmoschus esculentus* L. leaves; cytotoxic activity

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INTRODUCTION

Nowadays, the researchers are looking for alternative medicine which extracted from cultivated and wild medicinal plants due to the powerful effectiveness in the treatment of many diseases without causing side effects. *Abelmoschus*

esculentus L. (Okra) is one of the most widely known and utilized species of Malvaceae family, also, it is economically important vegetable crop grown in tropical and subtropical parts of the world [1-3].

Malvaceae is a flowering plants family; contain about 244 genera with 4225 known species. The principle economic use of Malvaceae plants is that they are sources of natural fibers, the family supplies possibly the world's 3 most important fiber crops. Plants of this family are also used for food, beverages, in traditional medicine, and in horticulture.

The entire plant is edible and is known to be used as a food [4,5]. Its origins are believed to be from East and/or South Africa, India or South-East Asia [6]. *Abelmoschus esculentus* L. has different names around the world. For example, its name is Bamia in Libya and Egypt [7], lady's finger in England (as it is slightly bent, and has a tapering end resembled the finger of a woman), gumbo in the United States of America, guino-gombo in Spanish and bhindin in India [8,9].

The plant has many benefits, it is used as fresh leaves, buds, flowers, pods, stems and seeds [10] (Figure 1). On the other hand, eating too much okra can have adverse effects on some people as gastrointestinal problems because it is rich in fructans, a type of carbohydrates that can cause diarrhea, gas, cramping and bloating in people with bowel problems. It also can cause oxalates and kidney stones as it contains high oxalate contents.

Figure 1. *Abelmoschus esculentus* L

Abelmoschus esculentus L. is a nutritional source of power used throughout history for both medicinal and culinary purposes. Okra is a good source of minerals, vitamins and nutrients that are responsible for the health benefits, as it contains potassium, sodium, magnesium and calcium, also, vitamin B, vitamin C and folic acid are present in it, in addition to iron and zinc are also present [11]. It is composed primarily of water, carbohydrates, and proteins with very little fat and a fair amount of dietary fiber. It is considered as a powerhouse of valuable nutrients, nearly half of which are soluble fibers in the form of gums and pectins which help to lower serum cholesterol and reducing the risk of heart diseases. The other fraction of okra is insoluble fiber, which helps to keep the intestinal tract healthy. Okra is rich in phenolic components which have remarkable pharmacological and antioxidant activities [12,13]. Thanks to fibers and other nutrients, okra is beneficial in normalizing blood sugar in the body by improving the rate as a result of which sugar is absorbed from the intestinal tract, okra is full of dietary fibers, which is essential for colon health and digestive health as a whole, plus it helps in promoting healthy skin. Vitamin C helps keeping the skin looking young and vibrant. Additionally, the presence of these fibers together with other nutrition manifests its advantages of lowering sugar levels in the body, hence improves the concentration of the blood sugar level by reducing sugar absorption through the intestines [14]. It is used in folk medicine in the management of diarrhea, inflammation and diabetes, furthermore, recently, a new benefit of including okra has been suggested to help manage blood sugar so, it has benefit for diabetic patients.

Actually, in addition to its vegetative importance, it has a good position in oil production due to its high content of oil in the seed (20-40%) [9,15], so, we can say that *Abelmoschus esculentus* L seeds are considered as oil and protein source. Recently, knowing the great importance of oil components in health benefits, leads to increasing the request for oil industries and thus, seeking for novel vegetable oil sources [13]. Okra pods are also rich in mucilage, which is usually concentrated in the pod wall, and chemically consists of acidic polysaccharides associated with proteins and minerals [16]. This mucilage binds cholesterol and bile acid carrying toxins dumped into it by the liver. Finally, we can conclude that okra is an important vegetable crop with a valuable nutritional quality and potential health benefits.

Phenolic compounds are considered as the most occurring phytochemical group, having strong antioxidant properties [17]. It is believed that the antioxidant, anti-inflammatory, anti-cancer, and antimicrobial activities of many plants, may be due to their phenolic content.

In the present work we have analyzed the polyphenolic profile compounds by using HPLC-MS, while, using GC/MS for the non-polar compounds, in addition to cytotoxic evaluation for three tested extracts of *Abelmoschus esculentus* L. leaves was recorded in this study. HPLC-MS is becoming a common choice in natural products and phytochemistry researches because it can identify and specify the nature of compounds from a small amount of plant extracts with low concentrations. On the other hand, MS is useful for determination the molecular masses of the isolated phenolic compounds.

