Studii şi Cercetări	Martie 2018	Biologie	27/1	35-43	Universitatea"Vasile Alecsandri" din Bacău

BIOACTIVITY AND ANTI-OXIDANTS ACTIVITIES OF THE ETHANOLIC EXTRACT OF FRUITS AND LEAVES OF *M. NIGRA* AND *M. ALBA* FROM OUED SOUF AREA (ALGERIA)

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Key words: *M. nigra , M. alba, nutrional content, phenols, flavonoïds, bioactivity*

INTRODUCTION

This study aims to compare between the nutrional content and anti-oxidants ,the bioactivity and the anti-oxidation that found in the ethanolic extract of fruits and leaves (*Morus nigra, Morus alba*) which found in the region of Qued Souf.

The fruit represent the primary source of nutritional elements and health-promoting compounds. It deserve special attention great. Mulberry is a wild plant that has a great importance in nutrition and traditional medicine. Which is the only food of silkworms (Maawn, 2011). It is widely grown (Ozrenk et al., 2010), is involved in the genus Morus, and the family Moraceae.

The cultivation of mulberry trees started recently in el oued region, which be located in the desert north, in grand erg oriental and characterized by desert climate. To the best of our knowledge, there have been no studies on The cultivation of mulberry trees and the antioxidant potential of the *M.alba* and *M.nigra*, grown under the desert climatic condition. Therefore, the aim of this work was to examine the comparative in contents of each of carbohydrate, protein, phenolic, flavonoid composition, antioxidant activity and antimicrobial activity of fresh fruit and leaves extracts of *M. alba* L and *M. nigra* from el oued region.

MATERIALS AND METHODS

Study area:

Oued souf is located between longitude 8 ° and 6 ° east and 30 ° and 34 ° north latitudes. In the south-east of Algeria (Najah, 1971). It is characterized by a hot dry desert climate in summer and cold in winter .

Plant material :

The study was conducted on two speises of mulberry (*M. nigra* and *M. alba*), which belong to the Moraceae family, where the mulberry tree is leafy. The branches are spread in a circular or pyramidal manner, with an average of 8-10 m and up to 20 m as trees Sparse and if they are in the form of intensive cultivation, the tree is about 25 years old (Leon and Mador, 2001; Fortier, 2003).

1. Preparation of Plant sample:

1- The leaves and fruits of the types of Mulberry plant of were collected in from the city of Hassi Khalifa during the fruiting period in April 2015 El-Oued, Algeria (33°25'0"N, 6°55'0"E).

A the collected plant material was air-dried in darkness at room temperature.

a grinded and dried the situation of powder samples in clean glass and symbolized by:

MA: fruits of *M. alba* .

MN: fruits of *M. nigra*.

FMA: leaves *M. alba* .

FMN: leaves *M. nigra*.

Figure 1. Photo powder dry fruits and leaves both white and black mulberry

2. Preparation and extracts:

The ethanolic extract was prepared for the four samples according to the method (**Fawzia and Al-Shanaw, 2009**). was prepared by maceration technique, Therefore, 25 mL 98% ethyl alcohol were added to 5g of each sample, Leave for 48 hours at room temperature in a dark place. After filtration, the extract was concentrated using a rotary evaporator at a maximum temperature of 40°C, a was stored in a freezer at -4°C until study.

Measurements:

Determination of pH

Total acidity of the samples was measured by Marx (1999) using a pH meter device.

Determination of the electric conductivity (EC)

Electrical conductivity was measured according to the method of (Jones, 2001) using a conductive meter device.

3. Determination of food content

Quantification of proteins

The measures of the protein container are made by Bradford's method. According to the method described by Snyder et Desborough, 1978 and the use of the standard curve of protein BSA (Kirchenbaum, 1970). Where it was obtained (Y = 0.076x - 0.009 and r2 = 0.998).

Quantification of carbohydrates

The method has been relied upon (Dubois 1956) in estimating the total sugar content which is mainly based on concentrated sulfuric acid, the use of the standard curve of glucose as a standard solution. According to the method described by Sailaja et al 2012 . Where it was obtained Y = 0.001x - 0.003 and r2 = 0.99.

4. Determination of bioactive substance:

Determination of total phenolice content :

Determination of total polyphenol contents of the extract was based on the method (Singleton et Rossi ., 2009). described by Chavanand Rekha (2013). Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of plant material.

Determination of total flavonoid content

Determination of total flavonoid of the extract was based on the method Mbaebie et al (2012). using AlCl3 as complexing color and Standard solution of quercetin .

