Zagazig J. Agric. Res., Vol. 50 No. (5) 2023 695-707

Biotechnology Research

Available online at http://zjar.journals.ekb.eg
http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

CHEMICAL ANALYSIS, ANTIOXIDANTS AND SUGARS IN WHITE MULBERRY FRUITS

Asmaa A. Ali[1*], H.T. Hefnawi[1], Faten M. Ibrahim[2] and Lamiaa M.M. El-Maghraby[1]

1. Biochem. Dept., Fac. Agric., Zagazig Univ., Egypt
2. Med. and Aromatic Plants Res. Dept., Pharm. and Drug Indust. Res. Inst., Nat. Res. Centre, PO Box 12622, Cairo, Egypt

Received: 29/08/2023 ; Accepted: 24/09/2023

ABSTRACT: The fruits of white mulberry are widespread fruit in Egypt and are eaten by most people with acceptable taste, so we tried in this research to show the chemical composition of the fruits and the effectiveness of white mulberry as an antioxidant and also know the quality of the polysaccharide that is found in white mulberry and study the properties of sugar many in terms of the quality of the structural units involved in the composition and effective groups of its molecular weight and also its ability as an antioxidant and to achieve this purpose we have collected the fruits of white mulberry from The local market of the city of Zagazig was washed and dried and then grinding and storage in appropriate conditions of temperature (4°C) and the chemical composition of moisture, total ash, crude protein, crude fat, fiber and carbohydrates was estimated by the difference and the results were as follows as a percentage of the dry sample of white mulberry fruits (12.02 - 14.42 - 21 - 4.83 - 15 - 59.75) respectively. Total phenols and total flavonoids were estimated through two types of extracts, aqueous extract and alcoholic extract to determine the effectiveness of white mulberry fruiting as an antioxidant, where the results were (23.5–13.9 mg gallic acid/gram total phenolat extract aqueous and alcoholic extract respectively As for the total flavonoids, the results were (1.43 – 2.54 mg Quercetin /gram aqueous and alcoholic extract, respectively, and finally. The antioxidant activity of white mulberry fruits was estimated by DPPH method and the results obtained were (6.66 – 1.97 mg vitamin C/gram aqueous and alcoholic extract respectively. The sugar was separated from many white mulberry fruits and purified and it became clear from the results to obtain two types of sugar many in the fruits of white mulberry differ in molecular weight and also in the type of structural units involved in the composition where the molecular weight sugar was reached 1.25×10^4 - 5.29×10^4 Dalton and called them fraction 1 and fraction 2 respectively The quality of monosaccharide was glucose – Mannose - Arabenose - fructose - Mannuronic acid in fractions WMP1 or fraction WMP2 It contains the following structural units (glucose - galactose - arabinose - manuuronic acid acid) Through the results obtained from the HPLC device, the quality of the effective groups in polysaccharide fractions in white mulberry fruits was also identified through the infrared spectrum, which showed peaks appear in 1728 - 1619.2 - 3345.6 cm^{-1} for samples Which indicates the presence of the CH_2 group and the C=O group hydroxyl group, respectively. Through the results obtained, we can say that the consumption of white mulberry fruits provides the human body with natural antioxidant compounds and also a high content of protein, salts and total carbohydrates.

Key words: Berry fruit sugars, antioxidant activities, infrared chemical composition.

INTRODUCTION

It has become known to the general public that eating fruits and vegetables provides the body with many natural compounds that protect against many diseases (such as type 2 diabetes, heart disease, cancer, etc.) and protect the body from many dangers, so the World Health

*** Corresponding author: Tel. :**+201066933702
E-mail address: Asmaa_555@gmail.com

Organization recommended for nutrition that a person eat at least 450 grams of fruits and vegetables daily (**Gao and Watanabe, 2011).**

The plant has many natural compounds called secondary products or secondary metabolism or secondary compounds and these compounds have a high ability to resist diseases (diseases of aging Kalzheimer's - viral diseases diabetes diseases of type-2) that result from increased oxidative stress, so you must eat fruits and vegetables daily and this is what the human realized a while ago

Therefore, humans and researchers began to search for plants (fruits and vegetables) and study their chemical composition and biological activities to introduce humans to the benefits of these plants and also their harms).

Fruits and vegetables are in a race for humans, as they have many effective compounds and vital activities in many diseases, and fortunately for humans, most of this plant is available in the environment in which it lives by a large percentage, examples (okra-mallow - pomegranate - white mulberry - lettuce - etc.)

White mulberry *Morus alba* L. A plant that follows the Moraceae family, which is a perennial plant and is grown in many types of agricultural land and can be used as windbreaks and fences on farms, so it is easy to spread and the plant contains many benefits, whether leaves or fruits

As it is known, the germination of white mulberry is grown for many purposes, including food for silkworm larvae, which is responsible for the production of natural silk and the mulberry plant is classified in Chinese medicine as a medicinal plant because it contains many active compounds (**Chandra *et al.*, 2014**).

The fruits of white mulberry contain a high nutritional value, whether wet or dry fruits, as reports and research have shown that the nutritional value and calories of wet white mulberry and dried white mulberry are as follows (calories (360 calories) and total protein total carbohydrates fats and salts total fiber are (9.5 - 65 - 2.6 - 10.4 - 12.4% respectively Wet fruits or dry fruits are (320 calories) (1.7 - 77.2 - 1.8 - 13.4 - 14.3% dry fruits.

White mulberry contain many vital compounds such as phenols, flavonoids and vitamins, which when consumed continuously protect the body from diseases.

White mulberry leaves are used in many directions, for example, silkworms are fed or dried and a packet of tea works for humans, in addition to the work of many extracts to obtain pure compounds or concentrated extracts that are useful in preventing diseases or treating diseases (**Ercisli and Orhan, 2007**).

Arabashahi (2006) show a chemical analysis of the fruits of white mulberry and found that white mulberry contain low calories up to 316 - to 350 calories and explained that they contain dietary fiber (known the importance of fiber in the diet of humans) White mulberry also contain vitamins such as vitamin C up to 300 mg and the fruits of white mulberry are characterized by a distinctive smell and flavor and attractive to humans as the human benefited from the color of the fruits of white mulberry in the extraction of dyes and add them to processed foods

The fruits of white mulberry contain dietary fiber for sure confirms that white mulberry benefits diabetics type 2 and also reduce cholesterol by a large percentage compared to other plants also known to many the importance of fiber in facilitating the digestion and absorption of many compounds **(Gunes, and Cekin, 2004**).

