Original Article

Cytotoxic, Antimicrobial Activities, and Phytochemical Investigation of Three Peach Cultivars and Acerola Leaves

Seham S. El-Hawary[1], Ola Mohamed Mousa[1,2], Rana Ahmed El-Fitiany[1], Rania A. El Gedaily[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Gizah, [2]Department of Pharmacognosy, Faculty of Pharmacy, Ahram Canadian University, 6th of October City, Egypt

Abstract

Background: Phytoconstituents of *Prunus persica* Linn. (Peach) and *Malpighia glabra* Linn. (Acerola) leaves were not thoroughly studied, although they are commonly incorporated in the food industry. **Aim:** Our aim is to explore metabolites and vitamins in three peach cultivars leaves; Desert red, Florida prince, Swelling and acerola. **Material and Methods:** Analysis was done using GC/MS (gas chromatography–mass spectrometry), HPLC (high-performance liquid chromatography), and spectrophotometry. Cytotoxicity was performed using MTT assay. **Results:** Total phenolic and flavonoid content varied from 79.54 to 121.51 μg gallic acid equivalent/mg dry weight and 31.05 to 39.77 μg quercetin equivalent/mg dry weight, respectively. Twenty-four flavonoids were identified; hesperidin was the major flavonoid in peach cultivars (3863.4 mg/100 g in Desert red, 2971 mg/100 g in Swelling, and 2624 mg/100g in Florida prince). Glucuronic acid (33.04%) and vitamin C (34 mg/100 g) were major in acerola. Thirty-four metabolites including supraene and sitosterol as well as 24 fatty-acid esters including linoleic and oleic acids were detected in the unsaponifiable and saponifiable matter, respectively. Antimicrobial activity against bacterial and fungal strains was screened in comparison with ampicillin and amphotericin B. All tested extracts significantly decreased cell viability against breast (MCF-7) and colon cell lines (HCT-116). *M. glabra* showed no significant difference from standard doxorubicin (0.1 μg/mL) which may suggest a strong anticancer activity against colon cell line. **Conclusion:** This study may highlight the magnitude of the leaves of these plants as rich sources of important metabolites and vitamin C.

Keywords: *Acerola, antimicrobial, cytotoxicity, GC/MS, HPLC, peach*

Introduction

Fruits and vegetables have a critical value in our nutrition and the human life. Due to the increasing world population and the changing dietary habits, the claim for such important food components has significantly increased.[1] Phytochemicals (mainly polyphenols), some vitamins (A, C, E, and folates), and dietary fibers are responsible for the great health benefits achieved by consuming vegetables, fruits, and other foodstuffs.[2] The wastes of fruits and vegetables can be used for the extraction and isolation of potentially bioactive compounds which can be incorporated in food, cosmetics, pharmaceutical, and textile industries.[1] The leaves of edible fruits can be considered as valuable byproducts which are wasted during harvesting of the fruits. These leaves have magnetized the attention of many researchers in the past few years in their search for new sources of valuable metabolites. For example, though rich in bioactive polyphenols, tons of berry leaves (blueberry, blackberry, raspberry, lingonberry, blackcurrant, bilberry, and cranberry) are wasted during harvesting each year.[3] Also surprisingly on comparing the phenolics of edible fruits and their leaves of seven selected species, *Malus domestica*, *Cydonia oblonga*, *Chaenomeles japonica*, *Ribes nigrum*, *Aronia melanocarpa*, *Vaccinium macrocarpon*, and *V. myrtillus*, the leaves contained notably higher polyphenol compounds compared to the fruits.[4] Polyphenols were also found abundant in strawberry leaves.[5] For our study, the leaves of two edible fruits were selected. *Prunus persica* Linn. and *Malpighia glabra* Linn. are two important edible plants belonging to families Rosaceae (2830−3100 species) and Malpigiaceae (1300 species),[6] respectively.

Prunus persica L. or peach[7] contains a diversity of phytochemical compounds such as alkaloids, glycosides, flavonoids, carbohydrates, fixed oils, steroids, tannins, phenols, amino acids, and proteins.[8] Peach has many important biological activities:

How to cite this article: El-Hawary SS, Mousa OM, El-Fitiany RA, El Gedaily RA. Cytotoxic, antimicrobial activities, and phytochemical investigation of three peach cultivars and acerola leaves. J Rep Pharma Sci 2020;9:221-34.

Received: 25 Aug 2019
Accepted: 15 July 2020
Published: 18 Aug 2020

Address for correspondence:
Dr. Rana Ahmed El-Fitiany, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Gizah, Egypt.
E-mail: marmarelfitiany@gmail.com.

Access this article online

Website: www.jrpsjournal.com

DOI:10.4103/jrptps.JRPTPS_88_19

Quick Response Code:

antidiabetic, antioxidant, antimicrobial, antitumor, antiallergic inflammatory, cholinesterase inhibitory, free radical scavenging, prokinetic, and polyphenol oxidase activities.[9] There are several cultivars of peach in Egypt; the most common cultivars are Florida prince (*P. persica cv.* Florida prince), Desert red (*P. persica cv.* Desert red), and Swelling (*P. persica cv.* Swelling).[10] According to the data acquired from Food and Agriculture Organization of the United Nations in 2016, 83 countries are cultivating peach worldwide; Egypt was ranked as the 11th country in peach production. Acerola is the common name assigned to the *M. glabra* L., also called "Barbados cherry" or "West Indian cherry."[11] Some of the reported active constituents in acerola's different organs are carotenoids,[12] fatty acids,[13] volatile constituents,[14] flavonoids,[15] and terpenes.[16] Acerola possess many reported significant pharmacological activities, for example, acetylcholinesterase inhibition,[17] antioxidant,[18,19] antipyretic,[20] anti-inflammatory,[21] and antihyperglycemic.[22] It is worth mentioning that no data are available concerning the chemical composition, cytotoxicity, and antimicrobial activity of the leaves of *M. glabra* Linn. and the three *P. persica* Linn. cultivars under study. This drove the authors to deeply investigate the chemical profile of these edible plant leaves to highlight the importance of these great sources of bioactive metabolites wasted as harvesting byproducts. High-performance liquid chromatography (HPLC) analysis was used to determine their flavonoid, carbohydrate, and vitamin content. GC/MS was used to analyze their saponifiable and unsaponifiable matter. Quantification of flavonoids and phenolic content was performed by applying Folin–Ciocalteu and aluminum chloride methods, respectively.

Materials and Methods

Plant material

Malpighia glabra L. leaves were collected from El-Orman Botanic Garden, Giza Governate, Egypt since May 2016 and it was kindly authenticated by the herbarium of El-Orman Botanic Garden. Samples of *P. persica* L. leaves of the three cultivars under investigation (*cv.* Florida prince, Desert red, and Swelling) were collected from Faculty of Agriculture, Cairo University and Manara-2 farm, Wadi El-Natrun, El-Behira Governate, Egypt in May 2015. They were kindly authenticated by Dr. Reem Samir Hamdy, associate professor of plant taxonomy, Department of Botany, Faculty of Science and Dr. Abdo Mohammed Abd El-Latef, associate professor in Fruit Orchards Department, Faculty of Agriculture, Cairo University, Egypt. Voucher specimens of *M. glabra* L. and *P. persica* Linn. cultivars were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University with serial numbers: 1.3.1.2019(1–4).

Preparation of plants extracts

Malpighia glabra L. leaves (450 g) and *P. persica* L. leaves (2 kg) of each of the three cultivars under investigation were separately air dried at 25°C and powdered in a household blender, then they were macerated in 80% ethanol for 1 week at room temperature, filtered, and the ethanol was evaporated using a rotary evaporator at 45°C. Maceration, filtration, and evaporation processes were repeated till exhaustion.

Phytochemical screening

The phytoconstituents of the peach cultivars and acerola leaves' 80% ethanolic extracts were screened for the presence of flavonoids,[23] cardiac glycosides,[24] alkaloids,[25] anthraquinone glycosides,[26] carbohydrates,[27] saponins,[28] sterols and/or triterpenes,[29] tannins,[30] and volatile constituents.[31]

Quantification of the plant phytoconstituents

Spectrophotometric determination of total phenolic content

The total phenolic content of the 80% ethanolic extract of the leaves of the plants under investigation was estimated using UV–visible spectrophotometer (Shimadzu UV-1650 PC, Kyoto, Japan) using Folin–Ciocalteu method.[32] The absorbance was measured at 765 nm. A calibration curve of gallic acid (Sigma, St. Louis, MO, USA), ranging from 80 to 280 µg/mL, was constructed (R^2 = 0.9856) [Figure 1A], and total content (%) of phenolics was calculated as gallic acid equivalents using the regression equation of the calibration curve. All determinations were repeated three times.

Spectrophotometric determination of flavonoid content

The total flavonoid content of the 80% ethanolic extract of the leaves of the plants under investigation was estimated using aluminum chloride colorimetric method.[33] The absorbance of the dilutions was measured at 415 nm expressed as quercetin equivalent (QE). A standard calibration curve (R^2 = 0.9964) was established with different aliquots (5–100 µg/mL) of standard quercetin (Sigma) [Figure 1B]. Each sample was done in triplicate.

High-performance liquid chromatography analysis for flavonoids

HPLC analysis of flavonoids (phenolics) was performed according to the method by Mattila *et al.*[34] using reversed-phase HPLC Agilent 1200 series (Agilent Technologies, Waldbronn, Germany) and ZORBAX ODS column 4.6 mm × 250 mm (Dupont Instrument, Wilmington, Delaware). Multiwavelength detector was set at 330 nm. Detailed analysis conditions are mentioned in Supplementary File (S1). All flavonoids were quantified using the external standard method. Quantification of samples and standards was based on peak area. Dilution of stock standards was done in methanol to give 2–20 µg/mL for the establishment of calibration curves.

