Extraction and Phytochemical screening of the root of mulberry in
local area of Ratchaburi

Soraya Thaivanich*
Paweena Pongdontri**

Abstract

Mulberry (Morus spp. L., Moraceae) is widely cultivated in Thailand. Mulberry bark, branches and root have been used in traditional medicine as having antimicrobial, antioxidant, anti-inflammatory, anti-hypotensive properties. The phytochemical analysis of Morus alba roots, which grown in Ratchaburi and adjacent provinces, were investigated using standard procedure. The root were dried under constant temperature and humidity in incubator and then extracted with different solvent extracts as well as partial identification of bioactive constituents. The extracts were concentrated using rotary evaporator and kept in desiccators for further analysis. The phytochemical screening revealed that the root contains flavonoids, alkaloids, tannins, and terpenoids in the local mulberry. Total phenolic and flavonoid contents were investigated by using Folin-Ciocalteu and colorimetric aluminium chloride assays, respectively. The results showed that methanolic extract obtained significantly higher total phenolic and flavonoid contents than other solvents.

Keywords: root, mulberry, phytochemical, extraction

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Extraction and Phytochemical screening of the root of mulberry in local area of Ratchaburi

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introduction

The Moraceae (Mulberry family) with approximately 68 species was mostly of pantropical distribution, widespread in tropical and subtropical regions. Mulberry is usually associated with sericulture, the production of silk through the silkworm (Bombyx mori). Genus Morus was mainly known for their edible fruits and used as fodder for silkworms of importance for sericulture in northeastern Thailand; and found widely in many regions including Ratchaburi province. Conventional medicines show of great interest to phytochemicals rich plant extracts to cure different maladies because nature sources are considered to be less toxic and free from undesirable effects. It is reported in Chinese medicine used in medicine and Chinese pharmacopoeia list the root bark, stem, fruits and leaves as a constituent in medicinal preparations (Kumar and Chauhan, 2008). There are chemical compound (phenolic compound, alkaloids, terpenoids, quinines, saponins, etc) with complex mixture of many medicinal plant metabolites. In recent years a number of studies have been reported, dealing with biological activities of extracts of medicinal plant such as chemical composition, therapeutic activities. Their alternative applications for the medical plant depends upon their chemical substances that generate a distinct physiological action. This study was to carry out preliminary phytochemical screening and to determine the total phenolic and flavonoid contents of root mulberry collected from Ratchaburi province.

Research Methodology

1. Plant Material

Root of Morus alba were collected in Ratchaburi province and adjacent province, Western Thailand. Taxonomic identification was performed and a collected specimen is deposited in the farm of The Queen Sirikit Department of Sericulture (Kanchanaburi), Thailand.

2. Extract Preparation

The plant materials separated were washed and cleaned thoroughly with tap water and then with distilled water and air-dried incubator for several days. They were then finely ground to powder using masala mill. Dried and powdered plant
tissues were extracted three times with the selected organic solvents. The phytochemical tests of mulberry roots were analysed after extraction by three solvents (hexane, ethyl acetate and methanol) at room temperature. Aqueous extracts of root were prepared by soaking powdered tissues in each of the solvents with intermittent shaking for 24 h. The extracts were then filtered through Whatman No.1 filter paper. The organic solvent extracts were evaporated by rotary evaporator while the aqueous extracts were lyophillized using nitrogen brow. We characterized chemical constituent for quantitative estimation of phytochemical constituents such as phenolics, flavonoids, tannins, alkaloids, terpenoid, saponins and anthraquinone.

2.3 Phytochemical screening

The crude methanolic extracts of root was tested for the presence of phenolics, flavonoids, tannins, alkaloids, terpenoid, saponins and anthraquinone. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Test for Alkaloids

About 15 mg of each extract was separately stirred with 1% HCl (6 mL) on a water bath for 5 min and filtered. These filtrates were divided into three equal parts. 

Dragendorff's test: To one portion of the filtrate, Dragendorff's reagent (Potassium bismuth iodide solution) (1 ml) was added; an orange red precipitate shows the presence of alkaloids. Mayer's test: To one portion of filtrate, Mayer's reagent (Potassium mercuric iodide solution) (1 ml) was added. Formation of cream colored precipitate gives an indication of the presence of alkaloids. Wagner's test: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 ml) and the solution was diluted to 100 ml with distilled water. Few drops of this solution were added to the filtrate; a brown colored precipitate indicates the presence of alkaloids. (Joshi et al., 2013 and Abdullahi et al., 2013).

Test for Terpenoid

An amount of 0.5 g of plant sample was taken in a test tube, then poured 10 ml of methanol in it, shaken well and filtered to take extract of the sample. Then 2
mL of chloroform were mixed in extract of plant sample and 3 mL of sulphuric acid were added in the sample extract. Formation of reddish brown color indicates the presence of terpenoids in the sample.

Test of Flavonoids

Methanolic crude extracts were mixed with few fragments of magnesium ribbon and concentrated HCl was added drop. After boiling a pink or red coloration appeared which indicated the presence of flavonoids.

Test of Saponins

Each of 0.5 g extract was added 5 mL of distilled water in the test tube. This solution was kept in a water bath at 60°C. It was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test of Tannins

Each of 0.5g extract was added in 5ml of distilled water in the rest tube. A few drop of ferric chloride solution was added and observed for blue-green to black coloration indicates the presence of tannins.

