An Overview of Correlations Between Therapeutic Uses and Chemical Composition of *Morus nigra* and *Morus alba* Species Fruits

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Abstract

The therapeutic effects of the *Morus nigra* L. and *Morus alba* L. fruits can be highlighted by their bioactive principles composition. The paper correlates recognized therapeutic uses of mulberries species in folk medicine with the chemical composition literature data. Phytotherapeutic treatments based on mulberry fruit extracts are highlighted, which have given very good results in many diseases such as, allergies, asthma, gout, rheumatism, chronic fatigue, growth disorders, circulation disorders, hoarseness, pharyngitis, tonsillitis, hepatitis viral stress and exhaustion, vitamin and mineral deficiencies, hypercholesterolemia, gastroduodenal ulcer, prostate cancer. In the present paper, applying specific methods to pharmacognostic studies, active principles have been identified in various concentrations of hydroalcoholic fruits extracts. Could be consider that the indigenous vegetable products *Morus nigra* L. and *Morus alba* L. fructus harvested from the Dobrudja area flora, eastern of Romania, region with a favorable climate for the accumulation of vitamins and active ingredients, represents an important source of total phenols, ascorbic acid, citric acid, niacin and riboflavin. The increased content of carotenoids pigments and phenolic compounds emphasize the valuable antioxidant activity, which could be corelated also, with their important minerals content such as, K, Na, Ca, Mg, Fe, Zn. In order for these natural substances to be properly accumulated by the human body, we recommend the second half of June as the optimal harvest period for ripe fruits and using in various phytotherapeutic forms, juices, syrups, teas, or as fresh food fruits.

Keywords: *Morus nigra* L., *Morus alba* L., phytotherapeutic, antioxidant activity, phenolic compounds

Introduction

The mulberries reside into the Morus genus, family Moraceae. Twenty-four species of Morus have been found to exist in the world with over one hundred different varieties. Morus species can be found from the Northern hemisphere to the Southern one and can grow in temperate and subtropical regions. They are widely spread from sea level to up to 4000 m altitude [1]. The name of the plant has its origin in Turkish *dut* <Persian (*tut*): mulberry, s.m. = the name of two species of trees with asymmetrically lobed leaves, with small, fleshy, white (*Morus alba* L.) or black-reddish (*Morus nigra* L.) fruits, with a sweet fad taste, whose leaves represent food for silkworms; acute; From the Turkish *dut*. = tree with small, fleshy, white or black fruits (*Morus alba*, *Morus nigra*). It has been suggested that the generic Morus name comes from the Latin word *mora* (delay) due to the late appearance of buds [2].

The genus *Morus* (mulberry) is represented by trees with a height of 12-15 m, originating from China and Japan (white mulberry) and from Persia (black mulberry). Mulberry displays a long history of therapeutic and medicinal use in China, with almost every part of the plant being exploited. It is currently widespread in Asia, Europe, Africa and North America. In Romania it is cultivated in the hill and plain areas. As a plant it belongs to the branch Magnoliophyta, class Magnoliopsida ordinal Rosales family Moraceae, genus Morus [3].

The black mulberry has been known throughout southern Europe since ancient times, and is thought to have been brought from Persia. It has been mentioned by most early Greek and Roman writers; Pliny observed it and described its use as a medicinal plant. Its leaves were used by the Romans to treat diseases of the mouth, trachea and lungs, and since the 17th century the bark of the roots has been used here as a dewormer, and also as a dye [4].

Mulberry leaves oftenly serve as food for silkworms. In ancient times, silk was considered a product of mulberry leaves, the activity of silkworms not being known and understood. The worm culture was introduced by Justinian of Constantinopole (527-565). In Italy, the black mulberry was used to grow silkworms until about 1434, when the white mulberry was introduced from the Levant, which was after that point the preffered choice [5]. Mullbery leaves have been used in the past as tea or powder juice [6]. In Korea, mulberry leaves are used to make ice-cream flavors and in India, they are used as a good nutritious, cheap food for breakfast and dinner [7]. Currently mulberry leaves have been approved as an excellent food source rich in protein, vitamins, microelements and dieteray fibers.

