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Chemical And Microbiological Studies On Black Mulberry Fruits (*Morus Nigra*, L.)

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Abstract:

The black mulberry fruits (*Morus nigra, L.*) is reported to possess antioxidant effect due to presence of phenolic compounds and anthocyanins. Chemical composition, mineral contents, total phenols, total flavonoids, and total anthocyanins of black mulberry were evaluated. Phenolic compounds were also measured using High Performance Liquid Chromatography (HPLC). Inhibitory effect of black mulberry as a powder and its alcoholic extract on some pathogenic microorganisms such as *Escherichia coli* (DSM 30083), *Staphylococcus aureus* (DSM 1104), *Bacillus cereus* (DSM 315), *Salmonella sp.* (DSM 347), mold *(Aspergillus niger*) and yeast *(Candida albicans)* were determined. The results showed that the values of protein, fat, fiber, carbohydrates and energy value contents were 10.27, 3.48, 9.17, 7.51% and 44.36 Kcal/kg, (on dry weight basis), respectively. Black mulberry fruit contains a high mineral content such as potassium, calcium, phosphorus and magnesium. The values of total phenols, total flavonoids and total anthocyanins contents of mulberry fruit were 13.80±0.93, 61.40±0.35 and 3.70±0.61 mg/g, respectively. On the other hand, mulberry fruit contain different amounts of phenolic compounds such as pyrogallol, gallic acid, protocatechuic acid, caffeic acid, vanillic acid, caffeine, ferulic acid and syringic acid. The values were 223.30, 9.97, 49.31, 19.66, 7.15, 13.13, 15.89 and 80.12 mg/100 g, respectively. The inhibitory effect of tested microorganisms increased as black mulberry fruits concentrations increased by different rates. The methanolic mulberry extract showed a higher inhibitory effect than that of its powder.

Key words: Black mulberry- Chemical composition – Mineral content - Phenolic compounds- Antimicrobial effect.

Introduction

Black mulberry *(Morus nigra,* L., family *Moraceae*) belongs to the genus *Morus* which is widely distributed in Asia, Europe, North and South America and Africa. Mulberry is an economically important plant used for sericulture, as a feed for the domesticated silkworm, *Bombyx mori* (Awasthi *et al.,* 2004), and has a long history of medicinal use in Chinese medicine as a herbal medicine called "Sang Bai-Pi". The root bark, twigs and fruits which contain phenolic compounds are used as refreshing substances, are prescribed to treat cough, asthma, other chest complaints and rheumatism(Kumar and Gupta, 1996). Wrolastad, (2001) mentioned that mulberry fruits are rich in anthocyanins and should be exploited for the industrial production of natural color to be used in the food industry. In particular, they are known to contain cyanin, which is the red pigment that gives the fruit a red to purple color. The major anthocyanins found are cyanidin-3-glucoside and cyanidin-3-rutinoside. These pigments hold potential for use as dietary modulators of mechanisms for various diseases, and as natural food colorants. As synthetic pigments are unsafe, there is a demand for natural food colorants in the food industry. Phenolic compounds, present in all plants, are of great importance for food and beverages derived from plants, since these compounds are responsible for their organoleptic properties. As a consequence, they are closely related to the quality of such products, and thus their analysis is of considerable interest. Moreover, in recent years, numerous research studies have associated the consumption of foods rich in polyphenols with the Prevention of cardiovascular diseases, certain type of cancer and other diseases related to aging, thanks to their antioxidant properties (Borbalan *et al.,* 2003). Kutlu *et al.,* (2011) mentioned that deep-colored fruits are good sources both of phenolics, including anthocyanins and other flavonoids, and carotenoids. Mulberry fruits are rich in phenolics and have a unique delicious fruity, sour and refreshing taste. They have been used as a folk remedy to treat oral and dental diseases, diabetes, hypertension, arthritis and anemia. With the aim of finding, new sources of natural antioxidants, plants, fruits, vegetables and other plant materials that are known to possess antioxidant activity have been investigated. Kostic *et al.,* (2013) reported that the mulberry plant is rich in phenolic compounds, macro-elements (K, Ca, Mg, Na) and microelements (Fe, Zn, Ni). Phenolic compounds are found in all parts of the mulberry plant. The mulberry plant is of significant biological importance for its antioxidant and antimicrobial properties.

