JJBS

Volume 6, Number 4, December .2013
ISSN 1995-6673
Pages 258 - 265

Jordan Journal of Biological Sciences

Evaluation of Antioxidant Properties of *Morus nigra* L. Fruit Extracts [II]

Najlaa K. Issa[1,*] and Rihan S. Abd-Aljabar[2]

[1]*Chemistry Department, Faculty of Science, Dohuk University;*

[2]*Chemistry Department, Faculty of Science, Zakho University, Kurdistan Region of Iraq,Iraq*

Recieved: March 3, 2013 *Revised: May 6, 2013* *Accepted: May 24, 2013*

Abstract

Antioxidant activity for *Morus nigra* L. fruit extracts ethanol (Eth. ext.), flavonoid (F. ext.) and anthocyanin (A. ext.) and the isolated pigment (Pig. a) was evaluated using different methods such as (β-carotene-linoleic acid assay, reducing power assay, scavenging of hydrogen peroxide and total antioxidant capacity). The results proved that Pig. (a) was the most potent antioxidant followed by A. ext. and Eth. ext. Black mulberry fruits contained the highest amount of total phenolic content followed by Eth. ext., whereas flavonoid ext. contained the highest amount of total flavonoid content followed by black mulberry fruits. The effect of pH, temperature and storage had been studied to evaluate the remaining antioxidant capacity of the prepared extracts and Pig. (a). The results showed that the antioxidant capacity was decreased with increasing the time of boiling, storage and extreme pH values.

Key words: *Morus nigra* Extracts, Antioxidant Activity , β-Carotene-linoleic acid, Reducing Power, Scavenging of H_2O_2, Total Antioxidant Capacity, Stability.

1. Introduction

Oxygen is an element obligatory for life where living systems have evolved to survive in the presence of molecular oxygen, which has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events (Shinde *et al.,* 2006). Oxidative stress results when the balance between the production of ROS exceeds the antioxidant capability of the target cell (Ahmad *et al.,* 2009). The antioxidant defense system in most living cells is composed of two components: antioxidant enzymes (endogenous antioxidants), and small molecule antioxidants (exogenous antioxidant) (Mugwerua and Rusling, 2006). Diet plays a vital role in the production of the antioxidant defense system by providing essential nutrient antioxidants such as vitamin C, vitamin E, and β-carotene. Other antioxidant plant phenols are flavonoids and essential minerals form important antioxidant enzymes. For example, superoxide dismutase (SOD) contains zinc and glutathione peroxidase that contains selenium (Willcox *et al.,* 2004; Kumar *et al.,* 2008). Plants are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates (Hamid *et al.,* 2011).

Under ideal circumstances the body would be in a steady state with free radicals produced and quenched by the endogenous antioxidants. When an endogenous and exogenous antioxidant system can't balance off the free radicals generated in human cells, oxidative stress occurs. Oxidative stress can cause oxidative damage to lipids, proteins and DNA (Jing, 2006). The antioxidants could attenuate this oxidative damage of a tissue indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radical species (Goldberg, 2003).

Antioxidant activity of flavonoid polyphenols and other small molecules is dependent on bioavailability and redox potential which is closely related to its chemical structure (Pokorny *et al.,* 2001).

The antioxidant activity of anthocyanins is attributed to their ability to scavenge free radicals, and the binding with heavy metals such as iron, zinc, and copper. Anthocyanins are inducers of antioxidant enzymes such as glutathione-S-transferase (GST) and SOD (Hosseinian *et al.,* 2008). Besides their color features, anthocyanins have recently attracted even more interest due to their possible health attributes, such as a reducing risk of coronary diseases, reducing risk of stroke,

* Corresponding author. e-mail :saminmamr@yahoo.com.

anticarcinogen activity, anti-inflammatory effects and improved cognitive behavior (Zhang and Demain, 2005). Laleh *et al.* (2006) showed that increasing in pH, temperature or exposure to light was able to spoil the anthocyanin molecule. Hurtado *et al.* (2009) proved that the isolated anthocyanins had higher capacity to capture free radicals than ascorbic acid and the hydroxylation degree of the isolated rutinosides had great influence on the antioxidant capacity.

The mulberry belongs to the genus *Morus* of the family *Moraceae.* Mulberry trees have historically been used for leaf yield in sericulture. In addition, their fruit have been used in folk medicine (especially in Chinese medicine) to treat diabetes, hypertension, anemia and arthritis. Also, black mulberry fruits are used for treating mouth lesions in Turkey. Recently, red and black mulberries have gained an important position in the food industry due to the presence of anthocyanins (Ozgen *et al.*, 2009).

The literature survey revealed that no work has been done to assess the antioxidant activity of black mulberry fruits *Morus nigra* L. prevailed in Kurdistan region. Thereby, this study was conducted to determine the antioxidant activity. Moreover, it was deemed of interest to investigate the antioxidant activity of the prepared fruit extracts by comparing various methods including β -carotene-linoleic acid assay, reducing power assay and total antioxidant capacity, besides the determination of total phenolics and flavonoids contents. It was also important to evaluate the effect of heat treatment, pH and storage on the stability of antioxidant activity.

