ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 7, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

EXTRACTION OF PHENOLIC COMPOUNDS AS ANTIOXIDANTS FROM SOME PLANTS AND THEIR CYTOTOXIC ACTIVITY AGAINST BREAST CANCER CELL LINE

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Received: 21 March 2017, Revised and Accepted: 20 April 2017

ABSTRACT

Objective: The objective of the study was to study the antioxidants and cytotoxic activities of phenolic extracts of some plants against breast cancer and normal cell lines.

Methods: Phenolics were extracted from different parts of some plants (15) such as seeds, fruits, leaves, and rhizomes using methanol: ethanol: HCl: Distilled water at a ratio 50:29:1:20 (v:v:v:v). The contents of phenolics and flavonoids were estimated using gallic acid and quercetin as standards, respectively. The antioxidant activity was determined by 2,2`-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay. Phenolic flavonoids were partially purified by adsorption chromatography using a silica gel column from selected plants and assayed their cytotoxic activity against breast cancer cell line Michigan Cancer Foundation-7 (MCF-7) and a normal cell line of non-tumorigenic fetal hepatic cell line (WRL-68).

Results: The *Cinnamomum zeylanicum* extract had the highest phenolic and flavonoid contents were followed by *Lawsonia inermis*, *Citrullus colocynthis, Syzygium aromaticum*, *Peganum nigrum,* and *Phoenix dactylifera*. The antioxidant activity of *Curcuma longa*, *P. dactylifera, C. colocynthis*, *Solanum melongena,* and *C. zeylanicum* extracts had the highest ability to scavenge the free radicals. The acetone fraction of *P. dactylifera* and *C. colocynthis* extracts had the minimum inhibition dose that kills 50% of cells inhibitory concentration 50 values 156.91 µg and 1055.06 µg against MCF-7 and 372.86 µg and 153.8 µg against WRL-68, respectively. While the *S. melongena* extract had less effect on both cell lines.

Conclusions: Phenolics as antioxidant substances had moderate or variable effectiveness on normal and cancer cell lines, and the highest concentrations were cancerous poison impact may be on normal cells over than cancer cells.

Keywords: Phenolics, Antioxidants, *Phoenix dactylifera*, *Citrullus colocynthis*, *Solanum melongena.*

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i7.18673

INTRODUCTION

Plants are very important sources of secondary metabolites such as phenolic compounds and antioxidants, which having variable functions in growth, photosynthesis, reproduction, and other primary processes are not known yet. In other hand, they are important in pharmaceutical industries, particularly in Asia [1]. Flavonoids and phenolic acids are the main antioxidant substance of plants have the ability to scavenge free superoxide radicals, anti-aging, reducing the risk of cancer, and enhance human immunity. Flavonoids are consisting a general variety of substances that play a key role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA [2].

Oxidative stress is very important, if not crucial, to initiate and develop many current conditions and diseases, including inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, aging, and arteriosclerosis. Antioxidants are the components that significantly retard or prevent the oxidation of an oxidizable substrate when present in low concentrations [2-4]. The main antioxidants are flavonoids, which proved to be more effective than Vitamin C, E and carotenoids; They protect human, animal, and plant cells against the damaging effects of free radicals (reactive oxygen species [ROS]). An imbalance between antioxidants and free radicals results in oxidative stress, possibly will consequence to cellular damage [2,3]. The antioxidant properties of phenolics are mediated by the following mechanisms: (1) Scavenging radical species like ROS/reactive nitrogen species (RNS), (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production, (3) up-regulating or protecting antioxidant defense. The reduced activity of phenolics depends on the number of free hydroxyl groups in the molecular structure, which would be supported by steric hindrance [2]. Luteolin is a flavone that acts as an antioxidant, free radical scavenger, cancer prevention agent, immune system modulator, and anti-inflammatory agent. Flavonoids from some plants such as tea, chocolate, cocoa, fruits, and vegetables are highly potent antioxidant compounds that help to decrease the occurrence of cancer, heart failure, and diabetes [2,4-6].

Cancer is a multifactorial disease, including genetic, metabolic, physical, environmental and chemical factors, in which each plays a direct and/or indirect role in the initiation and deterioration of cancers. A diet with high ingesting of antioxidant-rich vegetables and fruits reduces the risk of many cancer types, meaningfully suggesting that these antioxidants could be effective substances to inhibit or prevent cancer [1,7-9]. Polyphenols were isolated from different plants like young ginger (*Zingiber officinale*) including kaempferol, quercetin, rutin, and gallic acid, were inhibited the growth of human breast cancer cell lines such as MCF-7 and 3,4-methylenedioxyamphetamine (MDA)-MB-231 [10]. Furthermore, polyphenols were isolated from green tea and strawberry such as epicatechin, epigallocatechin, quercetin, kaempferol, coumaric acid, anthocyanins and ellagic acid, prevented the growth of human breast, prostate (LNCaP, DU-145) tumor cell lines, oral (KB, CAL-27) and colon (HT-29, HCT-116) [11,12].

The objective of this study was to extraction of phenolic compound as antioxidant agents from some local plants and study their ability to prevent breast cancer cell line growth.