Among foremost health problems, infectious diseases account for 41% of the global disease burden along with noninfectious diseases (43%) and injuries (16%) (Noumedem *et al.,* 2013). The main reasons of

these infectious diseases are the natural development of bacterial resistance to various antibiotics (Westh *et al.,* 2004). The development of multidrug-resistant (MDR) bacteria takes place because of the accumulation of different antibiotic resistance mechanisms inside the same strain. Although, in previous decades, the pharmacological companies have produced a number of new antibiotics, but even then drug resistance has increased (Nascimento *et al.,* 2000). This situation has forced the attention of researchers towards plant and fruit products, in search of development of better-quality drugs with improved antibacterial, antifungal, and antiviral activities (Parekh and Chanda, 2007). According to world Health Organization (WHO), 80%of the World's population is dependent on the traditional medicine (Maiyo *et al.,* 2010).The bark and fruits of mulberry was reported to be used to expel tape worm and its extracts have been reported to have antibacterial and fungicidal activity (Nomura, 1988). The phenolic compounds of black mulberry showed moderate anti-oxidant and anti-bacterial properties. The results add to the use of phenolic compounds presence in Mulberries to partially explain their reported pharmacological activities which include use as refreshing substances and as antibacterial (Ofentse *et al.,* 2011).The aim of the present study suggested the nutritional value of mulberry fruits and its antimicrobial effects.

Material & Methods:

1. Samples

Black mulberry fruits (*Morusnigra*) were obtained from local market, Menoufia Governorate, Egypt.

1.1. Chemicals:

Folin-Ciocalteu reagent and standard substances including gallic acid, sinapic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid and dihydroxy benzoic acid were purchased from Sigma chemical co., St. Louis, USA, vanillic acid, ferrulic acid, rutinand quercetin were purchased from Fluka (St. Gallen, Switzerland). All reagents and standards were prepared using Milli-Q deionized water (Millipore, Bedford, USA). All other chemicals and reagents were of analytical reagent grade.

Microbiological cultures:

Bacterial, fungal and yeasts cultures used in this study involved *Escherichia coli* (DSM 30083), *Staphylococcus aureus* (DSM 1104), *Bacillus cereus* (DSM 315), *Salmonela sp.* (DSM 347) were obtained from Microbiological Resource Center "MIRCIN", Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Mold (*Aspergillusniger*) & yeast (*Candida albicans*) were obtained from

Department of Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt.

Methods:

Analytical Methods:

Moisture, Protein (N x 6.25 Keldahl method), fat (hexane solvent, Soxhielt apparatus), fiber and ash were determined according to the method recommended by A O A C (2010).

Carbohydrates and energy value:

Carbohydrate calculated by differences as follows:

% Carbohydrates = 100 - (% moisture + % protein + % fat + % ash + % fiber).

Energy value was estimated by the sum of multiplying protein and carbohydrates by 4.0 and fat by 9.0 according to FAO (1982).

Microbiological methods:

Preparation of mulberry fruit samples for microbiological analysis:

Ten grams of each sample were homogenized with 90 ml. of distilled water so as to give 0.1 dilutions. Then different dilutions ($1:10^{-1}$ to $1:10^{-6}$) were prepared to be used for microorganisms tests.

Staphylococcus aureus determined on Paird parker agar basemedia (ICMSF, 1996), While Molds and yeast, enumerated in potato dextrose agar (ICMSF, 1996), Coliform bacterial (Oxoid) enumerated on Endo agar media (WHO, 1988), *Salmonella sp*. &*Shigella* SS agar modified Oxoid according to Bryan, (1991)and *Bacillus cereus* determined on *Bacillus cereus* selective agar medium with supplement SR99 (Roberts, 1991).

Biochemical analysis:

- **Determination of total phenolic contents:**

Total phenolic contents (TPC) were determined spectrophotometrically by using Folin-Ciocalteu reagent (Kahkonen*et al.,* 1999). The absorbance was measured at 765 nm and results were expressed as mg of chlorogenic acid equivalents (CAE) per gram of dry extracts.

- **Determination of total flavonoids contents:**

Total flavonoids contents (TFC) was performed using a modified colorimetric method (Jia *et al.,* 1999). The absorbance was measured at 510nm and results were expressed as mg of rutin equivalents(RE) per gram of dry extract. Triplicate tests were conducted for each sample. Spectrophotometric measurements were performed using a Vis spectrophotometer (Janwey6300, Germany).

