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Original Article

Functional And Nutraceutical Characterization of Mulberry Leaves

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ABSTRACT

Mulberry (Morus spp., Moracecae) is a notable medicinal and multi-functional plant. Distinct mulberry types are spread in subtropical, temperate and tropical areas all over the world. Mulberry leaves carry a number of bioactive compounds with it along with various pharmacological effects. It is a magnificent origin of nutrients, phytochemical and is been proven as nutraceutical. Mulberry leaves have a wide range of pharmacological effects having bacteriostatic, anti-hyperlipidemia, lowering blood glucose, anti-hypertensive, and antiviral properties. Mulberry leaves caloric content was determined as of carbohydrate (CHO), protein, fat, fiber, moisture and ash. Mulberry leaves contained the uppermost amount of carbohydrate (28.37%) in T1 and the lowermost amount of moisture (5.2%) in T1. The mineral content of macro-elements and micro-elements were determined from chromium (Cr), manganese (Mn), iron (Fe), zinc (Zn), magnesium (Mg), phosphorus (P), calcium (Ca), potassium (K) and sodium (Na). The content of minerals in mulberry leaves contained the highest amount of manganese (34.7) in T1 and the lowest sodium in T1. The content of vitamins in mulberry leaves contained the fat-soluble and water-soluble vitamins β-carotene, C, B3 and B2 were determined. Amongst the vitamins, mulberry leaves contained the uppermost value of β-carotene (14.0mg) and the lowermost value of vitamin B3(0.04). Bioactive compounds of mulberry leaves were determined are rutin, catechins, alkaloids and quercetin. Among the bioactive compounds in mulberry leaves contained the uppermost value of quercetin (763mg). The total phenolic content was (0.42-0.80 mg) Conclusively, mulberry leaves is enriched with nutritional values. So, it can be used in the treatment of various disease.

INTRODUCTION

Morus nigra L., sometimes called as mulberry's leaf, is a type of Traditional Chinese Medicine (TCM) is a sort of Chinese medicine that are being used from decades in clinical settings (Dhiman, S et al., 2020). Mulberry is a well-known herbal remedy (Morus spp., Moraceae). The mulberry leaf contains recognized amino acids, flavonoids, vitamins, minerals, and other active substances (Yadav, P et al., 2017). Mulberry is a shrub with several uses. Mulberry has made a name for itself as a useful food due to its high content of minerals and phytochemicals Bahmani, M et al., 2015). The mulberry tree's leaves, roots, bark, and fruits were used to treat coughs, fevers, hyperlipidemia, hypertension, and hyperglycemia have all been treated with them in traditional medicine for a long time (Devaraj, N. 2017). Mulberry leaves are light green in color and are slender and glossy. Even within the same tree, the leaf shape might vary considerably Zubair, F et al., 2018). Typically, the plantation is elevated and put out in a block configuration with Plant to plant and row to row, 6.5 feet or 7feet x 7 feet spacing. During the monsoon season (July-August), the plants are normally clipped 5-6 feet tall, with no more than 8-10 shoots at the crown, and let to mature (Abdulla, M. A et al., 2009). [6]. A leaf harvesting method is used 3-4 times a year to harvest the leaves. Peroxisome proliferator-activated receptor and Activated protein kinase activation expression by a Mulberry leaf extract rich in DNJ, quercetin, and kaempferol resulted in an increase in free fatty acid -oxidation and lipid breakdown (Abd el-Mawla et al., 2011). Mulberry is an edible fruit that has been used for centuries to heal fevers, protect,