MATERIALS AND METHODS

Plant raw materials

The plant raw materials, including 7 Iraqi plants, in addition to bananas which imported from Somalia were purchased from a grocery store, and the dill and myrtle leaves were collected from gardens in Babylon Province, Iraq, during April in 2014; The other plants (6 plant products) were purchased from local markets, which they were the imported products from India and China; Table 1 showed the plant materials, families, the source and part used in this study. The plant raw materials were washed with distilled water and dried in shade separately at room temperature. If most of the moisture has been removed, the plant material can be ground in a blender or mill to produce small particles (<2 mm). After that, these samples (100-500 g) were stored in glass containers at −24°C until extraction was performed.

Chemicals

All solvents, acids and some materials, including methanol (MeOH), ethanol (EtOH), acetone, hexane, dimethyl sulfoxide (DMSO), HCl, silica gel, and Na_2CO_3 were purchased from Gainland Chemical Co. Ltd., UK. The other materials were purchased from Sigma-Aldrich Co., USA, which including $AlCl_3$, quercetin, gallic acid, Folin–Ciocalteu reagent, 3-[4, 5 - dimethylthiazoyl]-2, 5-diphenyltetrazolium bromide (MTT dye), 2,2`-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and potassium persulfate.

Optimal phenolic extraction of plant samples

Optimum extraction method parameters will vary depending on the sample matrix. Each plant sample may require different temperatures and solvent mixtures [9,13]. When extracting antioxidants from plants, a good solvent mixture is alcohol-water (ratios will vary with different plant samples) which soaking for 24 h 30°C and shaking rate 120 rpm in shaker incubator. However, each plant sample (20 g) was extracted twice with different extraction systems (400 ml) at the ratio of raw material to solvent 1:20 by soaking for 24 h 30°C in the shaker incubator (JSSI-200 Series, JSR, Korean). The extraction systems were composed from H^2O-EtOH mixture (10-80%), the second extraction system was composed from EtOH:MeOH:HCl:H_2O at ratios 30:49:1:20; 40:39:1:20; 50:29:1:20; 60:19:1:20 and 70:9:1:20. The plant extracts were decanted, filtered under vacuum, concentrated in a rotary evaporator (IKA HB10, Germany) at 40°C, and then the concentrated extracts were stored at −24°C for further purification.

Partial purification of phenolics by adsorption chromatography

The resulting concentrated crude extract from selected plants was partially purified using adsorption chromatography by silica gel (mesh 60-120). The slurry of silica gel was prepared by soaking with hexane [14-16], subsequently with absolute EtOH and then poured into the column (25×2.5 cm) and washed with EtOH for one an hour to obtain better packing. Concentrated plant extract (5 ml) was loaded into the silica gel column and eluted successively with different solvents by using batch ways (500 ml for each) 95% EtOH, 70% acetone, 30% acetone and then distilled water. The solvent was run at 4 ml/minutes and 5 ml fraction volume were collected. Frequently each fraction was checked by absorbance at 275 nm for phenolic acids and isoflavonoids and 340 nm for flavonoids and coumestans using a spectrophotometer (PD-303 ultraviolet [UV], APEL Co., Ltd., Japan) [14-16], then estimating phenolic and flavonoid compounds content. Finally, the positive fractions for each solvent were combined together and stored at −24°C for further analysis.

Determination of total flavonoids

The total flavonoids were determined by the method described by Chaves *et al*. [17]. The extracts were diluted with distilled water. To the 2 ml of each test sample was added the same volume of 2% (w/v) $AlCl_3$ solution in methanol. This mixture remained undisturbed for 10 min before the UV spectrophotometric reading at 415 nm wavelength. The blank was prepared by replacing $AlCl_3$ solution by methanol. The total flavonoids were determined by the calibration curve using quercetin as standard at concentrations of 0, 6.25, 12.5, 25, and 50 µg/ml and expressed in µg equivalent of quercetin. The results were expressed as micrograms of quercetin/ml of the extract and mg quercetin equivalent per gram dry weight (mg QE/g DW) of plant.

Total phenolic content

The Folin–Ciocalteu method was used to determine the total polyphenols, using gallic acid as a standard [17], a 1 ml of diluted test sample was added to 1 ml of 1 mol/l Folin–Ciocalteu reagent. This mixture remained undisturbed for 2 minutes before the addition of 2 ml of 20% (w/v) Na_2CO_3 solution and left undisturbed for 10 min. After that, the reading was performed by a spectrophotometer (OPTIZEN POP - Korea) at 757 nm. The calibration curve was obtained with a stock solution of gallic acid (1 mg/ml), from which dilutions were made at concentrations of 5, 10, 15, 20, 25, 30, 35, and 40 µg/ml. The total content of polyphenols was expressed in microgram equivalents of the standard used and mg gallic acid equivalent (mg GAE/g DW) of plant.