Polysaccharides have recently shown many health benefits, especially those that have other effective groups other than the hydroxyl group, which has been researched to find out which plants contain polysaccharides

The fruits of white mulberry have a high percentage ofpolysaccharides, which have been shown through previous studies that they are responsible for resisting many diseases also oxidant-have the ability as an anti (**Zhao *et al.*, 2007**)

The polysaccharides are diverse and different among themselves in terms of molecular weight, effective totals and the type of interstitial units, and all these differences certainly affect the natural and chemical properties of polysaccharides, so each plant, whether fruit or vegetable, has polysaccharides that differ from polysaccharide

in the other plant, but in the same type variety (**Abdelhedi, 2016**).

Polysaccharides have shown many biological activities to resist many diseases Anti-tumor Anti-virus Antioxidant anti-bacterial starch Anti-cancer Discoveries are still made in the field of using polysaccharides in succession (**Zhang *et al.*, 2010**).

Polysaccharides consist of the association of many monosaccharides through the formation of bonds of the type glycoside bonds with the loss of a molecule of water from the two building blocks These bonds, including two types, alpha type and another beta type, and it is known to many researchers that the alpha type is in the human body and therefore will provide energy (starch) either beta type to metabolize them in the human body because of the lack of enzymes responsible for breaking the beta glucoside bond and therefore does not provide the body with energy, but is useful in facilitating the process Digestion and bowel movements slow down the absorption of sugars such as cellulose or dietary fiber (**Zeng *et al.*, 2014**).

Polysaccharides are used in many products such as cosmetics and pharmaceuticals as an active substance or filler used by industry Regulates blood glucose by reducing absorption or reducing release (**Wu *et al.*, 2020**).

It turns out that the fruits of mulberry metabolism contain polysaccharide has properties as an antioxidant and anti-bacterial activity and anti-inflammatory and antiviral and also cancer so we tried in this research to get polysaccharide and study the properties and complements in other research (**Jonsson *et al.*, 2020**).

Previous studies confirm that the quality of polysaccharide found in white mulberry has many uses and properties of low obesity and blood sugar regulation in the case of type 2 diabetics and antioxidants, whether polysaccharide extracted from the fruits of white mulberry or leaves of the mulberry plant and this led to the interest of many researchers where it was mentioned in Chinese folk medicine (**Wu *et al.*, 2020)**.

The mulberry plant is grown in a variety of climatic conditions and this is for human luck, as the production of white mulberry represents approximately 70% and does not need special care in the cultivation process and is used in Chinese folk medicine to increase immunity and prevent many diseases because it contains various vital properties and activities that make it characterized by these qualities and characteristics (**Kim *et al.*, 2018**).

Through the information that has been monitored above, it is clear that the aim of the research is to determine the chemical composition of white mulberry fruits as well as the content of total phenols, total flavonoids, antioxidant activity, and finally the extraction of polysaccharides and determine some properties by knowing its components and structural units through HPLC, its molecular weight, its activity as an antioxidant, as well as the active group through the infrared spectrum.

MATERIALS AND METHODS

Materials

White mulberry were collected *from M. alba* L., from the Abu Ahmed store located in Zagazig City, Sharkia Governorate - Egypt. The white mulberry were dried *and then powdered* using a blender. The samples were stored in airtight plastic bags at 0°° C analysis until use in the origin test.

Reagents and Chemicals

2,2-biphenyl-1-picrylhhydrazyl(DPPH), purchased from Sigma Aldrich (St. Louis, Missouri, USA). Volyn-Ciocalteo detector, TPTZ (2,4,6-tri- (2-pyridil)-s-triazine), and Kircetin Deville were obtained from Merck (Billerica, Massachusetts, USA). Hydrochloric acid, ethanol, acetone, methanol, formic acid, sodium carbonate and boric acid were also purchased from POCH (Gliwicz, Poland), and all reagents were analytical-grade. Finally, all other chemicals and solvents used in this research were obtained from El Gomhoria Chemicals and Pharmaceutical Company, Zagazig City, Sharqia, Egypt.

Determination of Chemical Compositions

Based on the analysis method (**AOAC, 2010**) the chemical composition of the basic compounds was estimated (total carbohydrates - moisture - crude fat, crude protein, total salts, total fiber,

and the previous estimates were expressed in units of 100 grams dry sample/gram (%). Total carbohydrates were estimated by a calculation shown as follows:

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

Energy value

According to **James (1995)**, the energy value was calculated mathematically by applying the following equation:

Energy value = (% carbohydrates × 4.1) + (% protein × 4.1) + (% fat × 9.1).

Preparation of White Mulberry Extract

A weight of 100 grams of dry white mulberry fruits was taken and 500 ml solvent (water - ethanol 80%) was added to it as a extraction ratio (1 : 5) and then shaking on the shaker for 24 hours at a speed of 50 rpm and then the filtration was made and the filtrate was taken (soluble) using Wattman filter paper No. 1 and then took the precipitate (insoluble part) and added to it the solvent and repeated steps and added The filtrate to the previous filtrate and finally the filtrate was focused on the vaporizer under vacuum and in the end the aqueous extract and the ethanolic extract were freezed on the cryophile device as mentioned in the methods of **Cao *et al.* (2021).**

Determination of Total Phenols

Constructively method for **Singleton and Rossi (1965)** using Folin-Ciocalteu (FC) detector. The total phenols were estimated as follows: First, in a test tube, take 0.5 grams with 2.5 ml of the essence of the Folin-Ciocalteu method reagent, shake well on the pipe shaker (VORTEX GENIE model, made in China) at a degree of 25°C, then add 2 ml sodium carbonate solution at a concentration of 8%, then mix and shake on the pipe shaker for an hour at a temperature of 25°C, then leave The sample for 30 minutes and the measurement at a wavelength of 760 nm on the spectrophotometer (UV/VIS Philips PU 8735 China) and to know the concentration of total phenols, a standard bass curve was used using gallic acid at concentrations from (5 to 125 mg/ml) as shown in the following equation = r = 0.0197 x - 2.231 (R^2 = 0.979).