Mulberry leaf contains tannins, flavonoids, glycosides. Recent studies have shown the presence of a substance - 1-Deoxynojirimycin - inhibitor of the enzyme glycosidase I with a role in the processing of oligosaccharides in the body, thus being responsible for the hypoglycemic effect of the leaf.

In the present paper, through applying specific methods to pharmacognostic studies, active principles have been identified in various concentrations of black and white mulberry hydroalcoholic fruits extracts.

Morus nigra L. and *Morus alba* L. in folk medicine

As a traditional folk medicine, mulberry was used as an analgesic, emollient, sedative, the leaves being considered antibacterial, astringent, diaphoretic, hypoglycemic, dental and ophthalmic. Bark tincture is used to relieve toothache. Romanian traditional medicine from the beginning of the 20th century used the peel of mulberry root with a hot and bitter taste, for its purgative action, e.g. Mororum syrup, is prepared from the fruit, with a laxative, slightly diuretic effect. The white mulberry had several uses in the popular tradition: the leaves represented food for silkworms, the fermented fruit was used to produce brandy and the mulberry leaf tea was used to cure liver disease. From the bark of the black mulberry branches, gathered in the spring, which was boiled and sweetened with sugar, a cure was obtained - which was taken in the morning, on an empty stomach, against tapeworm [8]. Mulberry is used as an excellent remedy in the treatment of: lung diseases, gastric and duodenal ulcers, sore throats, diarrhea (mulberry leaves), diabetes, chronic enteritis, stomatitis, tapeworms (bark), asthenia (mulberry leaves) [8].

In natural products mulberry leaves are used mainly due to their hypoglycemic action, being indicated in mild forms of diabetes. Mulberry leaves are also used for their valuable antioxidant properties.

Popular treatments with mulberry

Mulberry fruits, if eaten in the morning, 200 g on an empty stomach for 10 days, will cleanse the body of toxins. Also, the juice of ripe mulberry fruit, due to the citric acid it contains, is considered an excellent remedy in treating canker sores, angina and stomatitis.

Mulberry syrup is very effective in treating constipation. It is prepared with the help of a cup full of ripe mulberries, 2 cups of sugar and a liter of water. After boiling and reboiling several times, it is recommended, after cooling that the mulberry syrup be stored in a tightly closed container and kept in a cool place.

Mulberry leaf tea is drunk three times daily after each main meal and has an effect in treating diabetes and diarrhea. This mulberry leaf tea is also recommended to treat chronic enteritis and intestinal worms [9]. Mulberry root peel is very useful in treating ascites and intestinal worms. Thus, 2 crushed teaspoons of the root peel are boiled in 300 mL of water, until the liquid drops to 200 mL.

Material and method

The indigenous vegetable products *Morus nigra* L. and *Morus alba* L. fructus harvested from Dobrudja area flora, eastern of Romania, have been used. The plant material researched, mulberries represent the fruits of the black mulberry, *Morus nigra* L., and

the white mulberry *Morus alba* L. called drupes. Harvesting is done from Dobrudja spontaneous flora and crops, manually, from May to the end of June period.

The whole, healthy, well-ripened, black fruit have been harvested. The transport was done in paper bags, the plant material was protected from the direct action of the sun and high temperatures that could favor fermentation.

For the study, black mulberries were harvested from gardens and yards, which benefited during the development of different climatic and soil conditions in the Constanta area. These areas have brown and yellow-brown wild soils, with average annual temperatures of 9-11 °C. First, a general description for the botanical characterization of the plant was made.