Determination of antioxidant activity

Antioxidant activity was determined using ABTS radical scavenging assay, which was carried out following the method of Budrat and Shotipruk [18,19] with some modifications. The extract was diluted in series in water (from 5 µg/ml to 5 mg/ml) and each diluted samples were added to the $ABTS^{\bullet+}$ stock solution, which included 7 mM ABTS and 2.45 mM potassium persulfate, with the volume ratio of 1:10 (sample solution: $ABTS^{\bullet+}$ stock solution). The $ABTS^{\bullet+}$ stock solution had an absorbance of 0.70±0.02 units at 734 nm using the spectrophotometer. The solutions were mixed using a vortex and the

Table 1: Plant materials and their sources

Binomial name	Common name	Family	Source	Part used
A. ampeloprasum	Leek	Amaryllidaceae	Iraq	Leaves
A. graveolens	Dill	Apiaceae	Iraq	Leaves
C. sinesis	Tea plant	Theaceae	India	Leaves
C. annuum	Capsicums	Solanaceae	Iraq	Fruit
C. zeylanicum	Cinnamon	Lauraceae	China	Bark
C. colocynthis	Desert gourd	Cucurbitaceae	Iraq	Fruit
C. longa	Turmeric	Zingiberaceae	India	Rhizomes
L. inermis	Hina	Lythraceae	Iraq	Leaves
M. acuminata	Bananas	Musaceae	Somalia	Fruit Peels
M. communits	Myrtle	Myrtaceae	Iraq	Leaves
P. nigrum	Black pepper	Piperaceae	India	Fruit
P. dactylifera	Date palm	Arecaceae	Iraq	Seeds (pits)
S. longena	Aubergine	Solanaceae	Iraq	Fruit Peels
S. aromaticum	Cloves	Myrtaceae	India	Seeds
V. vinifera	Grapevine	Vitaceae	Iraq	Fruit

A. ampeloprasum: Allium ampeloprasum, A. graveolens: Anethum graveolens, C. sinesis: Camellia sinesis, C. annuum: Capsicum annuum, C. zeylanicum: Cinnamomum zeylanicum, C. colocynthis: Citrullus colocynthis, C. longa: Curcuma longa, L. inermis: Lawsonia inermis, M. acuminate: Musa acuminate, M. communits: Myrtus communits, P. nigrum: Piper nigrum, P. dactylifera: Phoenix dactylifera, S. melongena: Solanum melongena, S. aromaticum: Syzygium aromaticum, V. vinifera: Vitis vinifera

mixtures were incubated at room temperature for 10 min, and then the absorbance was taken at 734 nm using the spectrophotometer. For comparing, the antioxidant activity of the extracts obtained at various concentrations of sample producing 50% reduction of the radical absorbance inhibitory concentration 50 (IC_{50}) was used as an index. IC_{50} values, which defined as the concentration of test material needed to scavenge 50% of ABTS radical present in the test solution. Lower IC_{50} value reflects better ABTS radical scavenging activity. The IC_{50} values for various extracts were found from the plots of percent inhibition (PI) versus the corresponding concentration of the sample. The values of PI were calculated using the following equation:

$$PI(\%)=[1-\left(\frac{At}{Ar}\right)]\times 100$$

Where At and Ar are absorbance of test samples and absorbance of the reference, respectively.

Cytotoxic activity by MTT assay

To determine the cell viability by colorimetric assay using 3-[4, 5 -dimethylthiazoyl]-2, 5-diphenyltetrazolium bromide (MTT dye), two kinds of cells were employed in this work: The human breast cancer cell line Michigan Cancer Foundation-7 (MCF-7) and the Normal human hepatic cells (non-tumorigenic fetal hepatic cell line WRL-68). Briefly, 100 µl cell suspension was added onto the flat-bottomed micro-culture plate wells, each line in a separated plate, for the two cell lines and treated them with 100 µl partially purified plant extract, incubated for 24 h, centrifuged to remove the dead cells. An aliquot of 100 µl from 2 mg/ml MTT dye was added to each well and incubation was continued for a further 4 h, then 50 µl of solubilization solution of DMSO was added into each well. The experiment was performed in triplicate. After complete solubilization of the dye, the absorbance of the colored solution obtained from living cells was read at 620 nm with an ELISA reader. The mean absorbance for each group of replicates was calculated. The percentage viability of cells exposed to various treatments was obtained as follows [20,21]:

$$\%\ \text{cell viability} = \left(\frac{\text{Mean absorbance of treated sample}}{\text{Mean absorbance of control sample}}\right)\times 100$$

The control was the non-treated cultures in all experiments that contained cells in the medium only. This assay was held at the Centre for Natural Product Research and Drug Discovery, Department of Pharmacology, Faculty of Medicine, University of Malaya/Kuala Lumpur, Malaysia.

Statistical analyses

Data were analyzed using SPSS version 22 software Fisher's exact with a significant value of <0.05.

RESULTS AND DISCUSSION

Phenolic flavonoids were extracted from different plants (15 plants), including different plant parts such as leaves, fruits, bark, and rhizobium (Table 1). First, the extraction method was optimized using various percentages of H_2O-EtOH mixtures (0-80%) to extract phenolic compounds from 2 plants and then the best volume fraction was selected. The other system which composed from EtOH:MeOH:HCl:H_2O at various proportions was optimized to select the best solvent system to extract flavonoids from different plants.

The total flavonoids content was estimated using the method described by Chaves *et al*. [17], and total flavonoids were expressed as mg QE/gDW, through the calibration curve of quercetin (Fig. 1). Furthermore, the total phenolic content was determined using the Folin–Ciocalteu method which modified by Chaves *et al*. [17]. The principle of this method is based on phenolic substances reduces Folin–Ciocalteu reagent in the presence of sodium carbonate. This reduction causes a color change between 745 and 765 nm. Total phenolic content of plant extracts was expressed as mg GAE/gDW through the calibration curve with gallic acid (Fig. 2).