EXPERIMENTAL SECTION

Materials and Methods

Plant material

Abelmoschus esculentus L. leaves were collected from Giza farm in 2018 and identified by Mm. Tressa Labib, Taxonomist, at El-Orman Botanical Garden, Giza, Egypt. The plant samples were air-dried, powdered and kept for phytochemical and cytotoxic studies.

Extraction

500 g of *Abelmoschus esculentus* L. leaves was air dried and extracted with 70% methanol/water at room temperature three times (3x 1L). The aqueous methanolic extract (AME) was concentrated under reduced pressure in a rotary till dryness to give 90 g dried extract which was defatted by petroleum ether 40-60ºC (3x 250 ml) to give 20.50 g petroleum ether extract (PEE) and the residue, (defatted), aqueous methanolic extract (DAME, 69.5 g), preserved in -80ºC freezer until LC-ESI-MS analysis.

LC-ESI-MS analysis

The defatted aqueous methanol extract (DAME) of *Abelmoschus esculentus* L. leaves was analyzed using ESI-MS negative ion acquisition mode carried out on a XEVO TQD triple quadruple instrument. Waters Corporation, Milford, MA01757 U.S.A mass spectrometer Column: ACQUITY UPLC-BEH C18 1.7 µm-2.1 × 50 mm Column, Flow rate: 0.2 mL/min, solvent system consisted of (A) Water containing 0.1% formic acid, (B) Methanol containing 0.1% formic acid. The peaks and spectra were processed using the Maslynx 4.1 software and tentatively identified by comparing its retention time (R_t) and mass spectrum with reported data. The pure flavonoids obtained from Phytochemical and Plant Systematic Department were used as reference samples. Known peaks were identified

by comparing their retention time and mass spectrum with the flavonoids isolated in the present study. Unknown peaks were tentatively identified by comparing their mass fragmentation pattern with literatures.

Gas chromatography-mass spectrometry (GC/MS) analysis

The petroleum ether fraction (PEE) of *Abelmoschus esculentus* L. leaves was analysed using Thermo Scientific GC-MS equipped with AS 3000 autosampler, trace ultra GC and ISQ detector. A nonpolar column consisting of 5% phenylpolysilphenylene siloxane (Thermo Scientific TR 5MS) with dimensions of 30 m x 0.25 mm (internal diameter) x 0.25 µm (film thickness) was used for separation of the components. Helium, at a flow rate of 1 mL/min (constant flow mode), was used as carrier gas. A volume of 2 µL of sample extracts was injected in splitless mode. The injection port was set at 260^{o}C and temperature of oven was initially set at 50^{o}C for 2 minutes. Then it was ramped to 140^{o}C at rate of 5^{o}C/min for 2 minutes and finally to 280^{o}C at rate of 3^{o}C/min for 50 minutes. The maximum oven temperature was set at 330^{o}C. The mass spectrometer was operated in an electron ionization (EI) mode within the mass range of 50-700 amu with 20 scan times (min). The MS transfer line temperature and ion source temperature were kept at 290^{o}C and 300^{o}C respectively with electron multiplier voltage of 1 Kv. The mass spectra were interpreted using the reference library of the National Institute of Standards and Technology (NIST), US, along with Willey 5 and mass finder, as well as data reported by Adams [18]. The constituent percentages were measured based on the peak area.

Acid hydrolysis and paper chromatography

Acid hydrolysis was carried out for 2h at 100ºC using 10 mL hydrochloric acid (2 mol/L). The yielded aglycones were then extracted with ethyl acetate. Ethyl acetate layer was dried with anhydrous Na_2SO_4 then evaporated. The hydrolyzed extract was subjected to paper chromatography (PC) Whatman No. 1 (Whatman Ltd., Maidstone, Kent, England) using 15% AcOH/water (Acetic acid: H_2O, 15:85) and BAW (n-Butanol: Acetic acid: H_2O 4:1:5, upper layer) to detect the flavonoid aglycones. Also, the aqueous layer was carefully neutralized, then subjected to PC investigation using BBPW (Benzene: n-butanol: pyridine: water; 1:5:3:3, upper layer) to detect the sugars. The dried chromatograms were visualized by aniline phthalate reagent [19,20]. The sugar spots were observed in day light. The R_f values of tested sugars were compared with those of reference sugars [21,22].

Cell culture and *in vitro* cytotoxic activity

The cytotoxicity was carried out using sulphorhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara [23].

SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. The cells were seeded in 96-well microtiter plates at initial concentration of $3x10^3$ cell/well in a 150 µL fresh medium and left for 24 hours to attach to the plates. Different concentrations of extracts (0, 5, 12.5, 25, 50 µg/ml) were added. For each drug concentration, 3 wells were used and the plates were incubated for 48 hours. The cells were fixed with 50µl cold trichloroacetic acid 10% final concentration for 1 hour at 4 $^{\circ}$C. The plates were washed with distilled water using (automatic washer Tecan, Germany) and stained with 50 µL 0.4% SRB dissolved in 1% acetic acid for 30 minutes at room temperature then washed with 1% acetic acid and air-dried.

The dye was solubilized with 100 µL/well of 10 M tris base (pH 10.5) and optical density (O.D.) of each well was measured spectrophotometrically at 570 nm with an ELISA microplate reader (Sunrise Tecan reader, Germany). The mean background absorbance was automatically subtracted and mean values of each extract concentration was calculated.

The percentage of cell survival was calculated as follows:

Surviving fraction=O.D. (treated cells)/O.D. (control cells).

The IC_{50} values (the concentrations of drug required to produce 50% inhibition of cell growth) were also calculated.

Statistical analysis

All data were subjected to analysis of variance ANOVA to test the significance in the all experiments. The least significant difference (LSD) at $P<0.05$ level was calculated according to the statistical analysis method described by Casanova et al. [24].

RESULTS AND DISCUSSION

Acid Hydrolysis

The complete acid hydrolysis revealed that all glycosides were in O-glycoside form. Paper chromatography (PC) gave two components, their color reaction and R_f values resemble that of quercetin and kaempferol aglycones (as authentic references) in the organic phase. Glucose, arabinose, xylose and rhamnose were detected as sugar moieties in the aqueous phase, compared with authentic sugars.

Identification of Phenolic Compounds Using LC-ESI-MS Analysis

Liquid chromatography mass spectrometry (LC-MS) is a powerful and new technique for identification of complex extract, by which the structure of its components can be characterized. Therefore, in this study, the defatted aqueous methanolic extract (DAME) of *Abelmoschus esculentus L.* leaves was subjected to HPLC coupled with MS spectrometry in negative ion mode ESI (-ve), (Figure 2). The identification of the compounds of the methanolic fraction achieved by their retention times, molecular weights (MW), calculated m/z and major fragments produced under the ionization conditions, in addition to comparing these data with the previously reported data in the literature.

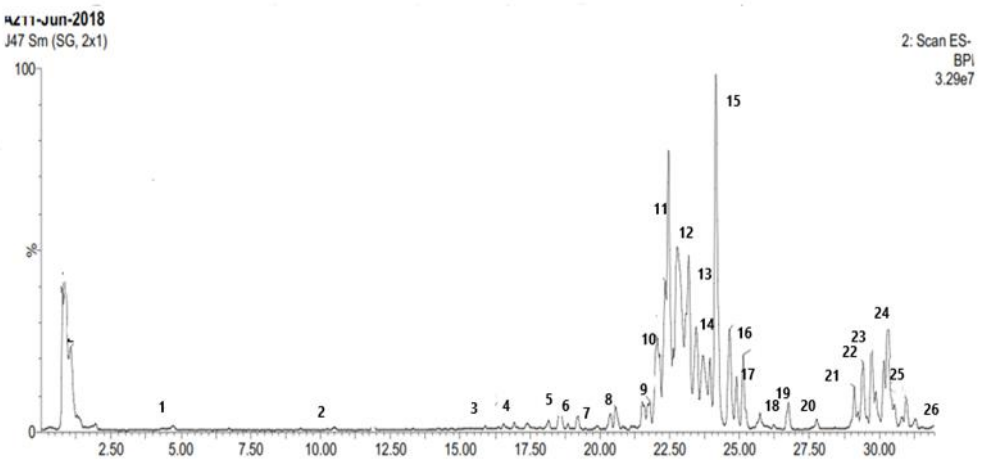

Figure 2. Total ion chromatogram of LC-ESI-MS of the DAME of the *Abelmoschus esculentus* L. leaves

Table 1. LC-MS analysis of phenolic compounds of DAME of the *Abelmoschus esculentus* L. leaves (R_t: Retention time; MW: Molecular weight)