The pharmacognostic study was performed using specific methods. In order to identify the active ingredients, we used the vegetable product remaining from the alcohol extraction, dried and extracted with 100 mL of water in a 90 °C water bath for 30 minutes. The solution is used for reactions, specific to groups of active principles soluble only in water (oozes and polyoozes) or not depleted by alcohol (tannins, alkaloids, etc.).

Etheric extractive solution

It was weighed 10 g of freshly sprayed vegetable product, which was first extracted with ethyl ether (2 x 50 mL), refluxing for 15 minutes. Each reflux was followed by filtration, the obtained solutions being collected in a ground flask. The ether solutions were concentrated on a water bath to 50 mL Solution A was obtained. It was used to carry out the reaction characteristic of lipophilic compounds. The ether-depleted vegetable product was stored for further alcohol extraction [8], [10].

Alcoholic extractive solution

The vegetable product depleted with ether, was brought to the water bath, in order to remove traces of ether. The vegetable product was extracted with methylic alcohol (2 x 100 mL) by refluxing for 30 minutes. The combined methanolic solutions have been concentrated to 50 mL by distillation of the solvent and have been divided into two parts. One part was used to identify the active principles on the non-hydrolyzed solution (25 mL), and the other part was subjected to hydrolysis with 15 mL 10% hydrochloric acid on the electric jacket, with water for 30 minutes (Fig. 4.2). Exhausted vegetable product is stored for water extraction.

Water extractive solution

It was used the remaining vegetable product from the alcohol extraction, dried and extracted with 100 mL of water in a 90 °C water bath for 30 minutes. The solution was used for reactions specific to groups of active principles soluble only in water (oozes and polyoozes) or not depleted by alcohol (tannins, alkaloids, etc.).

To identify the chemical compounds in the three extracts, we analyzed them separately, using methods corresponding to the physicochemical properties for each group of active principles.

Results

Botanical characterization

Mulberry are woody plants (trees, shrubs), herbaceous, that contain a latex, rich in rubber. They possess a straight, gray stem, branched from a low height, with a sparse crown. Mulberry leaves are thin, very different in shape (ovate or elliptical, acute or short acuminate), with a round base and an irregularly serrated edge; they are divided into 3-5 unequal or undivided lobes, glabrous, smooth, slightly glossy on the face and short pubescent at the ribs, on the back [11]. The unisexual flowers, grouped in cymose or amentiform inflorescences, are arranged monoeciously or dioeciously. The mulberry is a dioecious plant, so for 5-6 females a male is planted. There is also the possibility of grafting a male branch on a female crown. The flowers are unisexual, the male ones are cylindrical, and the female ones are oblong. It blooms in May. The plant bears fruit only from the age of 7-9 years.

Fig. 1. *Morus nigra* L. tree and leaves *(original)*

Fig. 2. *Morus alba* L. tree and leaves [23]

Fig. 3. *Morus nigra* L. leaves [24]

Fig. 4. Morus alba L. leaves [25]

Morus nigra is a deciduous tree that can grow up to 12 meters tall and up to 15 meters broad. Its leaves are 10-20 cm long and 6-10 cm broad, long, downy on the underside and short and rough on the surface, see Figs 1, 3 and 5.

Morus alba is a medium sized tree which grows up to 20 meters tall. It generally has a pretty small lifespan, although there are few exceptions that can live up to 250 years. If the tree is old the leaves are up to 12 cm long, sawlike on the edges, circular at the top and cordate at the base. The fruit is simple (achene or drupe) or compound, formed by the development of the receptacle, the perigon and the axis of the inflorescence. The fruit (mulberry) is composed of numerous false, small drupes, arranged on the axis of the inflorescence, which becomes fleshy. The multiple fruit is called sorose (mulberry, acute), see Figs 2, 4 and 6.