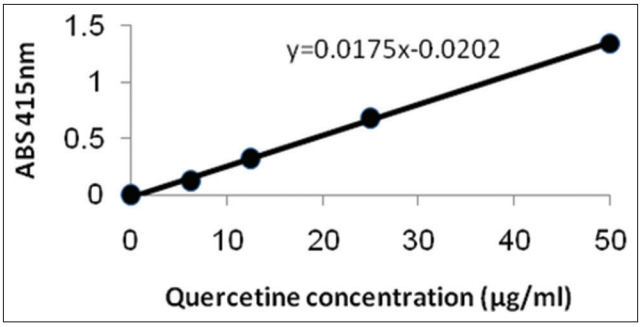

Fig. 1: The calibration curve of quercetin's standard

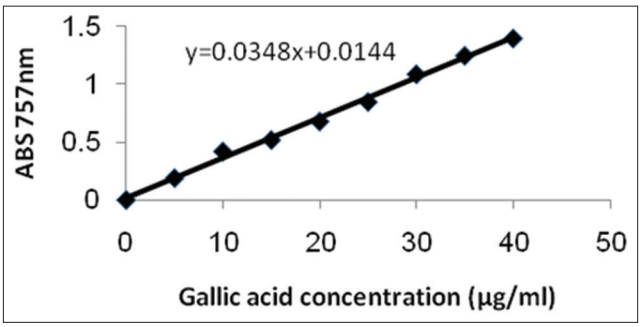

Fig. 2: The calibration curve of gallic acid's standard

Fig. 3 shows the extraction yield of total phenolic and flavonoids using various volume ratios of ethanol (0-80%) when other extraction conditions were as follows: Temperature 30°C, extraction time 24 h with shaking rate 120 rpm, the ratio of solvent to raw material was 20:1, and the extraction number 2. The extraction yield of flavonoids was ranged from 1.20±0.064 mg QE/gDW to 3.59±0.030 mg QE/gDW and the total phenolics was ranged from 15.82±0.034 mg GAE/gDW to 34.75±0.023 mg GAE/gDW from *A. ampeloprasum*. Whereas the extraction of flavonoids and total phenolics from *C. colocynthis* were ranged from 1.93±0.033 mg QE/gDW to 3.6±0.100 QE/gDW amg QE/gDW, and from 21.53±0.503 mg GAE/gDW to 41.1±0.100 mg GAE/gDW, respectively. The best flavonoid and phenolic extraction of both plant samples were occurred in the concentration 50% ethanol (p=0.008 and 0.001 at a level 0.05), and there was a nonsignificant variation of the phenolic extraction with increasing ethanol concentration over 50%. The highest yield of flavonoids was 3.59±0.030 mg QE/gDW and 3.6±0.100 mg QE/gDW obtained f from *A. ampeloprasum* and *C. colocynthis* respectively, using the percentage 50:50 of ethanol:water mixture, as well as the yield of phenolic compounds appeared the same extraction results. But increasing the concentration from 60% to 80%, slightly decreased the yield of flavonoids from both. Rostagno *et al.* [22] also found 50% as the best ethanol-water mixture for extraction of isoflavones by using the microwave, but in this study, we used shaker incubator at 30°C in the rate 120 rpm for 24 h. The highest phenolic concentration was obtained when ethanol-water mixture (≤50%) was used (Fig. 3). This is related to the dielectric properties of the solvent. Dielectric properties have an important role for interaction of the plant raw material with shaking. It is known that ethanol-water mixture has higher dielectric properties than pure water and absolute ethanol [23]. The synergistic effect that is revealed by ethanol-water mixture can be explained by the hydrogen bonding between water and ethanol which increases the dielectric properties. As a result, this solvent type can increase its efficiency by adding another solvent more polar like methanol, in addition to supplement this system with 1% HCl to release the bounded phenolic compound from cell or tissue structures. Hence, the other solvent mixture was used containing EtOH:MeOH:HCl:H_2O to obtain the best phenolics and flavonoids extraction from plants raw materials.