2.3.1. HPLC Analysis of phenolic compounds :

The HPLC system Perkin Elmer PE200 was composed of a binary pump, a column thermostat and an auto sampler. The mass spectrometer used was a 3200QTRAP MS/MS with ESI ionization (Applied Biosystems / MDSSciex, Foster City, USA). The experimental conditions where: mobile phase A: 50% acetonitrile, 50% acetic acid (0.5%); mobile phase B: 2% acetic acid; gradient elution: 0 min 30% A,70%B; 10 min 30% A,70% B; 30 min 100% A,0% B;35 min 100% A, 0% B; 40 min 30% A, 70% B for reconditioning of the system; flow rate: 0.7 mL/min; injection volume: 20 µL; ionisation: ESI negative; dwell time 50 ms; multi plereaction monitoring (MRM) transitions : gallic acid 169/125, dihydroxybenzoic acid153/109, sinapic acid 223/164, vanillic acid 167/123, caffeic acid 179/135, quercetin 301/151,chlorogenic acid 353/191,ferullic acid193//134,*p*-coumaric acid 163/119. Stock solutions of standards were diluted in the mobile phase to obtain working standard solutions. Concentrations of the compounds were calculated from chromatogram peak areas on the basis of calibration curves. The method linearity was assessed by means of linear regression of the mass of compounds injected vs. its peak area. All solvents were of HPLC grade and were filtered and degassed before use.

Statistical analysis:

Statistical analysis were performed by using Sigma Plot software SPSS (1998). Each experiment was performed 3times and the data are expressed as mean ± standard deviation (SD).

Results And Discussion

Data presented in table (1) show the chemical composition of black mulberry. It is clear to notice that moisture content recorded the highest content. The value was 87.35 % (on wet weight basis). While the values of protein, fat, fiber, carbohydrates and energy value contents were 10.27, 3.48, 9.17, 7.51and 44.36%,(on dry weight basis), respectively. On the other hand, black berry had low energy value, it being 129 Kcal/kg. The obtained data are in agreement with those of Singhal *et al.,* (2005 a,b). They reported that due to very high nutritional value, mulberry fruits are used for the health benefits of human beings. Also, a black mulberry (*M. nigra*) fruits contains nutrient elements of vital importance in human metabolism (Akbulut and Musazcan, 2009).

The minerals contents of black mulberry expressed as (mg/100g) are shown in table (2). The obtained data indicated that the highest mineral content of black mulberry recorded as potassium (K). The value was 1000 mg/100g. Black mulberry also contains high amounts of calcium (Ca), phosphorus (P) and Magnesium (Mg). The values were 135, 285 and 105mg/100g, respectively. The obtained data

are in agreement with those of Ercisli *et al.,* (2010) They found that black mulberry fruits contains essential macro-elements such as potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na), and micro-elements such as iron (Fe), and zinc (Zn).

Data given in table (3) show the total phenols, total flavonoid and anthocyanin contents of mulberry fruits expressed as (mg/g). It is clear to be mention that the values of total phenols, total flavonoid and anthocyanin contents of mulberry fruit were 13.80±0.93, 61.40±0.35 and 3.70±0.61 mg/g, respectively. These results are in agreements of Ozgen *et al.*,(2009). They reported that mulberry extract have higher content of phenolic compounds. Phenols possess a wide spectrum of biological activities and the results show that mulberry extracts could be good sources of these natural constituents. Imran *et al.* (2010), namely that the contents of total phenolics in mulberry fruits were 6.64 mg/100 g fresh mass (*Morus nigra*) and 7.55 mg/100 g fresh mass (*Morus alba*).Also, Danijela *et al.* (2013) mentioned that extracts of fresh mulberry fruits from South East Serbia, contain high levels of total phenols, flavonoids and anthocyanins, > 100 mg/100 g of fruits. The highest content of phenols was found in aqueous extract, flavonoids in ethanol extract and anthocyanins in ethanol / water extract. Extracts of mulberry have a high content of polymeric anthocyans.

Data presented in table (4) show the Phenolic compounds of mulberry fruits. It is clear to notice that mulberry fruit contain different amounts of phenolic compounds such aspyrogallol, gallic acid, protocatechuic acid, caffeic acid, vanillic acid, caffeine, ferulic acid and syringic acid. The values were 223.30, 9.97, 49.31, 19.66, 7.15, 13.13, 15.89 and 80.12mg/100g, respectively. On the other hand, sinapic acid did not detected .Zadernowski *et al.,* (2005) reported that the gallic acid, pyrocathehunic, vanillic acid, caffeic acid, o-coumaric acid, and p-coumaric acid, and ferulic acid acids in black mulberry fruits were as 27.3 mg/kg, 121.8 mg/kg, 6.5 mg/kg, 117.2 mg/kg, 212.7 mg/kg, 761.8 mg/kg, and 34.1 mg/kg, respectively. Also, these results are in agreements of Memon *et al.*,(2010).