Peak No.	R_t (min)	[M]	[M-H]-	Fragments m/z	Tentative Identification Compounds
1	4.57	170	169	-------	Gallic acid
2	10.21	180	179	135, 89, 78	Caffeic acid
3	15.24	342	341	179, 135	Caffeic acid glucoside
4	16.21	192	191	173, 128, 85	Qunic acid
5	17.51	354	353	191, 179,173, 135	cis 3-Caffeoylqunic acid
6	18.24	409	408	357, 193, 175	Unknown
7	19.23	302	301	----------	Quercetin
8	20.52	286	285	----------	Kaempferol
9	21.54	464	463	301	Quercetin-O-glucoside
10	22.53	626	625	463, 301	Quercetin-di-O-glucoside
11	23.74	610	609	301	Quercetin-O-rhamnoglucoside
12	23.84	834	833	729, 567, 463, 301	Quercetin-O-benzoylglucopyranoside
13	24.52	448	447	301	Quercetin-O-rhamnopyranoside
14	24.64	309	308	193, 134, 116	Feruloyl malate
15	25.1	448	447	285	Kaempferol-O glucopyranoside
16	25.12	516	515	353, 191, 179, 135	3,4,-Dicaffeoyl qunic acid
17	26.25	742	741	609, 447, 301	Quercetin-O-arabinoside-O-glucoside rhamnopyranoside
18	26.54	580	579	447, 433,261, 301	Quercetin-O-xyloside-O-rhamnopyranoside
19	27.12	435	434	301	Quercetin-O-arabinopyranoside
20	29.21	418	417	285	Kaempferol-O-arabinopyranoside
21	29.54	452	451	289	Catechin O-glucopyranoside
22	30.1	195	194	1,67,107	Unknown
23	31.54	812	811	463, 301, 169	Quercetin-galloyldiglucopyranoside
24	32.52	820	819	625, 447, 285, 193,134	Kaempferol-O-feruloyldiglucopyranoside
25	33.02	950	949	900, 814, 781, 670	Unkown

Twenty two compounds were identified from *Abelmoschus esculentus* L. leaves (Table 1). These compounds can be classified as seven phenolic acids and their derivatives, in addition to fifteen flavanol (as quercetin and kaempferol derivatives). Compounds 1 and 2 were eluted at 4.57 and 10.21 min which produced a molecular ions peaks $[M-H]^-$ at m/z 169, 179, which were identified as gallic acid and caffeic acid, and affirmed by the mass fragment and R_t of the authentic standards [25].

Compound 3 was eluted at 15.24 min and detected with $[M-H]^-$ peak at m/z 341 yielded fragment ions at m/z 179 and 135 which referring to caffeic acid after loss of hexose moiety, thus, identified as caffeic acid glucoside. Compound 4 recorded $[M-H]^-$ at m/z 191, giving fragments at m/z 173, 128 and 85; identified as quinic acid [26], while, compound 5 showed $[M-H]^-$ peak at m/z 353 and eluted at retention time 17.51 min which gave fragments at m/z 191, 179,173 and 135 thus, this compound was identified as *cis* 3-caffeoylqunic acid [27].

Peak 7 and 8 are identified as quercetin (compound 6) and kaempferol (compound 7) as two favanol aglycones with $[M-H]^-$ at m/z 301 and 285, respectively. Compound 8 revealed $[M-H]^-$ peak at m/z 463 with fragments at m/z 301 which corresponding to the aglycone (quercetin) after loss of glucose moiety and identified as quercetin-*O*-glucoside. Compound 9 produced a molecular ion peak at m/z 625 and specified as quercetin-di-*O*-glucoside, confirmed by mass fragments at m/z 463, 301, due to loss of one glucose moiety.

Quercetin-*O*-rhamnoglucoside (compound 10) with $[M-H]^-$ peak at m/z 609 gave fragment at m/z 301, corresponding to quercetin as aglycone after loss of glucose and rhamnose moieties. Peak 12 (compound 11) was eluted at retention time 23.84 min and identified as quercetin-*O*-benzoylglucopyranoside as appears with molecular ion peak at m/z 833 and fragment ions m/z 729, 567, 463, 301 that is due to loss of benzoyl group (102 amu), followed by loss of glucose unit (162 amu). Compound 12 identified as quercetin-*O*-rhamnopyranoside with molecular ion peak at m/z 447, gave fragment at m/z 301 which corresponding to quercetin as aglycone after loss of rhamnose moiety $[M-H-146]^-$. Compound 13 (peak 14) identified as feruloyl malate produced a molecular ion peak at m/z 308 which gave fragments at m/z 193, 134, 116 as ester of malic acid $[M-H-116]^-$. Compound 14 produced a molecular ion peak at m/z 447 which fragment at m/z 285 corresponding to the kaempferol as aglycone after loss of glucose and thus identified as kaempferol-*O*-glucopyranoside. 3,4,-dicaffeoylqunic acid (compound 15) as appears with molecular ion peak at m/z 515 and fragment ions m/z 353, 191, 179 and 135. Compound 16 showing the molecular ion peak at m/z 741 yielded fragment ions at m/z 609, 447, 301 due to loss of one arabinose unit (132 amu), one glucose unit (162) as well as one rhamnose unit (146 amu) thus, this compound identified as quercetin-O-arabinoside-*O*-glucosiderhamnopyranoside [28].