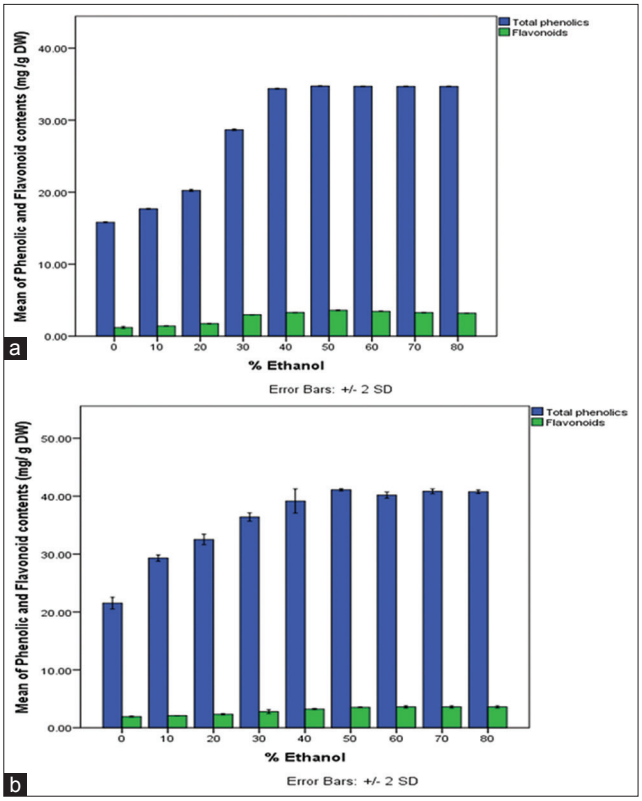

Fig. 3: Extraction total flavonoids and total phenolics from *Allium ampeloprasum* and *Citrullus colocynthis* using various ethanol ratios. (a) *A. ampeloprasum*, (b) *C. colocynthis.* Extraction conditions where temperature 30°C, extraction period of 24 h with shaking rate 120 rpm, solvent to raw material ratio 20:1, and the extraction number 2

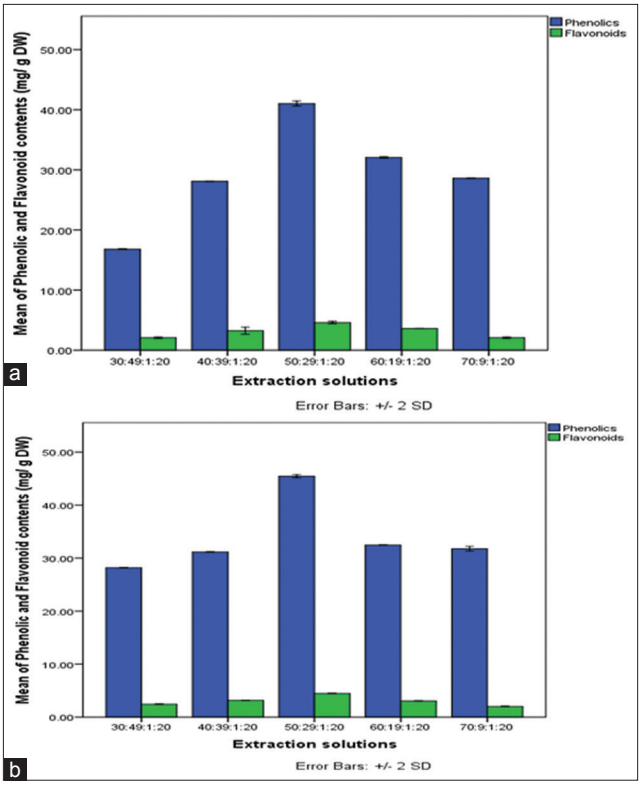

Fig. 4: Extraction total flavonoids and total phenolics from *Allium ampeloprasum* and *Citrullus colocynthis* using various ratios of EtOH:MeOH:HCl:H_2O. (a) *A. ampeloprasum*, (b) *C. colocynthis.* Extraction conditions where temperature 30°C, extraction period of 24 hrs with shaking rate 120 rpm, solvent to raw material ratio 20:1, and the extraction number 2

Table 2: Plants extracts and total phenolic and flavonoid compounds and antioxidant activity

Plant	Dry weight of extract (%) ± SD	Total phenolics (mgGAE/gDW) ± SD	Flavonoids (mgQE/gDW) ± SD	Antioxidant activity (µg/ml) ± SD
A. ampeloprasum	10.03±0.06*	40.75±0.05*	5.10±0.02*	92.00±1.00
A. graveolens	5.1±0.10	6.83±0.06	0.83±0.01	720.67±0.53
C. sinesis	4.1±0.10	15.10±0.10*	1.95±0.01	104.00±1.00
C. annuum	8.10±0.10	35.16±0.15	3.26±0.01	193.00±1.53
C. zeylanicum	9.10±0.10	148.26±0.15**	29.63±0.51*	95.33±0.58
C. colocynthis	5.04±0.05	45.5±0.15*	4.48±0.02*	87.33±1.53
C. longa	8.95±0.03	28.49±0.10	3.88±0.01	48.67±0.58
L. inermis	15.43±0.03**	66.62±0.11*	10.814±0.01	111.33±0.58
M. acuminata	8.80±0.10	2.06±0.10	0.613±0.01	162.67±0.58
M. communits	5.30±0.05	22.04±0.06	2.12±0.01	176.00±1.00
P. nigrum	9.20±0.10	41.08±0.10*	4.91±0.01	106.00±1.00
P. dactylifera	12.03±0.16*	49.75±0.05*	13.71±0.010*	52.00±1.00
S. longena	1.55±0.05	9.97±0.15	2.33±0.06	91.67±0.58
S. aromaticum	9.3±0.05*	43.86±0.24*	2.73±0.01	107.33±0.58
V. vinifera	8.25±0.05	7.58±0.08	0.914±0.01	101.67±0.58