The results were expressed as gallic acid mg/ gram extract.

Estimation of Total Flavonoids Content (TFC)

Constructively method for **Chen *et al.* (2016)** using aluminum chloride detector, the test was performed as follows in a test tube in which 500 µL of white mulberry extract (aqueous – ethanolic) was placed with 100 µL of aluminum chloride 10% and was shaken well by hand and then added 2 ml of methanol (95%) then 100 µl of potassium acetate and finally 3 ml of distilled water and the contents were shaken all together on a pipe shaker at a temperature of 25°C, then the samples were left for 45 minutes, after which the measurement was made at a wavelength of 430 nm on a spectrophotometer ((UV/VIS Philips PU 8735 China) and to find out the total flavonoid concentration, a standard curve was made using Quercetin with concentrations from (5 to 125 mg/ml) as shown in the following equation = Y = 0.0178x - 0.2023 (R^2 = 0.9878).

The results were expressed as quercetin mg/g extract.

Determination of Antioxidant Activity of Aqueous and Alcoholic Extract of White Mulberry Fruit by DPPH Method

DPPH· radical scavenging potential

Ibrahim *et al.* (2021) used the method to evaluate aqueous and alcoholic extract of white mulberry fruits extracts' free radical scavenging properties using DPPH· (0.1 mM) prepared in methanol. Consecutive concentrations, (50 - 600 µg/mL) of extracts, ascorbic acid at the same concentrations were prepared. The mixture of three milliliters of sample or standard at all concentrations individually and one milliliter of DPPH· solution was shaken vigorously and kept dark at room temperature for 50 min. Absorption at 517 nm was determined in a spectrophotometer (Jasco, serial No. C317961148, Japan). The following equation was used for the calculation of scavenging ability:

$$DPPH\cdot\ scavenging\ effect\ (\%) = \frac{(A_0 - A_1)}{A_0} * 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of a sample or standard.

Determination of Sugars

Based on the method of AOAC 2010, the total dissolved sugars and total reduced sugars were estimated practically by hydrolysis and then by the method of 3,5 dinitrosalicylic acid and the work of a standard curve of glucose at concentrations of 50 mg/(1 ml to 500 mg) and application in the equation of the standard curve, and then the total sugars were also estimated by a mathematical equation by subtracting the total reducing sugars from the total mixed sugars.

The fruits of white mulberry were prepared according to the method described by **Melgarejo *et al.* (2000)** with minor modifications. In short, a sample of 5 g fruit with water 50 ml and then centrifuged at 12,000 rpm for 2 minutes at 4°C. The floating material was then filtered with SEP-PAK C_{18} cartridges, transferred to a vial and used for analysis. The analysis of sugars was performed by HPLC (equal program) with μbondapak-NH_2 column and refractive index detector (R1) using 85% acetonitrile as a moving phase. The calculation of concentrations was based on laboratory standards.

Preparation of White Mulberry Polysaccharides

According to **Wang *et al.* (2017)**, polysaccharides and their sections were obtained in the fruits of white mulberry by following the following steps: The fruits of the white mulberry were mashed in the electric mixtures and then filtered through a piece of cloth (gauze) and the filtrate was dried and dried, then 10 grams of it were taken and 100 ml distilled water was added to it by extraction ratio (1: 10) and heating was done in the presence of an air reflector condenser to prevent steam loss for a period of 4 hours after the sample was cooled and centrifuged at the speed of 1000 rpm for 10 minutes to separate the filtrate from the precipitate and the previous steps were repeated with the precipitate by adding 100 ml of water headquarters and in the end the filtrate was collected and the work of evaporation on the rotavapour under to reduce the volume of water until it bends the addition of quantities of ethanol alcohol and the percentage of alcohol addition is absolute (1: 5) and was left for 24 hours at 0°C (refrigerator degree) and then was centrifuged at a speed of 15000 thousand rolls in minute for 15 minutes and the precipitate was collected and repeated the previous process by adding alcohol (in a ratio of 1: 10) to the filtrate and leaving it for 24 hours and then taking the precipitate (which was called sugar many raw) and the total precipitate was collected and dried at a temperature of 40°C under vacuum and kept until experiments on it. To purify the many raw sugar obtained, 5 grams of raw sugar were taken and dissolved in hot water in the amount of 500 ml distilled water was completely dissolved by continuous shaking on the shaker until complete dissolution and the temperature was raised to 50°C and then centrifuged at 15,000 rpm for 35 minutes to separate impurities and then 500 ml of sevage reagent was added (Chloroform: Butanol: 4: 1) in order to get rid of the protein that may be associated with polysaccharides in the form of gloprotein and was shaken well until the protein is deposited Balrj and then conducted centrifugation at a speed of 15000 rpm for 25 minutes The filtrate was taken and the precipitate (protein) was left The filtrate was collected, dried and dried in the form of pure polysaccharides

Analysis of Monosaccharide Structures

Monosaccharide formulations were determined by high-performance liquid chromatography (HPLC) after pre-column derivation. Purified polysaccharide powder (20 mg) was dissolved in trifluoroacetic acid at 2 mol/L and decomposed at 120 °C for 6 hours in a sealed tube. After hydrolysis, the excess acid was removed by co-distillation with methanol three times to produce dry hydrolysis. HPLC analysis was performed on the LC20A HPLC system (Shimadzu, Japan) equipped With UV detector SPD-20A and C18 column (250mm×4.6mm, 5μm, Shimadzu, Japan). The moving phase was a mixture of 0.1 mol/l NaH_2PO_4-Na_2HPO_4 buffer (pH 6.7) and acetonitrile (83:17), and a flow rate of 1.0 ml/min was used. The wavelength of the detection was 245 nm, and the shaft temperature was 30°C. Polysaccharides were determined in comparison with monosaccharides (mannose, glucose, d-ribose, rhanose, rhamnose, d-xylose, d-galactose, L-

arabinase, d-fructose). Molar ratios of monosaccharides were calculated based on the standard curve of each sugars Mono (**Chengcheng *et al.*, 2019)**.