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strengthen joints liver, and control blood pressure. The mulberry and tulsi, ashwagandha blends that function as antioxidants, immunomodulators, mood stabilizers, antiviral, and antibacterial. To promote human health, this product can be utilized in either dry or liquid form (Ahn, E et al., 2017). For better compliance, the flavor of This herbal bent can be made with or without sugar and created with FDA-approved natural ingredients. More study is needed to develop herbal mixtures for the treatment of various ailments (Banu, S et al., 2015). Flavonoids (quercetin and its homologues), sitosterols, and polysaccharides are among the chemical constituents of mulberry leaves (Hamid, H et al., 2017). Its active ingredient, quercetin, has been shown to lower blood lipids, lower postprandial blood glucose, reduce inflammation, and fight tumors. In a conventional clinic, the antioxidant quercetin has also influenced hepatic lipid metabolism (Hu, X. Q et al., 2017). Mulberry leaves, deliver trace elements, fiber, protein, vitamins, C, beta carotene, minerals, phosphorus, sodium, iron, potassium, calcium, as well as chemical compounds that are acknowledged to promote health facilities and prevent disease (Lee, D et al., 2018). The study aimed to recognized the mulberry leaves chemical analysis as well as mineral profile to assess its nutritional value, in human health it plays a prominent role in lipid profile reduction, so aimed to find the nutritional composition of mulberry leaves. Considering mulberry as a common fruit but it has many health benefits. Many different diseases can treat through the use of mulberry leaves and is responsible for biological functions and possible uses.

METHODS

Procurement of Raw Material: In this study, mulberry leaves will be procured from the local market of Lahore; they will be washed and dried at room temperature until further analysis at the laboratory of university.

Characterization of Mulberry Leaves: Mulberry leaves extract will be analyzed for will be examined for various quality traits, including proximate and mineral analysis according to the methods of AOAC 2006 (Katsube, T *et al.*, 2006).

Proximate Analysis: After delivering the samples to the same size, mulberry leaves were investigated for its chemical composition, i.e., crude protein, crude fat, crude fibre, moisture and ash was quantified consistent with its relevant procedures and Nitrogen Free Extract (NFE) of Mulberry leaves extract will be performed and determined according to the methods described by AOAC 2006 (Katsube, T *et al.*, 2006). Details as mentioned below.

Moisture Content: A sample was collected of mulberry leaves, and it was analysed for its moisture content using hot air oven (Memmet Germany) at a temperature of 105±5°C as stated by method No. 44-I5A (Sukandar, E. Y et al., 2016).

Crude Protein: Samples should be taken, and the content of protein in the mulberry leaves sample was obtained by using method of Kjeldahl's according to method No 46-10 (Lim, Y *et al.*, 2020).

Crude Fat: The fat content was observed, and a sample was taken for the determination of fat content. The instrument that was used is the Soxhlet apparatus (Model: J.P. SELECTA: 630086, Spain) according to the AOAC 2006 (Huang, J *et al.*, 2018).

Crude Fiber: The extracted crude fiber will being used for determing fiber content by using sulfuric acid and digesting fat free samples (1.25%) in acid and ceramic fiber filter using following the protocol of AOAC 2006 (Kobayashi, Y *et al.*, 2015).

Total Ash: The total ash content of resultant powder will be estimated by direct incorporation of powder in a Muffle Furnace (Model: COMECTA: 2200851, SNOL) at 550°C by adopting the mentioned protocol of AOAC 2006 (Iqbal, S *et al.*, 2012).

Nitrogen Free Extract: The nitrogen-free extract (NFE) was quantified by deducting the %ages of total ash, crude protein, crude fat, crude fibre and moisture from 100 (Srivastava, S et al., 2006).

Mineral determination: Mineral content of macro-minerals Magnesium (M), Potassium (K), Phosphorus (P), Calcium (Ca), Sodium (Na), micro-minerals Manganese (M), chromium (Cr), Zinc (Zn), Iron (Fe) of mulberry leaves was assessed with the use of atomic absorption spectrophotometer (Perken Elmer) by adopting the procedure given in AOAC 2006 (AOAC, 1990).

Vitamin Content

Water-soluble vitamins: Water-soluble vitamin for example niacin (B3), riboflavin (B2), ascorbic acid and beta carotene were obtained using the method of high-pressure liquid chromatography (HPLC) and Application Note: 1994 of Dionex Vydac as stated by Finglas and Foulks (1984) and Kamman, Wanthesen, and Labuza (1980) (Finglas PM et al., 1984).

Fat-soluble vitamins: Fat-soluble vitamins eg, vitamin E, A were obtained using method of high-pressure liquid chromatography (HPLC) according to Manz and Philipp (Manz, U et al., 1981).