A. ampeloprasum: Allium ampeloprasum, A. graveolens: Anethum graveolens, C. sinesis: Camellia sinesis, C. annuum: Capsicum annuum, C. zeylanicum: Cinnamomum zeylanicum, C. colocynthis: Citrullus colocynthis, C. longa: Curcuma longa, L. inermis: Lawsonia inermis, M. acuminate: Musa acuminate, M. communits: Myrtus communits, P. nigrum: Piper nigrum, P. dactylifera: Phoenix dactylifera, S. melongena: Solanum melongena, S. aromaticum: Syzygium aromaticum, V. vinifera: Vitis vinifera, **p=0.0005, *p=0.01 at a level 0.05, mg GAE/gDW: Mg gallic acid equivalents per gram of dry weight, mg QE/gDW: Mg quercetin equivalent per gram dry weight, antioxidant activity was estimated as BATS IC_{50} values, which defined as the concentration of test material needed to scavenge 50% of ABTS radical present in the test solution. Lower IC_{50} value reflects high antioxidant activity. SD values of a minimum of 3 replicates, ABTS: 2,2`-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), SD: Standard deviation, IC_{50}: Inhibitory concentration 50

The extraction system was used consisting of various ratios of ethanol and methanol by using the same previous extraction conditions, in addition to adding water:HCl at ratio 20:1 (v/v), that depending on previous studies [15,16] which reported the best water ratio for the flavonoids and the phenolic extraction was 20:1 of water:HCl. The phenolic extraction was occurred in acidic solvents for recovering the bounded phenolic compounds from tissues [15].

Fig. 4 appeared the extraction yield of flavonoids and phenolics from both plants, and the best extraction system was EtOH:MeOH:HCl:H_2O

at a ratio 50:29:1:20 which gave the highest yield of flavonoids were 5.10±0.02 and 4.48±0.02 mg QE/gDW and phenolics were 40.75±0.05 and 45.5±0.15 mg GAE/gDW from *A. ampeloprasum* and *C. colocynthis,* respectively (p=0.000 at a level 0.05). Hence, this solvent system was used to extract flavonoids and phenolics from the other plants.

Table 2 showed the extraction percentage per gram dry weight of plant (% of gDW) of 15 studied plants, which *Lawsonia inermis* had a highest percentage (15.43±0.03%) of extract and *S. melongena* had a lowest percentage (1.55±0.05%) for phenolic extractions. In addition, Table 2 revealed that the total phenolic compounds and flavonoids contents and the antioxidant activities of the extracts, which had ability to reduce the absorbance of ABTS free radical to half (IC_{50}) and referred as an inhibition percentage PI%. The results appeared that variation in phenolics and flavonoid contents and the antioxidant activities, however *C. zeylanicum* extract had highest phenolic content was 148.26±0.15 mg GAE/gDW, followed *L. inermis*, *C. colocynthis, Syzygium aromaticum*, *Piper nigrum,* and *Phoenix dactylifera* were 66.62±0.11, 45.5±0.15, 43.86±0.24, 41.08±0.10, and 49.75±0.05 mg GAE/gDW, respectively, and the lowest phenolic content was 2.06±0.01 mg GAE/gDW of *M. acuminate* extract. The extracts of *C. zeylanicum*, *L. inermis* and *P. dactylifera* had the highest flavonoid contents were 29.63±0.51, 13.71±0.010, and 10.814±0.01 mg QE/gDW, respectively, whereas the *M. acuminata* had lowest flavonoid content was 2.12±0.01 mg QE/gDW. The antioxidant activity assay of these extracts appeared that the lowest estimated values IC_{50} which indicated the higher ability to scavenge 50% of free radicals in respect to the PI value of µg/ml of plant extract, so *C. longa*, *P. dactylifera, C. colocynthis,* and *S. melongena* extracts (48.67±0.58, 52.00±1.00, 87.33±1.53, and 91.67±0.58 µg/ml of the extracts, respectively) had the highest ability to scavenge the free radicals, whereas *A. graveolens* (720.67±0.53 µg/ml) had the lowest ability.

Numerous published studies described significant and positive correlations between total phenolic content and the antioxidant activity [2,24]. However, Rivero-Pérez *et al*. [25] explain that antioxidant activity depends more on the structure and conformation of total phenolic compounds than their concentration. As the present study, the extraction results of *C. longa*, *P. dactylifera, C. colocynthis*, *A. ampeloprasum*, *S. melongena* which shown lower phenolic contents than *C. zeylanicum,* and they had highest antioxidant activities. Furthermore, the total phenolic compound was estimated according to the Folin–Ciocalteu method that may also determine other reducing compounds as reducing sugars [26] and react also with some nitrogen compounds as amino acids and amines [27]. The main disadvantage of spectrophotometric assays is that they only give an estimation of the total phenolic content. It does not separate nor does it give a quantitative measurement of individual compounds. Similarly, the molecular antioxidant response of phenolic compounds in plants varies remarkably, depending on their chemical structure [25]. Thus, the antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content.

