Review

A survey on macro- and micro-elements, phenolic compounds, biological activity and use of *Morus* spp. (Moraceae)

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Received 1 August 2012
Accepted 9 October 2012

Fruits, 2013, vol. 68, p. 333–347

DOI: 10.1051/fruits/2013079
www.fruits-journal.org

RESUMEN ESPAÑOL, p. 347

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Abstract – Introduction. Mulberry is the most medicinally important plant of the genus *Morus*. The mulberry fruit is used for many medical purposes. The species origin and adaptation, plant description, foliage, flowers and fruit, location, soil, and pests and diseases are summarized. **Composition of mulberry**. The studies from different countries show that the mulberry plant is rich in phenolic compounds, macro-elements (K, Ca, Mg, Na) and micro-elements (Fe, Zn, Ni). Phenolic compounds are found in all parts of the mulberry plant. **Uses**. Mulberry fruit can be used for making jam, jelly, pulp, fruit drinks, fruit sauce and cake. Mulberry fruits are rich in anthocyanins and should be exploited for the industrial production of natural color to be used in the food industry. **Conclusion**. The mulberry plant is of significant biological importance for its antioxidant and antimicrobial properties. Our study suggested the use of mulberry as a potential health food, or important antioxidant carrier in the food and pharmaceutical industries.

Serbia / *Morus* / fruits / proximate composition / polyphenols / antioxidants / microbiological analysis

Enquête sur les macro- et micro-éléments, la teneur en composés phénoliques, l'activité biologique et l'utilisation de *Morus* spp. (Moraceae).

Résumé – Introduction. Le mûrier est la plante médicinale la plus importante du genre *Morus*. Le fruit mûr est utilisé à de nombreuses fins médicales. L'origine et l'adaptation des espèces, la description de la plante, de son feuillage, de ses fleurs et fruits, sa localisation, ses ravageurs et maladies ont été synthétisés. **Composition du mûrier**. Les études effectuées dans différents pays montrent que le mûrier est riche en composés phénoliques, en macro-éléments (K, Ca, Mg, Na) et en micro-éléments (Fe, Zn, Ni). Les composés phénoliques sont présents dans toutes les parties de la plante adulte. **Utilisation**. Les fruits du mûrier peuvent être utilisés pour élaborer des confitures, gelées, pâtes de fruits, boissons aux fruits, sauces aux fruits et gâteaux. Les mûres sont riches en anthocyanes et aptes à être exploitées pour la production industrielle de colorants naturels à utiliser dans l'industrie alimentaire. **Conclusion**. Le mûrier est d'une importance biologique significative pour ses propriétés antioxydantes et antimicrobiennes. Notre étude suggère une utilisation de la mûre comme un aliment potentiel de santé ou une source importante d'antioxydants dans les industries alimentaires et pharmaceutiques.

Serbie / *Morus* / fruits / composition globale / polyphénol / antioxydant / analyse microbiologique

1. Mulberry presentation

Deep-colored fruits are good sources both of phenolics, including anthocyanins and other flavonoids, and carotenoids [1–4]. Mulberry fruits are rich in phenolics and have a unique delicious fruity, sour and refreshing taste [5]. They have been used as a folk remedy to treat oral and dental diseases, diabetes, hypertension, arthritis and anemia [6]. With the aim of finding new sources of natural antioxidants, plants, fruits, vegetables and other plant materials that are known to possess antioxidant activity have been investigated [7–12]. Our paper reports an overview of the studies on the mineral elements, phenolic composition, biological activity and uses of mulberry from different regions.

Morus, a genus of flowering plants in the family Moraceae (*table I*), comprises 10–16 species of deciduous trees commonly known as mulberries.

1.1. Origin

The more common *Morus* species are *M. alba* L., *M. rubra* L. and *M. nigra* L. The white mulberry (*Morus alba* L.) is native to eastern and central China. It became naturalized in Europe centuries ago. The tree was introduced into America for silkworm culture in early colonial times, naturalized, and hybridized with the native red mulberry. The red or American mulberry (*Morus rubra* L.) is native to the eastern United States from Massachusetts to Kansas and down to the Gulf coast. The black mulberry (*Morus nigra* L.) is native to western Asia and has been grown for its fruits in Europe since before Roman times [1, 6].

Table I.
Scientific classification of mulberry.

Kingdom	Plantae
(unranked)	Angiosperms
(unranked)	Eudicots
(unranked)	Rosids
Order	Rosales
Family	Moraceae
Tribe	Moreae
Genus	*Morus* L.
Species	*Morus alba* L., *Morus rubra* L., *Morus nigra* L.

