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ANTIMICROBIAL AND ANTICANCER ACTIVITY OF METHANOLIC EXTRACT OF DRIED MULBERRY FRUITS AND LEAVES ILLUSTRATED WITH THEIR CHEMICAL COMPOSITION

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ABSTRACT

This study was undertaken to determine some selected nutritive chemical composition of dried mulberry fruits and leaves (proximate composition: moisture, ash, fiber, protein, fat and carbohydrate, amino acids and minerals) and evaluation of antimicrobial and anticancer potential of their methanolic extract. The results indicated that the leaves have more nutritious quality than the fruits. The dried mulberry leaves recorded higher content of ash, protein, fiber, amino acids and minerals than that in fruits while moisture and fat content were higher in fruits than in leaves. The carbohydrate content was slightly higher in fruits than in leaves. However, both of them were nutritionally rich. The antimicrobial effect of different concentrations (5, 10 and 15 mg/ml DMSO) of mulberry fruits and leaves extract on growth and survival of *Staphylococcus aureus* strain and *Escherichia coli* strain in vitro and in mulberry juice were evaluated. Both fruits and leaves extract have attenuated effect on both bacteria. The concentration, 15 mg/ml of mulberry fruits extract represent the optimum concentration for decreasing *E. coli* and *Staph. aureus* counts in liquid medium such paid to decreasing them from $5X10^{10}$ and $5X10^{12}$ to $14X10^{2}$ and $4X10^{5}$ cfu/ml respectively. The different concentrations (10 and 15 mg/ml) of fruits and leaves extract induced completely elimination of *Staph. aureus* from mulberry juice and reduction of *E. coli* from $5X10^{10}$ to $4X10^{2}$ and $3X10^{3}$ cfu/ml respectively at concentration 15 mg/ml. The expression of p53 (tumor suppressor gene) from three types of cancer cell lines (Hep-2 (Larynx carcinoma), HepG2 (liver carcinoma) and CaCo2 (colorectal adenocarcinoma)) treated with fruits and leaves extract were evaluated to explore their anticancer effect. The results showed that the cancer cells treated with fruits and leaves extract were negative for p53 gene expression as the gene not detected comparing with positive cell control. The tested extracts were not anticancer agent.

Keywords: Mulberry fruits and leaves, chemical composition, antibacterial and anticancer activity.

INTRODUCTION

Plant-based foods such as fruits and vegetables, which are high in essential micronutrients, may potentially reduce the incidence of cancer and other deleterious diseases (Kris-Etherton *et al.,* 2002 and Liu, 2003). Research has indicated that the benefits of fruit and vegetable consumption are attributed to the presence of phytochemicals (Kris-Etherton *et al.,* 2002).

Plants are exemplary source of medicines and several drugs have been derived directly or indirectly from them. Mulberry is the most medicinally

important plant which belongs to genera *Morus*. It is a monoecious or dioecious plant up to 10 - 12 m high. This plant is widely distributed in India, China, Japan, North Africa, South Europe etc. It helps in treatment of many serious diseases like diabetes mellitus, arthrosclerosis, hyperlipidemia; hypertension etc. Mulberry can be grown both in tropics and in the temperate regions. It is also raised in rained and irrigated conditions. The optimum temperature ranges from 24 to 29°C, atmospheric humidity from 65 to 80% (Kumar and Chauhan, 2008). There are over 150 species found in genus *Morus*, among these *Morus alba L.* is dominate (Srivastava *et al.*, 2006).

Studies have been reported on the chemical composition and nutritional potentials of some mulberry species worldwide (Gerasopoulos and Stavroulakis, 1997; Elmacı and Altuğ, 2002; Darias- Martin *et al*., 2003; Arabshahi-Delouee and Urooj, 2007 and Ercisli and Orhan, 2007). Plants of this genus are known to be rich in flavonoids (Nomura, 1999 and 2001), a group of chemicals shown to have potent antiviral activities against herpes simplex virus, rhinovirus, rotavirus, human immunodeficiency virus, and various respiratory viruses (Alves *et al.*, 1999; Lin *et al.*, 1999; Bae *et al.*, 2000; Abdel-Kader, 2001 and Ma *et al*., 2002).

Morus alba L. contains an appreciable amount of proteins, carbohydrates, fats, fibers, mineral contents and some vitamins or their precursors (Butt *et al.*, 2008).