Determination of Molecular Weight

The molecular weights of white mulberry fruit fractions were determined by High Performance Gel Permeability Chromatography (HPGPC) using the Agilent 1200 HPLC system equipped with evaporative light scattering detector and TSK-gel 4000 PWXL column (7.8 mm×30 cm, TOSOH Corp., Japan). The column was extracted with double distilled water at a flow rate of 0.6 ml/min. Standard Dextrance (T10, T40, T70, T380, T500) The equation for the standard curve was log Mw = -0.514x + 7.809 (R^2 = 0.992), where Mw is the molecular weight and x is the retention time was used to determine the molecular weight (**Huang *et al.*, 2018)**.

Infrared Spectroscopy

Infrared spectra for Fourier conversion of sugars were obtained on the Bruker-Vector 22 spectrometer (Germany). The samples obtained from white mulberry were mixed with KPR powder, ground and pressed into 1 mm pellets, and the spectra were obtained in the frequency range 4000-500 cm-1. According to **Brooke (1957)**.

RESULTS AND DISCUSSION

Chemical Compositions of White Mulberry

Through the values and results in Table 1, it is shown that white mulberry fruits are high in carbohydrates and fiber, as well as ash and crude protein and low in crude fat extract. The chemical compositions of white mulberry fruit have been studied. The results in Table 1 reveal that white mulberry fruit can be considered a rich source of crude protein and total carbohydrates because it contains 21 and 59.75%, respectively.

In addition, it can be seen that the moisture content was 12.02% in white mul white mulberry. The results in Table 1 also showed that total carbohydrates were higher in white mulberry 59.75%, on the other hand crude fiber higher in white mulberry (15%). While the average ether extract was 6.34 and 4.83%, finally ash was recorded at 14.42% in white mulberry fruit.

Regarding the energy value of white mulberry 313.53 kcal/100 grams. The results obtained are consistent with **Ephemeral *et al.* (2007)** who stated that the content of the chemical composition of white mulberry. It was found that the moisture content ranged from 89.83 to 91.89%. Crude fiber ranged from 5.89 to 11.58%. Carbohydrates 34.42 to 42.94%. Protein 23.52 to 29.04%. Fat 3.48 to 5.08%. Ash 19.88 to 23.01%. On the other hand, the results obtained are consistent with (**Andallu *et al.*, 2003**) who found protein, fat, ash, crude fiber and total carbohydrates to be 23.10%, 7.92%, 15.43%, 13.85% and 39.70% respectively for white mulberry leaves. The results of the chemical composition of white mulberry are recorded in Table 1. From the tabular data, the results in Table 1 revealed that white mulberry can be considered a good source of crude protein, ether extract, crude fiber, ash and carbohydrates.

The moisture content of the raw specimen is often an important criterion for its quality, if the moisture content exceeds a certain value, decomposition of active principles may occur and microbial growth may occur. The moisture content of the white mulberry sample was determined and the results were obtained as shown in Table 1. These results are close to those reported by Butt *et al*. (2008) which found that white mulberry contains 15.31% crude protein, (2.09 - 7.92%) fat, (9.9 - 13.85%) crude fiber and (11.3-17.24%) ash. The results obtained are consistent with those reported by **Giampieri *et al.* (2012)**. The World Health Organization found that the moisture content of white mulberry was 90.95%. Of these results, white mulberry contained the highest level of protein (12.98%) and ash (6.36%).

Reduce Non-Reducing Sugars from White Mulberry with HPLC

Some sugars extracted from white mulberry were determined using high-performance liquid chromatography (HPLC) and the results are listed in Table 2. It was observed that fructose was 3.89 g/100 g, glucose 2.98 g/100 g and total

Table 1. Chemical composition of white mulberry fruit (g / 100 g based on dry weight)

Component	Concentration (%)
Moisture	12.02
Ether extraction	4.83
Crude protein	21
ashes	14.42
Total carbohydrates	59.75
Crude fiber	15
Carbohydrates available	44.75
Energy Value Kcal / 100 g	313.53

Total carbohydrates calculated by difference

Available carbohydrates = total carbohydrates - crude fiber.

Table 2. Reducing and non-reducing sugars of white mulberry using HPLC

Sample	Reduce sugars		Non-reducing sugar	Total sugar reduction
	Fructose	Glucose		
White mulberry	3.89	2.98	2.56	8.31

reducing sugars 8.31 g/100 g respectively. These findings are consistent with those reported by **Imran *et al*. (2010)** stated that the total reducing sugars in white mulberry were 6.87 g/100 g. These findings are consistent with those reported by **Giampieri *et al.* (2012).** The World Health Organization found that the total reducing sugars in white mulberry were 8.76 g / 100 g. From these results, it can be seen that white mulberry contain non-reducing sugar like sucrose. Higher than reducing sugars

Total Phenolic and Flavonic Content in Extracts (Ethanolic–Aqueous) of White Mulberry

Phenolic compounds are generally associated with antioxidant activity. White mulberry has been reported to be rich in polyphenols and possess the ability to inhibit fat-soluble antioxidants. The content of phenolic compounds in fruit can vary and is influenced by multiple factors, such as genetic differences, environmental conditions and/or temperature. Depending on the varieties, the phenolic content of white mulberry can also vary (**Flaczyk *et al.*, 2013**).

Given the content in phenols and flavonoids are the two main contributors that affect antioxidant activity, they have been examined more frequently than the various extracts of white mulberry fruits. The total phenolic content in aqueous white mulberry extracts appears to be much higher than in ethanolic extracts. Aqueous and ethanol extracts of white mulberry showed a total phenolic content of about 23.5 mg of/GAE g extract and 13.9 mg GAE/gextract, respectively (Table 4). Regarding the total flavonoid content, similar values were obtained (1.3 mg GAE/g) in aqueous extracts, while the content was significantly different in ethanolic extracts with a content of 2.54 mg. Thus the highest antioxidant activity measured with white mulberry extracts appears to have been associated with high phenol contents (**Swapana *et al.*, 2012**).