1.2. Adaptation

The white mulberry and, to a lesser extent, the red mulberry are quite tolerant of drought, pollution and poor soil. In the USDA, the white mulberry is considered a weed tree in many parts of the country, including urban areas[1] [13]. The black mulberry is more fastidious, faring less well in cold climates or areas with humid summers. The white mulberry is the most cold-hardy of the three species *Morus alba* L., *M. rubra* L. and *M. nigra* L., although this varies from one clone to another. Some are damaged at –3.8 °C, while others are unfazed at 31.6 °C. Red mulberries are hardy to sub-zero temperatures. The black mulberry is the least cold-hardy of the three *Morus* species, although again cold tolerance seems to depend on the clone. In general, it is limited to USA Hardiness Zone 7 (0 °C to 18 °C) average minimum) or warmer. It has been planted only to a limited extent in America, mostly on the Pacific Coast. The mulberry makes a good town tree that will grow well in a tub [3, 4].

1.3. Description

All mulberry species are deciduous trees of varying sizes[1] [13]. White mulberries can grow to 24.38 m and are the most variable in form, including drooping and pyramidal shapes. In South America, on rich soils, the red mulberry can reach 21.34 m in height. The black mulberry is the smallest of the three *Morus* species, sometimes growing to 9.14 m in height, but it tends to be a bush if not trained when it is young. The species vary greatly in longevity. Red mulberry trees rarely live more than 75 years, while black mulberries have been known to bear fruit for hundreds of years. The mulberry makes

[1] www.crfg.org/pubs/ff/mulberry.html.

an attractive tree which will bear fruit while still small and young.

1.4. Foliage

The white mulberry is so named for the color of its buds, rather than for the color of its fruit. The thin, glossy, light green leaves are variously lobed even on the same plant. Some are unlobed, while others are glove-shaped. The leaves of the red mulberry are larger and thicker, blunt-toothed and often lobed. They are rough on their upper surface and pubescent underneath. The smaller black mulberry leaves are similar to those of the red mulberry, but with sturdier twigs and fatter buds. The species vary in the time of year they begin to leaf-out. White mulberries generally come out in early spring, almost two months before black mulberries [13].

1.5. Flowers

Mulberry trees are either dioecious or monoecious, and sometimes will change from one sex to another. The flowers are held on short, green, pendulous, nondescript catkins that appear in the axils of the season's growth and on spurs of older wood. They are wind-pollinated and some cultivars will set fruit without any pollination [14]. Cross-pollination is not necessary.

1.6. Fruit

Botanically the fruit is not a berry but a collective fruit, in appearance like a swollen loganberry. When the flowers are pollinated, they and their fleshy bases begin to swell. Ultimately they become completely altered in texture and color, becoming succulent, fat and full of juice. In appearance, each tiny swollen flower roughly resembles the individual drupe of a blackberry. The color of the fruit does not identify the mulberry species [15]. White mulberries, for example, can produce white, lavender or black fruit. White mulberry fruits are generally very sweet but often lacking in needed tartness. Red mulberry fruits are usually deep red, almost black, and in the best clones have a flavor that almost equals that of the black mulberry. Black mulberry fruits are large and juicy, with a good balance of sweetness and tartness that makes them the best-flavored species of mulberry. The refreshing tart taste is in some ways reminiscent of grapefruit. Mulberries ripen over an extended period of time, unlike many other fruits which seem to come to ripeness all at once[1].

1.7. Location, soil, pests and diseases

Mulberries need full sun and also adequate space. The distance between trees should be at least 4.57 m. The trees should not be planted near a sidewalk. The fallen fruit will not only stain the walkway, but are likely to be tracked indoors. The trees are quite wind-resistant, with some cultivars used as windbreaks in the Great Plains region. Mulberries like a warm, well-drained soil, preferably a deep loam. Shallow soils such as those frequently found on chalk or gravel are not recommended. Mulberries are generally free of pests and diseases, although cankers and dieback can occur. In some areas “popcorn disease” is an occasional problem, in which fruits swell to resemble popped corn. *Musa alba / M. rubra* hybrids are particularly prone to this condition. The disease carries on from one season to the next, so collecting and burning infected fruits help control it. The ripe fruit is very attractive to birds, but there is usually enough fruit left over for harvesting [16].

2. Content of mineral elements

Several papers have previously been published on the composition of micro-, macro- and toxic elements in various mulberry species [3, 17]. Those studies were mainly focused on the investigation of the mineral fruit constituents of a range of the mulberry species, and showed that mulberry fruit contains essential macro-elements such as potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na), and micro-elements such as iron (Fe), zinc (Zn) and nickel (Ni) (*table II*) [3, 17, 18]. Sufficient quantities of essential macro- (K, Ca, Mg and Na) and micro- (Fe, Zn and Ni) elements were found in all the fruits. Investigation of constituents in various mulberry species performed in different countries gave the results of Ca content from (132 to 574) mg·100 g^{-1} in fresh fruit, Mg from (91 to 240) mg·100 g^{-1} standard procedure and Na from (45 to 280) mg·100 g^{-1} standard procedure. Potassium was the predominant element, with concentration ranging from (834 to 1731) mg·100 g^{-1}. Trace elements in these tested extracts of mulberry were in the range from (0.4 to 59.2) mg·100 g^{-1} fresh matter, with Fe and Zn being the most dominant elements and Cu the least dominant element in relation to all detected elements. Nitrogen (N), phosphorus (P), copper (Cu) and manganese (Mn) were not determined by Imran *et al.* in Pakistan [18].