2. Materials and Methods

2.1. Collection of Plant Materials

Black mulberry fruits *Morus nigra* L. were collected in June, 2008 from Duhok-dam, Duhok city- Kurdistan Region of Iraq. The ripe fruits were kept in plastic containers at -10 ºC until the time of use (Pantelidis *et al*., 2007; Spada *et al*., 2008). The plant was botanically authenticated in Agriculture College –Forestery Department, University of Duhok.

2.2. . Chemicals

All chemicals used were of analytical grade. Tannic acid, ammonium molybdate tetrahydrate, potassium ferricyanide and Tween 20 were obtained from Fluka; qurecetin dihydrate were obtained from ROTH; β-carotene from USP; Folin- Ciocalteu reagent from Ajax; linoleic acid from Merck; α-tocopherol from USD and others were obtained either from Fluka or Sigma chemicals.

2.3. Preparation of Plant Extracts

The ethanol extract (Eth. ext.) of black mulberry fruits was prepared according to the method of Laleh *et al*. (2006). The flavonoid extract (F. ext.) of black mulberry fruits was prepared according to the methods of (Peach *et al*., 1955; Harborne, 1984; Andersen and Markham, 2006). Anthocyanin extract (A. ext.) was prepared according to the method of (Harborne, 1984; Schofs, 2004; Andersen and Markham, 2006). Chromatographic techniques were applied for purification and isolation of pigment (a) (Pig.(a)) from anthocyanin extract.

2.4. Antioxidant Activity of Black Mulberry Extracts and Pig. (a)

2.4.1. Total Phenolic Content (TPC)

Total phenolics content of black mulberry fruits and extracts were measured according to the methods that were involving Folin- Ciocalteu reagent and tannic acid as a standard (Slinkard and Singleton, 1997; Kaur and Kapoor, 2002). All samples were assayed in three replications. The same procedure was applied to all standard tannic acid solutions (0–2500 mg/50 ml), and standard curve was obtained. Results were expressed as milligrams of tannic acid equivalent per gram of extract (mg TAE/g).

2.4.2. Total Flavonoid Content (TFC)

Total flavonoid content was determined according to the method described by (Chang *et al*., 2002; Hsu, 2006). All samples were assayed in three replications. The same procedure was applied to all standard quercetin solutions (12.5–100 µg /ml) in methanol and standard curve was obtained**.** Results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g).

2.4.3. Scavenging of Hydrogen Peroxide (H_2O_2)

The ability of the black mulberry fruits extracts and Pig. (a) to scavenge hydrogen peroxide was determined according to the method of (Ruch *et al*., 1989; Oktaly *et al*., 2003). All samples were assayed in three replications. Results were expressed as milligrams of ascorbic acid equivalent per gram of extracts (mg AAE/g) .

2.4.4. Total Antioxidant Capacity

The phosphomolybdenum assay was performed according to the methods of (Prieto *et al*., 1999; Delouee *et al*., 2007). All samples were assayed in three replications. The antioxidant capacity of extracts was expressed as micrograms of α-tocopherol equivalent per milliliter of extracts using extinction coefficient of (4 x 10^3 M^{-1} cm^{-1}) according to Beer's law.

2.4.5. β–Carotene-Linoleic Acid Assay

In β-carotene -linoleic acid assay, antioxidant activity of extracts and of some common antioxidants such as α-tocopherol was determined according to the method of (Marco, 1968; Barriere *et al*., 2001) with slight modifications. Antioxidant activity (AA) was calculated as percentage of inhibition relative to the control (α-tocopherol sample).

2.4.6. Reducing Power Assay

The ability of extracts to reduce iron (III) was assessed by the method of (Oyaizu, 1986; Yildirim *et al*., 2001; Tsasi *et al*., 2006; Su *et al*., 2009). The increase in absorbance of reaction mixture was interpreted as increase in reducing activity of the extract and the results were compared with ascorbic acid as (positive control). All samples were assayed in three replications.

2.5. Effect of Temperature, pH and Storage on the Antioxidant Capacity

The black mulberry fruits extracts and Pig. (a) were heated at 50 and 100 ºC for (60 and 120 min) and the residual antioxidant capacity was determined using total antioxidant capacity method as previously described. For pH stability, the extracts were pre-incubated at different pH values (3, 5, 7, 9 and 11) and the residual antioxidant capacity was evaluated. The extracts were also stored in the dark at 5ºC, and the antioxidant capacity was determined at intervals of 30 days over a period of 90 days. All samples were assayed in three replications. The effect of temperature, pH and storage on the antioxidant capacity of extracts was expressed as micrograms of α- tocopherol equivalent per milliliter of extracts using extinction coefficient of ($4x10^3$ M^{-1} cm^{-1}) (Arabshahi *et al.*, 2007).

