Review Article

The Fruit *Malus prunifolia* (*Malus micromalus* Mak.): A Minireview of Current Knowledge of Fruit Composition and Health Benefits

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The fruit *Malus prunifolia* (*Malus micromalus* Mak.), which belongs to the Rosaceae family, grows mostly in the upper-middle reaches of the Yellow River area. It has long been popular as a fruit commodity and as a natural remedy. Its main biologically active components include vitamin C, phenolics, flavonoids, polysaccharides, and triterpenic acids. Recent phytochemical studies on the fruit have shed some light on its biological activities, such as anticancer, immunomodulatory, antioxidant, immunostimulating, hepatoprotective, and gastrointestinal protective activities. A stronger focus on clinical studies and phytochemical characterization of the fruit will be essential for future research efforts. This minireview could be useful for predicting its other medicinal uses and its potential drug or food interactions, and it could be beneficial for people living in areas where the fruit is endemic and where healthcare resources are scarce.

1. Introduction

*Malus prunifolia* (*Malus micromalus* Mak.), which belongs to the Rosaceae family, is largely cultivated in the loess plateau in the upper-middle reaches of the Yellow River regions [1, 2]. The fruit has been cultivated for hundreds of years, and about 100 of its cultivars have been found in China [3]. In 2015, the fruit production reached 426,000 tons, and the total area for its culture reached approximately 0.3 million hectares, according to the statistics of the Ministry of Agriculture of the People’s Republic of China [4]. The *Malus prunifolia* fruit has a sweet and sour taste, crisp and juicy meat, and is becoming more and more popular with the local people in Fugu county of Yulin city [5].

The fruit is generally recognized as a very good source of biologically active compounds with nutritional and nutraceutical value [5]. The dried fruit has been commonly used as food, food additive, and flavoring for hundreds of years because of its high nutritional value. The fruit is made into juice, wine, paste, puree, syrup, and confection, which are consumed for digestion improvement and general health maintenance [6–9]. According to an ancient Chinese book on herbal medicine, *Huangdi Neijing* (475-221 BCE), consumption of the fruit is believed to promote digestion, relieve fatigue, lower blood lipid levels, and prolong the life span [2, 10]. Previous studies have revealed that the fruit contains several chemical constituents, including flavonoids, phenolic acids, polysaccharides, triterpenic acids, amino acids, mineral constituents, and volatile components [6, 10, 11]. To the best of our knowledge, there is no published review of investigations of primary and secondary metabolites of *M. prunifolia*. Hence, the aim of this minireview is to report the current knowledge of its composition and health benefits.

In order to elucidate the importance of the fruit to human health, the bioactivities of its extracts as measured by chemical model systems, together with the results of some *in vitro* and *in vivo* studies, are discussed. The use of the fruit extracts for nutraceutical purposes can add to the fruit’s
potential value. This minireview could be useful for predicting its other medicinal uses and potential drug or food interactions, and it may be beneficial for people living in places where *Malus prunifolia* species is endemic and where healthcare resources are scarce. Finally, it could encourage new research and further studies on postharvest processing of the fruit.

### 2. Nutrients

*Malus prunifolia* fruits may be considered as a healthy food choice. It has been of great interest because of its higher calcium content as compared with jujube, pear, peach, etc. [4], which makes it a more important source of the mineral [6, 7]. To a lesser extent, it is a source of several other minerals such as iron, zinc, manganese, and magnesium (Table 1). Great interest has developed in *Malus prunifolia* fruit because of their high content of vitamin C and B$_2$, which makes them an important source of this vitamin for human nutrition [8]. Glucose, fructose, and sucrose are its major sugars. Different organic acids such as malic, citric, succinic, and quinic acids have also been identified in the fruit.

### 3. Bioactive Compounds

#### 3.1. Total Phenolics

The fruit contains a higher amount of total phenolics as measured by Folin–Ciocalteu assay [13–15]. It has a total phenolics content (807.4–2275.3 mg GAE/100 g) higher than that of other common fruits with high phenolics content, such as jujube (275.6–541.8 mg GAE/100 g) [16], apple (74.0 mg GAE/100 g FW), sweetsop (405.4 mg GAE/100 g FW), and guava (194.2 mg GAE/100 g FW) [17]. The total flavonoids content of *M. prunifolia* is 20.7–105.1 mg rutin/100 g FW, which is similar to that of jujube [18] and other fruits [17]. The phenolics and flavonoids content of *M. prunifolia* vary with the cultivar [11, 12]. Genotypes, altitude, and annual precipitation have significant effects on the level of total phenolics and flavonoids in the fruit. Fruit grown in more severe drought and in higher altitude areas could produce a larger amount of phenolics and flavonoids, as well as exhibit higher antioxidant activities as compared with fruits grown in moderate environment conditions [12].