The leaves alone contain a wide variety of nutrients, including proteins, sugars, polyphenols, flavonoids, steroids, vitamins, and minerals (Andallu and Varadacharyulu, 2003). The antioxidative effects of mulberry leaves have been mainly attribuated to quercetin rutinoside (rutin), quercetin 3-glucoside (isoquercitrin) and quercetin 3-(6-malonylglucoside) (Katsube *et al.,* 2006). Mulberry leaves contain kuwanon C, mulberrofuran G and albanol B all shown strong antibacterial activity with minimum inhibitory concentrations (MIC's) ranging from 5 to 30 mg/ml (Sohn *et al.*, 2004 and Nomura, 2001).

The mulberry fruits are also known for its delicious taste and medicinal properties like vaso-tonic, antioxidant activity, anticancer, antiviral, anti-inflammatory etc (Kumar, and Chauhan, 2011). Mulberry fruits were found to serve as a potential source of food diet, natural antioxidants and high phenolic compounds (Imran *et al*., 2010).

Rich chemistry of mulberry extracts provides antimicrobial potential against harmful microorganism (Park *et al.*, 2003). Various fractions of mulberry such as chloroform extract have strong antimicrobial activities against *Bacilllus subtilis*, and fractions extracted with acetic acid against *Staphylococcus aureus, B. subtilis* and *Escherichia coli* (Kim *et al*., 1993).

During the last few years antimicrobial properties of plant extracts and natural products have been intensively investigated as demand for safe drugs which has increased due to misuse of antibiotics and an increase in immune-deficiency (Grayer and Harborne, 1994). Moreover dietary intake of natural antioxidants could be an important factor in body's defense mechanism against many mutagens and carcinogens, also many antioxidants are being identified as anticarcinogens. Many plant polyphenols, have been

shown to act as potent antimutagenic and anticarcinogenic agents (Yen and Chen, 1994).

The current study was conducted to investigate the chemical composition of dried mulberry fruits and leaves and evaluate the antimicrobial activity of methanolic extract of fruits and leaves against *Staphylococcus aureus* and *Escherichia coli* in vitro and in mulberry juice. The expression of p53 (tumor suppressor gene) from three cancer cell lines (Hep-2, HepG2 and CaCo2) treated with fruits and leaves extract were evaluated to explore their anticancer effect.

MATERIALS AND METHODS

Chemicals

All reagent and chemicals used in this study were of analytical grade and obtained from Sigma Chemical Co. (St Louis, MO, USA), unless stated otherwise.

Plant materials

Mulberry (*Morus alba L*.) fruits and leaves were bought from markets of Giza, Egypt. The mulberry leaves and fruits were washed with tap water and dried in a hot air oven at 40°C. The dried material was ground to a fine powder with electric blender, and kept at 4 °C until further use.

Extraction of mulberry fruits and leaves

The dried fruits and leaves of mulberry (15 g) were extracted overnight with 100 ml of 60% methanol in a mechanical shaker at room temperature. The extract was filtered with Whatman No. 1 filter paper. The filtrate was evaporated at 45 °C in a rotary evaporator to concentrate the solution, then lyophilized in order to obtain the dry extract and stored at 4 °C until use (Arabshahi-Delouee and Urooj, 2007).

Chemical analysis

Dried grounded plant materials were used for determination of proximate analysis, amino acids and minerals. Moisture contents, ash and fiber were determined by AOAC (2005) methods. Nitrogen content (N) of the sample was estimated by the method described by Kjeldahl (1983) and crude protein was calculated as N×6.25 (Imran *et al.*, 2008), while total fat from the samples were extracted with chloroform/methanol (2:1, v/v) and quantified gravimetrically (Christie, 1983). The amount of total carbohydrates was obtained by the difference between weight of the sample taken and sum of its moisture, ash, fat, protein, and fiber contents (Muler and Tobin, 1980). Amino acids were determined by high performance Amino Acid Analyzer, Model Beckman 7300 according to method of Becker *et al.* (1981).

The minerals content (K, Ca, Na, Mg, P, Fe, Se and Zn) was determined by AOAC (2005) method. The dried grounded samples (0.50 g) were taken and digested with 20 ml concentrated nitric acid. After adding 10 ml of perchloric acid, the contents were heated gently on a hot plate, followed by a vigorous heating till dryness (approximately 1–2 ml). After cooling, the digested samples were quantitatively transferred to a flask and diluted to 100

ml with deionized distilled water, and then filtered. ICP plasma Optima 2000 DV (Inductivity Coupled Plasma) was used for analysis of minerals.

Antibacterial activity techniques

Bacterial isolates:

Staphylococcus aureus strain No. 4 and *Escherichia coli* strain No. 5were obtained from Dr. Abdel Salam, A.F., Regional Center for Food and Feed, ARC, Giza- Egypt.

