

Screening of Phytochemical Constituents, Elemental Composition and Evaluation of Antioxidant and Antibacterial Activity of Leaf Extract of *Morus alba* L. (White Mulberry)

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Abstract

Leaf samples of *Morus alba* L. were collected from Sericulture Farm, Department of Agriculture, Myitkyina Township, Kachin State. The aim of this study is to analyze the phytochemical, elemental, antioxidant and antibacterial activities of mulberry leaf extract. The extract from mulberry leaves was extracted with water and ethanol. The research was performed in University Research Center, University of Myitkyina from June to October, 2019. The phytochemical analysis observed the presence of phenolic compound, polyphenol, glycoside, reducing sugar, saponin, tannin, lipophenol and steroid but the absence of alkaloids, flavonoid and terpene. Elemental analysis of mulberry leaves was performed by EDXRF spectrometry. According to the EDXRF spectrum data, the elements such as K, Ca, Si, S, Mn, Fe, P, Sr, Cu, Zn, Ti, Rb and Br were present as trace elements. The antioxidant activities of crude extract of mulberry leaves were studied by using DPPH free radical scavenging assay method. Six bacterial strains were taken for antibacterial analysis. The antibacterial studied against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella boydii* and *Pseudomonas aeruginosa*, each result in the zone of inhibition which was more in case of ethanol extract. *Bacillus cereus* was no more inhibition zone.

Key words: *Morus alba*, phytochemical, elemental, antioxidant, antibacterial

Introduction

Mulberry is known in Myanmar as “Poe sa” as all parts of the plant have many uses. It is essential to sericulture as the foliage constitutes the sole feed of the mulberry silkworm. *Morus alba* L. (white mulberry) belong to the family of Moraceae which is a family of flowering plants. The most acceptable species of *Morus* are 10 – 14. They are distributed into the sub-tropic regions of Asia such as Japan, India, Cambodia, Myanmar, Thailand, China and Korea. They are also present in other region of world like North America, an Africa (Zeng *et al.*, 2015). Most of these countries grow *Morus* species (mulberry plants) for different purposes like production of *Bombyx mori* L. (silkworm) or enhancing the foliage production in India and China (Vijayan *et al.*, 1997). White mulberry has a long history of medicinal use in Chinese medicine (Brown, 1995). Mulberry is a good source of minerals and provides nutritionally useful amounts of most of them, including K, Ca, P, Mg and S (Levickiene *et al.*, 2017). Over the years, medicinal plants have been found useful in the treatment and management of various health problems. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (WHO, 1993). Mulberries are a good source of vitamins and minerals and contain an especially high amount of anthocyanin (Gerasopoulos and Stavorulakis, 1997). In traditional medicine, white mulberry, next to the medicinal properties, is known for its antimicrobial properties (Ding, 2014). Mulberry leaves are traditional used for the treatment of several kinds of disease. The present research was to analysis the phytochemical constituents, element composition, antioxidant and antimicrobial activities of leaves of *Morus alba*.

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Materials and Methods

Leaves sample collection, identification and preparation

Morus alba L. (white mulberry), cultivar Buriram 60 (BR 60) leaves were collected from Sericulture Farm, Department of Agriculture, Myitkyina Township in July, 2019. Identification of this plant was carried out by referring to literature of (Backer and Brink, 1964). The leaves were kept away from sun rays and air dried at room temperature. They were milled to powder using a blender and stored in air tight container at room temperature until required for further analysis (Harbone, 1984).

Investigation of phytochemical constituents, determination of elemental composition, antioxidant and antibacterial activities of leaves of *Morus alba* L.

Preliminary phytochemical tests were carried out according to the appropriate reported methods (Tin Wa, 1972). Elemental composition was determined by Energy Dispersive X Ray Fluorescence (EDXRF) Spectrophotometer. It was carried out at Department of Chemistry, University of Monywa. Antioxidant activity was determined by radical scavenging assay (DPPH) using UV visible spectrophotometer. It was performed at Medical Biotechnology laboratory, Biotechnology Research Department (BRD), Kyauk-se. One solvent extract (ethanol) was determined by agar well diffusion method for antimicrobial activity. Six microorganisms; *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella boydii* and *Pseudomonas aeruginosa* were investigated in the antibacterial activity. This experiment was conducted at Department of Biotechnology, Technological University of Mandalay.

Results

Family name : Moraceae
 Scientific name : *Morus alba* L.
 English name : White Mulberry
 Myanmar name : Poe sa

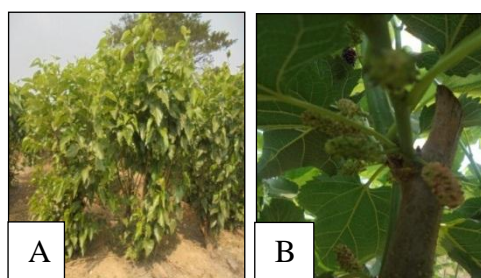


Figure 1.