Determination of Carotenoid:

Carotenoids were estimated according method by Mackinney (1941) . reading the absorbance extracted acetone at 470 nm, 647 nm and 663 nm and applying the following relationship:

Carotenoids = 5 × DO470nm + 2.846 × DO663 nm-14.876×DO647 nm.

Anthocyanins estimation

Anthocyanins were estimated by method (Mancinelli et al., 1975; Reay et al., 1998) modified, where 1 g of the sample was dissolved in a mixture of (HCl, Ethanol) by 1/9 v / v and left for 48 hours, Then separated by the centrifuge and read at the length of 530 nm and 657 nm and estimated the amount of entocyanins according to the following relationship:

A=A530 nm-(A657 nm /3).

5. Statistical analysis

Estimated by one-way analysis of variance (ANOVA) followed by least significance difference (LSD) test. Probability values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

1. Results of pH and electrical conductivity

The results indicated in (Table 1) shows that the acidity is ALMOST moderate to the extracts, Tend to base white mulberry leaves extract and acidic extract of the fruits of black berries. restricted between (5.71-8.11) and There is a great convergence of the leaf both types.

This finding is in accordance with observation of (Elmac et Altuq., 2002; Iqbal et al., 2010). as regards the values of electrical conductivity they were close to the fruit of the two types while moderate in the leaves of black and low in that *M. alba* leaves.

This difference is due To the genetic diversity factors and different parts of mulberry (Scalzo et al., 2005).

Table 1. pH, electrical conductivity values of the extract of leaves and fruits of plant berries (*M. alba* and *M. nigra*)

F M N	M N	F M A	M A	Plant part
7.94	5.71	8.11	7.42	**PH**
3.096	3.228	1.835	3.246	**(ms/CM2)EC**

Results of food content

Protein content :

The results figure 2 showed no significant difference in protein content of fruits and leaves both types (*M.alba, M. nigre*).

Where the amount of protein was close. Ranging from 2.678 to 2.774 mg / g dry matter. Also the results showed increase value in the *M.alba* leaves, and the lowest in the fruits of *M.alba*. These results agree with the findings of Vu et al (2011).

The slight difference is due in protein content to the environmental and dirt conditions and time of reaping the fruits (Guven., 2012). Leaf crude protein content varies according to variety, age of plant and growing conditions (Sanchez, 2001). Yao et al. (2000) found mulberry leaf CP values to be slightly higher in spring than in the fall (21.1% vs 20.9%).

Carbohydrate content :

Significant differences were found in values of carbohydrate in the two genotypes of fruits as compared with the leaves. was observed in dried leaf powder fairly low carbohydrate content.

Where an estimated (1.191 -1.179) mg/g derived from leaves *M.alba* and *M.nigra* respectively and estimated (1.383 - 1.488) mg/g derived from fruits *M. alba* and *M. nigra* respectively (figure 3). These results are in agreement with those obtained by (Iqbal et al., 2010).

The difference in the high carbohydrate content in the fruit is due to the fact that the plant stores the organic matter contained in the fruit, while the plant uses leaf content in its biological processes (Abeles et Takeda, 1990).

Polyphenol composition

The results figure 4 shows that the amounts of polyphenol are variable between two species in fruits and leaves. also show significantly polyphenol content in leaves *Morus nigra* compared with the fruits *M. alba*, fruits *M. nigra* and levaes *M. alba*. Significant increase in polyphenol content in fruits *M. alba* compared with content fruits *M. nigra* and levaes *M. alba*.

The results figure 5 showed significant differences in flavonoid content in the fruits and leaves of the two types of mulberry plant. Also The results show a better flavonoids contents in *M. alba* fruits. However, *M. alba* leaves present the minor quantity of flavonoids.

Where trend was quite different from that of polyphenol and flavonoids contents in the two types of mulberry plant. This is in agreement with what has been found by Kako (2011) and In a Previous reports, However, values noted in present study were diverant with what has been found by Thabti et al (2011).

This can be due to the various stress conditions that affect the chemical content of the leaves such as drought, heat, pollution and ultraviolet radiation, so that the production of flavonoids increases by the end of the plant to resist the thermal and water stress it is exposed to. Stress is responsible for the spread of free radicals OH Pincemail et al. 1986) or the effect of the climate of the region, the quality of the soil, the irrigation method, the period of harvesting in terms of the annual time, the age of the plant and the method of extraction are important factors affecting the richness and diversity of the chemical composition of the plant (Vasconcelos et al., 1999).