2.6. Statistical Analysis

The statistical analysis for all experiments was done by using (SAS, 2000). Means comparison was done by Duncan's Multiple Ranges Test under 1%, and figures were done by Graph Pad Prism 5 program.

3. Results and Discussion

3.1. Antioxidant Activity of Black Mulberry Extracts and Pig. (a)

3.1.1. Total Phenolic Content

Total phenolic content of black mulberry fruits, Eth., F. and A. ext. was determined using the calibration curve between concentration and absorbance at 760 nm of standard tannic acid as shown in Table 1 and Figure 1. Phenolic compounds are one class of antioxidant agents which considered as good proton donors resulting in free radical terminators and contributed to the antioxidant activities of plant (Garzon and Wrolstad, 2009). Mulberries are rich source of phenolics such as rutin, kaempferol, quercetin, isoquercetin and chlorogenic acid (Apak *et al.*, 2007; Turkoglu, *et al.*, 2007). A great variation in terms of total phenolics content was observed among black mulberry fruits and their extracts.

Table 1. Total phenolic content of black mulberry fruits, Eth., F. and A. ext.

Sample	Concentration (mg TAE/g)
Black mulberry fruits	20.19
Eth. ext.	14.26
F. ext.	5.36
A. ext.	3.38

The highest amount of total phenols was observed for black mulberry fruits (20.19 mg TAE/g) which was in agreement with the results that were obtained by Ercisli and Orhan (2007), where they reported that black mulberry fruits were rich in phenols. Also, Eth. extract was high in its phenols content (14.26 mg TAE/g).

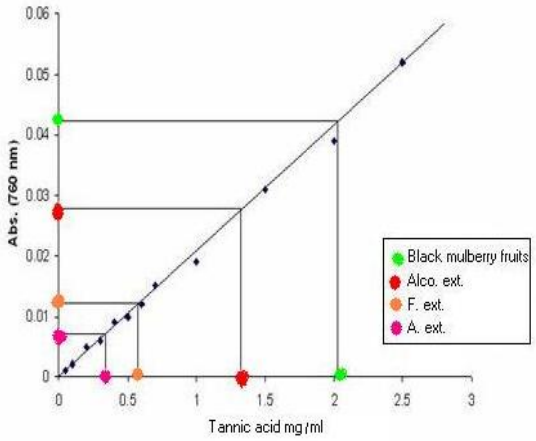


Figure 1. Total phenolic content of black mulberry fruits, Eth., F. and A. ext.

The high amount of phenols content in both black mulberry fruits and Eth. ext. might be due to their flavonoidal and non-flavonoidal phenolic compounds contents. Total phenols content of F.ext. had (5.36 mg TAE/g) due to the presence of flavonoid phenolic compounds only and A. ext. showed the least amount of total phenols content (3.38 mg TAE/g) due to the presence of anthocyanins only.

3.1.2. Total Flavonoid Content

Total flavonoid content of black mulberry fruits, Eth., F., A. ext. and Pig. **(**a**)** were determined using the calibration curve between concentration and absorbance at 415 nm of standard quercetin, as shown in Table 2 and Figure 2. Flavonoids are groups of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, and anticancer activities. They also act as inhibitors enzymes such as reducatase and xanthine oxidase. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidant (Chang *et al.*, 2002; Hsu, 2006).

Table 2. Total flavonoid content of black mulberry fruits, Eth., F. , A. ext. and Pig. (a)

Sample	Concentration (mg QE/g)
Black mulberry fruits	1.11
Eth. ext.	1.17
F. ext.	1.26
A. ext.	0.94
Pig. (a)	0.26

The presence of high phenol and flavonoid contents in black mulberry had contributed directly to the antioxidant activity by neutralizing the free radicals (Umamaheswari and Chatterjee, 2008). Aluminum chloride colorimetric method was used for flavonoids determination. Figure 2 was revealed that F. ext. had the highest total flavonoid content (1.26 mg QE/g), followed by black mulberry fruits and Eth. ext. which contained approximately equal amounts of total flavonoids content (1.11 mg QE/g) and (1.17 mg QE/g), respectively.

This could be due to that ethanolic solvent that can extract most plant components especially phenolic compounds (Harborne, 1984). These results are in agreement with those obtained by Ercisli and Orhan (2007) who reported that total flavonoids content in black mulberry fruits was (2.76 mg QE/g) of extract. Total flavonoids content of A. ext. (0.94 mg QE/g) was lower than the forgoing ones indicating the presence of anthocyanins only. The least amount of total flavonoids content was found in Pig. (a) (0.26 mg QE/g) of extract containing one pigment only.