Data given in table (5) shows the inhibitory effect of different concentrations of black mulberry fruits as a powder on some pathogenic microorganisms enumerated in liquid media. It is evident that the use of 0.4% black mulberry fruits powder recorded the highest inhibition value against *Candida albicans*, while the lowest recorded against *E. coli*. The values were 1.0×10^3 and 3.5×10^5cfu /g, respectively. In case of 0.8%, 1.2% and 1.6% black mulberry fruits powder, it could be indicated that the highest inhibition value was recorded against *Candida albicans*. The values were 3.0×10^2, 5.0×10^1 and 0.2×10^1cfu / g, respectively. While the lowest recorded against *Salmonella sp.* The values were 3.0×10^4,

2.0 x 10^3 and 1.5 x 10^3cfu / g, respectively. It could be concluded that the inhibitory effect of tested microorganisms increased as black mulberry fruits concentrations increased by different rats. The results are in agreement with the finding of Fukai et al., (2005). They found that black mulberry fruits powder had a markedly reduction of Staphylococcus aureus count.

The inhibitory effect of different concentrations of methanolic extract of black mulberry fruits on some pathogenic microorganisms enumerated in liquid media is shown in table (6). It is clear to evident that a complete inhibition (100%) of E. coli and Salmonella sp. was recorded with all tested methanolic extract of black mulberry fruits concentrations (0.4 %, 0.8 %, 1.2 % and 1.6 %). On the other hand, a complete inhibition (100 %) of Bacillus cereus was recorded at 1.2 % and 1.6 % methanolic extract of black mulberry fruits concentrations, respectively. But the lowest inhibition percentage was recorded with 0.4 % methanolic extract of black mulberry fruits. The value was 99.97 %.The maximum inhibition percentage of Staphylococcus aureus was recorded at 1.2 % and 1.6 % methanolic extract of black mulberry fruits being 99.99 % and 99.99 %, respectively. While the lowest one was recorded at 0.4 % methanolic extract of black mulberry fruits. The value was 99.96 %. On the other hand, a markedly reduction of Aspergillus niger and Candida albicans was observed especially at 1.6 % methanolic extract of black mulberry fruits concentration being 99.99 % and 100 %, respectively. While the lowest inhibition percentage was recorded at 0.4 % methanolic extract of black mulberry fruit. The values were 99.98 % and 99.99 %, respectively. These results are in agreement with Ofentse, (2011), who found that the methanolic extract of black mulberry had inhibitory activities against Staphylococcus aureus, Bacillus subtilis, Micrococusflavus, Streptococcus faecalis, Salmonella abony and Pseudomonas aeruginosa.

Table (1): chemical composition of mulberry fruits

Components	(W/W)%
Moisture	87.35
Protein	1.30
Fat	0.44
Fiber	1.16
Ash	0.95
Carbohydrates	8.80
Energy value (Kcal/g)	129

W/W = Wet weight D/W = Dry weight

Table (2): Minerals content of fresh black mulberry fruits

Minerals	Value (mg/100g^{-1})
Ca	135
Fe	6.0
Mg	105
P	285
K	1000
Na	60.0
Zn	3.0
Mn	7.0

Table (3):Total phenols, anthocyanin and flavonoid contents of mulberry fruits *(Morusnigra,* L.) expressed as (mg/g)

Total phenols	Total flavonoids	Anthocyanins
13.80±0.93	61.40±0.35	3.70±0.61

Each value is represented as mean ± standard deviation (n = 3).

Table (4): Phenolic compounds of mulberry fruits

Phenolic compounds	mg/100g of dry extract
pyrogallol	223.30
Gallic acid	9.97
Protocatechuic acid	49.31
Caffeic acid	19.66
Vanillic acid	7.15
Caffeine	13.13
Ferulic acid	15.89
Syringic acid	80.12
Sinapic acid	N.D

ND = Not Detected

Table (5): Inhibitory effect of different concentrations of black mulberry fruits powder on some pathogenic microorganisms enumerated in liquid media (Cfu/g)