Isolates maintenance

Staph. aureus and *E. coli* strains were maintained through monthly transfer on nutrient agar and stored at 4°C.

Standard inoculums

Standard inoculums were prepared by inoculation of conical flask (100 ml in volume) containing 50 ml of buffered peptone water (pH 7.2) for 24 hr at 37°C with loop of *Staph. aureus* and anther flask with loop of *E. coli*. Achieved viable cells counts were determined by a serial dilution and subsequent enumeration using Vojel Johnson medium for *Staph. aureus* and EMB medium for *E. coli.*

Screening of antimicrobial activity of mulberry fruits and leaves extract

The antimicrobial activity of mulberry activity against selected microorganisms was evaluated by the cup-plate agar diffusion method (Ebi and Ofoefule, 1997 and Ijeh, *et al.*, 2005). A 20 ml of nutrient agar was seeded with 0.2 ml of broth culture of the test organisms in sterile Petri dishes. The Petri dishes were rotated slowly to ensure a uniform distribution of microorganisms. The nutrient agar was left to solidify in the dish. With the aid of sterile cork borer, cups of 8.0 mm diameter were made in nutrient agar. The 5, 10 and 15 mg of dry lyophilized extracts were suspended in 1ml DMSO, and then were inoculated into the cups with the aid of micropipette (at ratio 100 µl of different concentrations). The dishes were allowed to stand for 30 min. at room temperature to allow proper diffusion of the extract to take place. The plate was then incubated for 24 hr at 37°C. At the end of incubation period, inhibition zones formed on the medium were measured in mm. The minimum inhibitory concentration (MIC) in mg/ml was determined by comparing the different concentrations of a particular extract that have different zones of inhibition and then selecting the lowest concentration for each extract (Ijeh *et al.*, 2005).

Effect of different concentration of mulberry fruits and leaves extract on growth of *E. coli* and *Staph. aureus* in vitro

Erlenmeyer flasks (250 ml) contained 50 ml of 0.1% buffered peptone water were divided into two groups (6 flasks in each group), the flask of first group were inoculated with 0.5 ml of *E. coli* inoculums containing about 10^{10} cfu/ml and other flask of second group were inoculated with 0.5 ml of *Staph. aureus* inoculums containing about 10^{12} cfu/ml then each different concentrations of fruits and leaves (5, 10 and 15 mg/ml DMSO) were added to the different flasks separately. The flasks were incubated at 37°C for 24 hr on rotary shaker (100 rpm). The controls were only inculcated with bacteria strains without adding any of tested extracts with the same experimental condition as mentioned before.

Effect of different concentrations of mulberry fruits and leaves extract on survival of *E. coli* and *Staph. aureus* in mulberry juice

Erlenmeyer flasks (250 ml) contained 50 ml mulberry juice were divided into two groups, first group inoculated with 0.5 ml of *E. coli* inoculums containing about 10^{10} cfu/ml. The second group was inoculated with 0.5 ml of *Staph. aureus* inoculums containing about 10^{12} cfu/ml then each different concentrations of fruits and leaves (5, 10 and 15 mg/ml DMSO) were added to the different flasks separately. The flasks were incubated at 37°C for 24 hr on rotary shaker (100 rpm). The controls were only inculcated with bacteria strains without adding any of tested extracts with the same experimental condition as mentioned before.

Anticancer activity techniques

Cytotoxicity

Cytotoxic effect of mulberry fruits and Leaves extract were evaluated to different cancer cell lines [Hep-2 cells (ATCC: CCL- 23), HepG2 (ATCC: HB-8065), and CaCo2 (ATCC: HTB-37**)** 24 hr post cell treatment using MTT assay (Cory *et al.*, 1991), where test extracts were cell culture media diluted (Biowhittaker-Belgium) to contain 1gm/ml, then sterile filtrated using 0.22 µm syringe filter (Millipore-USA).

96-well cancer cells precultured plates (Nunc-USA) were treated with descending double fold serially diluted extracts at 37°C for 24 hrs. Negative cell control was included. Residual living cells were treated with 20 µl of MTT (5 mg/ mL) (Sigma-Aldrich-USA) at 37°C for 4 hrs. MTT was discarded. Plates were PBS washed three times. DMSO (BDH-England) was added as 50µl / well. Plates were shacked on plate shaker (Staurt-England) for 30 min to dissolve the produced intracellular blue formazan complex. Optical densities (O.D) were measured at 570 nm using an ELISA plate reader (Dynatech -England). Data were reported for three independent experiments, (Berridge *et al.*, 2005). Viability percentage was calculated as follows: Cell viability percentage = (O.D of treated cells / O.D of untreated cells) X 100 Chen *et al.*, (2009).