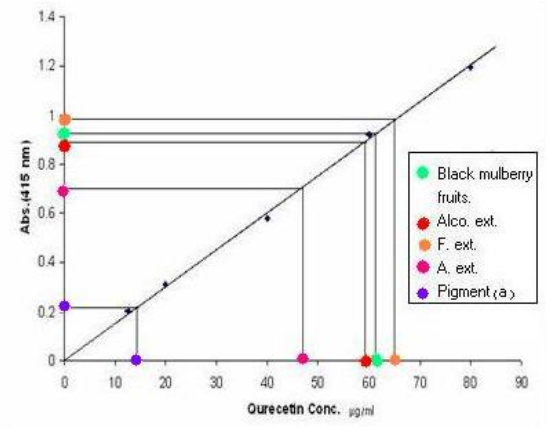


Figure 2. Total flavonoid content of black mulberry fruits, Eth., F., A. ext. and Pig. (a).

3.1.3. Scavenging of Hydrogen Peroxide

The ability of the Eth., F., A. ext. and Pig. **(a)** to scavenge H_2O_2 was determined using the calibration curve between concentration and absorbance at 230 nm of standard ascorbic acid, as shown in Table 3 and Figure 3. Since antioxidant compounds present in black mulberry extracts which are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O (Shon *et al.*, 2007). Figure 3 illustrates that the strongest anti - H_2O_2 activity was observed for F. ext. (227.22 mg AAE/g) followed by A. ext. (208.37 mg AAE/g) which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide and neutralizing it into water (Umamaheswari and Chatterjee, 2008). Eth. ext. and Pig. (a) exhibited weak scavenger H_2O_2 activity (199.10 mg AAE/g) and (191.90 mg AAE/g), respectively. The results showed that there was a strong correlation between total phenol content and scavenging of hydrogen peroxide.

Table 3. The ability of Eth., F., A. ext. and Pig. (a) to scavenge H_2O_2.

Sample	Concentration (mg AAE/g)
Eth. ext.	199.10
F. ext.	227.22
A. ext.	208.37
Pig. (a)	191.90

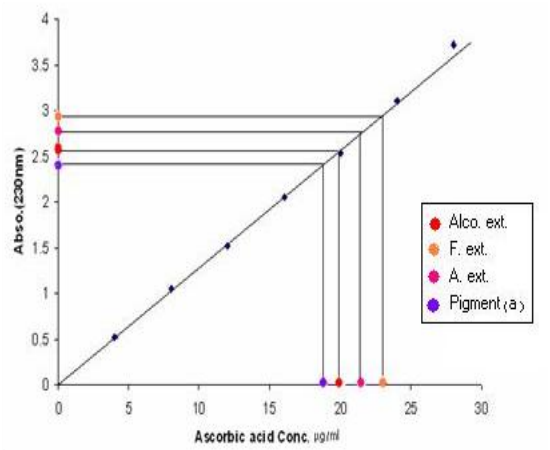
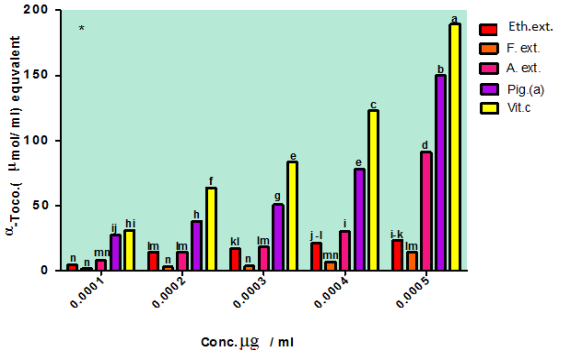


Figure 3. The ability of Eth., F. , A. ext. and Pig. (a) to scavenge H_2O_2.

3.1.4. Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the samples of Eth., F., A. ext. and Pig. **(a)**. The antioxidant capacity of the extracts was expressed as micromoles of α-tocopherol equivalent per milliliter of extracts using the calibration curve between micrograms of α-tocopherol equivalent and the concentration determined from Beer's law using extinction coefficient of (4×10^3 M^{-1} cm^{-1}) at 760 nm, as shown in Figure 4. The extracts were demonstrated electron- donating capacity and, thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products (Delouee *et al.*, 2007). Figure 4 illustrates that Pig. (a) was significantly exhibited the highest degree of activity at different concentrations especially at 0.0005 µg /ml followed by A. ext. compared to ascorbic acid. This activity might be attributed to the presence of phytochemicals (antioxidant secondary metabolites) (Banso, 2009). Eth. ext. exhibited significant degree of antioxidant capacity followed by F. ext. due to the presence of phenolic compounds which had redox properties allowed them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also had a metal chelating potential (Javanmardi *et al.*, 2003).



* Column followed by the same letter are not significantly different at 1% level based on Duncan's Multiple Rang Test.

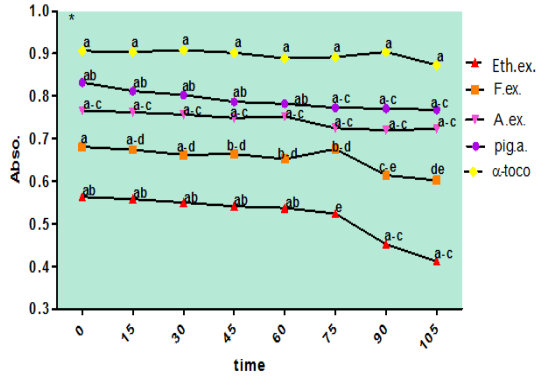
Figure 4. Total antioxidant capacity of Eth., F., A. ext. and Pig. (a).