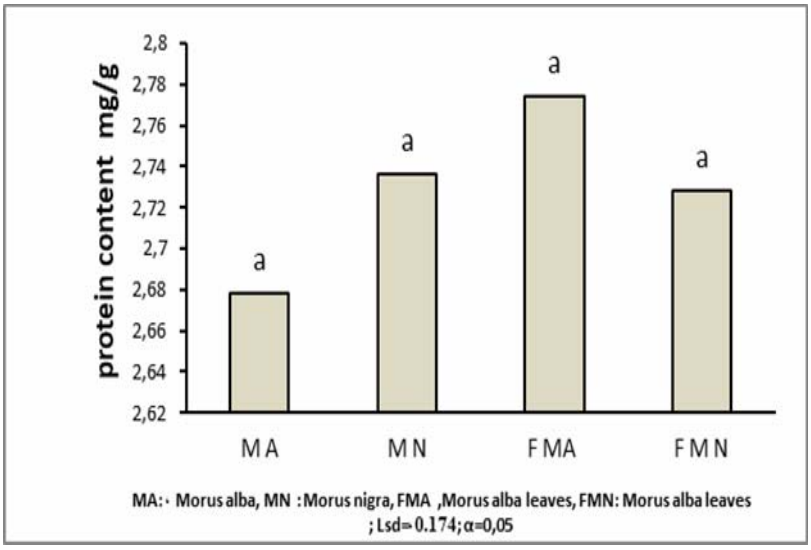

Figure 2. Protein content mg / g in dry matter to extract the fruits and leaves of the white mulberry and black

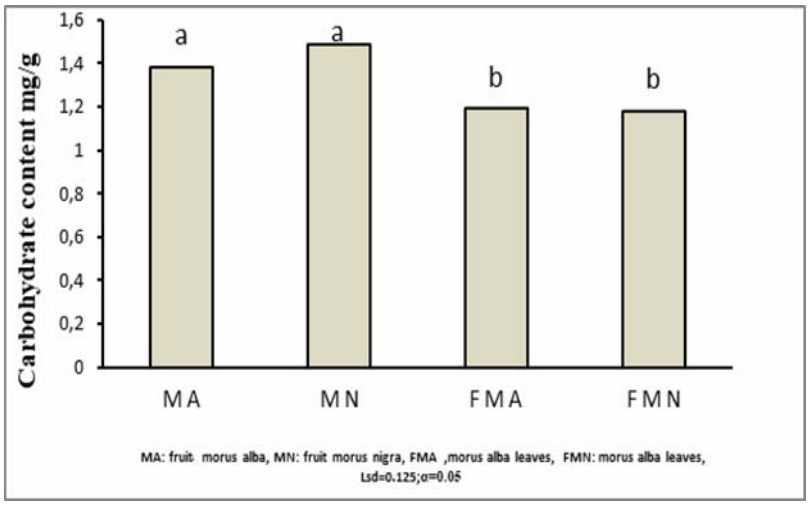

Figure 3. Carbohydrate content mg / g in dry matter to extract the fruits and leaves of the white mulberry and black

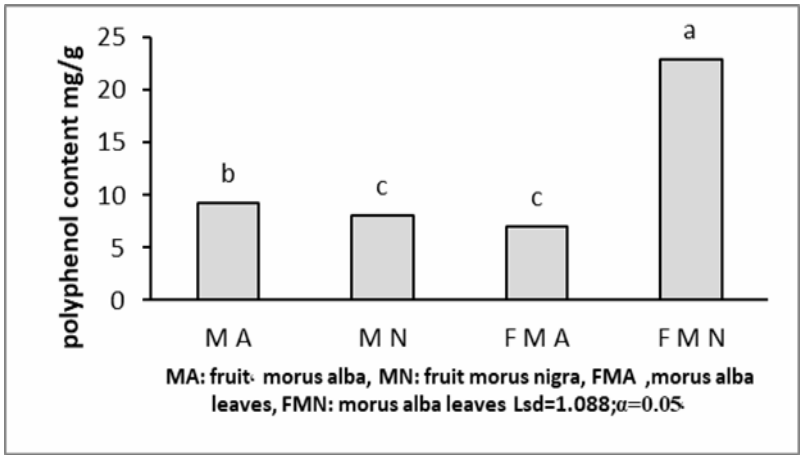

Figure 4. Polyphenol content mg / g in dry matter to extract the fruits and leaves of the white mulberry and black