Table 4. Content of total phenol, total flavonoids on extract (ethanol and aqueous) of white mulberry

Extracts	Total phenol (mg GAE / g extract)	Total flavonoids MG/g extract
Water	23.5	1.43
Ethanol	13.9	2.54

Antioxidant Activity of White Mulberry Extracts and Fraction Polysaccharides

Measuring antioxidant activity using a method called DPPH also revealed that antioxidant activity was higher in watery whiteberry fruit extracts. DPPH screening test is stable and generates radicals that can be dissolved in organic solvents such as methanol. Therefore, white mulberry were extracted by both ethanol and water. The antioxidant activity of DPPH of white mulberry extracts also appeared higher than reported activity (**Liao *et al.*, 2017**).

The antioxidant activity of ethanolic and aqueous extracts of white mulberry was evaluated, and these results indicated that whiteberry extracts showed different degrees of free radical cation activities where lower IC50 values indicate higher antioxidant activity. DPPH's IC50 was significantly higher in ethanolic extracts compared to aqueous extracts of white mulberry, indicating high antioxidant activity in aqueous extracts.

Similarly, water appeared to be more efficient at extracting antioxidant oxidation from white mulberry than ethanol. Furthermore, antioxidant activities were compared to standard ascorbic acid. The aqueous extract of white mulberry expressed the highest antioxidant activities of 6.74 mg ascorbic acid equivalent per gram extract (MG VCE/g extract), (Table 5). (**Me *et al.*, 2011**)

The results are presented in Fig.1 of all the crude sugars and purified fractions, this result showed that WMP had the strongest scavenging activities, and WMP1 showed the best activities among purified fractions. The radical scavenging rates of WMP and WMP1 increased significantly with increasing concentrations, while the WMP2 rate did not. The results suggest that the ability of free radicals DPPH to eliminate acidic sugars was higher than that of neutral sugars. The scavenging activity of sugars is largely due to the hydrogen supplied from the contact of the sugars with the radicals, determining their chemical properties and then terminating the radical chain reaction of free radicals. Another possibility is that sugars combine with key radical ions in a chain reaction that causes the free radical chain to expire (**Yu *et al.*, 2014**).

Purification and characterization of isolate and purification of white mulberry polysaccharides (WMP).

The raw polysaccharide was first fractionated using the DEAE-52 column. The main component of polysaccharide fractions extracted with 0.1 and 0.3 mol/l sodium chloride was WMP. After that, the fractions were further purified on the Sephadex G-100 column, and each fracture showed a single and symmetrical sharp peak (Fig. 2A and Fig. 2B). The main fractions were collected and dried by freezing. Thus, WMP1 and WMP2 purification was obtained.

Determination of Molecular Weight

Single peaks on HPLC for jelly permeation (Fig. 3) suggested that two sugars were homogeneous. The average molecular weight of WMP1 and WMP2 was determined by the titration curve performed by different standard dextrans and is estimated at 114.901 and 124.785 kDalton, respectively.

The Composition of Monosaccharides

The monosaccharide formulations of WMP1 and WMP2 were measured by HPLC, as shown in Table 6. The retention time and standard curve for each monosaccharide were determined by HPLC's analysis of individual ingredient parameters. The results indicated that WMP1 consists of glucose, mannose, galactose, arabinose and fucose in a molar ratio of 1.22: 0.96: 0.00: 1.00: 0.35 (Table 6), while WMP2 consists ofof glucose, mannose, galactose, arabinalose and fucose in a molar ratio of 1.51: 0.00: 1.00: 1.60: 0.00 (Table 6) (**Ercisli *et al.*, 2010**).

Table 5. Evaluation of Antioxidant Activities of White Mulberry Extracts from DPPH

Extracts	IC50(mg/ml)	Antioxidant activity (mg VCE / g extract)
Water	0.75	6.66
Ethanol	2.30	1.97

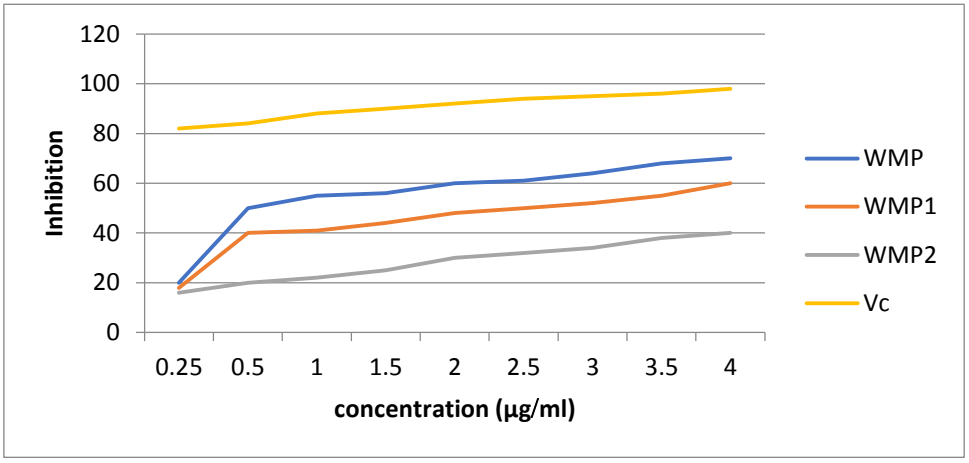

Fig. 1. Scavenging effects on DPPH root activity for WMP, WMP1, WMP2 and Vit C as laboratory control.

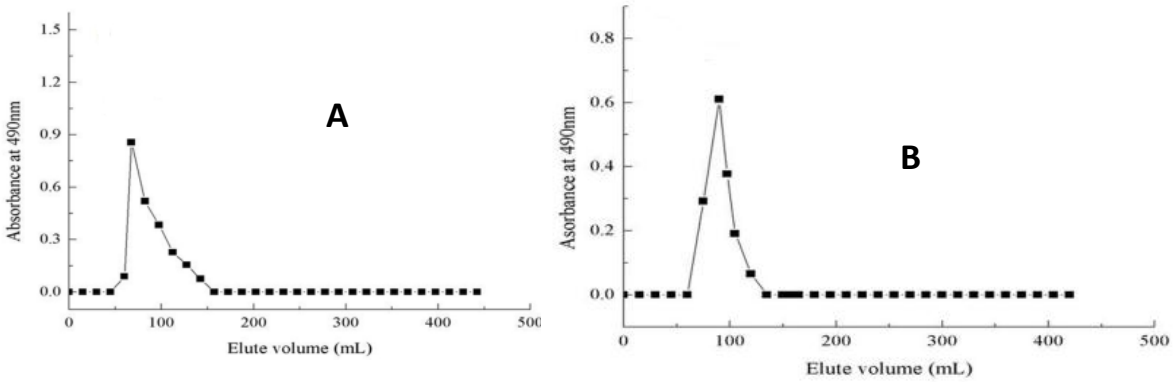

Fig. 2. Chromatology of rinsing from Sugars from white mulberry fruit. (a) Rinse curve WMP1 Fraction and (B) WMP2 Part of the Sephadex G-100 column