Quercetin-O-xyloside-O-rhamnopyranoside (compound 17) appears with molecular ion peak at m/z 579 and fragment ions m/z 447, 433, 261, 301, this is due to loss of one xylose and one rhamnose unit from quercetin as aglycone. Compound 18 and 19 are identified as quercetin-*O*-arabinopyranoside and kaempferol-*O*-arabinopyranoside showing molecular ion peaks at m/z 434 and 417 which gave quercetin 301 and kaempferol 285 as two aglycones after loss of arabinose moiety. Catechin glucopyranoside (compound 20) was observed with molecular ion peak at m/z 451, which fragments at m/z 289, corresponding to catechin moiety after loss of glucosyl moiety (162 amu) [29].

Finally, compounds 21 (peak 23) and 22 (peak 24) revealed molecular ion peaks $[M-H]^-$ at m/z 811 and 819, respectively. Compound 22 gave fragments at m/z 463, 301, 169, corresponding to quercetin as aglycone and loss of one glucose (162 amu) unit and gallic acid (169 amu) moiety, so, compound 21 was recorderd as quercetin-galloyldiglucopyranoside, while, compound 22 yieled fragments at m/z 625, 447, 285, 193,134, corresponding to kaempferol as aglycone, with a loss of two glucose moieties and ferulic acid (193 amu), thus, compound 22 was identified as kaempferol-*O*-feruloyldiglucopyranoside. From the above results, we can say that this work presents a more complete description of the phenolic compounds present in *Abelmoschus esculentus* L. leaves.

Identification of Non-Polar Compounds Using GC-MS Analysis

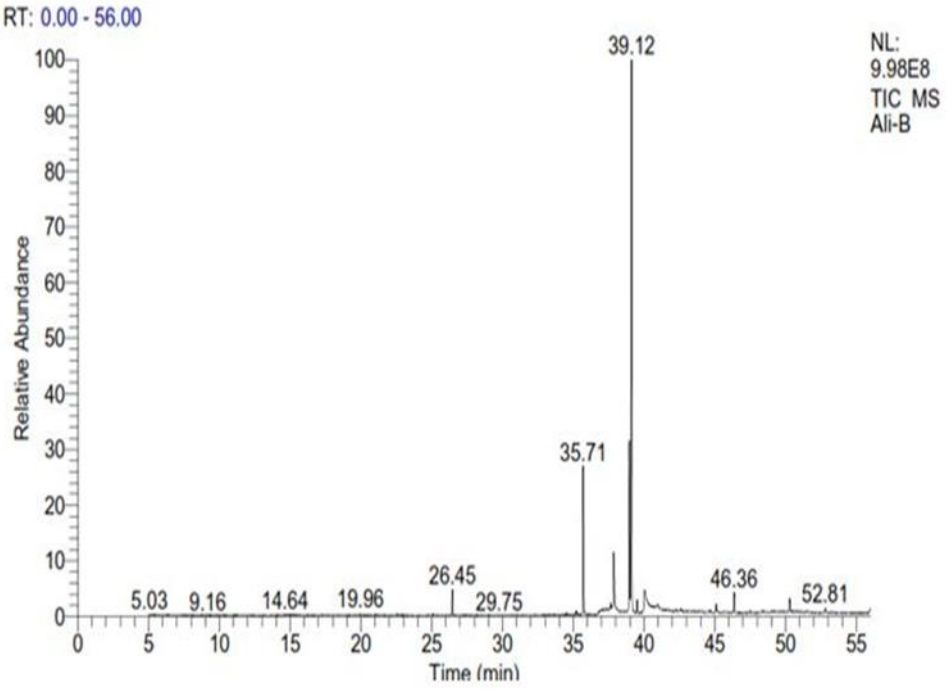

Figure 3. GC/MS analysis of PEE of *Abelmoschus esculentus* L. leaves

Table 2. GC/MS analysis of PEE of *Abelmoschus esculentus* L. leaves (R_t: Retention time; MW: Molecular Weight)