3.1.5.β–Carotene-Linoleic Acid Assay

In β-carotene-linoleic acid assay, antioxidant activity (AA) of Eth., F. , A. ext. and Pig. **(a)** was compared with antioxidant activity of some common antioxidants such as α-tocopherol when subjected to thermal auto oxidation at 50 ºC. The AA was shown in Table 5 and Figure 5.

Table 5. Antioxidant activity of Eth., F. , A. ext. and Pig.(a) as antioxidant compared to α-tocopherol.

Sample	A_i	A_t	A_i'	A_t'	AA%
Eth. ext.	0.496	0.412	0.379	0.207	51.20
F. ext.	0.681	0.602	0.379	0.207	54.10
A. ext.	0.766	0.723	0.379	0.207	75.00
Pig. (a)	0.832	0.768	0.379	0.207	62.8
α-tocopherol	0.906	0.873	0.379	0.207	80.9



*The same letters are not significantly different at 1% level based on Duncan's Multiple Rang Test.

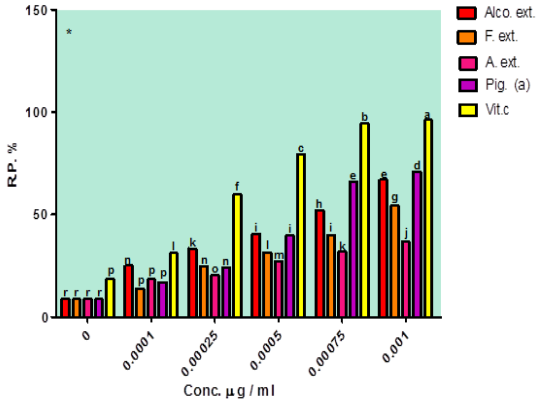
Figure 5. Antioxidant activity of Eth., F., A. ext. and Pig. (a).

The results indicated that the major contributors among individual extracts to AA could be Pig. (a) (62.8%) followed by A. ext. (75 %). Regarding the structure of these compounds, they possess a high degree of hydroxylation and methoxylation on their aromatic rings which suggests their higher contribution to AA of black mulberry antioxidant (Lachman *et al.*, 2009).

Flavonoid ext. showed significant AA (54.10%) especially at zero time which, then decreased when temperature elevated. The obtained results were in agreement with the reports of Zhshen *et al.* (1999) who confirmed the presence of total flavonoids espically rutin, quercetin, isoquercetin and luteolin in mulberry plants. These compounds are the major phytochemicals responsible for antioxidant activity of mulberry plants. Eth. ext. showed a moderate activity (51.20%) due to the presence of polyphenolic compounds in black mulberry fruits especially chlorogenic acid (Lin and Tang, 2007). Positive correlation between total phenol and antioxidant activity was found by many researchers (Brown *et al.*, 2005; Yang *et al.*, 2009; Garzon and Wrolstand, 2009).

3.1.6. Reducing Power Assay

In this assay, the ability of Eth., F. , A. ext. and Pig. **(a)** to reduce iron (III) to iron (II) was determined and compared to that of ascorbic acid, which is known to be a strong reducing agent. The percentage reduction (R.P. %) of the samples was shown in Figure 6.



* Column followed by the same letters are not significantly different at 1% level based on Duncan's Multiple Rang Test.

Figure 6. Reducing power of Eth., F. , A. ext. and Pig. (a) as a strong reducing agent compared to ascorbic acid.

Kaur and Arora (2008) determined reducing power of Eth., F., A. ext. and Pig. (a) and, compared to ascorbic acid, they considered it as a strong reducing agent. Data were shown that Eth., F., A. ext. and Pig. (a) possessed some degree of hydrogen donation capacity in a concentration dependent manner. The reducing power was increased with increasing concentration, but the capacities were inferior to that of ascorbic acid.

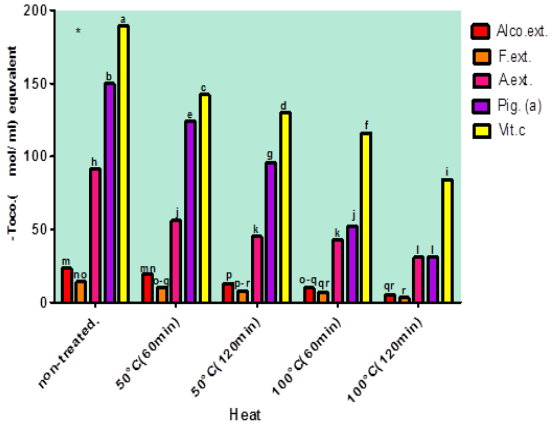
Pigment (a) was the most potent reducing agent followed by Eth. ext., F. and A. ext. due to the highest amount of total phenolics and phenolic acids. Phenols that have more number of hydrolysable groups (OH groups) attached to the ring acting as more powerful reducing agent considered as good proton donors resulting in the termination of free radicals chain reactions (Kaur and Arora, 2008). Hence, this may accelerate the conversion of H_2O_2 to H_2O (Ruch *et al.*, 1989). At high concentration, Pig. (a) showed a high potential for being a proton donor, wherever, A. ext. was the weakest in its activity due to the low content of total phenols. Similar relation between iron (III) reducing activity and total phenol content had been reported (Benzi and Szeto, 1999)