3. Determination of phenolic compounds of *Morus alba* L., *M. rubra* L. and *M. nigra* L. from different countries

Phenolic compounds are found in all parts of the mulberry plant. Methods for isolation of these compounds depend on the amount of plant material and the types of compounds that are tested. Flavonoids present in the waxes and oily surfaces can be obtained by scraping the surface or washing with a suitable solvent selected according to the polarity of the investigated compounds.

The results of studies carried out in various regions on the phenolic compounds extracted from mulberry (fruit, leaf, root) were recorded (*table III*). In one of these studies [19], five major white mulberry cultivars [Pachungsipyung (M-1), Whazosipmunja (M-2), Suwonnosang (M 3), Jasan (M-4) and Mocksang (M-5)] cultivated in Korea were assessed for their polyphenolic composition using spectrophotometric methodology and tested for antioxidant potential by some different assays. For the analysis, mulberry fruits were homogenized and extracted with 70% ethanol for 4 h at room temperature. The ethanolic extract showed a high and concentration-dependent antioxidant activity. Especially, the antioxidant activities of the extract from the M-2 and M-4 cultivars were higher than those of the others in a hemoglobin-induced linoleic acid system. The DPPH-scavenging ability of the ethanolic extract from mulberry fruit was 60.0% at (200 and 212) µg fresh weight of the M-2 and M-4 cultivars, respectively. The M-2 and M-4 cultivars also showed a sharp increase in hydroxyl scavenging ability with concentration of the extracts. IC_{50} values in scavenging abilities on hydroxyl radicals were 30 µg fresh weight and in a descending order of M-5 > M-3 > M-1 > M-4 > M-2 [19].

In a previous study, the chemical composition of white (*Morus alba* L.), red (*M. rubra* L.) and black (*M. nigra* L.) mulberry fruits grown in the East Anatolia Region of Turkey was investigated [3]. The highest total phenolic and flavonoid contents were observed in black mulberry (1422 mg gallic acid Eq·100 g^{-1} fresh matter and 276 mg quercetin Eq·100 g^{-1} fresh matter) (*table III*).

The ethanolic extract of black mulberry from Turkey [17] had a higher content of bioactive compounds than that of red mulberry [(169 and 215) mg gallic acid Eq·100 g^{-1} fresh fruit, respectively) [17]. On the other hand, in another investigation, *M. nigra* L. from Turkey showed an even higher content of phenolic compounds in the methanolic extract (1943–2237 mg gallic acid Eq·100 g^{-1} fresh fruit) [20] (*table III*).

In vitro antioxidant properties of three different extracts of black mulberry (*M. nigra* L.) from Turkey were investigated after extraction with acidified methanol, acidified water, and non-acidified methanol/water solution; the phenolic contents found were (450, 330 and 580) mg gallic acid Eq·100 g^{-1} fresh fruit, respectively [5] (*table III*).

Phytochemical and antioxidant properties of anthocyanin-rich mulberry species of *M. nigra* L. (black mulberry) and *M. rubra* L. (red mulberry) fruits harvested in Turkey were (273.7 and 160.3) mg of gallic acid

Table II.
Contents of mineral elements (mg·100 g^{-1}) in mulberry fruit from different countries.

Morus species	N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Ni	Zn	Reference
Morus nigra L. (Turkey)	800	289	1005	137	108	58	5.0	Not determined	7.0	Not determined	3.0	[17]
Morus rubra L. (Turkey)	690	242	929	143	91	45	5.0	Not determined	5.0	Not determined	3.0	[17]
Morus nigra L. (Turkey)	92	232	922	132	106	59	4.2	0.4	4.2	Not determined	3.2	[3]
Morus rubra L. (Turkey)	82	226	834	132	115	61	4.5	0.4	4.0	Not determined	3.2	[3]
Morus alba L. (Turkey)	75	247	1668	152	106	60	4.2	0.5	3.8	Not determined	2.8	[3]
Morus nigra L. (Pakistan)	Not determined	Not determined	1270	470	240	272	77.6	Not determined	Not determined	1.6	59.2	[18]
Morus alba L. (Pakistan)	Not determined	Not determined	1731	574	240	280	73	Not determined	Not determined	2.2	50.2	[18]

Table III.
Total phenol, flavonoid and anthocyanin contents and antioxidant activity of extracts of *Morus alba* L., *Morus rubra* L. and *Morus nigra* L. from different regions.