Fig. 5. *Morus nigra* L. fruits [24]

Fig. 6. *Morus alba* L. fruits [26]

Black mulberry is an edible fruit, its dark purple color is given by anthocyanins. The fruit contains small drupes that form a cluster, each drupe being 2-3 cm long. Black mulberry each drupe being 2-3 cm long [8]. Black mulberry fruits are richly flavoured in comparison to the white mulberry which are more insipid. White mulberry is

cultivated in many areas for the production of silk and for the fast release of pollen. Mulberry fruits are 1.5 cm long, in wilderness it can be of deep purple color, but usually the cultivated plants have white to pink fruits [8]. In comparison to black and red berry, fruit is sweet but bland.

Table 1 Differences between the two *Morus* varieties

***Morus alba* L.**	***Morus nigra* L.**
The leaves of the white mulberry have a length of 5-12 cm, are fragile, shiny, smooth and often lobed	The leaves of the black mulberry are larger (up to 20 cm), thick, rough and rarely lobed.
The white mulberry fruit is ripe in late spring	The fruit of the black mulberry is not ripe until early summer.
The white mulberry fruit has a long stalk (sometimes the length of the fruit)	The black mulberry fruit has practically no stalk or a very short stalk.
The white mulberry fruit is sweet before ripening, but not very sweet when it is ripe	The black mulberry fruit if very acid before ripening and it is very sweet when it is ripe
White mulberry fruit is generally unappreciated in some countries	The aroma of black mulberry fruit attracts almost the whole general public

Important bioactive compounds present in *Morus nigra* L. and *Morus alba* L. fruits

Following the reactions performed on the three extracts, etheric, alcoholic and aqueous obtained from the fruits of the species *Morus nigra* L. and *Morus alba* L., the many active principles were identified, see Table 2. The aglycones from the etheric extractive solution have been obtained through evaporation, after treating the obtained residue with CH_3OH, using the Shibata reaction [8].

Table 2. Active principles identified in the three extracts from the fruits of *Morus alba* L. and *Morus nigra* L. species

Analyzed solution	***Morus alba* L.**	***Morus nigra* L.**
Etheric extractive solution	Aglycones (flavonic, anthraquinone, coumarines and triterpenes)	Aglycones (flavonic, anthraquinone, coumarines and triterpenes)

	Volatile oils	Volatile oils
	Fatty acids and resin acids	Fatty acids and resin acids
	Alkaloid bases	Alkaloid bases
	-	Carotenoids
	Sterols	Sterols
Alcoholic extractive solution	Gallic tannins	Gallic tannins
	Resins and alkaloids	Resins and alkaloids
	Reducing compounds	Reducing compounds
	Flavonoides	Flavonoides
	Sterol and triterpene heterosides	Sterol and triterpene heterosides
	Alkaloids	Alkaloids
	Amino acids	Amino acids
	Polyphenols	Polyphenols
	-	Antocianosides
Water extractive solution	Polyholosides (mucilages)	Polyholosides (mucilages and pectins)
	Oozes and polyoozes	Oozes and polyoozes
	Gallic tannins	Gallic tannins

Volatile oils have been obtained using evaporation, treating the residue with alcohol, sterols and triterpenes using the Liebermann-Burchard reaction, carotenoids using the reaction with H_2SO_4 [8]. In the alcoholic extractive solution, the gallic tannin was identified using $FeCl_3$ reaction, alkaloids using the Mayer and Bertrand reaction and antocianosides were identified using an aqueous solution with acid pH [8]. The oozes and polyoozes from the water extractive solution have been identified using extract concentration and the residue was treated with H_2SO_4 and with Timol alcoholic solution [8].The ether extract contains valuable lipophilic, chemical compounds. The carotenoids that were found only in *Morus nigra* are highlighted. In the alcoholic extract there are hydrophilic chemical compounds. Table 2 highlights the existence of alkaloids in both etheric and alcoholic extractive solutions. In the aqueous extract, in addition to the alcohol-soluble compounds, except for the resins, there are polyholosides such as, pectins, mucilages, gums [8], [10].