High-performance liquid chromatography analysis for carbohydrates

HPLC analysis was done according to the method of Kiranmai *et al.*[35] The tested ethanolic extracts of the leaves were diluted to 1:10 (v/v) with deionized water and then filtered

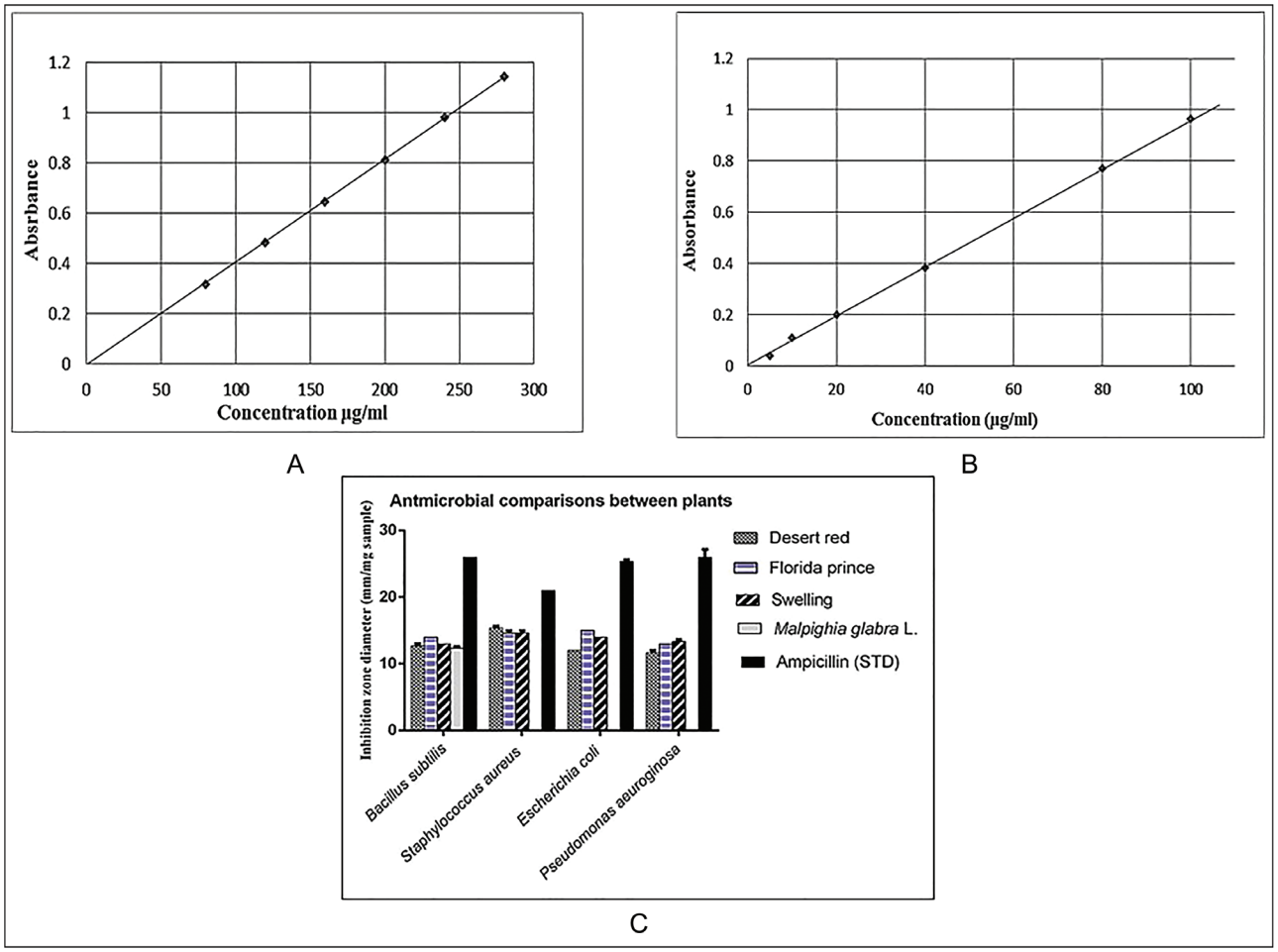

Figure 1: Calibration curve of (A) gallic acid (for phenolic) and (B) quercetin (for flavonoids). (C) Antimicrobial activity of the tested extracts against Gram-positive and Gram-negative bacteria

Table 1: Total phenolic and flavonoid content of the leaves of *Prunus persica* L. cultivars and *Malpighia glabra* L. expressed as gallic acid equivalent (µg of gallic acid/mg of sample) and quercetin equivalent (µg of quercetin/mg of sample), respectively

Plant name	Phenolic content (µg/mg) ± SD	Flavonoid content (µg/mg) ± SD
P. persica L. *cv.* Desert red	79.54 ± 0.140	31.05 ± 0.01
P. persica L. *cv.* Florida prince	118.74 ± 0.14	34.10 ± 0.06
P. persica L. *cv.* Swelling	121.51 ± 0.001	33.18 ± 0.01
Malpighia glabra L.	110.52 ± 0.14	39.77 ± 0.01

through a 0.22 μm filter membrane. An aliquot of 1.5 mL of each of these solutions was posited in vials for analysis. The analysis was performed using HPLC Agilent 1200 series (Agilent Technologies) using a Bio-Rad Aminex – carbohydrate HPX-87C column (300 mm × 7.8 mm). Detailed analysis conditions are mentioned in Supplementary File (S2). Sample detection was performed by comparing the retention time of analytes with standards. Quantification was based on peak area. Triplicate injections of seven different concentrations of each standard obtained by dilution in deionized water were performed. A calibration curve for each sugar was done by plotting the concentrations versus the peak area.

High-performance liquid chromatography analysis for vitamin C and E

Vitamin E and C quantification was performed according to the methods of Romeu-Nadal *et al.*[36] and Pyka *et al.*,[37] respectively, using reversed-phase HPLC Agilent 1200 series (Agilent Technologies). Multiwavelength detector was set at 254 and 292 nm for detection and quantification of vitamin C and vitamin E, respectively. The separation was carried out using ZORBAX ODS column 4.6 mm × 250 mm (Dupont Instrument). Detailed analysis conditions are mentioned in Supplementary File (S3). Ascorbic acid and α-tocopherol were recognized by comparing the retention time of the sample

peak with that of the standard. A calibration curve of different standard concentrations ranging from 0.5 to 100 µg/mL was plotted and quantification was carried out using external standard method.

Gas chromatography–mass spectrometry analysis of unsaponifiable and saponifiable matters

Air-dried powdered leaves of *M. glabra* Linn. (250 g.) and *P. persica* Linn. (171 g) cultivars (Desert red, Swelling, and Florida prince) were, separately, defatted in *n*-hexane. The solvents were evaporated under vacuum to give 6.07, 2.75, 1.81, and 3.01 g. residue, respectively. The unsaponifiable matters and the fatty acid methyl esters of the plants under investigation were prepared from the previously obtained hexane extracts.[38] The detection of saponifiable and unsaponifiable matters was carried out using gas chromatography coupled with mass spectroscopy (Shimadzu QP-5050 A, Japan) equipped with DB1-MS fused silica capillary column (30 m x 0.53 mm; film thickness 1.5 μm). Detailed analysis conditions are mentioned in Supplementary file (S4). All the standards were well resolved, and quantitative measures were obtained by correlating peak areas for all known compounds and relating them to standard curves of the standard compounds.

In vitro antimicrobial screening

Determination of the antimicrobial activity

The ethanolic extracts of the plants under investigation were screened for antimicrobial activity by implementing a modified Kirby–Bauer disk-diffusion method[39] against bacterial strains *Staphylococcus aureus* (*ATCC 12600*), *Bacillus subtilis* (*ATCC 6051*), *Escherichia coli* (*ATCC 11775*), and *Pseudomonas aeuroginosa* (ATCC 10145), *fungal strain* (*Aspergillus flavus* [*Link*]), and yeast (*Candida albicans* [*ATCC 7102*]), which were available in the micro-analytical center, Faculty of Science, Cairo University, Egypt. Standard disks of ampicillin (antibacterial agent supplied from Bristol-Myers Squibb, Switzerland) and amphotericin B (antifungal agent supplied from Bristol-Myers Squibb, Switzerland) served as positive controls for antimicrobial activity, whereas filter disks impregnated with 10 µL of solvent (distilled water, chloroform from El-Gomhouria Company for Trading Chemicals and Medical Appliances, DMSO from Loba Chemie) were used as a negative control. The agar used is Meuller-Hinton agar for bacteria and Czapek's Dox agar (sucrose-nitrate agar) for yeasts and fungi; they are rigorously tested for composition and pH. Determination of standard zones of inhibition was done for susceptible and resistant values. Blank paper disks (Schleicher and Schuel, Spain) with a diameter of 8.0 mm were impregnated with 10 μL of the tested concentration of the stock solutions of the plant extracts, which were dissolved in dimethyl sulfoxide. For the disk diffusion, slipping calipers of the National Committee for Clinical Laboratory Standards were used for the measurement of the zone diameters.[40] The test was performed in triplicates. Detailed analysis conditions are mentioned in Supplementary File (S5).

Determination of minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of the crude extracts under study were determined through performing the agar dilution method.[41] Stationary phase cultures of bacteria were prepared at 37°C and used to inoculate fresh 5.0 mL culture to an OD_{600} of 0.05. Incubation was done for the 5.0 mL cultures at 37°C until an OD_{600} of 0.10 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6 x 10^5 CFU/mL. Serial dilutions from the treatments (0–320 mg/mL) were prepared and mixed with 5.0 mL of the standardized bacteria suspension then added to the plates and incubated for 24 h at 37°C. The colony-forming units (CFU) were counted for each dilution.