(A) Plant of mulberry (B) Fruits

Outstanding characters of *Morus alba* L.

The plant is usually monoecious or dioecious. Perennial, small tree in sized. Stem is cylindrical. Leaves are variable in size and shape, broadly ovate, simple, alternate, stipulate, three lobed, margins dentate, apex acute, base truncate. Inflorescence is axillary, pendulous. Flowers are unisexual, actinomorphic, hypogynous, inconspicuous and greenish color; male spikes (catkins) are cylindrical, female spikes are ovoid and stalked. Fruit is syncarpous, white to pinkish white, purple or black when ripe. Seeds are endospermic (Figure 1 A and B).

Screening of phytochemical constituents of the *Morus alba* L. leaves

The phytochemical screening revealed the presence or absence of alkaloids, phenolic compound, polyphenol, flavonoid, glycoside, reducing sugar, saponin, tannin, lipophenol, steroid and terpene in mulberry leaves. The results were expressed in (Table 1 and Figure 2).

Table 1. Preliminary phytochemical tests of *Morus alba* L. leaves

No.	Test	Extract	Test Reagents	Observation	Result
1.	Alkaloid	1% HCl	Mayer's reagent	-	-
			Dragendorff's	-	-
			Wagner's	-	-
2.	Phenolic Compound	EtOH	10 % FeCl ₃	Brown colour	+
3.	Poly phenol	EtOH	1% FeCl ₃ and 1% K ₃ [Fe(CN) ₆]	Greenish blue colour	+
4.	Flavonoid	EtOH	A few pieces of Mg and few drops of concHCL	-	-
5.	Glycoside	H ₂ O	10% lead acetate	White ppt	+
6.	Reducing sugar	H ₂ O	Benedict's solution	Brick-red ppt	+
7.	Saponin	H ₂ O	Distilled water	Frothing	+
8.	Tannin	H ₂ O	10% FeCl ₃ and DilH ₂ SO ₄	Yellowish brown ppt	+
9.	Lipophenol	H ₂ O	0.5M KOH and NaOH	Deep Colour	+
10.	Steroids	EtOH	Acetic anhydride, concH ₂ SO ₄ and Chloroform	Green Colour	+
11.	Terpenes	EtOH	Aceticanhydride, Chloroform and concH ₂ SO ₄	Pink Color	-



(+) = presence (-) = absence

Figure 2. Phytochemical constituents of leaves of *Morus alba* L.

Elemental composition and antioxidant activity of leaves of *Morus alba* L.

Some elements present in leaves of *Morus alba* L. by EDXRF Spectrometry. X-ray spectrometer permits simultaneously analysis of light element to heavy elements from Na to U under vacuum condition was shown in (Table 2). The IC₅₀ value of the standard ascorbic acid was (500 µg/ml 94.63413% scavenging) µg/ml. From this experiment, it was found that ethanol extract (IC₅₀ = 341.92 ± 28.46 µg/ml) was observed to possess the most potent antioxidant activity. It was shown in (Table 3).

Table 2. Elemental composition of leaves of *Morus alba* L. by EDXRF

Elements	Relative abundance (%)
K	1.178
Ca	0.734
Si	0.639
S	0.134
Mn	0.009
Fe	0.009
P	0.009
Sr	0.002
Cu	0.002
Zn	0.002
Ti	0.001
Rb	0.001
Br	0.001

Table 3. Antioxidant activity of leaves of *Morus alba* L.

Sample (Concentration g/ml)	DPPH Scavenging (%) ±SD
1000	86.51±2.23
500	64.11±2.20
250	41.71±2.97
125	29.71±1.37
62.5	20.34±5.45
31.25	11.43±4.09
15.63	-
IC ₅₀ µg/ml±SD	341.92±28.46
Method	DPPH Radical Scavenging Assay

Antimicrobial activity

Antibacterial activity by using agar well diffusion method, EtOH extract of mulberry leaf was not found on the test microorganism *Bacillus cereus*. The remaining extracts were showed antibacterial activity against five microorganisms. It was shown in (Table 4 and Figure 3).

Table 4. Antibacterial activity of leaves of *Morus alba* L.