Sample	Country	Part of plant	Solvent	Total phenols	Total flavonoids	Monomeric anthocyanins	Antioxidant activities	Reference
Morus alba L.	Korea	Fruit	Ethanol	96–257[a]	0.56–6.54[b]	13.7–205.7[c]	90[d]	[19]
	Turkey	Fruit	Methanol	181[a]	29[e]	Not determined	Not determined	[3]
	Pakistan	Fruit	Methanol	1650[f]	Not determined	Not determined	95[h]	[18]
	Pakistan	Fruit	Methanol	775[a]	Not determined	Not determined	22.85[g]	[21]
	Pakistan	Leaf	Methanol	1416[a]	Not determined	Not determined	48.13[g]	[21]
	Serbia	Leaf	Ethanol	66.77[j]	33.30[k]	Not determined	Not determined	[23]
	Serbia	Root	Ethanol	170.2[j]	52.29[k]	Not determined	Not determined	[23]
	Serbia	Fruit	Ethanol/water	4.13[j]	0.89[k]	Not determined	Not determined	[23]
	Taiwan	Fruit	Water	1515[a]	250.1[e]	Not determined	Not determined	[25]
Morus rubra L.	Turkey	Fruit	Methanol	1035[a]	219[e]	Not determined	Not determined	[3]
	Turkey	Fruit	Ethanol	215[a]	Not determined	10.9[i]	442[h]	[17]
	Turkey	Fruit	Acetone/water	160.30[a]	Not determined	9.80[i]	Not determined	[1]
Morus nigra L.	Turkey	Fruit	Methanol	1422[a]	276[e]	Not determined	Not determined	[3]
	Pakistan	Fruit	Methanol	880[f]	Not determined	Not determined	30[d]	[18]
	Pakistan	Fruit	Methanol	661[a]	Not determined	Not determined	42.57[g]	[21]
	Pakistan	Leaf	Methanol	2004[a]	Not determined	Not determined	65.99[g]	[21]
	Turkey	Fruit	Ethanol	169[a]	Not determined	71.9[i]	748[h]	[17]
	Turkey	Fruit	Methanol	1943–2237[a]	Not determined	Not determined	Not determined	[20]
	Turkey	Fruit	Methanol	450[a]	Not determined	Not determined	95[d]	[5]
	Turkey	Fruit	Water	330[a]	Not determined	Not determined	75[d]	[5]
	Turkey	Fruit	Methanol/water	580[a]	Not determined	Not determined	80[d]	[5]
	Turkey	Fruit	Acetone/water	273.7[a]	Not determined	57.1[i]	Not determined	[1]
	Korea	Fruit	Ethanol	867[a]	Not determined	Not determined	Not determined	[22]
	Serbia	Leaf	Ethanol	115.23[j]	67.36[k]	Not determined	Not determined	[23]
	Serbia	Root	Ethanol	186.30[j]	67.11[k]	Not determined	Not determined	[23]
	Serbia	Fruit	Ethanol/water	6.37[j]	1.50[k]	Not determined	Not determined	[23]
	Brazil	Fruit	Methanol/water	373[a]	Not determined	Not determined	Not determined	[24]

[a] Total phenols expressed as gallic acid Eq·100 g^{-1} fresh fruit, [b] Total flavanols expressed as catechin Eq, [c] Total anthocyanins expressed as malvidin-3-glucoside Eq, [d] expressed as % of inhibition, [e] expressed as quercetin Eq·100 g^{-1} fresh fruit, [f] expressed as tannin acid Eq, [g] DPPH scavenging as quercetin Eq µmol·100 g^{-1}, [h] expressed as mg trolox Eq·100 g, [i] expressed as cyanidin-3-glucoside Eq·100 g^{-1}, [j] expressed as mg chlorogenic acid Eq·g^{-1} dry extracts, [k] expressed as mg rutin Eq·g^{-1} dry extract.

Eq·100 g^{-1} fresh fruit, respectively [1] (*table III*).

In a study on mulberries collected from northern regions of Pakistan, the fruits of *M. alba* L. and *M. nigra* L. were cleaned, dried, pressed, transferred to dark glass bottles before the analyses and stored in a deep freezer until further analyses were performed [18]. Then, the dried fruit samples were ground and extracted with methanol. The crude extracts obtained were filtered and evaporated in a vacuum to dryness. Total phenolic contents of mulberry fruits were determined by the Folin-Ciocalteu method using tannic acid as a standard. The potential antioxidant activity of the methanolic extracts was assessed based on the scavenging activity of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical [13]. For other work on the mulberry phenolic antioxidants, it is also useful to note that fresh fruit and leaf samples of *M. alba* L. and *M. nigra* L. from Pakistan were lyophilized at –30 °C and extracted with methanol [21] (*table III*).

For determination of total polyphenol content, the fruit of *M. nigra* L. from Korea was immersed in 70% ethanol solution for 4 h, then filtered. The process was repeated twice at room temperature [22].

Samples of leaves and roots of black mulberry from Serbia were dried naturally (in the shade, on a draft) for 1 month. Prior to extraction, samples were ground in a blender. Mulberry leaves and roots were extracted with 70% ethanol at a temperature of 30 °C, while the liquid-solid ratio was 15 mL·g^{-1} (solvent volume per g of sample). Samples of mulberry fruits were stored in polyethylene bags at –20 °C for up to 1 month until undertaking the analysis. Mulberry fruit samples were extracted with a ethanol:water:acetic acid (70:29.5:0.5, v/v/v) mixture, at a temperature of 30 °C with the same liquid-solid ratio as in the prior extraction [23].