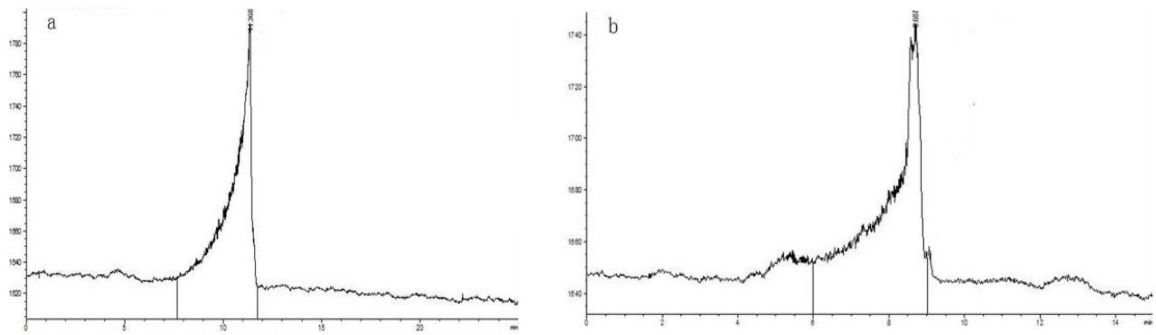

Fig. 3. HPGPC chromatogram charts for WMP1(a) and WMP2(b)

Table 6. Ratio of monomolar sugars for WMP1 and WMP2 by HPLC

Monosaccharides	WMP1	WMP2
Glucose	1.22	1.51
Manoz	0.96	0.00
Galactose	0.00	1.00
Arabinos	1.00	1.60
Fokuz	0.35	0.00
Manuronic	0.52	0.36

Infrared Spectrometer

The infrared spectrum is used to identify the quality of the effective aggregates in the polysaccharides under study and it is shown through the spectrum IR of the two types of sugar many detailed fruits of white mulberry WMP2 – WMP1 (Fig.4) the emergence of areas and vibration confirming the presence of certain effective groups, for example, the emergence of 3345.6 cm-1 resulting from the expansion vibration of hydroxyl groups, while the appearance of vibration at 2934 cm-1 indicates the presence of the link C – H. The signal was at 1653 cm-1 due to the bending vibrations of O-H, and the signals were at about 1400 cm-1 and 1240 cm-1 due to the bending vibrations of C-H and extended vibrations of C=O, respectively. The signal at about 1110 cm-1 is attributed to the extension of C-O-C links. Absorption bands at 880 and 826 cm-1 of WMP2 indicated the presence of galactopiranosil residues associated with β and α, respectively. In addition, the signal was in the spectrum of WMP 1 at 921 cm-1 of type β links (**Liu *et al.*, 2021**).

Conclusion

Through the results obtained from the research, it is found that the fruits of white mulberry contain natural nutrients and a high concentration, where the results of the chemical analysis indicate a high percentage of protein 21%, total carbohydrates 60%, high mineral salts 15%, and also dietary fiber 15% and also the content of total phenols, total flavonoids and antioxidant activity was estimated through two types of extracts, namely aqueous extract and alcoholic extract, and the results showed that white mulberry fruits contain a high percentage of total phenols compared to Total flavonoids and also aqueous extract are higher in the content of alcoholic extract and the high percentage of antioxidant activity such as vitamin C in the aqueous extract than the alcoholic extract, and this may be due to the high total phenols in the aqueous extract than the alcoholic extract, as it has a role in antioxidant activity.

WMP was extracted from the fruits of white mulberry with a yield of 6.49%, after that, two sugar parts WMP1 and WMP2. The raw saccharides and fractions obtained had good antioxidant activity. The results indicated that WMP could be used as a natural antioxidant for use in medicine or functional foods. This study provided some basic information on the chemical composition of the saccharides of white mulberry fruits for application and also the content of white mulberry fruits of nutrients compounds responsible for antioxidant activity (phenols and flavonoids total) so through this research we call to eat the fruits of white mulberry because they contain beneficial compounds for the body.

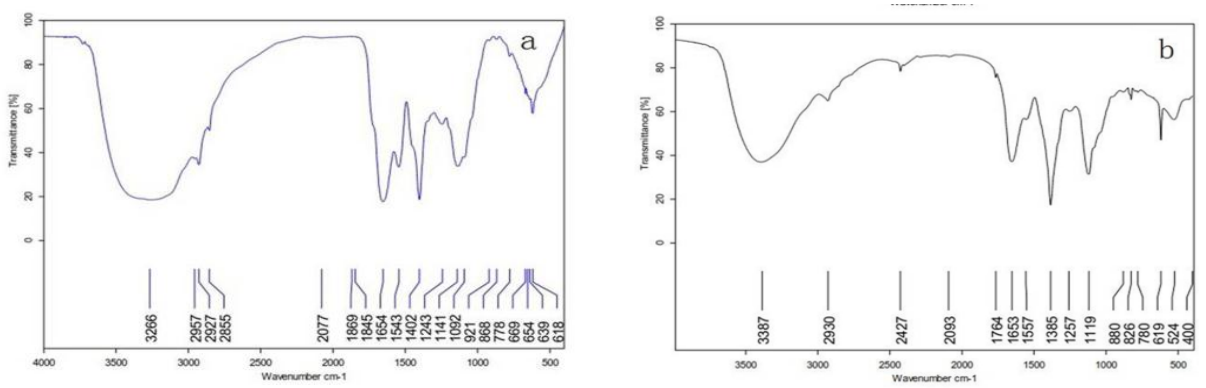

Fig. 4. Infrared spectra for WMP1(a) and WMP2(b)

REFERENCES

Abdelhadi, A., R. Nasri, N. Souissi, M. Nasri and M. Jereidi (2016). Sulphur sugars from common smooth fishing: extraction and evaluation of angiotensin, antioxidant and antibacterial activities. Carbohydrate Polymers, 152: 605-614

Andalusia, B., P. Radhika and V. Suriakantham (2003). The effect of aswagandha, ginger and mulberry leaves on hyperglycemia and hyperlipidemia. Plant Food for Human Nutr., 58:1-7

Arab, P., S. Deloy and A. Oruç (2006). Antioxidant properties of various solvent extracts of mulberry leaves (*Morus indica* L.), Food Chem. 102: 1233-1240.