Discussions

Phytotherapeutic treatments based on mulberry fruit extracts

The active principles of the black mulberry fruits *Morus nigra* L., *Morus alba* L. are synthesized in plant cells. In order for these natural substances to be properly utilised by the body, they must be found in various phytotherapeutic forms (juices, syrups, teas) [12]. The phytotherapeutic treatment based on mulberry fruit extracts gave very good results in the following diseases: allergies, asthma, gout, rheumatism, chronic fatigue, growth disorders, circulation disorders, hoarseness, pharyngitis, tonsillitis, viral hepatitis, conditions stress and exhaustion, vitamin and mineral deficiencies, hypercholesterolemia, gastroduodenal ulcer, prostate cancer. We consider that the vegetable product *Morus nigra* L. fructus harvested from the local flora is an important source of total polyphenols. We recommend the second half of June as the optimal harvest period, and as areas with a favorable climate for the accumulation of vitamins and active ingredients, from the the hilly and mountainous areas [13].

Phytotherapeutic treatments based on mulberry leaf extracts

Mulberry leaves contain mainly moranolin or L-deoxynojirimycin (DNJ), a piperidine alkaloid that inhibits the intestinal enzymes responsible for the breakdown of complex carbohydrate molecules into simple absorbable sugars. It is one of the strongest alpha-glucosidase. The concentration of DNJ in most mulberry leaf products is less than 0.2%. Extracts obtained from young mulberry leaves can contain between 0.5 and 3% DNJ. After oral absorption, moranoline is absorbed into the circulation and then rapidly excreted in the urine. Regarding the total alkaloid content found in mulberry fruits, it was reported for *Morus alba* L. 660 ± 5.25 mg / 100g FW and for *Morus nigra* L. 630 ± 5.93 mg / 100g FW [14], [15].

Hypoglycemic action

Mulberry leaves have been used in diabetes treatment since the earliest times. The effect of preventing and treating type II diabetes has been confirmed by recent studies by researchers in humans and laboratory animals. It is believed that this antiglycemic action is due to the synergistic effect of moralinoline, glycopeptides, flavonoids [16]. An important category of consumers prefer supplements from plant extracts to synthetic substances. In addition, mulberry leaf extract also contains compounds that stimulate insulin secretion and antioxidants.

Anti-inflammatory and anti-allergic action

Flavonoids and related compounds isolated from mulberry bark have anti-inflammatory effects. The extract obtained from the bark of Morus root in hot water possesses strong antihistamine and antiallergic activity [16].

Vasoactive action

Mulberry leaves can have a significant cardiovascular effect. Two main components of the

leaves, rutin and quercetin, induce considerable vasorelaxation.

Neuroprotective action

In diabetes there are subtle disturbances that include alterations in neurotransmissions, which generates learning disabilities and memory deficits. Indeed, the ability to learn and memorize is reduced in patients with type I diabetes, and those with type II diabetes have been observed to impair verbal memory or complex information processing [17]. The methanolic extract obtained from mulberry leaves has antidopamine activity, demonstrated experimentally in mice. The experiment confirms the potential of the clinical applications of mulberry in the management of psychiatric diseases. In addition, the mulberry possesses adaptogenic activity demonstrated on a model animal with chronic stress, with the possibility of its clinical use as an anti-stress agent [16].

Anticancer action

The methanolic extract of mulberry leaves shows strong cytotoxic action against cancer cells. A glycoside was isolated from the bark of Morus root, which significantly inhibited the proliferation of human ovarian cancer cells [16].

Anti-obesity action

Studies in rats with diet-induced obesity have shown that chronic treatment with ethanol extract obtained from mulberry leaves has an anti-obesity effect. This effect can be partly explained by the antagonistic action of mulberry extract on hormone receptors, which ultimately lead to a decrease in body weight [17].