RNA extraction

RNA was extracted from venom treated and untreated cells using SV total RNA isolation system (Promega-Germany) where cells were collected and PBS (ice-cold sterile) washed twice. 175 µl RNA lysis buffer and 350 µl RNA dilution buffer were added to cell pellet, mixed by inversion and heated for 3 min at 70°C. Cells were centrifuged at 14000 rpm for 10 min. The clear lysate was transferred to clean tube and 200 µl of 95 % ethanol was added. The mixture was transferred to spin basket assembly and centrifuged for 1 min. 600 µl of RNA wash solution was added, centrifuged for 1 minute followed by 50 µl of DNase incubation mix (40µl Yellow Core Buffer, 5µl 0.09M $MnCl_2$ and 5µl DNase I enzyme) and incubated at room temperature for 15 min. 200 µl of DNase stop solution was added and centrifuged for 1 minute. Each spin basket was treated with 600 µl then 250 µl of RNA wash solution and centrifuged for 1 and 2 min respectively. Finally 100 µl of nuclease free water was added to elute the extracted RNA which was stored at – 70 °C.

Reverse transcription- polymerase chain reaction (RT-PCR)

Extracted RNA was reverse transcripted to cDNA using revertaid first strand cDNA synthesis kit (Fermentas–Lithuania) where extracted RNA (1µg), random hexamer primer (1 µl) and DEPC-treated water (to 12 µl) were incubated at 65°C for 5 min. 4 µl reaction buffer (5X), 1 µl ribolock RNase inhibitor (20 µ/µl), 2 µl dNTP Mix (10 mM) and 1 µl revertaid reverse transcriptase (200 u/µl) were added and incubated at 25°C for 5 min followed by 42°C for 60 min. Reaction was terminated by heating at 70°C for 5 min. The produced (cDNA) were stored at -70°C till used. Verification of cDNA synthesis from extracted RNA was carried out using GAPDH specific internal control primers. The expression of proapoptotic genes (p53) was carried out using newly synthesized cDNA as templates for PCR. 25 µl dream Taq green master mix, 4 µl cDNA, 2 µl forward, 2 µl reverse primers and 17 µl nuclease free water were predenaturated at 94°C for 3 min. Amplification was performed (35 cycles) with each cycle consisting of denaturation at 94°C for 30 sec, annealing at 58°C (GAPDH), 57°C (p53), for 30 sec and extension at 72°C for 45 sec. The reaction was terminated by heating at 72°C for 5 min. 10 µl of RT-PCR product was loaded on 1% agarose gel and visualized using UV transillumiator after staining with ethidium bromide. Band intensities were measured using gel documentation system. Primer sequences and the PCR product size were described in Table (1).

Table (1): Primer sequences of apoptosis related genes and internal control.

Gene	Primer sequences	Size of PCR product (bp)
p53	F: 5 '-TCA GAT CCT AGC GTC GAG CCC-3 '	438
	R: 5 '-GGG TGT GGA ATC AAC CCA CAG-3 '	
GAPDH	F: 5 '-CAA GGT CAT CCA TGA CAA CTT TG-3 '	496
	R: 5 '-GTC CAC CAC CCT GTT GCT GTA G-3 '	

RESULTS AND DISSCUSION

Chemical composition

Proximate composition

The proximate composition of mulberry fruits and leaves illustrated in Table (2) revealed that the dried leaves recorded higher content of ash, fiber and protein than that in fruits while moisture, fat and carbohydrate content were higher in fruits than in leaves.

Table (2): Proximate composition of dried mulberry fruits and leaves.

Parameters / Mulberry part	Moisture%	Ash% DW	Fiber % DW	Protein % DW	Fat % DW	Carbohydrate% DW
Fruits	19.62	6.14	10.02	11.97	11.85	40.40
Leaves	9.19	13.76	11.83	24.20	2.73	38.29

***DW: on dry weight base**

The moisture, ash, fiber, protein, fat and carbohydrate content of mulberry fruits were 19.62, 6.14, 10.02, 11.97, 11.85 and 40.40% respectively. The results were higher than the results of Imran *et al.* (2010) for *Morus alba* genus and were in agreement with the ranges reported in various mulberry species by Ikhtiar and Alam (2007); Butt *et al.* (2008) and kumar and Chauhan (2011).