3.2. Effect of Temperature, pH and Storage on the Antioxidant Capacity

It is well known that many factors such as antioxidant concentration, temperature and pH of the media, processing treatment and storage are strongly influence the antioxidant capacity (Arabshahi *et al.*, 2007). In this study all extracts and Pig.(a) was subjected to thermal , pH and storage studies. Figure 7 shows the effect of temperature on the antioxidant stability of all extracts and Pig. (a).

The antioxidant capacity was decreased with increasing the time of boiling. This reduction was reached to 20.66% for Pig. **(a)** and to 34.06% for A. ext. when incubated at 100 ºC for 120 min.. This observation indicated that anthocyanins were not stable at high temperature which reasoned to the speed destruction of anthocyanins at high temperatures (Arabshahi *et al.*, 2007).

Antioxidant capacity of Eth. ext. was reduced to 81.70% followed by F. ext. 72.18% when incubated at 50 ºC for 60 min. The antioxidant capacity was decreased with increasing the time of boiling. The reduction activity was reduced for Eth. ext. and F. ext. when incubated at 100 ºC for 120 min which might be explained to the loss of naturally occurring antioxidants or phytochemicals present in the extract (Arabshahi *et al.*, 2007).

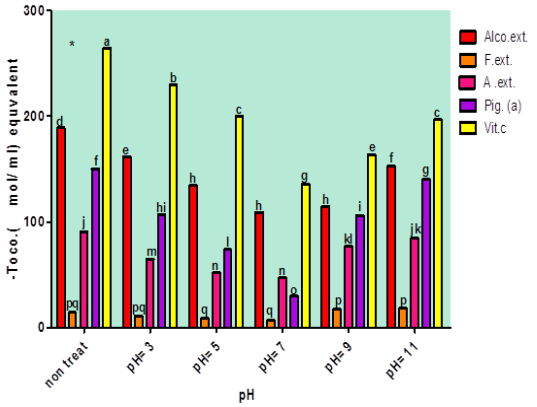


* Column followed by the same letters are not significantly different at 1% level based on Duncan's Multiple Rang Test.

Figure 7. Effect of temperature on the antioxidant capacity of Eth., F., A. ext. and Pig. (a).

The influence of pH on the antioxidant stability of all extracts and Pig. (a) is shown in Figure 8 . The antioxidant capacity of all extracts and Pig. (a) gradually decreased with minimum value at pH 7 followed by continuous increase at alkaline pH, indicating strong dependence of antioxidant capacity of extract to the pH of the system. At pH 3 no significant effect was noticed in Pig. (a) as well as A. ext. comparing to vit. C. Anthocyanin can be found in different chemical forms which depend on the pH of the solution. At low pH 1, the flavylium cation (red color) was the predominant species and contributed to the purple and red colors. The antioxidant capacity of Pig. (a) and A. ext. was reduced at pH 5-7. At pH 7 the antioxidant capacity of Pig. (a) and A. ext. was reduced to 19.80% and 51.86%, respectively.

It had been shown that simple anthocyanins were unstable and quickly decolorized by hydration at the 2-position of the anthocyanidin skeleton. At alkaline pH 11, the antioxidant capacity of Pig. (a) and A. ext. was increased to 93.30% and 93.07%, respectively, due to the increased amount of anhydrous base at alkaline pH (Furtado *et al.*, 1993). The antioxidant capacity of Eth. ext. was least effected by pH (87.23%, at pH 3) followed by F. ext. (75.35%, at pH 3).

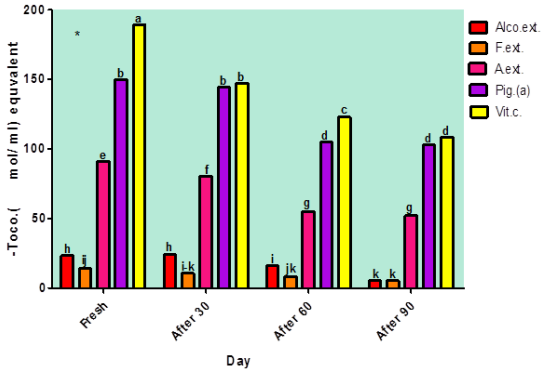


* Column followed by the same letters are not significantly different at 1% level based on Duncan's Multiple Rang Test.

Figure 8. Effect of pH on the antioxidant capacity of Eth., F., A. ext. and Pig. (a).