MTT assay

The cytotoxicity of the extracts under investigation was tested by performing MTT assay[42,43] against human breast adenocarcinoma (MCF-7) and human colon adenocarcinoma (HCT-116) cell lines, which were acquired from American type culture collection (ATCC, Wesel, Germany) and grown in the tissue culture lab of the Egyptian company for vaccines, sera, and drugs production (Vacsera, Giza, Egypt). Heat-inactivated fetal bovine serum was supplied from Invitrogen, Carlsbad, California. MTT solution/well was bought from Sigma Aldrich, Missouri.

Absorbance was measured at 570 nm using Epoch-2C plate reader (Bio Tek, Vermont). The cell viability was expressed relative to the untreated control cells and the concentrations induced 50% growth inhibition (IC_{50}) were calculated from the concentration-response curve using graph pad prism version 5 (GraphPad Software, California). The detailed analysis conditions are stated in Supplementary File (S6).

Statistical analysis

All the results were expressed as mean ± standard error of the mean (SEM). The inhibition zone diameters and cell viability were determined in triplicate for antimicrobial and cytotoxic activities, respectively, and the analysis of variance (ANOVA) was done, followed by Dunnett's and Tukey's multiple comparisons test for antimicrobial and cytotoxic activities, respectively, to determine the significance of difference among the studied groups. The statistical analyses were tested at 0.001 level of probability using the GraphPad Prism version 6 (GraphPad Software, San Diego, California).

Results and Discussion

Extraction yield

The resulted dry extracts of *M. glabra* L. was 74.24 g, whereas *P. persica* L. cultivars Florida prince, Desert red, and Swelling were 130.14, 242.38, and 75.91 g, respectively. They were kept at 4°C for further phytochemical and biological studies.

Phytochemical screening

The results of the phytochemical screening showed the presence of carbohydrates and/or glycosides, flavonoids, sterols and/or

triterpenes, tannins, and traces of volatile constituents in all tested plants. Alkaloids, saponins, anthraquinone, and cardiac glycosides were absent in all the extracts.

Determination of total phenolic and flavonoid content

The spectrophotometric analysis of the total phenolics revealed that *P. persica* L. *cv*. Swelling shows the highest total phenolic concentration (121.51 ± 0.001 μg of gallic acid equivalent/mg dry weight) followed by *P. persica* Linn. *cv.* Florida prince (118.74 ± 0.14 μg of gallic acid equivalent/mg dry weight) then *M. glabra* Linn. (110.52 ± 0.14 μg of gallic acid equivalent/mg dry weight) and *P. persica* Linn. *cv.* Desert red (79.54 ± 0.140 μg of gallic acid equivalent/mg dry weight).

Furthermore, the analysis of flavonoid content showed that *M. glabra* Linn. contains the highest total flavonoid content (39.77 ± 0.01 μg of QE/mg dry weight), followed by *P. persica* Linn. *cv.* Florida prince (34.10 ± 0.06 μg of QE/mg dry weight) then *P. persica* Linn. *cv.* Swelling (33.18 ± 0.01 μg of QE/mg dry weight) and *P. persica* Linn. *cv.* Desert red (31.05 ± 0.01 μg of QE/mg dry weight) as shown in Table 1. No previous data were found concerning the total phenolic and flavonoid content of the leaves. Previous studies reported that the total phenolic and total flavonoid content in the pulps and peels of five Chinese peach cultivars were found in the range of 24.83–163.54 mg GAE/100 g and 17.76–299.86 mg Rutin Equivalent/100 g, respectively,[44] whereas the peel and pulp extracts from different varieties of peach from Pakistan showed an considerable amount of total phenolics and total flavonoids, ranging from 1209.3 to 1354.5, 711.7 to 881.3 mg GAE/100 g and 599.7 to 785.5, 301.3 to 499.7 mg catechin equivalents (CE) /100 g on a dry weight basis, respectively.[45] Cantin *et al.*[46] found out that the total phenolics of Spanish peach fruit among varied genotypes were in the range of 12.7–71.3 mg of GAE/100 g and the total flavonoids content ranged from 1.8 to 30.9 mg of CE per 100 g, with an average of 8.8 mg of CE per 100 g. Also, previous reports regarding *M. glabra* L. showed that the contents of polyphenol in fruit extracts in India were 355.74 mg GAE/100 g.[19] Moreover, the total phenolics of the fruits of *M. glabra* L. from Thailand was determined as 723.83 ± 36.94 mg GAE/100 g and 195.36 ± 0.14 mg QE/100 g.[47]

High-performance liquid chromatography analysis for flavonoids

HPLC analysis of the flavonoids in the tested leaf extracts led to the identification and quantification of a total of 24 flavonoids [Table 2]. Hesperidin was the most abundant flavonoid in the three peach cultivars (3863.4 mg/100 g dry extract in Desert red,

Table 2: HPLC analysis of flavonoid content of the 80% ethanolic extracts of the leaves of *Prunus persica* L. cultivars Desert red, Florida prince, and Swelling and *Malpighia glabra* L.

No.	RRt. (min)	Flavonoid	Content (mg/100 g of dry sample)			
			Prunus persica L.			
			Desert red	Florida prince	Swelling	*M. glabra* L.
1	0.743	Luteolin 6-arbinose 8- glucose	3786.94	2275.53	–	548.83
2	0.841	Luteolin 6- glucose 8-arabinose	150.6	122	562.7	49.81
3	0.919	Apigenin 6-arbinose 8-glactose	42.35	37.05	267.18	134.68
4	0.953	Apigenin 6-rhamnose 8-glucose	126.15	92.72	146.79	284.17
5	0.966	Apigenin 6-glucose 8-rhamnose	520.94	163.64	–	409.93
6	0.969	Luteolin	22.89	35.53	–	–
7	0.971	Luteolin 7-glucose	15.26	25.22	112.1	–
8	0.984	Naringin	211.08	128.19	–	632.69
9	0.992	Rutin	48.68	48.94	–	127.14
10	0.993	Quercetin-3-O-Glucoside	–	–	–	24.15
11	1	Hesperidin	3863.4	2624.92	2971	336.06
12	1.038	Kaempferol 3,7-dirhamoside	28.99	120.92	221.26	127.86
13	1.058	apigenin 7- glucose	13.65	203.89	292.66	17.36
14	1.070	Quercetrin	31.58	17.69	56.38	300.72
15	1.195	Quercetin	298.02	502.73	238.05	37.62
16	1.198	Narengenin	–	–	–	6.74
17	1.199	Kaempferol 3-(2-p-coumaroyl) glucose	398.09	243.03	714.46	178.22
18	1.200	Acacetin neo. rutinoside	–	–	–	60.56
19	1.242	Hispertin	31.64	37.83	32.46	75.17
20	1.302	Kaempferol	7.66	47.85	18.66	12.75
21	1.315	Rhamnetin	12.4	38.34	2.69	10.32
22	1.322	Apigenin	5.42	10.53	11.23	6.14
23	1.374	Apigenin 7-O-neohespiroside	–	–	–	81.82
24	1.510	Acacetin	136.92	426.85	95.19	205.22
Total identified flavonoids			9793.12	7230.73	5742.81	3671.5

RRt. = relative retention time (calculated relative to hesperidin as a reference)

2971 mg/100 g dry extract in Swelling, and 2624.92 mg/100 g dry extract in Florida prince); according to previous recorded preclinical studies and clinical trials, hesperidin is a bioflavonoid which showed several therapeutic effects in various diseases including neurological, psychiatric, cardiovascular disorders and others owing to its antihypertensive, anti-inflammatory, antioxidant, lipid-lowering, and insulin-sensitizing effects.[48] On the contrary, narengin (632 mg/100g dry extract) was the abundant flavonoid in acerola leaves. Luteolin 6-arbinose 8-glucose showed appreciable concentrations (3786, 2275, and 548 mg/100 g dry extract) in Desert red and Florida prince cultivars, as well as acerola, respectively.

Also, rutin was detected in reasonable concentrations ranging from 48.68 to 127.14 mg/100g in all the tested extracts except Swelling cultivar of peach; it is worth mentioning that rutin was previously determined in peach kernel oil using HPLC analysis and it was found to be the major flavonoid in it beside (–)-epicatechin gallate.[49] Furthermore, quercetrin was identified and quantified in all the crude extracts under investigation and it was found in concentrations ranging from 17.69 to 300.72 mg/100 g. It is noteworthy that quercetin 3-rhamnoside (quercetrin) was detected during the investigation of the phenolic compounds of 25 peach and nectarine fruit cultivars by HPLC–DAD–ESI-MS.[50] Other previous studies regarding HPLC analysis of flavonoids in peach different organs included the identification of multiflorin A in the methanolic extract of the leaves of the edible peach,[51] in addition to the determination of four kaempferol glycoside derivatives *viz.*, multiflorin B, trifolin, afzelin, and astragalin from the peach flowers extracts.[52]

High-performance liquid chromatography analysis for carbohydrates

HPLC-RI resulted in the identification and quantification of 11 different sugars and sugar acids [Table 3]. Glucuronic acid was detected in a relatively higher percentage (33.04%) in acerola, whereas sorbitol was abundant in peach cultivars (ranging from 6.19% to 12.27%). Maltose, Lactose, Xylose, and Rhamnose were absent in all the tested extracts. From recent studies concerning peach kernels and fruits, sucrose, glucose, and fructose were detected as the most important sugars in peach kernels.[53] Also sucrose, glucose, fructose, sorbitol, malic acid, citric acid, and quinic acid were identified in the fruits of 106 peach cultivars from different breeding programs at Catalonia, Spain.[54]

High-performance liquid chromatography analysis for vitamin C and E

Vitamin C and E were characterized and quantified in the 80% ethanolic extract of the leaves of the plants under investigation using reversed-phase HPLC analysis. vitamin C and E were present in the tested extracts with reasonable concentrations. As the analysis results showed that all the examined extracts are rich in vitamin C and E at which their contents are ranging from 11.4 to 34 and from 0.01 to 0.14 mg/100 g, respectively. *Malpighia glabra* Linn. contains the highest content of vitamin C (34.00288 mg/100g), whereas *P. persica* Linn. *cv.* Florida prince owns the highest content of vitamin E (0.1388673 mg/100 g). On the contrary, *P. persica* Linn. *cv.* Swelling possesses the lowest concentration of both vitamins. The detailed results are shown in Table 4. The total amount of vitamin-E-active compounds in peach kernel oil from Turkey was previously estimated to be 62.9 mg/kg.[55]

Also previous studies regarding peach reported that the total vitamin C of western red nectarine of France (*P. persica* L. batsch) fruits is 5.34 ± 0.51 mg / 100 gram[56]; moreover, the Italian peach fresh fruit contains 7 mg vitamin C/100 g and 3 mg vitamin C/100 in canned peach fruit.[57] Also the ranges of total vitamin C in peach were determined from California

Table 3: HPLC-RI data of sugar contents of the 80% ethanolic extracts of the leaves of *Prunus persica* L. cultivars Desert red, Florida prince, swelling, and *Malpighia glabra* L.