Bioactive compounds: Quercetin, catechins, total phenols, total flavonoids and deoxynojirimycin were determined. Using a method of gas chromatographic-mass spectrophotometric (GC-MS) aimed at the extraction as well as analysis for components of mulberry leaves (Yuan, Q et al., 2017).

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Total Phenolic Content (TPC): Total phenolic content (TPC) was performed using FC (Folin- Ciocalteu reagent) and by the procedure adjusted with small scale (Mena, Martí, Saura,2013).Using a spectrophotometer, the total phenolic content test was determined (Yan, F et al., 2017).

Ferric-Reducing Antioxidant Power (FRAP): Ferric reducing antioxidant power of extracts will be used to determine the concentration of estimated total antioxidant (Sevindik, E. at el., 2018).

Statistical Analysis: Data attained from each parameter was subjected to statistically analyzed using SPSS 25-version.

Results and Discussion: This study was supervised to attained the nutritional amount of leaves of mulberry found locally. Determination of mulberry leaves proximate analysis was done by using standard procedures then presented in Table 1.

Treatments	Moisture %	Protein %	Fat %	CHO %	Fiber %	Ash %
T1	5.2	18.40	6.46	28.37	25.12	8.7

Table.1 Proximate analysis of mulberry leaves

Results of Table 1 revealed that proximate analysis of mulberry leaves include content of fat, carbohydrate, protein, ash, and fiber range were 5.2%, 18.40%, 6.46%, 28.37%, 28.37%, 25.12 and 8.7%. The results obtained revealed that mulberry leaves contain high content of carbohydrate. The content of protein and fat was lower as compared to carbohydrate. Correspondingly, another study by (Shahid et al., 2012) found in mulberry leaves, the content of fat, carbohydrate, protein, ash, and fiber were range as 3.6%, 3.5%, 6.05%, 1.2%, 13.9, 28.38% and respectively.

Mineral Content: Determination of mineral content of mulberry leaves was done by using the standard procedures then presented in Table 2.

Sr.	Micro Minerals				Macro Minerals				
1	Iron	Zinc	Nitrogen	Manganese	Calcium	Magnesium	Potassium	Sodium	Phosphorus
2	4.8	24.8	2.0	34.7	1.5	2.8	3.7	0.02	0.4

Table 2. Mineral Content of Mulberry Leaves.

The table 2 showed that the mulberry contains iron, zinc, chromium, manganese, calcium, magnesium, potassium, sodium and phosphorus the mineral contents range were 4.8, 24.8, 2.0, 34.7, 1.5, 2.8, 3.7, 0.02, 0.4. Mineral's content of mulberry leaves includes the uppermost value for manganese whereas the lowermost value for sodium. In contrast, iron, calcium and phosphorus content are lesser than the stated values by (Sanchez-Salcedo et al., 2017) they found that mulberry leaves contain mineral content iron, zinc, calcium, magnesium, potassium, sodium, and phosphorus were range as 119.3, 21.8, 1.6, 1.3, 3.6, 0.01 and 0.2 correspondingly.

Vitamins Content: Determination of vitamin content of spice cinnamon was done by using the High-Pressure Liquid Chromatography (HPLC), then presented in Table 3

Vitamin Content	
Ascorbic acid	11.30 -15.37 mg/100g
Riboflavin	0.58 mg/100g
Niacin	0.04 mg/100g
b-carotenes	14.0 mg/100g

Table 3. Vitamin Content of Mulberry Leaves.

This table showed that mulberry leave contains β-carotene, ascorbic acid and B3, B2 were range as 14.0 mg/100g, 0.04 mg/100g, 0.58 mg/100g and 11.30-15.37 mg/100g. Among the vitamins mulberry leaves contained the uppermost value of β-carotene and the lowermost value of vitamin B3. A study conducted by (Dhiman et al., 2020), who found mulberry leaves contain vitamin B3, B2 beta-carotene then vitamin C were range as 12.40-14.25mg, 0.45mg, 0.06mg and 15.5mg correspondingly.

Bioactive Compounds: Using gas chromatographic-mass spectrophotometric (GC-MS), mulberry leaves bioactive contents were determined and presented in Table 4.