This observation could be attributed to the presence of antioxidant secondary metabolites other than anthocyanins like polyphenolic compounds in black mulberry fruits, especially chlorogenic acid (Lin and Tang, 2007).

The effect of storage on the antioxidant stability of all extracts and Pig. (a) was also studied up to 3 months at intervals of 30 days as shown in Figure 9. The antioxidant capacity of all extracts and Pig. (a) were gradually reduced with minimum value during 90 days period. The antioxidant capacity of Pig. (a) and A. ext. were unaffected by storage for 30 days and slightly reduced after 60 days only. Their antioxidant capacity after 90 days period were reduced to 68.66% and 57.14%, respectively, which was approximately similar to the antioxidant capacity of vit. C. with a reduction value of 57.25% after 90 days period storage.



* Column followed by the same letter are not significantly different at 1% level based on Duncan's Multiple Rang Test.

Figure 9. Effect of storage on the antioxidant capacity of Eth., F., A. ext. and Pig. (a).

Antioxidant capacity of Eth. and F. ext. was significantly affected by storage for 30 days and reduced after 60 days and their antioxidant capacity after 90 days period were reduced to 23.40% and 36.97%, respectively. These results were in agreement with the reports of Arabshahi *et al.*, (2007) who noticed that the

remaining activity of anthocyanin black mulberry extracts was about 65% after three months storage indicating that it could be still considered as a source of natural antioxidants.

4. Conclusions

Based on the obtained results, it may be concluded that *Morus nigra* fruits are a well recognized source of secondary metabolites like flavonoids and anthocyanins which considered as an excellent antioxidants. The antioxidant activities of *Morus nigra* fruits extracts varied with the test method . Pigment (a) showed a strong total antioxidant capacity , reducing power ability and inhibition of ß-carotene bleaching. Flavonoid ext. exhibit a suitable H_2O_2 scavenging activity which may be correlated with the high amount of polyphenolic content especially flovonoids and anthocyanins. Increasing the time of boiling, storage , and extreme pH values decreased total antioxidant capacity of all extracts.

References

Ahmad IM , Abdalla MY, Mustafa NH, Qnais EY and Abdulla FA. 2009. *Datura* aqueous leaf extract enhances cytotoxicity via metabolic oxidative stress on different human cancer cells. *Jordan J Biol Sci.,* **2**: 9-14.

Andersen QM and Jordheim M .2006. The anthocyanins . In: Andersen QM and Markham KR (Eds), **Flavoniods Chemistry, Biochemistry and Applications.** New York, pp. 471-553.

Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B, Berker I and Ozyurt D. 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *J Molecules*, **12**: 1496-1547.

Arabshahi DS, Devi DV and Urooj A. 2007. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. *Food Chem.,* **100**: 1100–1105.

Banso A. 2009. Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *J Med Plants Res.,* **3**: 082-085.

Barriere C, Centeno D, Lebert A, Leroy-Setrin S, Berdague, JL and Talon R. 2001. Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. *Microbiol Lett.,* **201**: 181–185.

Benzie IF and Fand Szeto YT. 1999. Total antioxidant capacity of tea by the ferric reducing antioxidant power assay. *J Agri Food Chem*., **47**: 633–636.

Brown C R. 2005. Antioxidant in potato. J *Amer Potato,***82**: 163–172.

Chang C, Yang M, Wen H and Chern J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*., **10**: 178-182.

Delouee SAW and Urooj A. 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chem.,* **102**:1233-1240.

Ercisli S and Orhan E. 2007. Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. *Food Chem.,* **103:** 1380–1384.

Furtado P, Figueiredo P, Neves, HC and Pina F. 1993. Photochemical and thermal degradation of anthocyanidins. *J Photochem Photobiol A: Chem*. ,**75**:113-118.

Garzon GA and Wrolstad RE. 2009. Major anthocyanins and antioxidant activity of Nasturtium flowers (*Tropaeolum majus*). *Food Chem*., **114**: 44–49.

Goldberg G. 2003. **Plants: Diet and Health**. Blackwell Science, USA.

Hamid K, Sultana S, Urmi KF, Obayed Ullah M, Zulfiker AM and Hossain A . 2011. *In vitro* Free radical scavenging and brine shrimp lethality bioassay of aqueous extract of *Ficus racemosa* seed. *Jordan J of Biol Sci.,* **4**: 51-54.

Harborne JB.1984. **Phytochemical Methods**. New York ,Wiley, pp. 1-70.

Hosseinian FS, Li W and Beta T. 2008. Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chem.,* **109**: 916–924.

Hsu C. 2006. Antioxidant activity of extract from *Polygonum aviculare* L. *Biol Res.,* **39**:281-288.

Hurtado NH, Morales AL, Gonzalez-Miret LG, Escudero-Gilete, ML and Heredia FJ. 2009. Color, pH stability and antioxidant activity of anthocyanin rutinosides isolated from tamarillo fruit (*Solanum betaceum* Cav.). *Food Chem.,* **117**: 88–93.