No.	Sugars/sugar derivatives	Retention time (min)	Content (%)			
			Prunus persica L.			*Malpighia glabra* L.
			Desert red	Florida prince	Swelling	
1	Glucuronic	5.105	–	0.68	–	33.04
2	Stachyose	5.5	–	4.57	2.57	5.87
3	Galacturonic	5.607	4.74	0.51	0.6	2.11
4	Sucrose	6.351	–	–	3.95	1.53
5	Maltose	6.355	–	–	–	–
6	Lactose	6.578	–	–	–	–
7	Glucose	7.5	5.88	5.15	–	13.43
8	Xylose	8.499	–	–	–	–
9	Galactose	8.772	2.07	0.62	1.69	1.85
10	L-Rhaminose	9.018	–	–	–	–
11	Mannose	9.041	0.96	0.45	–	0.98
12	Arabinose	10.415	2.84	–	–	–
13	Fructose	10.621	5.39	3.59	1.47	5.54
14	Mannitol	14.259	0.24	–	–	0.9
15	Sorbitol	18.73	8.51	6.19	12.27	0.10
Total			30.63 %	21.76%	22.55%	65.35%

(in mg/100 g of fresh weight) as follows: 5–14 (white-flesh nectarines), 6–8 (yellow-flesh nectarines), 6–9 (white-flesh peaches), and 4–13 (yellow-flesh peaches).[2] Therefore, our study exposed that the leaves of the Egyptian peach cultivars under study contain more vitamin C than those previously reported in the literature. The variation in vitamin C content detected may be attributed to variation in climatic conditions. Other previous studies concerning acerola fruits stated that Brazilian acerola fruits residues contain 170.73 ± 0.46 mg vitamin C/ 100 g and 506.00 ± 11.00 mg vitamin C/ 100 g in acerola edible portion of the fruit without residues.[58] Also São Paulo and Ceará aqueous fruit extracts contain 900.0 mg vitamin C/ 100 g and 4,447.6 mg vitamin C/ 100 g, respectively.[59] Moreover, it was mentioned that Italian acerola fresh fruit contains 1.677 mg vitamin C/ 100 g,[57] whereas other report stated that western Mexico acerola contains 1000–4500 mg/100 g of fruit.[60]

Gas chromatography–mass spectrometry analysis of unsaponifiable and saponifiable matters

From Table 5, the percentage of the total identified constituents in the unsaponifiable matter represented 47.84%, 58.19%, 10.45%, and 28.92% of the total lipoidal content of the leaves of *M. glabra* Linn. and *P. persica* Linn. cultivars (Desert red, Swelling, and Florida prince), respectively. Sitosterol was detected in *P. persica* Linn. *cv.* Desert red at a concentration of 4.25%. Supraene (squalene) was the only triterpene found in *M. glabra* Linn. (11.78%), *P. persica* Linn. *cv.* Swelling (2.79%) and Florida prince (2.57%). It is important to mention that *β*-sitosterol was previously reported in peach leaves to be 1.12%.[61] Hentriacontane, heptacosane, bicyclohexanone, and cyclopentane were the major identified compounds in the unsaponifiable matter of the leaves of *M. glabra* Linn. and *P. persica* Linn. cultivars (Desert red, Swelling, and Florida prince) representing 20.43%, 20.78%, 4.25%, and 14.39%, respectively.

Also as shown in Table 6, the total identified fatty acids represented 90.85% in *M. glabra* Linn. and 87.01%, 29.31%, and 67.11% in *P. persica* Linn. cultivars (Desert red, Swelling, and Florida prince), respectively. Oleic acid (0.7%) was detected only in *M. glabra* Linn., whereas Linolenic acid (9.75%) was identified only in *P. persica* Linn. *cv.* Swelling and linoleic acid (1.88%) was detected only in *P. persica* Linn. *cv.* Desert red. It is worth mentioning that oleic acid was previously determined in fruit residues including seeds and peels of *M. glabra* L. (23.2%).[58] Also it was determined in the total oil yield of seeds of three *M. glabra* Linn. genotypes ranging from 5 to 34%.[13]

Octadecatrienoic acid and octadecenoic acid methyl esters were the major identified compounds in the saponifiable matter of the leaves of *M. glabra* Linn. and *P. persica* Linn. *cv.* Florida prince representing 52.47% and 23.65%, respectively. Although pentadecanoic acid methyl ester was the major compound in *P. persica* Linn. *cv.* Desert red representing 36.36%, linolenic acid and octadecatrienoic acid methyl esters were the major components in *P. persica* Linn. *cv.* Swelling representing 9.75%. The fatty-acid components of kernel oils were determined from different Tunisian *P. persica* varieties including peach and nectarine at which the oleic acid (67.7%–75.0%) was found to be the predominant fatty acid, followed by linoleic (15.7%–22.1%) and palmitic (5.6%–6.3%) acids.[62] Furthermore, evaluation of the fatty-acid content was executed for the fleshes and peels of three *P. persica* cultivars at the Regional Centre of Agricultural Research in the Experimental Farm of Sidi Bouzid in Tunisia during two maturation stages; the results showed that palmitic (26.58–45.78%), oleic (2.23–31.33%), and linoleic acids (2.85%–10.14%) are the most copious fatty acids in *P. persica* cultivars.[63]

In vitro antimicrobial screening

Antibacterial activity

One-way ANOVA showed significant differences in the resulted antibacterial activities among the tested plant extracts on the Gram-positive bacteria (*B. subtilis, S. aureus)* ($P < 0.0001$) and on the Gram-negative bacteria (*E. coli*, *P. aeuroginosa*) ($P < 0.0001$). The average activities were reported as inhibition zone diameter in Table 7and Figure 1C. Ampicillin (standard antibacterial drug) displayed a strong antimicrobial activity against Gram-positive bacteria (*B. subtilis, S. aureus*) and Gram-negative bacteria (*E. coli, P. aeuroginosa*). The inhibitory activity of ampicillin against all the tested pathogens ranged from 21 to 26 mm/mg. Plant extracts where inhibition zone diameter values were 11.67–15.33 mm/mg are considered as having low activity compared to the reference drug ampicillin ($P < 0.001$). *Malpighia glabra* 80% ethanolic extract showed no antimicrobial activity against the Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*, *P. aeuroginosa*); this agrees with previous reports which stated that the fruits of *M. glabra* Linn. had no antimicrobial activity.[64,65] Moreover, according to the performed MIC tests on the active extracts, it was noticed that *P. persica* Linn. *cv.* Florida prince 80% ethanolic extract was the most potent tested sample with a concentration of

Table 4: HPLC—multiwavelength detector data of vitamin C and vitamin E content in the 80% ethanolic extracts of the leaves of *Prunus persica* L. cultivars Desert red, Florida prince, swelling, and *Malpighia glabra* L.

No.	Vitamins	Retention time (min)	Content (mg/100 g)			
			Prunus persica L.			*Malpighia glabra* L.
			Desert red	Florida prince	Swelling	
1	Vitamin C	2.066	26.11832	12.42739	11.41296	34.00288
2	Vitamin E	9.563	0.1211825	0.1388673	0.01044932	0.03366323

Table 5: Results of GC/MS analysis of unsaponifiable matter from the leaves of *Prunus persica* L. cultivars Desert red, Florida prince, swelling, and *Malpighia glabra* L.