Bioactive Compounds	
Alkaloids	660 mg/100g
Rutin	226 mg/100 g
Quercetin	763 mg/100 g
Catechins	432 mg/100 g
Total flavonoids	33.89 mg/ 100 g
Total Phenols	12.26 mg/ 100 g
Hemicellulos e	10.2 mg/100g

Table 4: Bioactive Compounds of Mulberry leaves.

The table showed that mulberry leaves contain bioactive compounds alkaloids, rutin, quercetin, catechins, total flavonoids, total phenols and hemicellulose was range as 660 mg/100g, 226 mg/100 g, 763 mg/100 g and 432 mg/100 g, 33.89 mg/100g, 12.26 mg/100g and 10.2mg/100g. Among the bioactive compounds, quercetin contained the uppermost value as well as the lowermost value of rutin. A study evaluated by Katsube T et al., 2015, who assessed bioactive compounds in alkaloids, rutin, quercetin, and catechins were range as 650mg/100g, 220mg/100g, 765mg/100g and 430mg/100g.

Estimation of Total Phenolic Content (TPC): Reagent named as Folin-Ciocalteu (FC) was used to determine the phenolic content of mulberry leaves by the help of assay

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root on spectrophotometric. Firstly, solution of Na2co3 that is 7.5% (2ml) was added in mixed solution of 200 microliter extract and reagent (1.10) diluted FC. Deionized H2O was added to raise the volume of solution and then to place it for 2 hours in dull black environment. Phenolic amount was measured by the absorbance that was calculated by spectrophotometrically on 765nm and tpc was determined by gallic acid correspondent (mg/g), gallic acid criterion was used for calculation (Katsube T et al., 2015)

Estimation of Total Flavonoid Content (TFC): Flavonoid content was determined by previously outlined procedure. Sodium nitrite of 0.3ml (5%) was mixed with extract of leaves (1ml) and deionized H2O of 4ml placed in flask that has space for 10 ml and solution was mixed together. 0.3 ml later 5mins the aluminum chloride with 10% was poured and then 1 mole sodium hydroxide of 2 ml was poured at 6 min. For proper mixing procedure the deionized H2O 2.5ml was mixed so that content dilute properly. Flavonoid amount was measured by the absorbance that was calculated by spectrophotometrically on 510nm and tfc was determined by rutin correspondent (mg/g), rutin criterion was used for calculation.

Ferric Reducing Antioxidant Power (FRAP) Assay: FRAP was determined by previously outlined procedure with few changes. Mixture of 2,4,6-Tripyridyl-S-triazine in 40 nm in sodium chloride with 10 nm and 300nm acetate buffer with ferric chloride hexahydrate 20nm was added to prepare a solution. Different concentrations were used for accurate solution preparation like 2.5ml ferric chloride hexahydrate, 2.5ml 2,4,6-Tripyridyl-S-triazine and 25ml acetate buffer was then placed at heating to a optimum temperature of 37 °C to stabilize the 3.6pH. Sample of 25µl was assorted with functioning mixture of 2 milliliter and at 593nm absorbance of the solution was noted for 4 minutes opposed to blank solution. The results were calculated by using this method in micromoles per gram. (Katsube T et al., 2015).

Physiochemical parameter	
FRAP	117.83 µmolAAE/g
DPHH	41.49 µmol TE/g
TPC	0.42 -0.80 mg GAE/g
TFC	276 mg QE/100g

Table 5: Physiochemical Parameters of Mulberry Leaves.

CONCLUSION

This study spotlights the therapeutic activity of mulberry leaves in healthcare. It is proven in previous studies that mulberry leaves are safe to use and possesses medicinal properties. Mulberry leaves contains nutritive values of carbohydrates, protein, fat, fiber moisture and ash. The mineral content of macro elements magnesium, sodium, potassium, phosphorus and microelements iron, zinc, chromium and manganese were determined. Mulberry leaves includes vitamins B2, B3, C and beta carotene. The bioactive compounds of mulberry leaves are rutin, catechins, alkaloids and quercetin. Mulberry leaves has a very strong capacity for antioxidants. Morus nigra has just few anti-nutrients but high amount of protein and ash content so it can be used for nutraceutical purposes too. Studies suggest that mulberry leaves have effective potential for prevention of diseases.

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