Jayaprakasha GK, Singh RP and Sakariah KK. 2001.Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. *Food Chem.,* **73**:285-290.

Jing P. 2006. Purple corn anthocyanins: chemical structure chemoprotective activity and structure/ function relationships. Ph.D. Thesis. Graduate School. Ohio State University, USA.

Kaur C and Kapoor HC. 2002. Antioxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Technol*., **37**:153-161.

Kaur R and Arora S. 2008. Investigations of antioxidant activity of methanol extract of *Chukrasia tabularis* A. Juss. leaves. *J Clin Med.,* **3**: 200-205.

Kumar V and Chauhan S. 2008. Mulberry: Life enhancer. *J Med Plants Res*., **2**: 271-278.

Lachman J, Hamouz K, Sulc M, Orsak M, Pivec V, Hejtmankova A, Dvorak P and Cepl J. 2009. Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity. *Food Chem.,* **114:** 836–843.

Laleh GH, Frydoonfar H, Heidary R, Jameel R and Zare S. 2006. The effect of light, temperature, pH and species on stability of anthocyanin pigments in four *Berberis* species. *Pakistan J Nut.,* **5**:90-92.

Lin JY and Tang CY. 2007. Determination of total phenolics and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.,* **101**: 140–147.

Marco GJ. 1968. A rapid determination method for evaluation of antioxidants. *J Ame Oil Chem Soc*., **45**: 594-598.

Mugweru A and Rusling J. 2006. Studies of DNA damage inhibition by dietary antioxidants using metallopolyion / DNA sensors. *J Electro.,* **18**: 327 – 332.

Oktay M, Gulcin I and Kufreviogl U. 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebnsm- Wiss. U.- Technol. **33**:263-271.

Oyaizu M. 1986. Antioxidative activity of browning products of glucosamine fractionated by organic solvent and thin-layer

chromatography. *Nippon Shokuhin Kogyo Gakkaishi.* **35**:771–775.

Ozgen M, Serc S and Kaya C. 2009. Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *Morus rubra* fruits. *Scientia Horticulturae*, **119**: 275–279.

Pantelidis GE, Vasilakakis M, Manganaris GA and Diamantidis G. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chem*., **102**:777-783.

Peach K and Tracey MV. 1955. **Modern Methods of Plant Analysis.** Springer-Verlag Bertin ,Germany.

Pokorny J, Yanishlieva N and Gordon M. 2001. **Antioxidants in Food: Practical Applications**, England, pp. 88.

Prieto P, Pineda M, and Aguilar M. 1999. Spectrophotometric quantization of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*., **269**: 337–341.

Ruch RJ, Cheng SJ and Klaunig JE. 1989. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogen*. **10**:1003-1008.

Schofs B. 2004. Determination of pigments in vegetables .*J Chrom*., 1**045**: 217-226.

Shinde A, Abd Elmouttale AT, Ashraf T and Mostafa U . 2012. Effect of tomato and guava juices on oxidative stress in rats after strenuous exercise. *Jordan J of Bio Sci.*, **5**: 167 – 174.

Shon MY, Lee J and Choi JH. 2007. Antioxidant and free radical scavenging activity of methanol extract of chungkukjang. *J Food Composition and Analysis*, **20**: 113-118.

Slinkard K, and Singleton VL. 1997. Total phenol analysis: Automation and comparison with manual methods. *Amer J. Enol. Viticult.*, **28**: 49–55.

Spada PDS, Souza GGN, Bortolini GV, Henriques JAP and Salvador M. 2008. Antioxidant, mutagenic, and antimutagenic activity of frozen fruits. *J Med Food*, **1**: 144-151.

Su XS, Wang ZY and Liu JR. 2009. *In vitro* and *in vivo* antioxidant activity of *Pinus koraiensis* seed extract containing phenolic compounds. *Food Chem.*, **117**: 681–686.

Tsasi SY, Huang SJ and Mau JL. 2006. Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. *Food Chem.*, **98**:670–677.

Turkoglu A, Duru ME and Mercan N. 2007. Antioxidant and antimicrobial activity of *Russula delica* Fr: An Edidle wild mushroom. *Eurasian J of Anal Chem.*, **1**: 54-67.

Umamaheswari M and Chatterjee TK. 2008. *In vitro* antioxidant activities of the fracions of *Coccinia grandis* L. leaf extract. *Afr J Tradi.*, **5**:61-73.

Willcox J K, Ash SL. and Catignani GL. 2004. Antioxidants and prevention of chronic disease. *Critical Rev Food Sci Nut.*, **44**:275–295.

Yang J, Martinson TE and Liu RH. 2009. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem.*, **116**: 332–339.

Yildirim A, Mavi A and Kara AA. 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J Agr Food Chem*., **49**: 4083–4089.

Zhang L and Demain AL. 2007. **Natural Products: Drug Discovery and Therapeutic Medicine**, Springer,USA.

Zhishen J, Mengcheng T and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*., **64**: 555–559.