Lipid-lowering action

Diabetes is associated with many lipid abnormalities. The extract obtained from mulberry leaves in butanol inhibited the growth of cholesterol, preventing atherosclerosis. Treatment with traditional Chinese herbs, including *Morus* sp., significantly reduces the accumulation of alcohol-related liver lipids, restores normal liver lipid levels, after four weeks of treatment and can be used as a remedy to prevent or treat alcohol-induced fatty liver [18].

Antioxidant and antimicrobial action

Lipid peroxidation of cell membranes can lead to cell necrosis and is thought to be involved in many pathophysiological conditions such as type I diabetes. Free radicals and reactive oxygen species also predominate in diabetes [18].

Mulberry contains several antioxidants compounds: polyphenols, carotenoids, vitamins A, C, E, which increase the antioxidant status of the body and modulate oxidation through various mechanisms [19].

The total polyphenols content has been determined using the Folin-Ciocalteu method and results may be different in the two species such as, in *Morus nigra* it has been identified 880±7.20 mg/100 g FW and in *Morus alba* 1650±12.25 mg/100 g FW [20]. Among the vitamins, mulberry fruits contain a significant amount of ascorbic acid, in *Morus nigra* 15.37±0.89 mg/100 g FW has been identified and in *Morus alba* 15.20±1.25 mg/100 g FW [20]. Other vitamins found were Niacin, for *Morus alba* 3.10 ± 0.60 mg / 100 g FW, and for *Morus nigra* 1.60 ± 0.1 mg / 100 g FW were reported.

A diet rich in plant antioxidants has been inversely correlated with mortality from coronary heart disease. Mulberry contains as main flavonoids quercitin-3-(6-maloniglucoside), the most abundant flavonol-glycolose in mulberry fruits. These have shown antioxidant activity in various experimental animal models. In order to ensure the stability of the antioxidant components and to preserve the antioxidant activity of the mulberry preparations, it is important that the leaves are dried at a controlled temperature, 60 °C [19].

Also, mouthwashes based on mixtures black mulberry, bitter cherry and cornelian cherry fruits hydroalcoholic extracts, present a significant antioxidant and antimicrobial activity and could be recommended in the oral cavity affections treatment, gum inflammations, dental pains, preventing of bacterial plaque formation, halitosis and would be a possible new antimicrobial alternatives, with lesser side effects that are often associated with synthetic antimicrobials [21, 22].

Minerals content

The *Morus* sp. minerals content it is also highlighted in the literature [20]. Thus the most potassium content is found in *Morus alba* 1731 ± 11.5 mg / 100 g FW and in *Morus nigra* 1270 ± 9.36 mg / 100 g FW, followed by calcium 576 ± 7.37 mg / 100 g FW for *Morus alba* and 470 ± 6.95 mg / 100 g FW, sodium and magnesium are comparable values. For sodium in *Morus alba* 280 ± 3.5 mg / 100 g FW and in *Morus nigra* 272 ± 5.32 mg / 100g FW, and for magnesium in *Morus alba* 240 ± 3,905 mg / 100 g FW and in *Morus nigra* 240 ± 3.51 mg / 100 g FW. Iron was also identified in *Morus alba* 73.0 ± 2.6 mg / 100 g FW and in *Morus nigra* 77.6 ± 1.98 mg / 100 g FW. It can be mentioned that all the values shown are highlighted to argue the antioxidant and nutritional action of *Morus* species. The registered values are different depending on several factors such as the type of soil, the area where the plant is grown, the local climate, etc.

Conclusions

The bioactive principles identified in the species *Morus alba* L. and *Morus nigra* L. make this plant particularly useful. Along with its importance as food for silkworms, knowing the beneficial actions for the human body is an important step to capitalize

on the health benefits of Morus. Phytotherapeutic treatments based on extracts from leaves and fruits of *Morus nigra* L. and *Morus alba* L. species are supported by the chemical compositions of the vegetal products and are verified by their use over time.

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