Peak No.	R_t (min)	MW	Area %	Identified compounds
1	26.25	206	0.21	Phenol, 2,4bis (1,1dimethylethyl) C14H22O
2	26.45	212	0.65	Pentadecane C15H32
3	29.75	226	0.52	Hexadecane C16H34
4	30.85	310	1.2	Heptadecane C17H36
5	33.56	256	1.12	Tetradecanoic acid, 12methyl, methyl ester C16H32O2
6	34.55	278	0.26	1,2Benzenedicarboxylic acid, dibutyl ester C16H22O4
7	35.15	268	6.45	9Hexadecenoic acid, methyl ester, (Z) C17H32O2
8	35.71	270	12.75	Hexadecanoic acid, methyl ester C17H34O2

9	36.41	468	0.5	Lup20(29)en3ol, acetate, (3á) C32H52O2
10	36.83	490	2.03	17Pentatriacontene C35H70
11	37.13	310	4.7	Docosane C22H46
12	37.35	280	1.2	Methyl 9,12heptadecadienoate C18H32O2
13	37.47	282	2.13	cis10Heptadecenoic acid, methyl ester C18H34O2
14	37.54	284	0.42	Heptadecanoic acid, methyl ester C18H36O2
15	38.86	294	13.54	9,12Octadecadienoic acid, methyl ester C19H34O2
16	39.12	296	10.54	9Octadecenoic acid (Z), methyl ester C19H36O2
17	39.51	298	2.03	Octadecanoic acid, methyl ester (Methyl stearate) C19H38O2
18	39.72	328	0.51	6,8Bisdehydro4,5:10.11dibenzopentatridecafulval-ene C26H16
19	40.14	280	7.05	9,12Octadecadienoic acid (Z,Z) C18H32O2
20	40.86	354	3.79	8,9,10,11Tetrahydro8,8,11,11,15pentamethylanchraceno [1,2c]1,8-naphthridin C25H26N2
21	42.38	215	2.2	Octanoic acid, 2dimethylaminoethyl ester C12H25NO2
22	42.67	592	1.16	Octadecanamide, N,N'1,2ethanediylbis C38H76N2O2
23	42.73	320	2.18	11,14,17Eicosatrienoic acid, methyl ester C21H36O2
24	43.31	392	2.22	Cholan24oic acid, 3,12dihydroxy, (3à,5á,12à) C24H40O4
25	44.65	354	10.2	9,12Octadecadienoicacid (Z,Z),2hydroxy1(hydroxy methyl) ethyl ester C21H38O4
26	45.08	131	1.2	Acetic acid, 2(dimethylamino) ethyl ester C6H13NO2
27	46.46	540	1.58	8'OEthyláalectoronic acid C30H36O9
28	47.48	314	0.24	Delta.1(2) tetrahydrocannabinol C21H30O2
29	49.32	378	2.21	Cyclohexane,1,3,5trimethyl2octadecyl C27H54
30	49.86	412	1.45	Tetracosapentaene,2,6,10,15,19,23hexamethyl C30H52
31	50.31	410	2.2	Squalene C30H50

Petroleum ether extract (PEE) of *Abelmoschus esculentus* L. leaves was analyzed by GC/MS which consists of 31 compounds (Figure 3 and Table 2). These compounds belonging to the classes of hydrocarbons, including oxygenated, non-oxygenated and nitrogenous compounds. The total peak areas of the detected compounds represent (98.44%), The major peak areas (represent 47.03% of the total peak) are 9,12-octadecadienoic acid, methyl ester $C_{19}H_{34}O_2$ (13.54%), hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$ (12.75%), 9-octadecenoic acid (Z)-, methyl ester $C_{19}H_{36}O_2$ (10.54%), 9,12-octadecadienoicacid (Z,Z)-,2-hydroxy-1 -(hydroxy methyl) ethyl ester $C_{21}H_{38}O_4$ (10.20%), which were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC/MS instrument.