The moisture, ash, fiber, protein, fat and carbohydrate content of mulberry leaves were 9.19, 13.76, 11.83, 24.20, 2.73 and 38.29% respectively. The results were in agreements with the reported literature in *Morus alba* and other mulberry species (Srivastava *et al.*, 2006; Butt *et al.*, 2008 and kumar and Chauhan, 2011).

The overall results showed that the mulberry fruits and leaves could be a potential source of fiber, protein, fat, carbohydrate and hence energy. Our results supported by the result obtained by Andallu and Varadacharyulu (2003) and Imran *et al.* (2010).

Amino acids

Data in Table (3) indicated that the dried mulberry leaves contain higher quantity of amino acids than that in fruits. The mulberry leaves are considered as a good source of amino acids. These results run in agreement with the data of Al-kirshi *et al.* (2009) who indicated that the dry mulberry leaves is a good source of essential amino acids especially lysine 1.88% and leucine 2.55%. There are several places where mulberry is utilized traditionally as a feed in mixed forage. Excellent results have been obtained with mulberry leaves as ruminant feed (Oviedo *et al.,* 1994; Esquivel *et al.,* 1996 and Gonzalez, 1996).

Table (3): Amino acids content of dried mulberry fruits and leaves.

Mulberry parts / Amino acids (mg/100mg dry sample)	Fruits	Leaves
Aspartic acid	1.24	2.36
Threonine	0.31	0.84
Serine	0.43	0.85
Glutamic acid	1.34	2.13
Glycine	0.44	1.00
Proline	0.36	1.36
Alanine	0.43	1.03
Valine	0.50	1.11
Isoleucine	0.35	0.84
Leucine	0.60	1.53
Tyrosine	0.35	0.75
Phenylalanine	0.41	1.02
Histidine	0.19	0.41
Lysine	0.29	1.12
Arginine	0.77	1.05
Total	8.25	17.84

Minerals

Sufficient quantities of essential macro-(K, Ca, Mg, Na and P) and micro-(Fe, Se and Zn) elements were found in fruits and leaves (Table 4). Ca

was the predominant element (1748.00mg/100g sample) in leaves followed by K, P, Mg and finally Na, while K was the predominant element (1116.00mg/100g sample) in fruits followed by Ca, P, Mg and finally Na. The decreasing order of micro-elements was Fe> Se> Zn in both fruits and leaves. The content of minerals was higher in leaves than that in fruits except for Na and P. Mulberry fruits and leaves were consider as rich source of minerals and may act as better supplements of these minerals (Srivastava, *et al.*, 2006; Butt *et al.*, 2008 and Imran *et al.*, 2010).

Table (4): Minerals content of dried mulberry fruits and leaves.

Mulberry parts / Elements (mg/ 100g sample)	Fruits	Leaves
Potassium (K)	1016.00	1164.00
Calcium (Ca)	622.60	1748.00
Magnesium (Mg)	89.80	150.10
Sodium (Na)	43.47	34.50
Phosphorus (P)	285.70	245.20
Iron (Fe)	26.41	73.56
Selenium (Se)	6.88	8.115
Zinc (Zn)	1.78	2.38

Antimicrobial activity

Inhibitory effect of mulberry fruits and leaves extract on growth of *E. coli* and *Staph. aureus*

The recorded results in Table (5) showed that *Staph. aureus* was unsusceptible for different concentration of both extracts, while *E. coli* was more susceptible for these concentrations especially at 15 mg fruits extract powder/ml DMSO which inhibited E. coli with diameter zone inhibition 1.9 mm.

Table (5): Inhibitory effect of mulberry fruits and leaves extract on growth of *E. coli* and *Staph. aureus* (mm)

Concentration (mg/ ml) / Microorganism	5		10		15	
	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves
E. coli	1.6	1.3	1.7	1.6	1.9	1.7
Staph. aureus	-	-	-	-	-	-

Effect of mulberry fruits and leaves extract on growth of *E. coli* and *Staph. aureus* in broth medium

Data represented in Table (6) Cleary showed the effect of different concentration of mulberry fruits and leaves extract (5, 10 and 15mg/ml DMSO) on survival of *E. coli* and *Staph. aureus* in vitro. Mulberry fruits extract at concentration of 15mg/ml resulted in decreased of *E. coli* and *Staph. aureus* counts from $5X10^{10}$ to $14X10^{2}$ cfu/ml and from $5X10^{12}$ to $4X10^{5}$ cfu/ml respectively. The concentration of 15 mg/ml of mulberry fruits extract represented the optimum concentration for decreasing *E. coli* and *Staph. aureus* in liquid medium.