In this study, all studied plant extracts exhibited different phenolic and flavonoid contents for example *A. ampeloprasum* had a higher total phenolic content (40.75 mg GAE/gDW) than the total phenolic contents reported by Garcia-Herrera *et al.* [28] in the whole leek plant was 5.77 mg GAE/g fresh weight, whereas Ben Arfa *et al.* [29] found that the wild species of *A. ampeloprasum* had variable bioactive compounds such as the total polyphenol (16.64-48.22 mg GEA/gDM), flavonoid (1.01-5.84 mg CE/gDM), and tannin (3.47-7.62 mg CE/gDM) contents and antioxidant activities (2,2-diphenyl-1-picrylhydrazyl [DPPH] and iron chelating power) were strongly affected by the environmental condition on these characteristic have been scarcely known. Whereas our result revealed that the total phenolic and flavonoid contents of the *C. zeylanicum.*

The bark was slightly lower (148.26±0.15 mg GAE/gDW and 29.63±0.51 mg QE/gDW) than Varalakshmi *et al.* [30] results who reported that *C. zeylanicum* bark is a nutraceutical rich in phenolic antioxidants, and the total phenolic content was 153.33±23.09 mg of pyrocatechol equivalents/g, and flavonoids was 33.66±1.15 mg of catechin equivalents/g of powdered bark. Whereas the phenolic and flavonoid contents and antioxidant activity of *C. longa* were lower than the contents that reported by Alafiatayo *et al.* [31] who found 42.71 mg GAE/gDW, 741.36 mg NGN/gDW and it had the highest free radical scavenging capacity of 270.07 mg TE/gDW, respectively. The differences of results were related to the extraction solvent type and conditions which extracted by 100% methanol with refluxed at 60°C, in addition to the estimation methods of flavonoid and antioxidant activity were different which, depending on mg naringenin equivalents (Ng)/g samples and used DPPH to determine the free radical scavenging capacity. In addition, the plant age is very important due to its effects on phenolic contents; Alafiatayo *et al.* [31] used fresh and young rhizomes which were harvested after 1 month of growth, whereas our results depending on different extraction solvents and conditions, also the rhizomes not fresh; they were imported from original countries and stored under conventional conditions. Similar results reported by Sahu and Saxena [32] how found the total phenolic content was 260±0.25 mg GAE/gDW and 79.36±0.01 mg CE/gDW of the total flavonoid content which extracted from *C. longa* by methanol. The other results of the phenolic and flavonoid contents of the rest studied plants such as *P. dactylifera, A. graveolens, C. sinensis, C. annuum, C. colocynthis, L. inermis, M. acuminata, M. communits, P. nigrum, S. melongena, S. aromaticum,* and *V. vinifera* were different or in agreement with previous results depending on extraction methods, extraction solvents and conditions, plant age, the estimation methods of phenolics, flavonoids and antioxidant activities, so on [33-45].

Overall, the variations in the chemical composition of the selected plants, as in other plant tissues may be due to the multiple influences of different factors such as temperature, age, storage conditions, precipitation, sun exposure, soil composition, growing status, and the interaction of the other plants or animals in the ecosystem [30]. Unfavorable environmental conditions (salinity, drought, heat/cold, luminosity, and other hostile conditions) may trigger oxidative stress in plants, generating the formation of ROS, leading to cellular damage, metabolic disorders, and senescence processes [46,47].

The extracts of *P. dactylifera, C. colocynthis,* and *S. melongena* were selected depending on antioxidant activity to study their effects on

Table 3: Partial purification of plant extracts by adsorption chromatography using a silica gel column

Plant	Steps	Total phenolics (mg GAE±SD)	Total flavonoids (mg QE±SD)
P. dactylifera	Crude extract	3578±2.577	284±1.012
	95% ethanol	530±1.201	30±0.577
	70% acetone	441±1.120	190.5±1.130
	30% acetone	80±1.021	24.5±0.577
	H_2O	1075±2.155	0
	Recovery %	59.42	85.91
C. colocynthis	Crude extract	277.2±1.577	37.8±0.577
	95% ethanol	72.35±0.577	2.9±0.012
	70% acetone	148±1.051	20±0.577
	30% acetone	39±0.152	3.61±0.012
	H_2O	4.48±0.122	0
	Recovery %	95.18	70.13
S. melongena	Crude extract	434.50±2.012	96.55±0.577
	95% ethanol	228.40±1.055	10.60±0.012
	70% acetone	154.50±1.557	60.28±1.150
	30% acetone	21.06±0.577	0.78±0.012
	H_2O	15.35±0.252	0.51±0.011
	Recovery	96.50	74.75

C. colocynthis: Citrullus colocynthis, S. melongena: Solanum melongena, P. dactylifera: Phoenix dactylifera, GAE: Gallic acid equivalents, SD: Standard deviation. Total phenolics and total flavonoids were expressed as mg GAE±SD and mg QE±SD which resulted from mg GAE/ml x total volume (ml) of fractions and mg QE/ml x total volume of fractions (ml), H_2O: Distilled water. Each fractionation was replicated three times

Table 4: The cytotoxic activity of partially purified plant extracts against the breast cancer cell line MCF-7 and the non-tumorigenic fetal hepatic cell line WRL-68

Partially purified phenolics	Concentration (µg/ml)	Total amount		% Cell viability mean±SE		T-test value (p<0.05)
		Phenolics (µg AGE/ml)	Flavonoids (µg QE/ml)	MCF-7	WRL-68	
P. dactylifera	100	77.68	33.56	60.79±3.45	72.38±2.35	0.000
	50	38.84	16.78	77.27±3.97	91.08±3.68	
	25	19.42	8.39	84.97±2.64	93.93±3.57	
C. colocynthis	100	62.40	8.43	94.93±3.01	68.22±2.21	0.000
	50	31.20	4.22	98.55±1.08	95.82±3.54	
	25	15.60	2.11	101.36±0.98	101.77±4.01	
S. melongena	100	98.94	38.6	88.23±1.20	88.25±0.20	0.265
	50	49.47	19.3	92.47±2.35	89.83±2.34	
	25	24.735	9.65	93.74±3.87	92.23±1.25	