Tested organisms	Control	Mulberry concentration %			
		0.4	0.8	1.2	1.6
Escherichia coli	1.0×10^6	3.5×10^5	2.0×10^4	9.0×10^2	3.8×10^1
Salmonella sp.	1.0×10^6	1.0×10^4	3.0×10^4	2.0×10^3	1.5×10^3
Bacillus cereus	1.0×10^6	3.6×10^4	1.4×10^3	1.4×10^3	8.5×10^2
Staphylococcus aureus	1.0×10^6	2.0×10^3	1.5×10^3	1.0×10^2	1.0×10^2
Aspergillusniger	1.0×10^6	2.5×10^3	1.4×10^3	1.2×10^2	0.5×10^2
Candida albicans	1.0×10^6	1.0×10^3	3.0×10^2	5.0×10^1	0.2×10^1

Table (6): Inhibitory effect of different concentrations of black mulberry fruits extract on some pathogenic microorganisms enumerated in liquid media (Cfu/g)

Tested organisms	Control	Mulberry concentration %			
		0.4	0.8	1.2	1.6
Escherichia coli	1.0×10^6	N.D	N.D	N.D.	N.D
Salmonella sp.	1.0×10^6	1.0×10^1	N.D	N.D	N.D
Bacillus cereus	1.0×10^6	3.0×10^2	4.0×10^1	N.D	N.D
Staphylococcus aureus	1.0×10^6	4.5×10^2	2.1×10^2	1.0×10^1	1.0×10^1
Aspergillusniger	1.0×10^6	2.0×10^2	1.5×10^2	1.0×10^2	0.7×10^2
Candida albicans	1.0×10^6	1.0×10^2	1.0×10^1	0.6×10^1	N.D

N.D.= Not detected.

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دراسات كيماوية وميكروبيولوجية على ثمار التوت الأسود

محمد مصطفى السيد ـ عادل عبد المعطى أحمد ـ عماد محمد الخولى
نفين محمد اسماعيل على شريف
قسم التغذية وعلوم الأطعمة- كلية الأقتصاد المنزلى ـ جامعة المنوفية

الملخص :

فى هذه الدراسة تم تقدير التركيب الكيميائي، والأملاح المعدنية والفينولات الكلية total phenols، الفلافونويدات الكلية total flavonoids، وصبغة الأنثوسيانين total anthocyanins لثمار التوت الأسود (*Morus nigra L.*). كما تم تقدير المركبات الفينولية بأستخدام جهاز الكروماتوجرافى السائل عالى الأداء (HPLC). ودراسة التأثير المثبط لثمار التوت الأسود فى صورة مسحوق ومستخلص على بعض الميكروبات المرضية مثل *Escherichia coli , Staphylococcus aureus, Bacillus cereus , Salmonella sp., Aspergillus niger and Candida albicans* وأظهرت النتائج أن قيم البروتين والدهون والألياف والكربوهيدرات ومحتوي قيمة الطاقة كانت ١٠.٢٧، ٣.٤٨، ٩.١٧، ٧.٥١٪ (على أساس الوزن الجاف) و٤٤.٣٦سعر حرارى/ كجم، على التوالي. وكانت قيم الفينولات الكلية والفلافونويدات الكلية وصبغة الأنثوسيانين لثمار التوت الأسود ١٣.٨٠ ± ٠.٩٣، ٦١.٤٠ ± ٠.٣٥ و ٣.٧٠ ± ٠.٦١ ملجم / جم على التوالي. ومن ناحية أخرى، وجد أن ثمار التوت تحتوي على كميات مختلفة من المركبات الفينولية مثل بيروجالول pyrogallol، حمض الجاليك gallic acid وحمض الكافيك caffeic acid، حمض بيوتوكاتشيوريك proto catechuic acid، وحامض فانيليك vanillic acid، والكافيينcaffeine، وحمض الفيريولك ferulic acid وحمض سيرينجيك syringic acid. وكانت القيم ٢٢٣.٣٠، ٩.٩٧، ٤٩.٣١، ١٩.٦٦، ٧.١٥، ١٣.١٣، ١٥.٨٩ و ٨٠.١٢ ملجم / ١٠٠جم، على التوالي. كذلك وجد أنه كلما زادت تركيزات ثمار التوت الأسود سواء فى صورة مسحوق أو مستخلص أدى ذلك لزيادة تثبيط الكائنات الحية الدقيقة المختبرة بنسب مختلفة .كماأظهرت النتائج المتحصل عليها أن مستخلص التوت له تأثير مثبط على الكائنات الحية الدقيقة بنسبة أعلى من ثمار التوت على هيئة مسحوق.

الكلمات الافتتاحية: التوت الاسود - التركيب الكيميائى- المحتوى من المعادن – المركبات الفينولية – التأثير المثبط على الكائنات الدقيقة.