Table (6): Effect of mulberry fruits and leaves extract on growth of *E. coli* and *Staph. aureus* in broth medium (cfu/ml)

Concentration (mg/ ml) / Microorganism	5		10		15		control
	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	
E. coli	$13X10^{6}$	$6.5X10^{9}$	$6X10^{4}$	$9X10^{7}$	$14X10^{2}$	$11X10^{4}$	$5X10^{13}$
****Staph. aureus***	$2X10^{8}$	$5X10^{9}$	$6X10^{6}$	$4X10^{7}$	$4X10^{5}$	$3X10^{6}$	$2X10^{15}$

***The used inoculums of *E. coli* was $5X10^{10}$ cfu/ml**
****The used inoculums of *Staph. aureus* was $5X10^{12}$ cfu/ml**

Mulberry leaves extract decreased *E. coli* and *Staph. aureus* counts especially at concentration level 15 mg/ml. This concentration was able to diminish density of pathogenic bacteria as *E. coli* from $5X10^{10}$ to $11X10^{4}$ cfu/ml and density of *Staph. aureus* from $5X10^{12}$ to $3X10^{6}$ cfu/ml. The broth medium without addition of any extracts encouraged growth of pathogenic bacteria such paid to increasing of *E. coli* counts from $5X10^{10}$ to $5X10^{13}$ cfu/ml and *Staph. aureus* counts from $5X10^{12}$ to $2X10^{15}$ cfu/ml.

Effect of mulberry fruits and leaves extract on growth of *E. coli* and *Staph. aureus* in mulberry juice

The obtained results from Table (7) revealed that the different concentrations (10 and 15mg/ml DMSO) of mulberry fruits and leaves extract induced completely elimination of *Staph. aureus* in mulberry juice while the concentration of 5mg/ml of mulberry fruits and leaves extract decreased *Staph. aureus* counts from $5X10^{12}$ to $6X10^{12}$ and $5X10^{3}$ cfu/ml respectively in mulberry juice, comparing with the same extract concentration in broth medium. In addition the concentration of 15mg/ml of mulberry fruits and leaves extract revealed higher antimicrobial effect in decreasing density of *E. coli* counts in mulberry juice from $5X10^{10}$ to $4X10^{2}$ and $3X10^{3}$cfu/ml respectively, comparing with the same concentration in broth medium. Mulberry juice alone without addition of any tested extracts didn't induce approximately increasing or decreasing in *E. coli* and *Staph. aureus* counts. These results were in agreement with those reported by several investigations i.e. inhibitory effect of raspberry juice was demonstrated against *E. coli*, *Salmonella typhimurium* and *Staph. epidermidis* (Ryan *et al.*, 2001; Lee *et al*., 2003).

Table (7): Effect of mulberry fruits and leaves extract on growth of *E. coli* and *Staph. aureus* in mulberry juice (cfu/ml)

Concentration (mg/ ml) / Microorganism	5		10		15		control
	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	
E. coli	$5X10^{4}$	$2X10^{7}$	$6X10^{3}$	$9X10^{5}$	$4X10^{2}$	$3X10^{3}$	$2X10^{10}$
****Staph. aureus***	$6X10^{2}$	$5X10^{3}$	-	-	-	-	$3X10^{12}$

***The used inoculums of *E. coli* was $5X10^{10}$ cfu/ml**
****The used inoculums of *Staph. aureus* was $5X10^{12}$ cfu/ml**

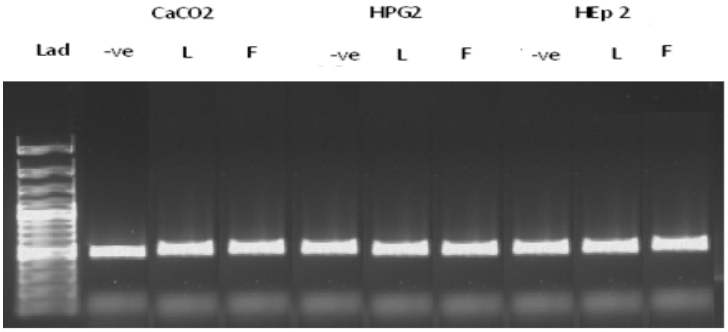
Blackberry juice had no inhibitory effect on growth of *Salmonella* species (*S. California, S. enteritidis, S. typhimurium*) but strongly inhibited *Klebsiela pneumonia* (Cavanagh *et al*., 2003). Blackcurrant juice and extracts

were more efficient against Gram-positive bacteria than against Gram-negative ones (Puupponen-Pimiä *et al.*, 2001). It is worthy to note, that the Gram-negative and Gram-positive organisms showed different sensitivity to antibacterial agent because the former possess of outer membrane surrounding the cell wall (Ratledge and Wilkinson, 1988). Also no correlation between Gram-negative and Gram-positive bacteria status and susceptibility to berries (Cavanagh *et al*., 2003).