A sample of black mulberry from Brazil was extracted three times in 100 mL of methanol/water/acetic acid (70:25:5 v/v) for 2 min in an ice bath, then the content of total phenols was determined in the extract [24]. Total phenolic contents of *Morus alba* L. from Taiwan were determined by the Folin–Ciocalteu method [25].

4. Compounds isolated by HPLC analysis

The phenolic compounds isolated from mulberry (fruit, leaf, root, stem) and identified by HPLC analysis were registered together with the literature references (*table IV*). It appears that the HPLC separation of the phenolic compounds of *M. alba* L. fruit from China allowed the isolation of cyanidin-3-*O*-β-D-glucopyranoside, cyanidin 3-*O*-β-D-galactopyranosides and cyanidin 7-*O*-β-D-glucopiranosides [26]. Cyanidin-3-glucoside and quercetin-3-O-rutinoside were isolated from *M. nigra* L. from Spain [27], while HPLC-PDAESI-MS analyses performed on black and white mulberries from Poland allowed the isolation of cyanidin-3-glucoside and kaempferol-3-O-rutinoside [28].

Flavonoids from wild black mulberry from Brazil were separated by HPLC and identified as two peaks of cyanidine derivates, one peak of a pelargonidin derivate and one of a quercetin derivative, in the methanol elute. On the other hand, in the methanol: ammonia elute, two peaks of hydroxycinnamic acid were obtained. The variation in the flavonoid content between harvests was significant ($p < 0.05$) for cyanidin [(256 and 138] mg·100 g^{-1} fresh weight], pelargonidin [(3.6 and 2.7) mg·100 g^{-1} fresh weight], quercetin [(14.1 and 15.3) mg·100 g^{-1} fresh weight] and hydroxycinnamic acid [(12.4 and 14.1 mg·100 g^{-1} fresh weight], respectively [24].

Red and white mulberry from Tunisia were analyzed and quantification was achieved by injection of solutions of known concentrations of chlorogenic acid (250 µg·mL^{-1}), caffeic acid (100 µg·mL^{-1}), rutin (235 µg·mL^{-1}) and quercitrin (200 µg·mL^{-1}) [29]. Chlorogenic acid and caffeic acid were chosen because they had already been identified together with their derivatives in these extracts of red and white mulberries. Rutin was used, as an equivalent, to quantify the quercetin and

Table IV.
Compounds isolated from mulberry (fruit, leaf, root, stem bark) by HPLC analysis.

Compound	*Morus nigra* L.	*Morus rubra* L.	*Morus alba* L.	Reference
Cyanidin 3-*O*-β-D- glucopyranoside	–	–	+	[26]
Cyanidin 3-*O*-β-D-galactopyranoside	–	–	+	[26]
Cyanidin 7-*O*-β-D-glucopiranoside	–	–	+	[26]
Cyanidin-3-glucoside	+	–	–	[27]
	+	–	+	[28]
	+	–	–	[24]
Quercetin-3-*O*-rhamnoside-7-*O*-glucoside	–	+	+	[29]
Quercetin-3-7-D-*O*-β-*O*-glucopyranoside	–	+	+	[29]
Kaempferol-7-O-glucoside	–	+	+	[29]
Kaempferol-3-O-rutinoside	+	–	+	[28]
Kaempferol	–	–	+	[30]
Rutin	+	+	+	[31]
	–	–	+	[30]
	+	+	+	[29]
	+	+	+	[32]
	+	–	+	[23]
Quercetin	+	+	+	[31]
	+	–	+	[23]
	–	–	+	[30]
	+	+	+	[32]
Catechin	+	+	+	[31]
Quercetin-3-O-glucoside	+	+	+	[29]
Quercetin-3-O-rutinoside	+	–	–	[27]
Taxifolin	+	–	–	[33]
Chlorogenic acid	+	–	+	[21]
	+	+	+	[31]
	–	–	+	[30]
	+	–	+	[23]
p-Coumaric acid	+	+	+	[31]
	+	–	+	[21]
m-Coumaric acid	+	–	+	[31]
	+	–	+	[21]
o-Coumaric acid	+	+	+	[31]
Vanillic acid	+	+	+	[31]
	+	–	+	[21]
Gallic acid	+	–	+	[23]
	+	+	+	[31]
	–	–	+	[30]
	+	–	+	[21]
Caffeic acid	–	–	+	[30]
	+	+	+	[31]
	–	+	+	[29]
Ferulic acid	+	–	+	[30]
	+	–	+	[23]
1-Caffeoylquinnic acid	–	+	+	[29]
4-Caffeoylquinnic acid	–	+	+	[29]
5-Caffeoylquinnic acid	–	+	+	[29]

kaempferol diglycosides. In fact, the kaempferol derivatives differ from the quercetin derivatives only by the presence of a hydroxyl group in the 3' position of the flavan nucleus, and rutin had already been identified in the extracts. Quercitrin was used, as an equivalent, to quantify the monoglycoside derivatives of quercetin and kaempferol [29].