Brooke Nelly, W. (1957). Infrared spectra of carbohydrates. Advances in carbohydrate chemistry. Acad. Press Comp., Publishers.

Cao, W., W. Jiang, H. Bai and S. Wang (2021). Study on the active components of raspberry leaves for the prevention and treatment of cardiovascular complications of diabetes. J. Functional Food, 83: 104549

Chandra, S., S. Khan, B. Avola, H. Lata and M.H. Yang (2014). Evaluation of total phenol and flavonoid content, antioxidant properties, yield of leafy vegetables and aerobically grown fruit crops and conventionally: a comparative study, Evid-based Alternat Med. Supplement, 253875.

Chao, M.M., N. Yang, P.I. Yang, Y.M. Jiang and J.H. Zhang (2007). Structural characterization of water-soluble sugars of Opuntia monacantha cladodes in relation to their anti-polysaccharide activities. Food Chem., 105: 1480-1486.

Chen, H., J. Bo, D. Liu, W. Yu, Y. Shao, J. Yang, Z. Xiang and N. Hu (2016). Anti-inflammatory and anti-allergic properties of flavonoids from the fruits of black white mulberry (*Morus nigra* L.). PLOS ONE, 11.

Ersesley, S., M. Toson, V.B. Doraliga and M. Singul (2010). Phytochemical content of some lions (*Morus nigra* L.) and purple (*Morus rubra* L.) white mulberry genotypes. Food technology. Biotechnol., 48 (1): 102-106.

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

Gao, P.F. and K. Watanabe (2011). Introduction of the WHO project for the international classification of traditional medicine. J. Chin. Integrative Med., 9 (11): 1161-1164

Giampieri, F., S. Tulipani, J.M. Alvarez Soarez, J.L. Quiles, B. Mezzetti and M. Patino (2012). Straw white mulberry : composition, nutritional quality and impact on human health. Feed., 28 (1): 9-19.

Gunness, M.M. and C. Secken (2004). Some chemical and physical properties of the fruits

of various raspberry varieties commonly grown in Anatolia, Turkey, Asian J. Chem., 16: 1849-1855.

Huang, S.H., M.S. Shen, T.J. Yoon and K. Shane (2018). Immunoadjuvant activity of sugars isolated from *Portulaca olearaceae* leaves. Int. J. Biol. Macromol., 107: 695-700.

Ibrahim, F.M., R. Fouad, S. El-Hallouty, S.F. Hendawy, E.S.A. Omer and R.S. Mohammed (2021). Egyptian *Myrtus communis* L. Essential oil Potential role as in vitro Antioxidant, Cytotoxic and a-amylase Inhibitor. Egypt. J. Chem., 64: 3005–3.

Imran, M., H. Khan, M. Shah, R. Khan and F. Khan (2010). Chemical composition and antioxidant activity of some types of Morus. J. Zhejiang Univ. Sci. B., 11: 973–980.

James, C.S. (1995). Analytical chemistry of food. Chapter 6, General Nutr. Studies, I. Alden Press, Oxford, UK.

Johnson, M., L. Allah Gully, R.R. Sardare, G.O. Hreggviðsson and K.E. Nordberg (2020). Extraction and modification of macrosaccharides for current and next generation applications. Particles, 25: 930 - 935.

Kim, D.S., K. Wai-M, J. Wai, S. Wai, C. Ji and K.H. Kong (2018). Antioxidant activities and polyphenol content of *Morus alba leaf extracts* collected from different regions. Biomed Rip., 2: 675–680.

Lee, Y.J., E.O. Kim and S.W. Choi (2011). Isolation and determination of antioxidant polyphenolic compounds in raspberry seeds (*Morus alba* L.). J. Korean Soc. Food Sci. Notre., 40: 517-524.

Liao, P.Y., Zhu, D.Y., Thakur, K., Li, L., Zhang, JJ, & Wei, Z.J. (2017). Thermal and antioxidant properties of sugars sequentially extracted from raspberry leaves (*Morus alba* L.). Particles, 22 (12): 2271.

Liu, Y.Q, X. Wang, D.H. He and Y.X. Cheng (2021). Protection from side effects from chemotherapy and radiation therapy: a review based on therapeutic mechanisms and opportunities for phytochemicals. Plant Med., 80: 153402

Milgarrejo, B., D.M. Salazar and F. Artis (2000). Organic acids and sugars the composition of harvested pomegranate fruits. Eur. Food Res. Technol., 185-190.

Oak (2010). Official methods of analysis. Association of Official Analytical Chemists, Washington, D.C., USA.

Singleton, V.L. and J.L. Rossi (1965). Colorimetry of total phenols with phosphomolybdic-phosphotungstic acid reagents. Ame. J. Biol. and Vitic., 16: 144-158.

Swapana, N., T. Gotenkumar, C.P. Devi, M.S. Singh and C.T. Singh (2012). Otoal Phenolic, Total flavonoid contents and antioxidant activity of native fruits grown in Manipur. Bioscan., 7: 73–76.

Vlazek, E., J. Kobus Sisovska, M. Przyor, J. Kurczak, M. Remezewsky, E. Korbas and M. Buchowski (2013). Chemical characterization and antioxidant properties of the Polish variety of aqueous extracts *Morus alba* L. Leaf from the laboratory and large-scale experimental processes. Agric. Sci.,4:141–147.

Wang, X., Q.F. Ding, H.G. Chen and X. Zhu (2017). Characterization and effect of activity on ADH of polysaccharides of mori fructos. Chin J. Chen. Mother. Mead., 42: 2329 – 2333.