Mulberry juice showed no effect on growth of *Salmonella typhimurium* and *Campylobacter jejuni*. Water and ethanol extracts or dark and white mulberry, had no difference in inhibitory effect (Galgoczy *et al.,* 2009). Fukai *et al.* (2005) reported significant antibacterial activity of nine 2-arylbenzofurans isolated from *Morus* species including moracin C and M against methicillin- sensitive *Staph. aureus* (MSSA), methicillin- resistant *Staph. aureus* (MRSA), *Bacillus subtilis, Micrococcus luteus* and *E. coli*. Moreover, mulberry leaves extracts of five cultivars, could inhibit the growth of *Staph. aureus, Bacillus cereus* and *Pseudomonas flurescens* (Suwansri *et al.*, 2008). It was found that *E. coli, Salmonella dysenteriae, Salmonella typhimurium, Pseudomonas aeruginosa* and *Bucillus cereus* were inhibited by *Morus mesozygia* stem bark (Kuete *et al.,* 2009). Mulberrofuran showed strong antibacterial activity with 5-30µg/ml of MICs (Sohn *et al.*, 2004). MLL_1 isolated from leaves of *Morus alba* inhibited growth of pathogenic bacteria (*Staph. aureus* and *E. coli*) in liquid medium (Ratanapo *et al.,* 2001). Also, the isolated compounds from *Morus nigra L.* showed activities against *Staph. aureus*, *Bacillus subtilis*, *Micrococus flavus*, *Streptococcus faecalis*, *Salmonella abony*, *Pseudomonas aeruginosa* (Mazimba *et al.*, 2011).

Anticancer activity

Fig. (1) shows GAPDH gene expression results (specific internal control primers) which used as standard gene because it found in all cells. The GPDH gene was detected in all cells (control cancer cell line, fruit (F) and leaf (L) extracts treated cancer cell line).

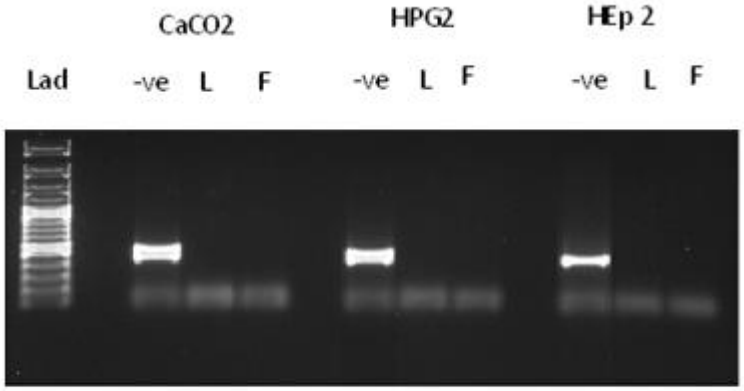


Detection of GAPDH positive control gene in different cancer cell lines treated with leaf and fruit extracts

Fig. (1): GAPDH gene expression

CaCo2: colorectal adenocarcinoma cell line **HPG2: liver carcinoma**
HEP2: larynx carcinoma **L: mulberry leaves extract**
F: mulberry fruits extract

The expression of p53 gene (proapoptotic gene) (Fig. 2) which act as tumor suppressor extracted from mRNA of three types of cancer cell lines (Hep-2 (Larynx carcinoma), HepG2 (liver carcinoma) and CaCo2 (colorectal adenocarcinoma)) treated with mulberry fruits and leaves extract was used as a detector of anticancer effect of mulberry. The results showed that the cancer cells treated with fruits and leaves extract were negative for p53 gene expression as the gene not detected comparing with positive cell control. The tested extracts not anticancer agent.