Cytotoxic Activity

The in vitro cytotoxic activities of aqueous methanolic extract (AME), petroleum ether extract (PEE) and defatted aqueous methanol extract (DAME) of *Abelmoschus esculentus* L. leaves were evaluated for any cytotoxic activity

against four carcinoma cell lines: HELA (cervix carcinoma cell line), MCF7 (breast carcinoma cell line), PC3 (prostate carcinoma cell line) and HEPG2 (liver carcinoma cell line) at different concentrations [0-50 µg/ml] in comparison with standard doxorubicin (SD). Results recorded in Table 3; Figures 4-7 concluded that the DAME at concentration 50 µg/ml showed an obvious high antitumor activity (0.298, 0.289, and 0.452) against HepG2, PC3, and MC-7, respectively, with IC_{50} (5.2, 6.84, and 7.4 µg/ml), while it has moderate activity against HELA at the same concentration, with IC_{50} (15.54 µg/ml), compared with doxorubicin standard. Also, it was observed that AME at concentration 50 µg/ml presented significant antitumor activity (0.487, 0.297, and 0.323) against MC-7, PC3, and HepG2, respectively, with IC_{50} (19.78, 22.71, and 14.51 µg/ml), while it has moderate activity against HELA with IC_{50} (18.82 µg/ml) at the same concentration. On the other hand, it was shown that PEE (IC_{50} 27.24, 33.84, and 12.57 µg/ml, on MC-7, PC3, and HepG2, respectively), generally has moderate activity at 50µg/ml against the above three tested cell lines, in addition to its moderate to weak activities at the other concentrations against the four tested cell lines. Finally, the results (Table 3 and Figures 4-7) revealed that the three tested extracts (AME, PEE, and DAME) at the lower concentrations, have antitumor activities ranging from moderate to weak against the four tested cell lines (HELA, MC-7, PC3, and HepG2). All the previous results were compared with the standard doxorubicin (SD).

Table 3. Cytotoxic activity of AME, PEE and DAME, of *Abelmoschus esculentus* L. leaves, compared with SD

Cell line		**Sample**	**Conc. of the extract (µg/ml)**	**Conc. of the extract (µg/ml)**			
			0	**5**	**12.5**	**25**	**50**
Surviving fractions of the four tested cell lines	HELA	SD	1	0.415	0.378	0.287	0.192
		AME	1	0.887	0.758	0.582	0.412
		PEE	1	0.951	0.795	0.632	0.567
		DAME	1	0.864	0.617	0.524	0.321
	MC-7	SD	1	0.471	0.466	0.424	0.421
		AME	1	0.725	0.612	0.492	0.487
		PEE	1	0.812	0.691	0.526	0.518
		DAME	1	0.685	0.584	0.458	0.452
	PC3	SD	1	0.342	0.231	0.283	0.221
		AME	1	0.712	0.627	0.481	0.297
		PEE	1	0.845	0.756	0.534	0.346
		DAME	1	0.521	0.464	0.312	0.289
	HepG2	SD	1	0.421	0.354	0.337	0.277
		AME	1	0.486	0.384	0.381	0.323
		PEE	1	0.735	0.532	0.465	0.397
		DAME	1	0.481	0.344	0.341	0.298

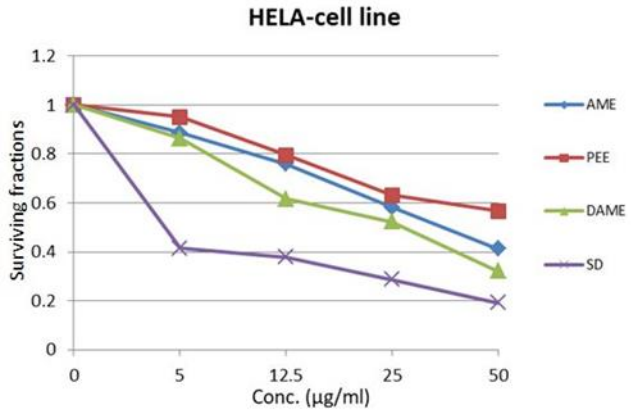

Figure 4. Cytotoxic activity of SD, AME, PEE and DAME of *Abelmoschus esculentus* L. leaves plant on HELA-carcinoma cell line

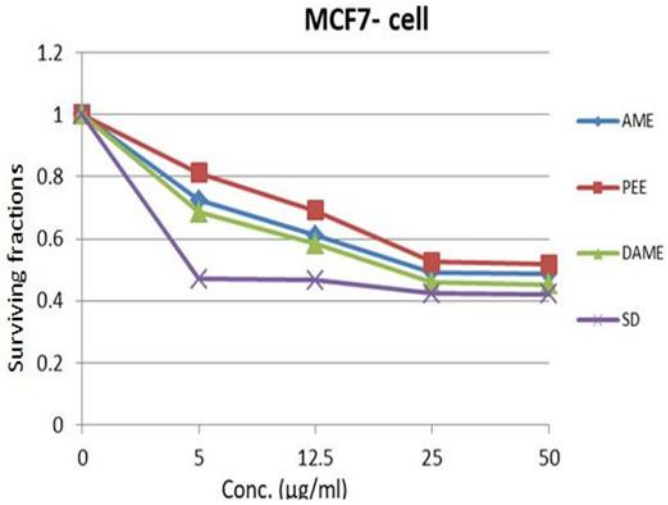

Figure 5. Cytotoxic activity of SD, AME, PEE and DAME of *Abelmoschus esculentus* L. leaves plant on MCF7-carcinoma cell line.