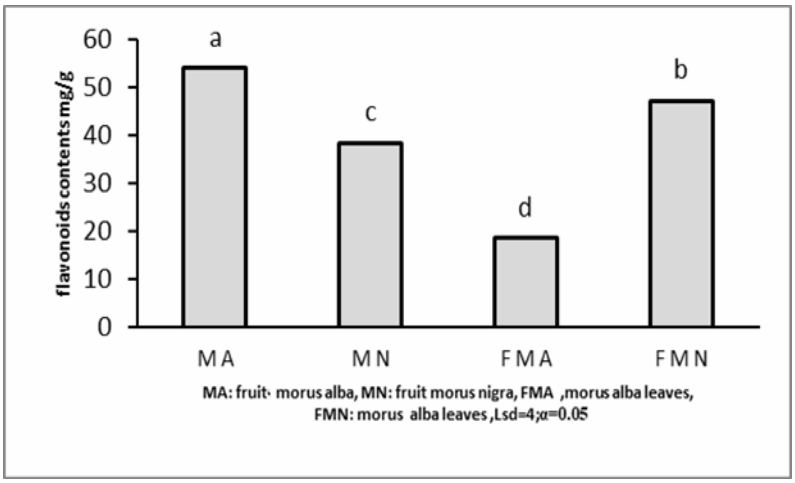

Figure 5. Flavonoid content mg / g in dry matter to extract the fruits and leaves of the white mulberry and black

Quantitative differences in the anthocyanins values were observed among the studied mulberry samples figure 6. The anthocyanins values obtained from the black-coloured mulberries were several times higher than the content of anthocyanins found in the white-coloured mulberries. It shows the color of the fruit purple.

Also show significantly anthocyanins content in fruits and leaves morusnigra compared with the fruits M. alba and levaes *M. alba*. is consistent with that reported by Syvacy et Sokmen (2004) and Song (2009).

The results figure 7 showed significant differences in cartenoide content between fruits and leaves two species mulberry (*M.alba , M.nigra*). total carotenoïds (TC) clearly showed that fruits of mulberry had low contents.

The highest total carotenoids content is detected in *M. alba* leaves (227.27 mg /g FM), Which exceeds the content of blackberry leaves by (9%). while the lowest amount is recorded in M. alba fruits (1.63 mg /g FM). These results are in agreement with those obtained by Aljane and Sdiri (2016).

We interpret the high content of carotenoids in leaves as an important part of the synthesis of chlorophyll, which is the seat of the bio-structure (Han et al., 2004).

Antioxidant capacity DPPH in mulberry

The total antioxidant capacity of whole fruits and leaves extracts of the two mulberry species was quantified by methods DPPH and we found significant differences among pecies. The black mulberry showed the highest total antioxidant activity. Where he recorded the lowest value for IC50 (0.0769) mg/ml, whereas, the lowest total antioxidant capacity values were found in *M.alba*, Where he recorded the highest value for IC50 (1.822). Ozgen et al. (2009) determined that *M.nigra* species had the highest total antioxidant activity (fig. 8).

We return the difference in antioxidant activity by specie and anthocyanins content. Furthermore, for comparison, antioxidant property of different extracts may not only be attributed to contents of anthocyanins, phenolics and flavonoids rather the involvement of many other phytochemicals is also taken into account by many authors (Sengulf et al 2009). however, no correlation was found between radical scavenging activity and the total phenols (Khan et al., 2006).

We can also explain the high antioxidant activite to the structure and quality of phenolic compounds and the concentration and quantity of these compounds within the plant tissues (Rice al., 1997), and explains the difference in the antioxidant activity between samples in different behavior in giving the proton and electron (Miliauskas et al., 2004). These differences, however, could be due to maturity of the plant.

Methanol extract of *M. nigra* fruits showed significantly higher antibacterial activity against various gram positive and negative bacteria than the other extracts, the diameter of inhibition zone ranged 20.35 mm with *Serratia marcescen* bacterial, whereas, the diameter in *M. alba* (8.8ml). The results showed of the *M.alba* extract had no inhibitory effect on the bacteria growth (*Escherichia coli, Micrococcus luteus, Bacillus anthracis*) (figures 9, 10). The *M. nigra* extract has a significant inhibitory effect for the growth of the bacterial strains imilardetermin by Dhaouadi et al (2010) .