HPLC, as a prime analytical method, was applied to analyze the flavonoids, cumarins and chlorogenic acid in fruits, bark and leaves of *M. alba* L. from China and allowed to isolate kaempferol, rutin, chlorogenic and gallic acid [30]. This analytical method also allowed the isolation of rutin, quercetin, catechin, chlorogenic acid, caffeic acid, gallic acid, vanillic acid, and m- and o-coumaric acids from the extracts of black, red and white mulberries from Turkey [31]. In another study, quercetin and rutin were also found in *M. nigra* L. *M. rubra* L. and M. *alba* L. from China [32].

Morus alba L. and *Morus nigra* L. from Serbia contained rutin, quercetin and the phenolic acids: gallic, dihydroxy benzoic, sinapic, vanillic, caffeic, chlorogenic, ferulic and *p*-coumaric [23]. Chlorogenic, p- and m-coumaric, vanillic, gallic and ferulic acids were isolated from the fruit and leaves of *M. alba* L. and *M. nigra* L. from Pakistan [21].

5. Antimicrobial activity

The antimicrobial activities of compounds isolated from mulberry were ranged according to the parts of the plant from the *Morus alba* L. or *M. nigra* L. species (*table V*).

Oxyresveratrol, arylbenzofuran moracin M2, cyclomorusin, morusin, kuwanon C5, kuwanon C6, betulinic acid, α-amyrin acetate and β sitosterol-3-O-β-D-glucoside compounds were isolated from stem and trunk bark of *M. nigra* L. from Botswana [33]. Oxyresveratrol and arylbenzofuran moracin M2 showed bactericidal activity against *Staphylococcus aureus* [minimal bactericidal concentration (MBC) = (125 and 62.5) $\mu g \cdot mL^{-1}$, respectively]; arylbenzofuran moracin M2 also showed bactericidal activity against *Streptococcus faecalis* [MBC = (500 and 250) $\mu g \cdot mL^{-1}$, respectively]) (*table V*). The structure-activity relationship of cyclomorusin and morusin showed that the cyclization of the prenyl unit at C-3 in 3 reduced activity, whereas the presence of a free prenyl unit at C-3 in 4 enhanced the activity [33]. By comparing the activities of 4 morusin and kuwanon C6, it was observed that, when the prenyl unit at C-3 was cyclized (kuwanon C6), then, to have better activity, the C-8 attached prenyl unit should be open. Cyclomorusin has both prenyl units cyclized, and kuwanon C5 has both prenyl units open; these have lower activities than the compounds morusin and kuwanon C6, which have one of the prenyl units open. Though it is inconclusive, the results show that the electron donating group (-OH) at positions 7 and/or 2' was vital for increased activity, as observed for morusin and kuwanon C6 [33].

The microorganism used for bioassay-guided fractionation of antibacterial compounds of root bark was *Staphylococcus mutant* ATCC 25175, a well-known oral pathogen [34]. An antibacterial assay was also performed for various microorganisms such as *Streptococcus sanguis* ATCC 35105, *Streptococcus sobrinus* ATCC 27351, *S. aureus* ATCC 10231, *Candida albicans* ATCC 10231, *Actinobacillus actinomycetemcomitans* ATCC 33384, *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus casei* ATCC 4646 and *Porphyromonas gingivalis* W50.

To isolate and identify the active compound of root bark of *M. alba* L. from South Korea, its ethyl acetate fraction, exhibiting strong antibacterial activity against *Staphylococcus mutant*, was further separated using silica gel column chromatography, and the purification procedures of the active *S. mutant* compound were monitored by MICs (Minimum Inhibitory Concentration) [34].

The antibacterial activity of kuwanon G was investigated in terms of the MIC in comparison with some commercially available agents used for caries control. The MIC of kuwanon G against *Streptococcus* was 8.0 $\mu g \cdot mL^{-1}$ (*table V*). It was much lower than the 32 $\mu g \cdot mL^{-1}$ of sanguinarine,

Table V.
Antimicrobial activity of compounds isolated in different parts of *Morus alba* L. and *M. nigra* L. (MIC = Minimum Inhibitory Concentration).