C. colocynthis: Citrullus colocynthis, S. melongena: Solanum melongena, P. dactylifera: Phoenix dactylifera, MCF: Michigan Cancer Foundation-7, SE: Standard error. Each measurement was replicated 3 times

cancer cell lines after flavonoids were partially purified by adsorption chromatography using a silica gel column. In addition to these plants is rarely studied and abundant in Iraq, particularly *P. dactylifera*.

Partial purification was accomplished by adsorption chromatography using a silica gel column the loading solvents were 95% ethanol, and the elution solvents were 70% acetone followed 30% acetone and then pure water respectively, using the batch ways method. Each solvent was added at 500 ml or more depending on the reading value of the absorbance at 280 nm when it became between 0.000 and 0.001, then the next followed solvent was added. After that, the total flavonoids and phenolics estimated for each fraction. In this study, we focused on hydrophobic phenolics especially that related to flavonoids because the members of this group have antioxidant, anti-inflammatory, and anticarcinogenic effects and it decreases the fragility of blood vessels like rutin, also known as vitamin P [2,6,48,49].

In the present results, the purification of phenolic and flavonoid extracts of the selected plants *P. dactylifera, C. colocynthis*, and *S. melongena* by adsorption chromatography using the silica gel column appeared varied amounts of phenolics and flavonoids that separated using different concentrations of solvents as shown in Table 3. From these results, the most of the phenolic content of *P. dactylifera* was eluted by DW (soluble in water), and the most flavonoid content was eluted by 70% acetone, whereas the most phenolics and flavonoids of *C. colocynthis* were eluted by 70% acetone. Most of phenolics and flavonoids of *S. melongena* were eluted by 95% ethanol and 70% of acetone, respectively (Table 3). These results were in agreement with previous studies that used a silica gel column in phenolics purification [15,16,49].

The cytotoxic activity of the partial purified extracts was assayed using two cell lines, including breast cancer cell line MCF-7 and the normal human hepatic cells (non-tumorigenic fetal hepatic cell line WRL-68). The selected partially purified extracts (70% acetone fractions which mostly contain flavonoids) were used in the concentrations ranged from 25 to 100 µg/ml for *in vitro* evaluating to select the best effective plant extract against cell lines, and subsequent study will focus on its preventive effects against induced mammary cancer in a rat model. The results revealed that all selected extracts had variable effects on % cell viability (Table 4). From the results, the partially purified seed extract of *P. dactylifera* had the best cytotoxic effect on the cancer cell line MCF-7 at concentration 100 µg/ml followed 50 µg/ml and it had less effect on the normal cell line WRL-68 especially at 50 µg/ml ($p<0.05$), and the minimum inhibition dose that kills approximately 50% of cells (IC_{50}) at 24 h was 202 µg/ml (156.91 µg phenolics) for MCF-7 and 480 µg/ml (372.86 µg phenolics) for WRL-68. Whereas the extracts of *C. colocynthis* had cytotoxic effects on the normal cell line more than breast cancer cell line ($p<0.05$). The IC_{50} value for MCF-7 was 1690.8 µg/ml (1055.06 µg phenolics) of *C. colocynthis* extract, and the IC_{50} value for WRL-68 was 246.51 µg/ml (153.8 µg phenolics) respectively. While the extract of *S. melongena* had the same effects on cancer and normal cell lines ($p=0.265$), and the IC_{50} was very wide which was 58528 µg/ml (57907.6 µg phenolics) for both cell lines. The results indicate the phenolic extract of *P. dactylifera* was the best compared with other plant extracts because it had cytotoxic effects against cancer cell line more than the normal cell line.

These results were in agreement with [50] who showed that the *P. dactylifera* seed extract had anticancer activity against Ehrlich ascites carcinoma cells.

The antiproliferative effect of cucurbitacin glycosides extracted from *C. colocynthis* leaves was studied in human breast cancer cell growth. The leaves were extracted and cucurbitacin B/E glycosides were isolated from the extract. The cucurbitacin glycoside combination (1:1) inhibited growth of ER+ MCF-7 and ER- MDA-MB- 231 human breast cancer cell lines [51,52] in comparison with our study that we used the *C. colocynthis* fruits. Whereas, *S. melongena* extract had a low cytotoxic effect against cell lines, and these results were in agreement with the previous studies [43,53].

CONCLUSION

The phenolic compounds act as antioxidant substances which had variable effectiveness on normal cell and cancer cell lines, and the highest concentrations were toxic to normal cells and cancerous poison impact may be on normal cells over than the cancerous cells.

ACKNOWLEDGMENT

The authors are thankful to Pioneer Projects Department, Research and Development Directorate, Ministry of Higher Education and Scientific Research, Iraq, for funding this research which was done at the Laboratory of Biotechnology and Genetic Engineering, Department of Biology, College of Science, University of Babylon, Iraq.

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