#### 3.2. Individual Phenolic Compounds

According to their number of phenol rings and the structural elements that bind these rings together, phenolics are divided into several classes, such as flavonoids, phenolic acids, tannins (hydrolyzable and condensed), stilbenes, and lignans [19–21]. Different types of flavonoids such as flavonols and flavan-3-ols are found in *M. prunifolia* [12]. Flavonoids may vary significantly with its variety, maturity level, and location. The fruit contains the following flavonoids: rutin, catechin, epicatechin, quercetin, and kaempferol [11, 22].

Wang et al. applied a high-performance liquid chromatography (HPLC) method to simultaneously separate and determine eleven phenolic compounds in the fruit, namely, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, ellagic acid, caffeic acid, fenugreek acid, ferulic acid, chlorogenic acid, and cinnamic acid [11, 12, 23]. The content of phenolic acids in the fruit ranges from 106.9 mg gallic acid/100 g FW (peel) to 129.2 mg gallic acid/100 g FW (pulp). In the peel, the phenolic acids are mainly found in the insoluble, bound form; in the pulp, they are in the glycosidic form [11]. Free phenolic acids constitute 4.7% (peel) to 32.5% (pulp) of the total phenolic acids present in the fruit. Gallic acid and vanillic acid are the dominant phenolic acids in both pulp and peel and even in the whole fruit. Protocatechuic acid, p-hydroxybenzoic acid, and quercetin are present only in the pulp, whereas chlorogenic acid is present in large amounts in the peel. Hydroxybenzoic acids in the fruit include caffeic, fenugreek, ferulic, chlorogenic, and cinnamic acids (Table 2).

#### 3.3. Polysaccharides

Currently, the popularity of polysaccharides has received remarkable attention, partly because polysaccharides are the major structural component of cells and have a variety of physiological functions [24, 25]. Polysaccharides are a major group of biologically active constituents of the fruit [26, 27]. Polysaccharides from the fruit could be obtained by hot water extraction followed by ethanol precipitation, deproteinization, and dialysis and purification using DEAE-52 and DEAE-Sepharose CL-6B anion exchange, Sephadex G-100, and Sepharose CL-6B column chromatography [7, 28, 29]. The main physiochemical and structural characteristics of polysaccharides in the fruit are their monosaccharide composition, molecular weight, sequence of monosaccharides, configuration of glycosidic linkages, types of glycosidic linkages, and positions of glycosidic linkages [25–27].

Hao et al. extracted polysaccharides from *M. prunifolia* harvested in Hohehot Municipality, using distilled water as the extraction solvent [30]. Optimal extraction conditions for extraction include an extraction time of 6 h, an extraction

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**Table 1: Nutrient composition of *Malus prunifolia* fruits (adapted from Shanxi Food Industrial Research Institute) [12].**

<table>
<thead>
<tr>
<th>Type</th>
<th>Nutrient (units)</th>
<th>Content (per 1000 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (%)</td>
<td></td>
<td>81.25</td>
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<tr>
<td>Titratable acid (%)</td>
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<td>1.59</td>
</tr>
<tr>
<td>Protein (g)</td>
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<td>3.4</td>
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<tr>
<td>Total lipid (g)</td>
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</tr>
<tr>
<td>Carbohydrate (g)</td>
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<td>Zinc, Zn (mg)</td>
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<td><strong>Vitamins</strong></td>
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<td>Vitamin B-1 (mg)</td>
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<td>Vitamin B-2 (mg)</td>
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<table>
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<th>Main class</th>
<th>Subclass</th>
<th>Name</th>
<th>Chemical structure</th>
<th>Content (µg/g FW)</th>
<th>Reference</th>
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<td></td>
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<td>Protocatechuic acid</td>
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<td>37.20–76.07</td>
<td>[11]</td>
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<td>p-Hydroxybenzoic acid</td>
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<td>[12]</td>
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<td>[13]</td>
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<td>Ferulic acid</td>
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<td>[12]</td>
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<td>[12]</td>
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<td></td>
<td>Cinnamic acid</td>
<td><img src="image" alt="Cinnamic acid" /></td>
<td>3.55</td>
<td>[13]</td>
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temperature of 90°C, a water/material ratio of 10:1, and three rounds of extraction. Another extraction method, microwave-assisted extraction, is also frequently used. To increase the yield of *M. prunifolia* polysaccharides, a response surface methodology design is applied during the process of extraction. According to Wu et al. the ideal extraction conditions include a microwave power of 90 W, during 50 min at 55°C, using water/material ratio (w/w) of 15:1 [28]. The ideal favorable extraction conditions for the fruit harvested in Shanxi province, which produced a final yield of 8.33%, included an extraction time of 4 h, an extraction temperature of 86°C, and a water/material ratio of 28:1 [29]. The crude polysaccharides were then further purified by DEAE-52 cellulose column chromatography and Sephadex G-100 column chromatography to yield four fractions. The molecular weights of *M. prunifolia* polysaccharides were found to be $6.78 \times 10^5$, $8.23 \times 10^5$, $3.34 \times 10^4$, and $1.31 \times 10^6$ Da [29]. Molecular weights in the range of $10^4$–$10^6$ Da have been observed in various *M. prunifolia* fruits under different experimental conditions [31]. Among the purified polysaccharides, a neutral polysaccharide fraction and acidic polysaccharide fractions were found to be more effective at scavenging superoxide anions than hydroxyl radicals [30]. The acidic polysaccharide fraction has also been shown to have substantial activity in chelating ferrous ion, depending on its concentration.