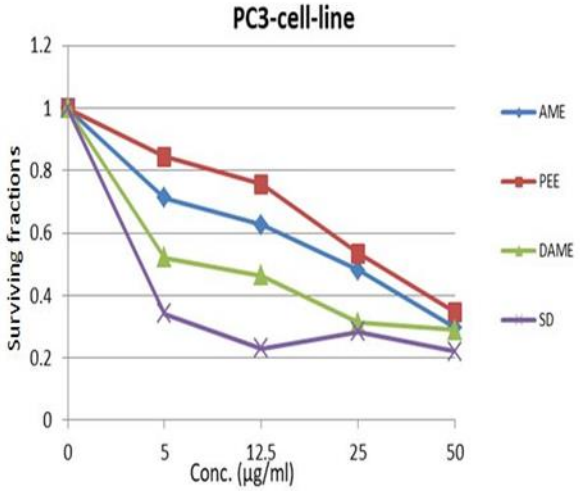

Figure 6. Cytotoxic activity of SD, AME, PEE and DAME of *Abelmoschus esculentus* L. leaves plant on PC3-carcinoma cell line

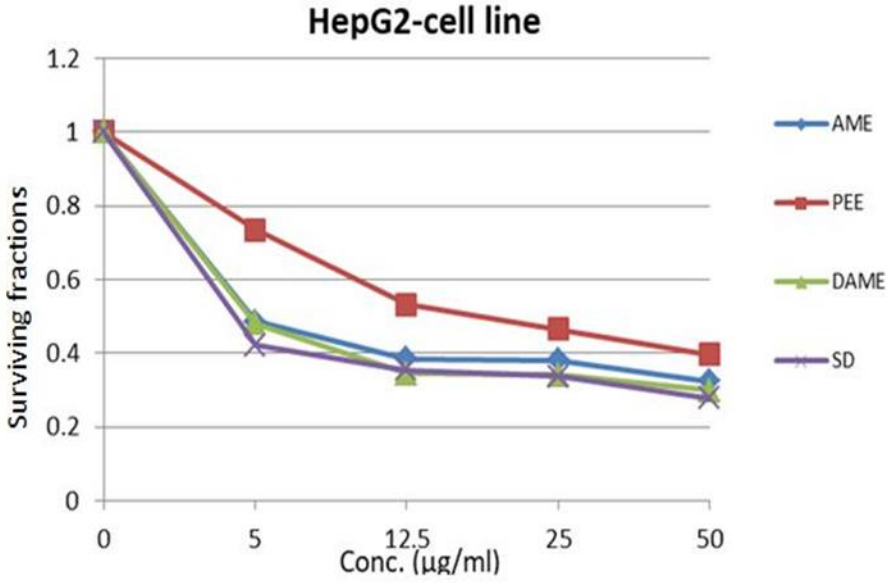

Figure 7. Cytotoxic activity of SD, AME, PEE and DAME of *Abelmoschus esculentus* L. leaves plant on HepG2-carcinoma cell line

CONCLUSION

In the present study, chemical fingerprint of *Abelmoschus esculentus* L. leaves by using LC-ESI-MS and GC/MS analysis was recorded. Twenty two phenolic and flavonoid compounds were identified from defatted aqueous methanolic extract (DAME), thus, the obtained results indicate that *Abelmoschus esculentus* L. leaves could be considered as a rich source of bioactive phenolic compounds. On the other hand, thirty one compounds were recorded from the petroleum ether extract (PEE). Furthermore, cytotoxic activities of the aqueous methanolic extract, petroleum ether and defatted aqueous methanolic extracts against four carcinoma cell lines: HELA (cervix carcinoma cell line), MCF7 (breast carcinoma cell line), PC3 (prostate carcinoma cell line) and HepG2 (liver carcinoma cell line), were also evaluated in this study.

Conflicts of Interest

The authors declared that present study was performed in absence of any conflict of interest.

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