Wu, X., L. Huang, X. Zhu and J. Liu (2020). Curcumin protects cardiomyopathy damage by inhibiting the production of reactive oxygen species in mice with type 2 diabetes. Biochem. and Biophysical Res. Communic., 530: 15-21.

Yu, L., W. Sun, L. Cheng, Z. Jin, W. Yang, M. Chai and W. Duan (2014). Melatonin receptor mediated protection against myocardial ischemia/ perfusion injury: the role of SIRT1. J. Conif. Res., 57 (2): 228-238.

Zawli, A., T.D. Xuan and S. Tawata (2007). Essential oils, kava peronates and phenolic compounds of Alpinia zerumbet leaves and roots and their antioxidant activity. Food Chem., 103: 486–494

Zeng, W.C., Z. Zhang and L.R. Jia (2014). Antioxidant activity and characterization of antioxidant polysaccharides of pine needle

(*Cedrus deodara*). Carbohydrate polymers, 108: 58-64

Zhang, N.W., J.F. Li, Y.X. Hu, J.L. Cheng, X.Y. Zhou and F.Q. Liu (2010). Effects of ofastragalus polysaccharide on the immune response to thrush fevervaccine in rats. Carbohydrate Polymers, 82: 680-686.

Zhengcheng, L., Z. Jingying, L. Peng, Y. Tingshu, X.F.X. Fanxing, W. Bo, B. Kaixun and J. Weing(2019). Okra polysaccharide (*Abelmoschus esculentus* L. Moench.) improves antioxidant capacity across PI3K/AKT pathways and Nrf2 overflow in the type 2 diabetes model. Particles, 24: 17 – 29.

التحليـــل الكيميـــائـــي ومضـــادات الأكســـــــدة والســـــكريات فـي ثمـــار التـوت الأبيـض

أسماء أبو بكر علي[1*] - حفناوي طه حفناوي[1] - فاتن محمد إبراهيم[2] - لمياء محمد مصطفي المغربي[1]

1- قسم الكيمياء الحيوية - كلية الزراعة- جامعة الزقازيق - مصر

2- قسم بحوث النباتات الطبية و العطرية- معهد بحوث الصناعات الصيدلية و الدوائية– المركز القومي للبحوث- مصر

ثمار التوت الابيض فاكهة منتشرة في مصر ويتناولها اغلب الناس ذات الطعم المقبول لذا حاولنا في هذا البحث ان نبيين التركيب الكيميائي للثمار ومدي فاعلية التوت الابيض كمضاد للاكسدة وايضا معرفة نوعية السكر العديد الذي يتواجد في التوت الابيض ودراسة خواص السكر العديد من حيث نوعية الوحدات البنائية الداخلة في تركيبة والمجاميع الفعالة الوزن الجزيئي له وايضا قدرته كمضاد اكسدة ولتحقيق هذا الغرض قمنا بجمع ثمار التوت الابيض من السوق المحلي لمدينة الزقازيق وتم غسلها وتجفيفها ثم الطحن والتخزين في ظروف ملائمة من حرارة (4 درجة مئوية) وتم تقدير التركيب الكيميائي من رطوبة ورماد كلي وبروتين خام ودهن خام والياف ثم الكربوهيدرات عن طريق الفرق وكانت النتائج كالاتي كنسبة مئوية للعينة الجافة من ثمار التوت الابيض (12.02 – 14.42 – 21 – 4.83 – 15 – 59.75) علي الترتيب. تم تقدير الفينولات الكلية وايضا الفلافونيدات الكلية من خلال نوعين من المستخلصات هما المستخلص المائي والمستخلص الكحولي للوقوف علي فاعلية اثمار التوت الابيض كمضاد للاكسدة حيث كانت النتائج هي (23.5 – 13.9 ملجم حمض جاليك / جرام مستخلص فينولات كلية مستخلص مائي وكحولي علي التوالي اما بالنسبة للفلافونيدات الكلية فكانت النتائج هي (1.43 – 2.54 ملجم كراستين /جرام مستخلص مائي وكحولي علي التوالي واخيرا بالنسبة للنشاط المضاد للاكسدة لثمار التوت الابيض تم تقديرها بطريقة DPPH وكانت النتائج المتحصل عليها هي (6.66 – 1.97 ملجم فيتامين سي / جرام مستخلص مائي وكحولي علي الترتيب. تم فصل السكر العديد من ثمار التوت الابيض وتنقيته واتضح من النتائج الحصول علي نوعين من السكر العديد في ثمار التوت الابيض يختلفان في الوزن الجزيئي وايض في نوع الوحدات البنائية الداخلة في التركيب حيث تم التوصل الي سكر وزنة الجزيئي 1.25 * 10 ^{4} - 5.29 * 10 ^{4} دالتون واطلق عليهم كسر 1 وكسر 2 علي التوالي اما نوعية السكر الاحادي فكانت جلوكوز – مانوز -ارابينوز -فركتوز حمض المانورونيك اسد في الكسر 1 ام الكسر 2 فحتوي علي الوحدات البنائية التالية (جلوكوز – جالاكتوز – ارابينوز – حمض المانويورنيك اسد) وذلك من خلال النتائج المتحصل عليها من جهاز HPLC تم ايضا التعرف علي نوعية المجاميع الفعالة في كسور السكر العديد لدي ثمار التوت الابيض من خلال طيف الاشعة التحت الحمراء والتي بينت تظهر قمم في.4 1728 – 1619.2 - 3345.6 سم-1 للعينات والتي تشير الي وجود مجموعة CH2 ومجموعة C=O مجموعة الهيدروكسيل علي التوالي، من خلال النتائج المتحصل عليها يمكننا القول ان استهلاك ثمار التوت الابيض يمد جسم الانسان بمركبات مضادة للأكسدة طبيعية وايضا محتوي عالي من البروتين والاملاح والكربوهيدرات الكلية.

المحكمـــون:

1- أ.د. أيمن يحيى الخطيب أستاذ ورئيس قسم الكيمياء الحيوية – كلية الزراعة – جامعة المنصورة.

2- أ.د. علي عثمان محمـد أستاذ الكيمياء الحيوية – كلية الزراعة – جامعة الزقازيق.