The isolation, structural characterization, and bioactivity determination of polysaccharides from the fruit have been investigated in published studies [29–31]. However, both the higher order structural features of these active components, as well as the relationships between their bioactivities and chemical structures, are still not well established because of the great structural diversity and complexity of the polysaccharide molecules [25, 32]. Further research into the exact higher order structures and the structure-bioactivity relationships of *M. prunifolia* polysaccharides is required in order to extend our understanding of the functional effects of these macromolecules [29].
3.4. **Volatile Components.** Volatile components are one of the most important qualities of *Malus prunifolia* fruit and could influence its flavor. Yang et al. employed gas chromatography-mass spectrometry (GC-MS) to investigate volatile components in the fruit [33]. They identified sixty compounds accounting for 97% of the dichloromethane extract of the fruit, including seventeen alcohols, seventeen aldehydes, four esters, five ketones, five alkenes, and six alkanes, and they quantified their relative amounts by area normalization method. In their study, benzaldehyde and 2-hexenoic aldehyde were the main volatile components, present at amounts significantly higher than those other components.

3.5. **Other Components.** Plant pigments are amongst the most common additives incorporated to foodstuff, not only for improving their nutritional status but also for coloration, preservation, and even therapeutic purposes. Hema-tochrome is a mixture of carotenoid pigments and their derivates. Ren extracted the hematohrome from *M. prunifolia* fruit harvested in Shaanxi province, using 95% ethanol as solvent and optimal extraction conditions, including an extraction time of 1.5 h, an extraction temperature of 70°C, a pH of 7.0, and three rounds of extraction [34]. Under these conditions, the yield of hematohrome was 7.1%. The pigment was stable in an acidic environment and deepened in color under light and heat. Tong et al. identified the following optimal extraction conditions for anthocyanins from the fruit: an extraction time of 25 min, an extraction temperature of 50°C, a water/material ratio of 4:1, a pH of 4.0, and an extractant of 0.1% hydrochloric acid-methanol solution [35, 36]. The relationships between the extraction conditions and anthocyanins properties and structures described in *M. prunifolia* fruit are difficult to determine and their structure are difficult to determine. Therefore, details of the structure of these anthocyanins require further investigation with advanced technologies.

### 4. Health Benefits

*M. prunifolia* has long been studied for its biological activity using *in vitro*, *in vivo*, and animal model studies; however, evidence from human epidemiologic and interventional studies is still lacking. The health benefits hypothesized to be related to its consumption include their role in antioxidant, anti-inflammatory, antitumor, antiaging, and myocardial protection activities.

4.1. **Antioxidant Activity.** Natural materials are a highly promising source of antioxidants; a wide range of bioactive constituents of fruit have antioxidant activities [37]. Antioxidant activities have been the focus of much research into the mechanisms underlying the nutraceutical and therapeutic effects of traditional Chinese medicines. These studies are based on various assay methods and activity indices [38, 39]. The antioxidant capacity of fruit is closely correlated with the levels of efficient oxygen radical scavengers, such as phenolic compounds, polysaccharides, and vitamin C [40]. A group of Chinese investigators compared the antioxidant capacities of extracts from sixteen cultivars of Chinese *M. prunifolia* and found that the antioxidant capacity differed among cultivars [12]. A positive correlation was seen between the total phenolics content and antioxidant capacities of extracts from the fruit. Later, Wang evaluated the antioxidant capacities of various tissues of *M. prunifolia* [12]. They found that the pulp has higher antioxidant capacity, reflecting the higher content of total phenolics and flavonoids of this part. They also found that the predominant phenolic acids in the fruit are vanillic acid and rutin, followed by gallic acid and epicatechin.