No.	Identified compound	Rt. (min)	RRt.	% Area			
				Prunus persica Linn.			*Malpighia glabra* Linn.
				Desert red	Swelling	Florida prince	
1	Cyclooctatetraene	5.03	0.10		1.09	0.25	
2	Phenylpropane	5.43	0.11			0.11	
3	Cyclophanene	5.93	0.12		1.52		
4	Docosane	19.06	0.39	1.05			0.92
5	Nonacosane	19.06	0.39	1.05			0.19
6	Dodecanol	20.16	0.42	7.35			
7	Octanedione	20.16	0.42	7.35			
8	Tridecanone	21.94	0.45	0.21			
9	Cyclobutane	24.52	0.51			0.05	
10	Cycloheptatriene	25.14	0.52			0.33	
11	Pentadecanone	27.11	0.56	2.46			2.34
12	Undecanone	27.11	0.56				2.34
13	Pentacosane	28.31	0.59	1.08			
14	Dodecane	28.81	0.60	0.25			
15	Octene	29.64	0.61			3.6	
16	Butylindenone	30.87	0.64			0.05	
17	Cyclopentane	31.46	0.65			14.39	
18	Bicyclohexanone	31.83	0.66		4.25		
19	Phytol	32.72	0.68				8.55
20	Heptane-7-One	32.73	0.68			7.35	
21	Diethylheptadecane	34.31	0.71	0.17			
22	Nonadecane	34.31	0.71	0.17			
23	Heneicosane	37.29	0.77				0.59
24	Tetracosane	37.89	0.78	0.72			
25	Hexacosane	41.17	0.85	0.68			
26	Heptacosane	42.8	0.89	20.78			0.7
27	Pentatriacontene	46.63	0.96	0.34			
28	Supraene	48.36	1.00		2.79	2.57	11.78
29	Chlorocholestene	48.75	1.01			0.11	
30	Pyranone	48.75	1.01			0.11	
31	Hentriacontane	50.12	1.04	5.14			20.43
32	Hexatriacontane	50.12	1.04	5.14			
33	Sitosterol	54.56	1.13	4.25			
34	Phenanthrenone	55.85	1.15		0.8		
% of total identified hydrocarbons				53.94	7.66	26.24	36.06
% of total identified sterols and triterpenes				4.25	2.79	2.68	11.78
% of total identified unsaponifiable matter				58.19	10.45	28.92	47.84

Rt. = retention time, RRt. = relative retention to Supraene with Rt. = 48.36 min

96 mg/mL. In general, it was observed that the studied extracts are more potent against Gram-positive bacteria than Gram-negative bacteria; these results especially for peach cultivars are similar to that which was previously reported for the ethanolic extracted seeds of peach,[66] in addition to other studies which determined the antibacterial activity of the leaves' ethanolic extract of peach against both Gram-positive and Gram-negative bacteria and the bark methanolic extract too.[67,68]

Antifungal activity

One-way ANOVA showed significant differences in the antifungal activities among the groups of different tested plant extracts on the *C. albicans* (F [2, 6] = 61.23 $P < 0.001$). The average activity was reported as the inhibition zone diameter in Table 7. The average inhibitory activity of amphotericin B (antifungal standard drug) displayed a strong antifungal activity reported as 16 mm/mg sample and 19 mm/mg sample against *A. flavus* and *C. albicans*, respectively. It is noteworthy to know that all the tested plant extracts had neither antifungal activity against *A. flavus* nor on the *C. albicans* except Swelling 80% ethanolic extract where inhibition zone average diameter value was reported as 9 mm/mg sample, and therefore it is considered as having low activity against *C. albicans* as compared to the standard drug amphotericin B ($P < 0.001$). It is clear that the antifungal activities were very week, which is not in coordinance with a previous reported study, which showed that

Table 6: Results of GC/MS analysis of the fatty acid methyl esters of the saponifiable matter from the leaves of *Prunus persica* L. cultivars Desert red, Florida prince, swelling and *Malpighia glabra* L.

No.	Identified compound	Rt. (min)	RRt.*	% Area			
				Prunus persica Linn.			*Malpighia glabra*
				Desert red	Swelling	Florida prince	Linn.
1	Tridecanoic acid	11.1	0.661				4.12
2	Hexadecanoic acid	15.99	0.952	2.83		1.28	15.88
3	Pentadecanoic acid	15.99	0.952	36.36		19.69	4.12
4	Oleic acid	16.79	1				0.7
5	Octadecynoic acid	18.35	1.093				1.47
6	Tetradecanoic acid	18.37	1.094	1.58			
7	Octadecadienoic acid	19.94	1.188	10.79		9.64	5.28
8	Octadecatrienoic acid	20.17	1.201	17.36	9.75		52.47
9	Linolenic acid	20.28	1.208		9.75		
10	Octadecanoic acid	20.66	1.23	8.67	4.37	9.51	2.64
11	Linoleic acid	21.57	1.285	1.88			
12	cis-Eicosenoic acid	21.67	1.291				0.21
13	Hexadecenoic acid	22.39	1.334	0.25			0.39
14	Eicosanoic acid	25	1.489	4.87		3.34	0.92
15	Heneicosanoic acid	27.05	1.611				0.22
16	Docosanoic acid	29.02	1.728				0.68
17	Hexanoic acid	30.44	1.813		1.38		
18	Tricosanoic acid	30.92	1.842				0.19
19	Octadecanoic acid 2-(hexadecyloxy)	31.04	1.849	0.22			
20	Prostenoic acid	32.55	1.939		2.72		
21	Octadecenoic acid	32.58	1.94	0.59		23.65	1.56
22	Tetracosanoic acid	32.77	1.952	0.78			
23	Heptadecanoic acid	33.05	1.968	0.83			
24	Prostenoic acid	40.1	2.388		1.34		
% of total identified saturated fatty acids				85.13	19.56	67.11	90.15
% of total identified unsaturated fatty acids				1.88	9.75	0	0.7
% of total identified fatty acids				87.01	29.31	67.11	90.85

Rt. = retention time. RRt. = relative retention to Supraene with Rt. = 48.36 min

the antifungal activity was confirmed in the *M. glabra* Linn. but not in all parts; the most active organs were the leaves and bark.[69] Also, the antifungal activity was confirmed before in the methanolic crude extract of *P. persica* bark in a recent study as it showed a considerable antibacterial activity against *Enterococcus faecalis* and *Klebsiella pneumonia*.[70]

MTT assay

We first examined the effects of the plant extracts which are under investigation on the viability of two human cancer cell lines: human breast cancer cell line (MCF-7) and human colon cancer cell line (HCT-116). We then compared them to doxorubicin standard. As shown in Table 8, treatment with plant extracts of different concentrations significantly decreased the viability of all cell lines with IC_{50}s ranging from 302 to >1000 µg/mL against colon cancer cell line and IC_{50}s ranging from 249.5 to >813 µg/mL against breast cancer cell line; these results are agreed with a recent study that showed *in vivo* tumor growth inhibition and antimetastatic effects of the polyphenolics content of peach using a xenograft model and MDA-MB-435 breast cancer cells in a dose ranging from 0.8 to 1.6 mg/day.[71] It is known that the lower the IC_{50} value, the higher the potency of the tested sample. Therefore, the resulted values of IC_{50} of the tested extracts versus colon cancer cell line indicated that the arrangement of potency of the tested extracts will be as follows: *Malpighia glabra* 80% ethanolic extract > Swelling 80% ethanolic extract > Florida prince 80% ethanolic extract > Desert red 80% ethanolic extract, whereas regarding breast cancer cell line, the potency is as follows: Desert red 80% ethanolic extract > *M. glabra* 80% ethanolic extract > Swelling 80% ethanolic extract > Florida prince 80% ethanolic extract.

One-way ANOVA showed significant differences in the cytotoxicity among each group of the tested plant extracts on both HCT116 and MCF-7 cell lines ($P < 0.0001$). All the plant extracts with the lowest concentration (0.1 µg/mL) had significantly reduced the viability of HCT116 cell line at $P < 0.001$ [Table 9]. Tukey's multiple comparisons test displayed that there was no significant difference between 0.1 µg/mL of the standard drug "doxorubicin" vs. 0.1 µg/mL of *M. glabra* 80% ethanolic extract ($P < 0.0946$) which may suggest a strong anticancer activity against HCT116 cell line. *Malpighia glabra* L. Brazilian leaves' cytotoxicity was studied before it showed cell growth inhibition against breast and colon cell lines (64.4 and 16 cell growth inhibition percentage, respectively) with IC_{50}

Table 7: The antimicrobial activity and minimum inhibitory concentration of the leaves' total 80% ethanolic extract of *Prunus persica* L. cultivars Desert red, Florida prince, swelling, and *Malpighia glabra* L.

Microorganism				*Prunus persica* L.			*M. glabra* L.	Ampicillin (antibacterial agent)	Amphotericin B (antifungal agent)
				Desert red	Florida prince	Swelling			
Inhibition zone diameter (mm/mg sample) (%potency relative to standard drug)	Bacterial species	(G+)	*Bacillus subtilis*	12.67 ± 0.3333 [a] (48.7%)	14 ± 0.0 [a] (53.85%)	13 ± 0.0 [a] (50%)	12.33 ± 0.3333 [a] (47.42%)	26 ± 0.0 (100%)	--
			Staphylococcus aureus	15.33 ± 0.3333 [a] (73%)	14.67 ± 0.3333 [a] (69.86%)	14.67 ± 0.3333 [a] (69.86%)	0	21 ± 0.0 (100%)	--
		(G-)	*Escherichia coli*	12 ± 0.0 [a] (47.37%)	15 ± 0.0 [a] (59.22%)	14 ± 0.0 [a] (55.27%)	0	25.33 ± 0.3333 (100%)	--
			Pseudomonas aeuroginosa	11.67 ± 0.3333 [a] (44.88%)	13 ± 0.0 [a] (50%)	13.33 ± 0.3333 [a] (50%)	0	26 ± 1.155 (100%)	--
	Fungal species		*Aspergillus flavus*	0	0	0	0	--	16.33± 0.8819 (100%)
			Candida albicans	0	0	9± 0 [a] (47.37%)	0	--	19± 1.155 (100%)
MIC (mg/mL) "minimum inhibitory concentration"			*Bacillus subtilis*	0	0	0	178	--	--
			Staphylococcus aureus	118	96	108	0	--	--

Data were expressed as mean ± SEM (n = 3)

Statistical analysis was carried out by one-way ANOVA followed by Dunnett's multiple comparisons test

[a] Significantly different from the standard drug at $P < 0.001$

<1000,[72] whereas the Egyptian *M. glabra* leaves in our study showed cell growth inhibition with IC_{50} 490 and 302 against breast and colon cell lines, respectively, which proves that our results suggest that the Egyptian *M. glabra* leaves are more potent than the Brazilian leaves.

Florida prince 80% ethanolic extract ($P < 0.001$), Desert red, and Swelling 80% ethanolic extracts ($P < 0.01$) and *M. glabra* 80% ethanolic extract ($P < 0.05$) at the lowest tested concentration (0.1μg/mL) had significantly reduced the viability of MCF-7 cell line [Table 10]. Tukey's multiple comparisons test showed that there was no significant difference between 10 μg/mL of doxorubicin vs. 1000 μg/mL 80% ethanolic extract of Florida prince ($P = 0.8701$) on MCF-7 cell lines. This could suggest that the 80% ethanolic extract of Florida prince may show anticancer activity against breast cancer cell lines at high concentration.