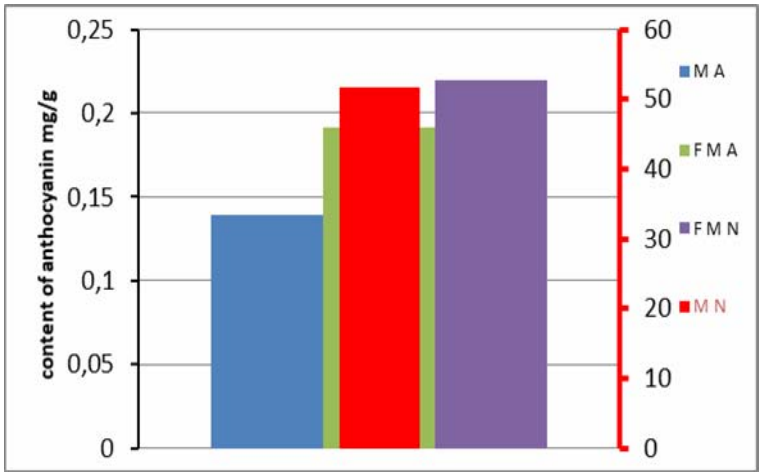

Figure 6. Anthocyanins content mg/g in dry matter to extract the fruits and leaves of the white mulberry and black

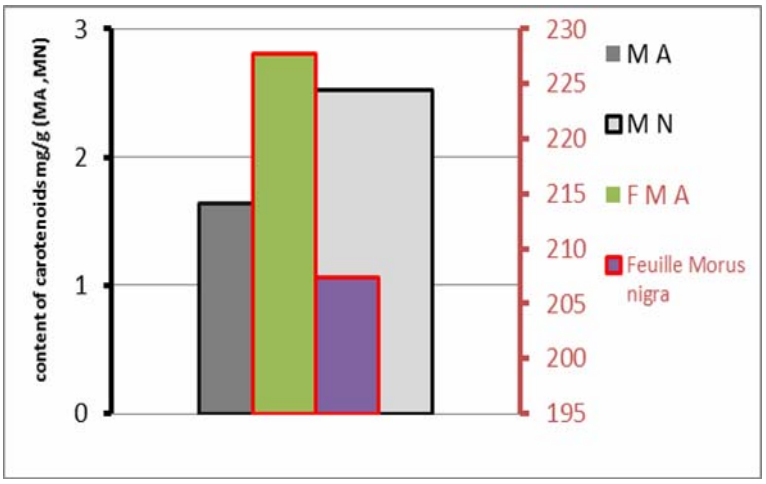

Figure 7. Carotenoids content mg / g in dry matter to extract the fruits and leaves of the white mulberry and black

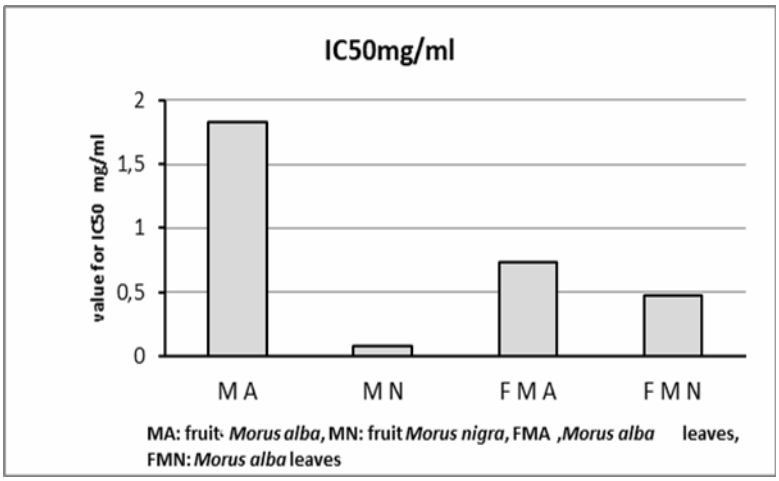

Figure 8. Value IC50 mg / ml in dry matter to extract the fruits and leaves of the white mulberry and black