Detection of P53 gene in cancer cell lines post treatment with Leaf and Fruit extracts

Fig. (2): p53 gene expression
CaCo2: colorectal adenocarcinoma cell line **HPG2: liver carcinoma**
HEP2: larynx carcinoma **L: mulberry leaves extract**
F: mulberry fruits extract

Such results may be owed to that active phytochemicals in purified form may be powerful and have anticancer effect than whole extract. So fractionation of mulberry could be useful in protection against cancer. Many studies recorded anticancer effect of active substance extracted from mulberry. Kofujita *et al.* (2004) isolated 7, 20, 40, 60-tetrahydoroxy-6-geranylflavanone, a prenylated flavanone, from ethyl acetate extracts of *Morus alba* root. This prenylated flavanone exhibited cytotoxic activity against rat hepatoma cells. Chen *et al.* (2006) observed that the cyaniding 3-rutinoside and cyanidin 3-glucoside (anthocyanins extracted from *Morus alba* fruit*)* exert dose-dependent inhibitory effect on the migration and invasion, of highly metastatic A549 human lung carcinoma cells. Moreover, flavonoids (papyriflavonol A, kuraridin, sophoraflavanone D, sophoraisoflavanone A and broussochalcone A) isolated from medicinal plants (*Morus alba*, *Morus mongolica*, *Broussnetia papyrifera Vent*, *Sophora flavescens Ait* and *Echinosophora. koreensis Nakai*) showed cytotoxic activity against HepG2 cell line (Sohn *et al,.* 2004).

In conclusion, the results of this study indicate that, the dried mulberry fruits and leaves were nutritionally rich. Meanwhile, their extract

especially at high concentration showed strong antibacterial activity against *Staph. aureus* and *E. coli* in vitro and in mulberry juice. While their extract exhibit no anticancer activity.

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النشاط المضاد للميكروبات و للسرطان للمستخلص الميثانولى لثمار و أوراق التوت المجففة مع الأشارة لتركيبهم الكيميائي.

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أجريت هذه الدراسة لتقدير بعض المركبات الكيميائية الغذائية فى ثمار و أوراق التوت (الرطوبة, الرماد, البروتين، الألياف ,الدهون، الكربوهيدرات, الأحماض الأمينية والمعادن) وتقييم تأثير مستخلصهم الميثانولى كمضاد للميكروبات ومضاد للسرطان. أشارت النتائج إلى أن أوراق التوت لها جودة غذائية أكثر من الثمار. سجلت أوراق التوت المجفف محتوى أعلى من الرماد, البروتين، الأحماض الأمينية ، الألياف والمعادن من الثمار في حين كانت نسب الرطوبة والدهون أعلى في الثمار مما كانت عليه في الأوراق. بينما سجلت الكربوهيدرات ارتفاع طفيف فى الثمار عن الأوراق. ومع ذلك، كل منهما يعتبر غني من الناحية التغذوية. تم تقييم التأثير المثبط من التركيزات المختلفة (5 و 10 و 15 مجم / مللىDMSO) من ثمار وأوراق التوت على نمو و بقاء الإستافيلوكوكس أوريس و الإشيرشيا كولاى فى بيئة النمو السائلة وعصير التوت. أظهرت النتائج أن كلاً من مستخلصى الثمار والأوراق أظهرا تأثير مثبط على البكتيريا المستخدمة. أوضحت النتائج أن تركيز 15 مجم / مللى من مستخلص ثمار التوت يمثل التركيز الأمثل لتقليل الإشيرشيا كولاى و الإستافيلوكوكس أوريس فى بيئة النمو السائلة حيث أدى إلى خفض أعدادهم من $10^{10}X5$ و$10^{12}X5$ إلى $10^{2}X14$ و $10^{5}X4$ (خلية/ مللى) على التوالي. أحدثت التركيزات المختلفة (10 و 15 مجم / مللى) من مستخلص الثمار والأوراق إزالة تامة لميكروب الإستافيلوكوكس أوريس من عصير التوت و كذلك إنخفاض أعداد ميكروب الإشيرشيا كولاى من $10^{10}X5$ إلى $10^{2}X4$ و $10^{3}X3$ خلية/مللى على التوالي على تركيز 15 مجم/مللى. تم تقييم تمثيل جين p53 (الجين المثبط للأورام) من ثلاثة أنواع من الخلايا السرطانية (Hep-2(سرطان الحنجرة)، HepG2 (سرطان الكبد) وCaCo2 (سرطان القولون والمستقيم)) التى تم معاملتها بمستخلص ثمار و أوراق التوت لأستكشاف تأثيرهم المضاد للسرطان . واظهرت النتائج ان الخلايا السرطانية المعاملة بمستخلص الثمار والأوراق كانت سلبية لتمثيل جين p53 حيث أن الجين لم يتم رصدة مقارنة بالخلايا السرطانية الغير معاملة. المستخلص قيد البحث ليس له تأثير مضاد للسرطان.

الكلمات الدالة: ثماروأوراق التوت ، التركيب الكميائى, النشاط المضاد لكل من البكتريا و السرطان .

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