Table 8: The IC_{50} of the tested leaves' total ethanolic extract of *Prunus persica* Linn. cultivars and *Malpighia glabra* Linn. against colon and breast cancer cell lines

Sample		IC_{50} (µg/mL)	
		Breast cell lines MCF-7	Colon cell lines HCT-116
Doxorubicin (standard anticancer agent)		2.1	2
Prunus persica L. *cv.*	Desert red	249.5	>1000
	Florida prince	813	617
	Swelling	617	490
Malpighia glabra Linn.		490	302

Table 9: Cytotoxicity for the tested leaves' total ethanolic extract of *Prunus persica* Linn. cultivars *and Malpighia glabra* Linn. cultivated in Egypt against colon cancer cell lines "HCT116" ($n = 3$)

Extract Conc. (µg/mL)	Doxorubicin (standard anticancer drug)	*Prunus persica* L. *cv.*			*Malpighia glabra* L.
		Desert red	Florida prince	Swelling	
0	100 ± 0.874608	100 ± 0.874608	100 ± 0.874608	100 ± 0.874608	100 ± 0.874608
0.1	76.59576 ± 1.357711###	86.56217 ± 0.23952###,***	88.12992 ± 0.346661###, ***	83.98658 ± 0.648179###,***	81.07505 ± 0.201844###
1	55.0952 ± 0.88883	83.98658 ± 1.464356	83.8746 ± 0.783875	78.38748 ± 0.223964	78.8354 ± 0.874608
10	44.68086 ± 0.88883	82.75478 ± 1.068241	78.8354 ± 0.223964	75.13999 ± 0.734316	77.6036 ± 1.58762
100	12.76596 ± 0.335946	81.74694 ± 0.956775	77.15567 ± 0.296277	72.78837 ± 0.403757	68.30909 ± 0.916613
1000	–	80.96306 ± 0.513166	38.63383 ± 1.026333	31.80292 ± 0.111982	8.286676 ± 0.447928

Data were expressed as mean ± SEM ($n = 3$)
Statistical analysis was carried out by one-way ANOVA followed by Tukey's multiple comparisons test
*** Significantly different from the standard drug at $P < 0.001$
Significantly different from the Zero concentration of the same plant extract at $P < 0.001$

Table 10: Cytotoxicity for the tested leaves' total ethanolic extract of *Prunus persica* Linn. cultivars and *Malpighia glabra* Linn. cultivated in Egypt against breast cancer cell lines "MCF7" ($n = 3$)

Extract Conc. (µg/mL)	Doxorubicin (standard anticancer drug)	*Prunus persica* L. *cv.*			*Malpighia glabra* L.
		Desert red	Florida prince	Swelling	
0	100 ± 1.077699	100 ± 1.077699	100 ± 1.077699	100 ± 1.077699	100 ± 1.077699
0.1	72.50509 ± 0.44388###	95.31568 ± 0.555015##,***	90.32587 ± 0.705072###,***	92.36253 ± 1.848385##,***	96.13035 ± 0.994224#,***
1	57.63748 ± 0.203666	92.66803 ± 0.269425	89.61304 ± 0.566982	88.49287 ± 0.269425	93.38086 ± 0.795341
10	40.52953 ± 1.002939	86.65988 ± 0.53885	87.47454 ± 0.971425	84.21589 ± 1.238852	89.61304 ± 0.44388
100	21.89409 ± 0.44388	84.82688 ± 0.566982	85.94705 ± 0.712831	83.70672 ± 0.982042	86.25255 ± 0.44388
1000	–	17.20978 ± 0.44388	43.38086 ± 0.8819	26.68024 ± 0.887759	6.720978 ± 0.17638

Data were expressed as mean ± SEM ($n = 3$). Statistical analysis was carried out by one-way ANOVA followed by Tukey's multiple comparisons test
*** Significantly different from the standard drug at $P < 0.001$
# Significantly different from the zero concentration of the same plant extract at $P < 0.05$
Significantly different from the zero concentration of the same plant extract at $P < 0.01$
Significantly different from the zero concentration of the same plant extract at $P < 0.001$

Conclusion

This work appeared to be the first detailed study on the chemical profile, antimicrobial, and cytotoxic activities of the leaves of the Egyptian cultivated *M. glabra* Linn. and *P. persica* Linn. cultivars (Desert red, Swelling, and Florida prince). This study clearly confirmed that the leaves under investigation are a tremendous source of nutritional and bioactive metabolites, as carbohydrates, flavonoids, phenolics, sterols, and vitamin C and E which may be responsible in part for their anticancer activity.[71,73,74] Also it could be concluded that their flavonoid and fatty-acid content may rationalize their antimicrobial activity.[75-77] In conclusion, the findings of this study indicated that the investigated plants are promising to continue the isolation of their bioactive compounds and to assess extra detailed biological studies.

Acknowledgement

Our sincere thanks go to Youssef Shalaby (teaching assistant in Pharmacology Department at Ahram Canadian University) for performing the antimicrobial and cytotoxicity statistical analysis, Dr. Mohamed El-Gebaly (plant classification specialist at Orman Botanical Garden, Egypt), and Prof. Dr. Mohamed EL-Khashab (professor in Faculty of Agriculture, Cairo University, Egypt) for their help in providing *M. glabra* Linn. leaves, as well as, Dr. Mohamed Alaa (Faculty of Agriculture, Cairo University, Egypt) for providing us the plant material of peach cultivars for free, Dr. Reem Samir Hamdy (associate professor of plant taxonomy, Department of Botany, Faculty of Science, Cairo University, Egypt) for her authentication of peach plant material and Dr. Abdo Mohammed Abd El-Latef (associate professor in Fruit Orchards Department, Faculty of Agriculture, Cairo University, Egypt) for his identification of peach cultivars.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Sagar NA, Pareek S, Sharma S, Yahia EM, Lobo MG. Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. Compr Rev Food Sci Food Saf 2018;17:512-31.
2. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Kader AA. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. J Agric Food Chem 2002;50:4976-82.
3. Ferlemi A-V, Lamari F. Berry leaves: An alternative source of bioactive natural products of nutritional and medicinal value. Antioxidants 2016;5:1-17.
4. Teleszko M, Wojdyło A. Comparison of phenolic compounds and antioxidant potential between selected edible fruits and their leaves. J Funct Foods 2015;14:736-46.
5. Kårlund A, Salminen JP, Koskinen P, Ahern JR, Karonen M, Tiilikkala K, *et al*. Polyphenols in strawberry (fragaria × ananassa) leaves induced by plant activators. J Agric Food Chem 2014;62:4592-600.
6. Smith N, Mori SA, Henderson A, Stevenson DW, Heald SV, editors. Flowering Plants of the Neotropics. Princeton, NJ: Princeton University Press; 2004.
7. Terry L. Health-Promoting Properties of Fruits and Vegetables. Wallingford, UK: CABI; 2011.
8. Nitin K, Chaudhary A. Pharmacognostic and phytochemical evaluation of *Prunus persica* (L.). Int J Res Dev Pharm L Sci 2017;6:2806-12.
9. Kant R, Shukla RK, Shukla A. A review on peach (*Prunus persica*): An asset of medicinal phytochemicals. Int J Res Appl Sci Eng Tech 2018;6:2186-200.
10. Ahmed EA, El-Habashy S, Maklad MF. Trend of vegetative growth and fruiting of some peach cultivars budded on Okinawa and Nemaguard rootstocks. Middle East J 2017;6:1346-58.
11. Engels G, Brinckmann J. Acerola: *Malpighia glabra*. HerbalGram; 2014;104:64-9.
12. Azevedo-Meleiro CH, Rodriguez-Amaya DB. Confirmation of the identity of the carotenoids of tropical fruits by HPLC-DAD and HPLC-MS. J Food Compost Anal 2004;17:385-96.
13. Egydio AP, Dos Santos DY. Fatty acid composition of seeds of three *Malpighia glabra* L. genotypes (Malpighiaceae). Boletim de Botânica da Univerdiade de São Paulo (Brazil) 2012;30:1-4.
14. Bicas JL, Molina G, Dionísio AP, Barros FFC, Wagner R, Maróstica MR, *et al*. Volatile constituents of exotic fruits from Brazil. Food Res Int 2011;44:1843-55.
15. Kawaguchi M, Tanabe H, Nagamine K. Isolation and characterization of a novel flavonoid possessing a 4,2”-glycosidic linkage from green mature acerola (*Malpighia emarginata* DC.) Fruit. Biosci Biotechnol Biochem 2007;71:1130-5.
16. Liu JQ, Deng YY, Li TZ, Han Q, Li Y, Qiu MH. Three new tetranorditerpenes from aerial parts of acerola cherry (*Malpighia emarginata*). Molecules 2014;19:2629-36.
17. Morais SM, Lima KS, Siqueira SM, Cavalcanti ES, Souza MS, Menezes JE, *et al*. Correlaçao entre as atividades antiradical, antiacetilcolinesterase e teor de fenóis totais de extratos de plantas medicinais de farmácias vivas. Rev Bras Plantas Med 2013;15:575-82.
18. Nunes Rda S, Kahl VF, Sarmento Mda S, Richter MF, Costa-Lotufo LV, Rodrigues FA, *et al*. Antigenotoxicity and antioxidant activity of acerola fruit (*Malpighia glabra* L.) At two stages of ripeness. Plant Foods Hum Nutr 2011;66:129-35.
19. Singh DR, Singh S, Salim KM, Srivastava RC. Estimation of phytochemicals and antioxidant activity of underutilized fruits of Andaman Islands (India). Int J Food Sci Nutr 2012;63:446-52.
20. Araújo CL, Bezerra IWL, Dantas IC, Lima TVS, Oliveira AS, Miranda MRA, *et al*. Biological activity of proteins from pulps of tropical fruits. Food Chem 2004;85:107-10.
21. Dartsch PC, Kler A, Kriesl E. Antioxidative and antiinflammatory potential of different functional drink concepts in vitro. Phytother Res 2009;23:165-71.
22. Hanamura T, Mayama C, Aoki H, Hirayama Y, Shimizu M. Antihyperglycemic effect of polyphenols from acerola (*Malpighia emarginata* DC.) Fruit. Biosci Biotechnol Biochem 2006;70:1813-20.
23. Geissman TA. The Chemistry of Flavonoid Compounds. Oxford, London, New York, Paris: Pergamon Press; 1962.
24. Fieser LF, Fieser M. Steroids. Vol 28. New York, NY: Reinhold Publishing Corporation; 1959. p. 253.
25. Shellard EJ. Practical Plant Chemistry [M]. London, UK: Pitman Medical Publishing; 1957.
26. Farnsworth NR, Fong HH, Blomster RN, Draus FJ. Studies on vinca major (apocynaceae). II. Phytochemical investigation. J Pharm Sci 1962;51:217-24.