In studies by Jia et al., water-soluble polysaccharide fractions isolated from *M. prunifolia* have definite antioxidative activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydroxyl radical, and superoxide anion systems. The polysaccharides showed significant inhibitory effects on DPPH, with an IC50 of 1.2 mg/mL at polysaccharide concentrations of up to 1.0 mg/mL [8, 13]. The scavenging capacity reached 87.2%, which is close to the 94.5% capacity reported for vitamin C. Deng et al. found that the total flavonoids from *M. prunifolia* have significant scavenging superoxide anion capacity and moderate antioxidant and anti-inflammatory activities of ethanol extracts of the fruit and found that the extract can be used in chemoprevention and therapy for oxidative stress and inflammation [43].

Although *M. prunifolia* is a potential source of natural antioxidants for the food industry, we should be aware of the essential metabolic functions of reactive oxygen species. The removal of too much ROS can upset cell signaling pathways and actually increase the risk of chronic disease. The antioxidant activities of *M. prunifolia* evaluated *in vivo* are reported to be weak and thus should be further investigated [44].

4.2. **Antitumor Activity.** Many studies have shown that functional components have strong antitumor activity, which has different proposed mechanisms [45–47]. The currently accepted mechanism by which fruit components exert their antitumor effects could be summarized as follows: (1) prevention of oncogenesis by oral administration, (2) improvement of the immune response to tumors, (3) direct antitumor activity that induces the apoptosis of tumor cells, and (4) prevention of the spread or migration of tumor cells in the body [48–50]. Xu and Wang reported that crude flavonoids from *M. prunifolia* can inhibit HeLa cell proliferation *in vitro*. Their results show that the total flavonoids significantly inhibited the HeLa cell growth *in vitro*, with an IC50 of 109.9 mg/L [51].

4.3. **Antiaging Activity.** Only a few studies have demonstrated the direct antiaging activity of the bioactive compounds from *M. prunifolia*. Therefore, further detailed studies are required to clarify the bioactive compounds and antiaging properties of the fruit. Huang et al. reported that polysaccharides from the fruit can protect brain cells of aging-model mice [52]. The authors found that different
groups that studied *M. prunifolia* polysaccharides could observe brain cell neuron dendrites getting right inordinately, neuron volume increasing gradually, nuclear form recovering regulation, organelles becoming abundant, chromatinis distributing regularly, aging improving, and the apoptosis rate decreasing markedly in a dose-dependent manner. The results showed that *M. prunifolia* polysaccharides can improve the brain structure of aging mice models, restrain apoptosis in the brain, reduce the apoptotic rate, and resist brain aging [52].

4.4. Myocardial Protection Activity. Some research groups have investigated the myocardial protection activity of *M. prunifolia* bioactive compounds in animal models. Ge et al. reported the protective effect of its flavonoids on acute myocardial ischemia [53]. Their results showed significantly antagonized changes in the T-wave, point S, and P-P period of model rats administered with different concentrations of *M. prunifolia* flavonoids, as well as the remarkably counteracted changes in SOD and MDA concentrations in the serum. Their experimental results suggested that the fruit’s flavonoids have a protective effect against myocardial ischemia in rats.

5. Discussion and Perspective

Phytochemical data combined with biological activity information confirm that *M. prunifolia* has potential medicinal and dietary values for humans. However, other unidentified phenolics or other compounds in these fruits, including saponins, polysaccharides, and nonpolar constituents, should also be investigated for their biological effects. This minireview compiles ethnobotanical and nutritional compositions of the fruit for interpreting data from phytochemical investigations; it is thus useful for predicting the biological activities of under-researched natural foods or medicines, such as *M. prunifolia*. Studies on this fruit may be especially important to people in areas where such natural resource may be endemic and where proper healthcare is scarce. Information about the phenolics, flavonoids, and polysaccharides may also serve as a springboard for studies on their bioavailability, as well as the mechanisms of anti-oxidant and immunostimulating activities.

In *vivo* studies on animals and, when possible, on humans are necessary to better understand the effect of the *M. prunifolia* fruit’s metabolites on human health because the effect of these compounds in *M. prunifolia* on human cells and tissues as measured by *in vitro* tests cannot represent their actual effect *in vivo*.

There are still opportunities and challenges for studies on the chemical composition and biological activity of the *M. prunifolia* fruit. The increasing number of modern studies of its bioactive constituents suggests that these chemical compounds in the fruit have great medical significance in the future.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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