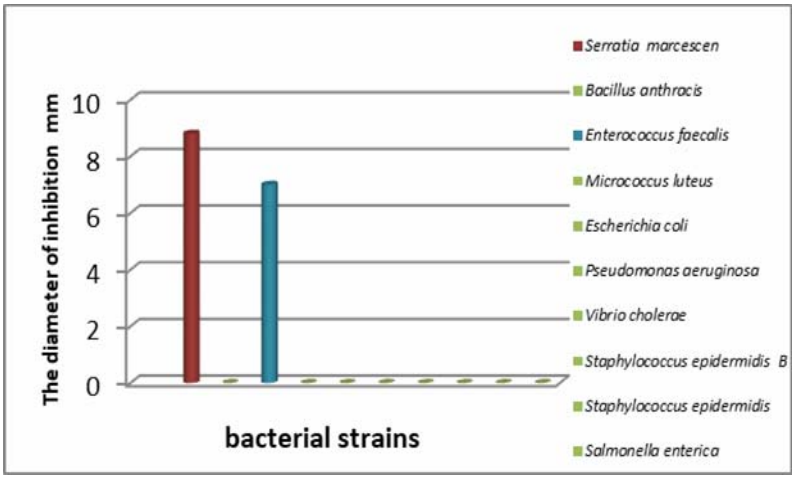

Figure 9. Antioxidant activity to extract the fruits and leaves of the white mulberry

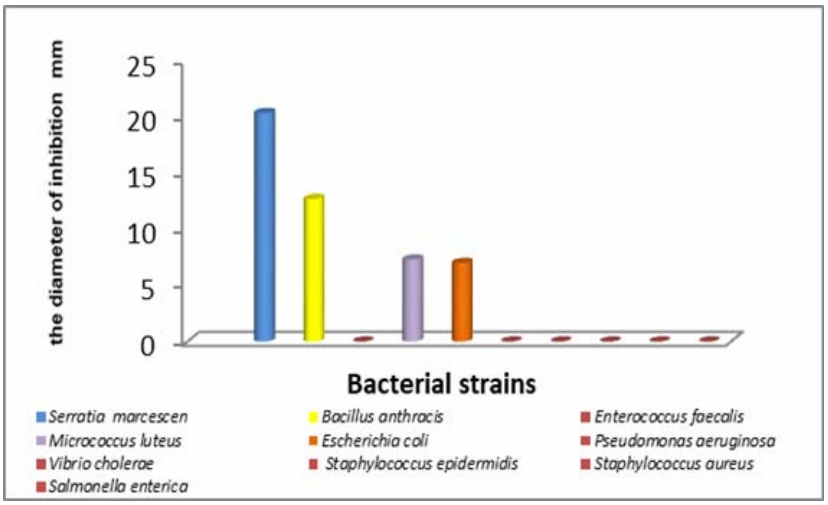

Figure 10. The bioactivities to extract the fruits and leaves of the black mulberry

CONCLUSION

In the present study, analytical investigations were carried out to compare the chemical composition (nutritional values, antioxidant potentials of morus species of south Algeria (el-Oued). The fruits and leaves of these two mulberry species (white and black) showed significant different in some traits (phytochemical components). Also The results showed that the studied mulberry fruits and leaves were nutritionally rich and may be useful in a balanced diet. Higher Polyphenol composition contents with antioxidant activity further increase their nutritive as well as phytomedicinal potentials. *M.nigra* reducing total phenolics and total Flavonoïds. while more Total anthocyanins content, sugars and protein This has a high antioxidant activity and antimicrobial. These variations may be due to different species, genetic potential and maturation period. The study suggested that white Mulberry and Black Mulberry can be exploited for commercial. also a step to-wards the standardization of these fruits as potential healthy foods, which may also be used in food and pharmaceutical industries.

ABSTRACT

The study was about the estimation of proteins, carbohydrates, phenolic and flavonoid compounds, Anthocyanins pigment and carotene, also measuring the acidity (pH) and electrical conductivity, as well as we carried out the counter effectiveness test of oxidation and the biologic effectiveness test. The results obtained showed that the fruits and leaves of both of the two types of the berries ,Have nearly the same amount of proteins and carbohydrates, also the leaves of *M.nigra* and the fruits of the *M.alba* have bigger amount of phenols and flavonoid . and as for the pigments , the fruits *M. alba* and the leaves *M. nigra*, the amount of anthocyanins and there was a high amount of carotene in both types. Regarding the acidity (pH), it was mostly moderate where it tends to alkalinity in the leaves of *M. alba*, and tends to acidity in the fruits of *M. nigra*. while the electrical conductivity was nearly the same amoung the two types of fruits and leaves of *M. nigra*. A low salinity was observed in the leaves of *M. alba*. *M. nigra* have higher bacterial inhibition activity and anti-oxidant activity compared to *M. alba*

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