27. Balbaa S, Hilal S, Zaki A. Medicinal Plant Constituents. 3rd ed. Cairo, Egypt: General Organization for University and School Books; 1981. p. 190-255.
28. Wall ME, Krider MM, Krewson C, Eddy CR, Willaman J, Corell D, *et al*. Steroidal sapogenins VII. Survey of plants for steroidal sapogenins and other constituents. J Am Pharm Assoc 1954;43:1-7.
29. Claus EP. Pharmacognosy. 5th ed. London, UK: Henery Krimpton; 1967. p. 168.
30. Trease G, Evans W. Text Book of Pharmacognosy. London, UK: BailliareTindall; 1983;12:336.
31. Health Mo. Egyptian Pharmacopoeia [M]. Cairo, Egypt: General Organization for Governmental Printing Office; 1984.
32. Saboo S, Tapadiya R, Khadabadi S, Deokate U. *In vitro* antioxidant activity and total phenolic, flavonoid contents of the crude extracts of Pterospermum acerifolium wild leaves (Sterculiaceae). J Chem Pharm Res 2010;2:417-23.
33. Kiranmai M, Kumar C, Ibrahim M. Comparison of total flavanoid content of Azadirachta indica root bark extracts prepared by different methods of extraction. Res J Pharm Biol Chem Sci 2011;2:254-61.
34. Mattila P, Astola J, Kumpulainen J. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. J Agric Food Chem 2000;48:5834-41.
35. Zielinski AAF, Braga CM, Demiate IM, Beltrame FL, Nogueira A, Wosiacki G. Development and optimization of a HPLC-RI method for the determination of major sugars in apple juice and evaluation of the effect of the ripening stage. Food Sci Technol 2014;34:38-43.
36. Romeu-Nadal M, Morera-Pons S, Castellote AI, López-Sabater MC. Rapid high-performance liquid chromatographic method for vitamin C determination in human milk versus an enzymatic method. J Chromatogr B Analyt Technol Biomed Life Sci 2006;830:41-6.
37. Pyka A, Sliwiok J. Chromatographic separation of tocopherols. J Chromatogr A 2001;935:71-6.
38. Mohammed F, Amer M. Oils, Fats, Waxes and Surfactants. Cairo, Egypt: The Anglo Egyptian Bookshop; 1965. p. 41.
39. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
40. Jorgensen HJ. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. National Committee for Clinical Laboratory Standards Antimicrobial Susceptibility Testing. Wayne, PA: CLSI; 1993:NCCLS-M7.
41. Waynne P. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically. Approved standard, 4th ed. National Committee for Clinical Laboratory Standards. Wayne, PA: CLSI; 1997:NCCLS M7-A4.
42. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
43. Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, *et al*. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res 1988;48:4827-33.
44. Liu H, Cao J, Jiang W. Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars. LWT-Food Sci Technol 2015;63:1042-8.
45. Manzoor M, Anwar F, Mahmood Z, Rashid U, Ashraf M. Variation in minerals, phenolics and antioxidant activity of peel and pulp of different varieties of peach (*Prunus persica* L.) Fruit from Pakistan. Molecules 2012;17:6491-506.
46. Cantín CM, Moreno MA, Gogorcena Y. Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine [*Prunus persica* (L.) Batsch] breeding progenies. J Agric Food Chem 2009;57:4586-92.
47. Anantachoke N, Lomarat P, Praserttirachai W, Khammanit R, Mangmool S. Thai fruits exhibit antioxidant activity and induction of antioxidant enzymes in HEK-293 cells. Evid Based Complement Alternat Med 2016;2016:6083136.
48. Li C, Schluesener H. Health-promoting effects of the citrus flavanone hesperidin. Crit Rev Food Sci Nutr 2017;57:613-31.
49. Wu H, Shi J, Xue S, Kakuda Y, Wang D, Jiang Y, *et al*. Essential oil extracted from peach (*Prunus persica*) kernel and its physicochemical and antioxidant properties. LWT-Food Sci Technol 2011;44:2032-9.
50. Tomás-Barberán FA, Gil MI, Cremin P, Waterhouse AL, Hess-Pierce B, Kader AA. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J Agric Food Chem 2001;49:4748-60.
51. Shirosaki M, Goto Y, Hirooka S, Masuda H, Koyama T, Yazawa K. Peach leaf contains multiflorin a as a potent inhibitor of glucose absorption in the small intestine in mice. Biol Pharm Bull 2012;35:1264-8.
52. Heo MY, Jo BK. The extract of the flowers of *Prunus persica*, a new cosmetic ingredient, protects against solar ultraviolet-induced skin damage in vivo. J Cosmet Sci 2002;53:27-34.
53. Stanojević M, Trifković J, Akšić MF, Rakonjac V, Nikolić D, Šegan S, *et al*. Sugar profile of kernels as a marker of origin and ripening time of peach (*Prunus persicae* L.). Plant Foods Hum Nutr 2015;70:433-40.
54. Reig G, Iglesias I, Gatius F, Alegre S. Antioxidant capacity, quality, and anthocyanin and nutrient contents of several peach cultivars [*Prunus persica* (L.) Batsch] grown in Spain. J Agric Food Chem 2013;61:6344-57.
55. Matthaeus B, Oezcan MM. Fatty acids and tocopherol contents of some Prunus spp. kernel oils. J Food Lipids 2009;16: 187-99.
56. Aubert C, Bony P, Chalot G, Landry P, Lurol S. Effects of storage temperature, storage duration, and subsequent ripening on the physicochemical characteristics, volatile compounds, and phytochemicals of western red nectarine (*Prunus persica* L. Batsch). J Agric Food Chem 2014;62:4707-24.
57. Bounous G, Beccaro GL, Mellano MG, Novello V. Nutraceutical content of berries and minor fruits. Proceedings of the International Symposium on Minor Fruits and Medicinal Plants for Health and Ecological Security (ISMF & MP); 2011; West Bengal, India: Bidhan Chandra Krishi Viswandyalaya.
58. Sancho SdO, da Silva ARA, Dantas ANdS, Magalhães TA, Lopes GS, Rodrigues S, *et al*. Characterization of the industrial residues of seven fruits and prospection of their potential application as food supplements. J Chem 2015;2015:1-8.
59. Da Silva Nunes R, Silva Kahl VF, Da Silva Sarmento M, Richter MF, Abin-Carriquiry JA, Martinez MM, *et al*. Genotoxic and antigenotoxic activity of acerola (*Malpighia glabra* L.) extract in relation to the geographic origin. Phytother Res 2013;27:1495-501.
60. Farías-Larios J, López-Aguirre J, Miranda J, Bayardo-Vizcaino L. 018 use of commercial rhizobacteria for root-knot nematodes (Meloydogyne arenaria and M. incognita) management in acerola plants. HortScience 2000;35:391 A-391.
61. Chandra S, Sastry M. Chemical constituents from *Prunus persica* leaves. Fitoterapia. 1990;61:379.
62. Chamli D, Bootello García MÁ, Bouali I, Jouhri S, Boukhchina S, Martínez-Force E. Chemical characterization and thermal properties of kernel oils from Tunisian peach and nectarine varieties of *Prunus persica*. Grasas Aceites 2017;68:211.

63. Dabbou S, Lussiana C, Maatallah S, Gasco L, Hajlaoui H, Flamini G. Changes in biochemical compounds in flesh and peel from *Prunus persica* fruits grown in tunisia during two maturation stages. Plant Physiol Biochem 2016;100:1-11.
64. Araújo IMCd. Caracterização bioativa de resíduos de frutas tropicais. Universidade Federal do Rio Grande do Norte; 2017.
65. Schmourlo G, De Morais-Filho Z, De Oliveira D, Costa S, Miranda A, Mendonca-Filho R, *et al*. Antioxidant and antimicrobial activity of edible plants and their potential use as nutraceuticals. Acta Hortic 2007.
66. Aljamali NM. Study effect of medical plant extracts in comparison with antibiotic against bacteria. J Sci Innov Res 2013;2:843-5.
67. Raturi R, Singh H, Bahuguna P, Sati S, Badoni P. Antibacterial and antioxidant activity of methanolic extract of bark of *Prunus persica*. J Nat Appl Sci 2011;3:312-4.
68. Edrah S, Alafid F, Kumar A. Preliminary phytochemical screening and antibacterial activity of pistacia atlantica and *Prunus persica* plants of libyan origin. Int J Sci Res 2013;6:14.
69. Cáceres A, López B, Juárez X, del Aguila J, García S. Plants used in guatemala for the treatment of dermatophytic infections. 2. Evaluation of antifungal activity of seven american plants. J Ethnopharmacol 1993;40:207-13.
70. Aziz S. Biological activities of *Prunus persica* L. batch. J Med Plants Res 2013;7:947-51.
71. Noratto G, Porter W, Byrne D, Cisneros-Zevallos L. Polyphenolics from peach (*Prunus persica* var. Rich lady) inhibit tumor growth and metastasis of MDA-MB-435 breast cancer cells in vivo. J Nutr Biochem 2014;25:796-800.
72. dos Santos Júnior HM, Oliveira DF, de Carvalho DA, Pinto JM, Campos VA, Mourão AR, *et al*. Evaluation of native and exotic brazilian plants for anticancer activity. J Nat Med 2010;64:231-8.
73. Block G. Dietary guidelines and the results of food consumption surveys. Am J Clin Nutr 1991;53:356-7S.
74. Grattan BJ Jr. Plant sterols as anticancer nutrients: Evidence for their role in breast cancer. Nutrients 2013;5:359-87.
75. McGaw L, Jäger A, Van Staden J, Houghton P. Antibacterial effects of fatty acids and related compounds from plants. S Afr J Bot 2002;68:417-23.
76. Iranshahi M, Rezaee R, Parhiz H, Roohbakhsh A, Soltani F. Protective effects of flavonoids against microbes and toxins: The cases of hesperidin and hesperetin. Life Sci 2015;137:125-32.
77. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005;26:343-56.