Mulberry species	Compound isolated	Part of plant	Antimicrobial activity	MIC ($\mu g \cdot mL^{-1}$)	Reference
Morus alba L.	kuwanon G	Root	*Streptococcus mutans*	8	[34]
			Streptococcus sanguis	8	
			Streptococcus sobrinus	8	
			Porphyromonas gingivalis	8	
			Staphylococcus aureus	125	
			Actinobacillus actinomycetemcomitans	1000	
			Candida albicans	1000	
			Lactobacillus acidophilus	> 1000	
			Lactobacillus casei	> 1000	
	Mulberofuran G	Root	*Candida albicans*	> 60	[35]
			Saccharomyces cerevisiae	> 60	
			Escherichia coli	30	
			Salmonella typhimurium	7.5	
			Staphylococcus epidermis	6.25	
			Staphylococcus aureus	5	
	Albanol B	Root	*Candida albicans*	> 60	[35]
			Saccharomyces cerevisiae	> 60	
			Escherichia coli	20	
			Salmonella typhimurium	5	
			Staphylococcus epidermis	5	
			Staphylococcus aureus	5	
	Halkomoracin	Leaf	*Micrococcus luteus*	1.56	[35]
			Bacillus subtilis	3.13	
			Escherichia coli	> 100	
			Klebsiella pneumoniae	> 100	
			Pseudomonas aeruginosa	> 100	
Morus nigra L.	Total extract	Stem	*Staphylococcus aureus*	125	[33]
			Bacillus subtilis	125	
			Micrococcus flavus	500	
			Streptococcus faecalis	500	
			Salmonella abony	250	
			Pseudomonas aeruginosa	500	
		Trunk	*Staphylococcus aureus*	62.5	[33]
			Bacillus subtilis	62.5	
			Micrococcus flavus	250	
			Streptococcus faecalis	250	
			Salmonella abony	125	
			Pseudomonas aeruginosa	500	
		Fruit (juice)	*Bacillus spizizenii*	19.68	[37]
			Bacillus subtilis	18.46	
			Corynebacterium diphtheriae	15.57	
			Enterococcus faecalis	16.03	
			Staphylococcus aureus	17.37	
			Escherichia coli	9.98	
			Pseudomonas aeruginosa	19.87	
			Salmonella typhimurium	11.73	

125 µg·mL^{-1} of carvacrol, and 500 µg·mL^{-1} of thymol and eucalyptol, which are commercial agents with antibacterial activity. This indicates that kuwanon G confers much stronger antibacterial activity than other commercial agents. The antibacterial activity of the kuwanon was comparable with the antibiotics vancomycin and chlorhexidine, which, however, possess detrimental side effects such as discoloring of teeth, reducing immune defense, disrupting the normal ecology of plaque, diarrhea, vomiting, etc. [34]. Other antimicrobial activity of compounds isolated from mulberry from different countries were registered (*table V*) [35–37].

6. Medicinal properties of mulberry

Globally, the main use of mulberry is as feed for the silk worm, but, depending on the location, it is also appreciated for its fruit (consumed fresh, in juice or as preserves), as a delicious vegetable (young leaves and stems), for its medicinal properties in infusions (mulberry leaf tea), for landscaping and as animal feed [38].

The mulberry fruit is used for many medical purposes such as to nourish the skin and blood, and benefit the liver and kidney. It is also used to treat urinary incontinence, tinnitus, dizziness, constipation in the elderly and the anemic, sore throat, depression and fever [38]. The raw mulberry juice is squeezed out of the mulberry fruit with delicate fragrance and taste. This juice will enhance the health, such as yin nourishing, enriching the blood, notifying the liver and kidney, calming the nerves, promoting the metabolism of alcohol, balancing internal secretions, and enhancing immunity. Besides, the mulberry fruit can be brewed into wine and used as a resin substitute. This wine has a sweet and sour taste and it is taken for cleaning the blood. Many people believe that a glass of mulberry wine daily helps in getting rid of impurities and coprostasis (fecal residue in the intestines) in the body and enhances the health. The fruits of *M. alba* L. are eaten fresh or made into juice, stews and tarts. They may be squashed and fermented to yield liquor. Besides, fruits are also a potent source of anthocyanins, which play a key role in antioxidant activity. This also offers the possibility of industrial use of mulberry as a source of anthocyanins as a natural food colorant, which could enhance the overall profitability of sericulture and holds promise for tropical sericulture countries for profiting from industrial anthocyanin production from mulberry through better anthocyanin recovery [39–45]. Active biomolecules of different *Morus* species are kuwanon I, kuwanon I hexamethyl ether, kuwanon I octamethyl ether, 2'-hydroxy-2,4,4'-trimethoxychalcone and 2'-hydroxy 3'prenyl-2,4,4'-trimethoxychalcone, mulberrofuran T and kuwanon E, morusin, mulberrofuran D, G, K, kuwanon G, H, mulberroside A, cis-mulberroside A, oxyresveratrol, isoquercetin, kuwanon G, moracin E, F, G and H, kuwanon D, E, F, deoxynojirimycin-1, etc. from the root, stem, leaves and fruits of *M. alba* L. [46]. These molecules have medicinal properties: astringent, anthelmintic, anti-HIV, antitussive, anti-inflammatory, exudative, anti-high blood pressure, anti-diaphoretic, purgative, emollient, anti-diarrhea, anti-diabetic, anti-atherosclerosis, anti-tumor, anti-hypoglycemia, etc. [41–44]. Rubraflavones A, B, C and D were isolated from the root of *M. rubra* L.; they act as an anti-dysentric, laxative, purgative, vermifuge and help with urinary problems and weakness, etc. Deoxynojirimycin was isolated from the root, leaves and fruits of *M. nigra* L.; it is used in treatment of diabetes, AIDS, cancer and high arterial pressure. It is also used as a purgative and vermifuge, etc. [34, 47–49].