Test Microorganism	Inhibition zone (mm)
<i>Bacillus cereus</i>	-
<i>Escherichia coli</i>	10
<i>Staphylococcus aureus</i>	10
<i>Salmonella typhi</i>	10
<i>Shigella boydii</i>	10
<i>Pseudomonas aeruginosa</i>	12

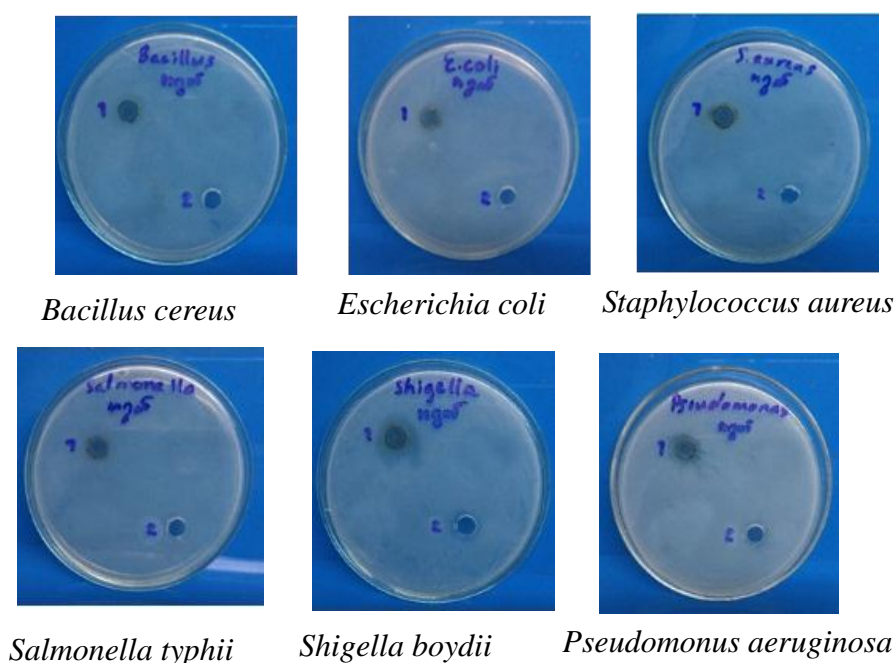


Figure 3. Antimicrobial activity of leaf extract of of *Morus alba* L.

Discussion and Conclusion

Guo *et al.* (2003) stated that several compounds which act as antioxidant agents include phenolic compounds, flavonoids, steroid, glycoside, terpenoid, saponins, alkaloids and tannins. In the preliminary phytochemical tests, it indicated that phenolic compound, polyphenol, glycoside, reducing sugar, saponin, tannin, lipophenol and steroids were present. Alkaloids, flavonoid and terpene were absent.

According to Yigit *et al.* (2010) the elemental composition and concentration of some mulberry leaves were evaluated by wavelength-dispersive X ray fluorescence (WDXRF) analyses. Elements such as P, K, Ca, Mg, Na, Al, Si, S, Cl, Cr, Fe, Mn, Sr and Zn were determined in leaf samples.

In this research, the qualitative elemental composition of leaves of *Morus alba* L., BR 60 was determined by applying EDXRF spectrometry. The high amount of the major elements K and Ca and trace amount of Cl, Si, S, P, Fe, Mn, Sr, Ti, Cu, Zn, Br and Rb were observed that were present in mulberry leaves.

The scavenging activity of the methanol extracts against DPPH radical is concentration dependent and the concentration to scavenge 50% of DPPH present in the reaction mixture (IC_{50}) is the minor in *M. rubra*. The study of (Baea and Suh, 2007) showed IC_{50} DPPH exerted by leaves of *M. alba* between 225.9 and 537.6 μ g. In the experiment, the antioxidant activity of ethanol extract of the leaves of *Morus alba* was investigated by using DPPH assay method. Ascorbic acid was used as standard antioxidant activity. It was found that ethanol extract ($IC_{50}=341.92 \pm 28.46$ μ g/ml) was observed to possess the most potent antioxidant activity in leaves of mulberry.

Paiva *et al.* (2010) indicated that among the substances present in white mulberry leaf extract with antimicrobial effects is present of saponins and tannins. Ayoola *et al.* (2011) studied that the antibacterial activity of ethanol extracts from the leaves of *Morus alba*. They showed that the ethanol extract inhibits the growth of Gram negative bacteria *E. coli*, *P. aeruginosa*, *Neisseria gonorrhoea* and Gram positive bacteria *Proteus vulgaris*, *Staphylococcus aureus* and *Streptococcus faecium*. In this research, antibacterial studied against *Escherichia coli* (10 mm), *Staphylococcus aureus* (10 mm), *Salmonella typhii* (10

mm), *Shigella boydii* (10 mm) and *Pseudomonas aeruginosa* (12 mm), each result in the zone of inhibition which was more in case of ethanol extracts. *Bacillus cereus* was no more inhibition zone.

Leaves of white mulberry are containing phytochemical constituents, elemental composition, antioxidant and antibacterial activity. So, they are suitable for health because of a rich source of vitamins, minerals and nutrients.

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