Test of Anthraquinone

Each of 0.5g extract was taking in separate test tube and 10 mL of chloroform was added. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of anthraquinones (Manga and Oyeleke, 2008).

Determination of total phenolics and total flavonoid contents

The total phenolics content was estimated using Folin-Ciocalteau reagent by the method (Velioglu et al., 1998; Cai et al., 2004; Chlipicka et al., 2012) with some modifications. About 20 µg of root extracts were taken and it was made up to 100 µl with distilled water. Then 40µl of Folin-phenol reagent and 40µl of sodium carbonate (10%) were added. The mixture was shaken well and incubated in dark condition for 40 min for the development of color. After incubation, the absorbance was measured at 765 nm. A calibration curve of gallic acid was constructed and
linearity was obtained in the range of 10-50 µg/ml. The total phenolics content in the plant extracts were expressed as mg of gallic acid equivalent (mg GAF/g extract) by using the standard curve.

The total flavonoids content was estimated using the procedure described by Zhishen et al. A total of 100 µl of plant extracts were diluted with 100 µl of distilled water separately followed by the addition of 150 µl of sodium nitrite (5%) solution. This mixture was incubated for 10 min and then 150 µl of aluminium chloride (5%) was added and allowed to stand for 10 min. The mixture was shaken well and left it for 15 min at room temperature. The absorbance was measured at 425 nm. The concentration of flavonoids was calculated based on a calibration curve using apigenin (Sigma, USA).

Statistical analysis

The values are represented as mean ± standard error of mean (SEM) for triplicate set of experiments.

Research Results
Preliminary qualitative phytochemical analysis

Root of Morus alba were collected during July 2015-April 2016 at the Western Thailand in order to obtain material as for locality and environmental conditions. There were no morphological differences among mulberry plants from individual localities. The phytochemical analysis of ethyl acetate and methanol extraction of root of Morus alba were presented in table1. The results revealed the presence of medically active compound in the plant studied. Important medicinal phytochemicals such as flavonoid, alkaloid, tannin and terpenoid were present in the sample. Flavonoids are rich in Morus alba roots have been reported for antidiabetic activity, antimicrobial activity, anticancer activity, antioxidant activity, antihyperglycemic hypotension, anemia, antistress and arthritis (Ozgen M. 2009). Theses phytochemical exhibit diverse pharmacological and biochemical actions. The root part has been used in folk medicine (especially in Chinese medicine). Alkaloids are reported to antidiabetic activity, antioxidant, hypoglycemic activity. The phytoconstituents tannins and triterpenes are also reported as the main active
principles. The methanolic extraction was done by taking root wood and root bark. It was found that the phytochemical substances in root bark are higher than substances in root wood (Table 2). Total phenolic contents obtained were 11.4 mg/gm of the extract and total flavonoid contents obtained were 18 mg/gm of the extract for the mulberry root.

**Table 1** phytochemical analysis of methanolic extract of *Morus alba*

<table>
<thead>
<tr>
<th>Bioactive component</th>
<th>methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = positive, - = negative

**Table 2** Qualitative phytochemical analysis of methanol extracts

<table>
<thead>
<tr>
<th>Tree</th>
<th>Part</th>
<th>Phytochemical Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>alkaloid</td>
</tr>
<tr>
<td><em>Morus</em></td>
<td>root</td>
<td></td>
</tr>
<tr>
<td>alba</td>
<td>wood</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>root bark</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = positive, - = negative

**Discussion**

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to potent medicinal as well as physiological activities. Analysis of the plant extracts revealed the presence of phytochemicals such as flavonoids, alkaloid, tannins, terpenoid and phenolic compound. The results of this investigation are indicative of possible active principle of natural origin from the extract with possible high potency which could serve as a
lead to the isolation of chemotherapeutic agents. Some of these active principles are alkaloids and triterpenes whose presence in some plant species are noted to have antidiabetic effects. The phytochemical information indicated the rightfulness of the traditional use of the studied plants as antidiabetics. The mulberry root reportedly possesses anthemicntic and astringent properties. The root is astringent and bark is anthelminthic (Bhattari, 1992). Root is one of the constituent of potent drug named which reduces the plasma sugar level in mice (Hikino et al., 1985). The methanolic extract of the root bark displayed activity HIV which contains flavnoids like Morusin, Mulberrofuran D, G, K and Kwanon G, H., of which Morusin and Kwanon H showed positive activity against HIV (Shi-De et al., 1995). Morusin also inhibits tumor promotion (Shigeru et al., 1989). New antifungal phytoalexins- Moracin E, F, G and H, Kwanon D, E, F were isolated from root bark of mulberry plant. The root bark of M. alba contains Sanggenon alkaloid which inhibited plaque formation. Morusin 4’- glycoside and Kwanon H show positive activity against HIV. The root bark also contain an alkaloid, Deoxynojirimycin-1 inhibited glycogenlyses, glycoprotein, processing and saccharide hydrolysis enzymes whereas its derivatives have great therapeutic potential for the treatment of viral infections, diabetes, obesity and cancer (Hughes and Rudge, 1994).

Conclusion

The results revealed the presence of medicinally important constituents in the plant studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as therapeutic properties to the plant studied in the treatment of different ailments. Therefore, extracts from these plant could be seen as a good source for useful agents.
References