7. Uses of mulberry

In the world, mulberry is generally used as forage in animal production. However, besides the leaves, mulberry bears sweet fruit. The full-bodied flavor of this fruit is a good balance of sweetness and tartness with nutrient elements of vital importance for the human metabolism. If these fruits are industrially exploited for various commercially valuable products, mulberry can become an important crop throughout the world.

Mulberry fruit can be used for making jam, jelly, pulp, fruit drinks, fruit sauce and cake. Many desserts are made from Persian mulberries along with sauces, pie-making, cakes and jelly[2], and fruit tea. In Chinese markets, mulberry is often provided in the form of a paste called "sangshengao". This paste is mixed in hot water to make a tea to improve the liver and kidney, sharpen the hearing and brighten the eyes[3] [52]. Mulberry fruits can be dried and stored as a powder. About 10 g of dried fruits provides about 100 mg of anthocyanins. As it contains resveratrol, the fruit powder works as an anti-mutagen that can inhibit the mutation of healthy normal cells into cancerous cells[3]. It is believed to prevent heart disease, cancer and other diseases associated with chronic inflammation. The fruit powder has an anti-aging effect on cells because it combats free radical damage. The fruit powder promotes maintaining a normal level of cholesterol and controls carbohydrate digestion in the human body [50]. Over-ripened and sour fruits can be converted into mulberry wine. This wine has a sweet and sour taste. A glass of mulberry wine a day helps get rid of impurities and coprostasis (fecal residue in the intestines) in the body that can help make the body slim. The wine is made by immersing the mulberry in rice wine or in grape wine; it works as a medicine for weakness after diseases and can be used to tonify masculine vitality and benefit overall vitality.

In Azerbaijan, Georgia and Armenia, a potent liqueur, "Tut araghi", made from mulberry fruit juice is very popular. It is one of the national Azerbaijani versions of vodka. It is believed that a small dose of the drink protects against stomach and heart diseases. In Greece, mulberry fruits are used for the production of the traditional aromatic "mouro distillate". Mulberry fruit wine is very popular as a woman's drink in Europe[4].

Mulberry fruits are rich in anthocyanins and should be exploited for the industrial production of natural color to be used in the food industry. In particular, they are known to contain cyanin, which is the red pigment that gives the fruit a red to purple color. The major anthocyanins found are cyanidin-3-glucoside and cyanidin-3-rutinoside. These pigments hold potential for use as dietary modulators of mechanisms for various diseases, and as natural food colorants. As synthetic pigments are unsafe, there is a demand for natural food colorants in the food industry [51]. Mulberry fruits are reported to be anti-diabetic with antioxidative properties as they can check the accumulation of thiobarbituric reactive substances (TBARS) [52]. Mulberry fruits can also be used as ruminant livestock feed and in the pharmaceutical industry [53]. The sole use of mulberries in modern medicine is for the preparation of a syrup: to add flavors and natural color to medicines. The mulberry fruit is used for many medical purposes such as for balancing internal secretions and enhancing immunity. It is used to treat urinary incontinence, tinnitus, dizziness, constipation, sore throat, depression and fever. The fruits of *M. alba* have a cooling and laxative property and are used in throat infection, dyspepsia and melancholia. The juice, which is a refrigerant, is used as a drink in febrile diseases. It checks thirst and cools the blood. The fruit juice is commonly used for reducing high fever as a febrifuge. This is the first treatment normally given to any patient with symptoms of fever during endemic malaria [54]. It opens up a new vista for industrial exploitation of mulberry fruits worldwide. Such a use of the mulberry has been overlooked for the sake of using only mulberry leaves for the sericulture industry.

Acknowledgments

We thank the Serbian Ministry of Education and Science, Project No. ON 172047, for financial support of this work.

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Estudio sobre los macro y microelementos, el contenido en compuestos fenólicos, la actividad biológica y la utilización de *Morus* spp. (Moraceae).

Resumen – **Introducción**. La morera es la planta medicinal más importante del género *Morus*. La fruta madura se utiliza para numerosos fines médicos. Se sintetizaron el origen y la adaptación de las especies, la descripción de la planta, de su follaje, de sus flores y de sus frutas, así como su localización, sus plagas y enfermedades. **Composición de la morera**. Los estudios efectuados en diferentes países muestran que la morera es rica en compuestos fenólicos, en macroelementos (K, Ca, Mg, Na) y en microelementos (Fe, Zn, Ni). Los compuestos fenólicos están presentes en todas las partes de la planta adulta. **Utilización**. Las frutas de la morera pueden utilizarse para elaborar mermeladas, gelatinas, dulces de fruta, bebidas de fruta, salsas de fruta y bollería. Las moras son ricas en antocianas y aptas para su explotación en la producción industrial de colorantes naturales de la industria alimentaria. **Conclusión**. La morera cuenta con una importancia biológica significativa por sus propiedades antioxidantes y antimicrobianas. Nuestro estudio sugiere utilizar la morera como alimento potenciador de la salud o fuente importante de antioxidantes en las industrias alimentaria y farmacéutica.

Serbia / *Morus* / frutas / composición aproximada / polifenoles / antioxidantes / análisis microbiológico