Supplementary File

S1: HPLC analysis for flavonoids

The 80% ethanolic extracts of the tested leaves were analyzed using HPLC according to.[1] HPLC analysis of phenolic compounds was carried out on reversed phase HPLC Agilent 1200 series (Agilent Technologies) using ZORBAX ODS column 4.6x250 mm (Dupont Instrument). Multiwavelength detector was set at 330 nm. Temperature of the column compartments was set at 35 °C. The mobile phase consisted of 50 mm H_3PO_4, pH 2.5 (solution A) and acetonitrile (solution B) and eluted as follows: Isocratic elution 95% A/5% B, 0-5 min; linear gradient from 95% A/5% B to 50% A/ 50% B, 5-55 min; isocratic elution 50% A/50% B, 55-65 min; linear gradient from 50% A/50% B to 95% A/5% B, 65-67 min; post-time 6 min before next injection. The flow rate was 1 mL/min and the injection volumes were 10 µL of the standards and sample extracts. The samples were prepared and analyzed in triplicates. All standards were prepared as stock solutions at 5 mg/50 mL in MeOH, except for luteolin and apigenin (5 mg/50 mL in DMF/MeOH, 1:6, v/v), as well as rhamnetin (5 mg/50 mL in DMF/MeOH, 1:10, v/v). Working standards were made by diluting stock solutions in methanol to yield 2-4 µg/mL. Stock and working solutions of the standards were stored in darkness at -18 °C. All flavonoids were quantified using the external standard method. Quantification of samples and standards was based on peak area. Calibration curves of the standards were made by diluting stock standards in methanol to yield 2-20 µg/mL.

S2: HPLC analysis carbohydrates

HPLC analysis was done according to.[2] The tested ethanolic extracts of the leaves were diluted to 1:10 (v/v) with deionized water and then filtered through a 0.22 µm filter membrane. An aliquot of 1.5 mL of each of these solutions was placed in vials for analysis. The analysis was performed using HPLC Agilent 1200 series (Agilent Technologies) using a Bio-Rad Aminex - carbohydrate HPX-87C column (300 mm x 7.8 mm). Deionized water was used as the mobile phase. Temperature of the column compartments was set at 85 °C. The flow rate of the mobile phase during run was 1 mL/min and the injection volumes were 5 µL of the standards and sample extracts. Refractive index detector was set for detection and quantification of sugars. Sample detection was performed by comparing the retention time of analytes with the retention time of the standards. Quantification was based on peak area. Triplicate injections of seven different concentrations of each standard, obtained by dilution in deionized water were performed. A calibration curve for each sugar was done by plotting the concentrations versus the peak area.

S3: HPLC analysis for vitamin C and E

Vitamin E and C quantification was performed according to,[3,4] respectively, using reversed phase HPLC Agilent 1200 series (Agilent Technologies). Multiwavelength detector was set at 254 nm and 292 nm for detection and quantification of vitamin C and vitamin E, respectively. The separation was carried out using ZORBAX ODS column 4.6x250 mm. (Dupont Instrument). Temperature of the column compartments was set at 35 °C. The mobile phase was distilled water with acetic acid (0.1%, v/v) and methanol in a relative proportion of 95:5 (v/v) for the identification of vitamin C and methanol–water (9:1, v/v) for vitamin E. The flow rate of the mobile phase during the run was 1 mL/min and the injection volumes were 5 µL of the prepared standards and sample extracts. Ascorbic acid and α- tocopherol were identified by comparing the retention time of the sample peak with that of the standard. A calibration curve of different standard concentrations ranged from 0.5 to 100 µg/mL was plotted and quantification was carried out using external standard method.

S4: GC/MS analysis of the unsaponifiable and saponifiable matters

Air-dried powdered leaves of *M. glabra* Linn. (250 g) and *P. persica* Linn. (171 g) cultivars (Desert red, Swelling, Florida prince) were, separately, defatted in n-hexane. The solvents were evaporated under vacuum to give 6.07 , 2.75, 1.81, and 3.01 g residue, respectively. The unsaponifiable matters and the fatty acid methyl esters of the plants under investigation were prepared according to[5] from the previously obtained hexane extracts. The detection of saponifiable and unsaponifiable matters was carried out using Gas Chromatography coupled with Mass spectroscopy (Shimadzu QP-5050 A, Japan) equipped with DB1-MS fused silica capillary column (30m x 0.53mm; film thickness 1.5 µm), using Helium as the carrier gas, injector temperature was 280 º C, detector temperature was 300 º C, the temperature program for unsaponifiable matter was 30º C (1 min) - 150 º C (1 min) at 7.5 º C/min - 250 º C (5 min) at 2.5 º C/min - 270 º C (2 min) at 3.5 º C/min and for saponifiable matter was 115º C (1 min) - 200 º C (1 min) at 7.5 º C/min - 240 º C (2 min) at 5 º C/min - 260 º C (2 min) at 3.5 º C/min. All the standards were well resolved and quantitative measures were obtained by correlating peak areas for all known compounds and relating them to standard curves of the standard compounds.

S4: Determination of the antimicrobial activity

Antimicrobial activity of the ethanolic extracts of the plants under investigation were determined using a modified Kirby-Bauer disc diffusion method[6] against bacterial strains {*S. aureus (ATCC 12600)*, *B. subtilis (ATCC 6051)*, *E. coli (ATCC 11775)* and *P. aeuroginosa* (ATCC 10145)}, *fungal strain* {*A. flavus (Link)}* and yeast {*C. albicans (ATCC 7102)}* which were available in the micro-analytical center, Faculty of science, Cairo University, Egypt. Briefly, 100 µl of the tested bacteria and fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL for bacteria and 10^5 cells/mL for fungi.[7] 100 µl of microbial suspension were spread onto agar plates. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for

susceptibility by disc diffusion method.[8,9] Disc diffusion method for filamentous fungi was tested by using approved standard method (M38-A) developed by the[10] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts was developed by using approved standard method (M44-P) by the.[11] Plates inoculated with the tested microorganisms according to the method of.[6] Standard discs of Ampicillin (Antibacterial agent supplied from Bristol-Myers Squibb, Switzerland) and Amphotericin B (Antifungal agent supplied from Bristol-Myers Squibb, Switzerland) served as positive controls for antimicrobial activity, whereas filter discs impregnated with 10 ul of solvent (distilled water, chloroform from El-Gomhouria Company for Trading Chemicals and Medical Appliances, DMSO from Loba Chemie) were used as a negative control. The agar used is Meuller-Hinton agar for bacteria and Czapek's Dox agar (sucrose-nitrate agar) for yeasts and fungi; they are rigorously tested for composition and pH. Standard zones of inhibition have been determined for susceptible and resistant values. Blank paper disks (Schleicher & Schuel, Spain) with a diameter of 8.0 mm were impregnated with 10ul of the tested concentration of the stock solutions of the plant extracts, which were dissolved in dimethyl sulfoxide. For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards.[9] The test was performed in triplicates.

S6: MTT assay

The cytotoxicity of the extracts under investigation was tested by performing MTT assay according to[12,13] against Human breast adenocarcinoma (MCF-7) and human colon adenocarcinoma (HCT-116) cell lines which were originally purchased from American type culture collection (ATCC, Wesel, Germany) and grown in the tissue culture lab of the Egyptian company for production of vaccines, sera and drugs (Vacsera, Giza, Egypt). Heat-inactivated fetal bovine serum was obtained from Invitrogen, Carlsbad, CA. MTT solution / well was purchased from Sigma Aldrich, MO.

Briefly, exponentially growing cells were trypsinized, counted and seeded at the appropriate densities (5000 cells/0.33 cm^2 well) into 96-well microtiter plates. Cells were incubated in a humidified atmosphere at 37°C for 24 hours. Then, cells were exposed to the tested samples and the standard drug at the desired concentrations, (0.1, 1, 10, 100, and 1000 µg/mL) for 72 hours. At the end of the treatment period, media were removed; cells were incubated with 200 µl of 5% MTT solution/well (Sigma Aldrich, MO) and allowed to metabolize the dye into a colored-insoluble formazan complex for 2 hours. Medium was discarded from the wells and the formazan crystals were dissolved in 200 µl/well acidified isopropanol for 30 min, covered with aluminum foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance was measured at 570 nm using Epoch-2C plate reader (Bio Tek, VT). The cell viability was expressed relative to the untreated control cells and the concentrations induced 50% growth inhibition (IC_{50}) were calculated from the concentration response curve using graph pad prism version 5 (GraphPad software inc., CA).