Phytochemical Analysis of Black Mulberry Tree (*Morus nigra*)

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Abstract *– This study aimed to determine the phytochemical constituents of the different parts of Morus nigra such as the leaves, bark, roots, and fruits. It made use of the Qualitative Method Design and Laboratory Method Analysis. The samples were collected and prepared in Alcala, Pangasinan. Plant samples were brought to the University of the Philippines at Diliman, Quezon City for identification and authentication. Plant materials were submitted to the Industrial Technology Development*

In terms of phytochemical analysis, the roots and bark is abundant in triterpenes and lacks flavonoids. The bark is also abundant in saponins. The leaves has abundant sterols and the fruits are rich in flavonoids. The beneficial phytochemical constituents are the sterols that are abundant in the leaves of Black Mulberry Tree; flavonoids that are abundant in the fruits and alkaloids that are found in traces in all the parts of Black Mulberry Tree. The hazardous phytochemical constituent are the tannins and saponins which were found in traces and moderate amounts in all of the parts of Black Mulberry Tree. The bark and roots of the tree could be used to create insect-repellent and anti-microbial solutions because of abundance of triterpenes. The leaves can be used as anti-cancer and immunity booster since it has a lot of sterols. Flavonoids in fruits could be used as anti-oxidant and heart disease preventer. However, abundant amount of saponins in the bark of the tree can cause destructive consequences in the body. It is hereby recommended that Black Mulberry Tree be cultivated and be propagated on a larger scale because of its medicinal value. The phytochemical constituents must be isolated and proximate analysis must be performed.

Keywords *– Black Mulberry, Herbal, Medicinal Uses, Phytochemical Analysis*

INTRODUCTION

All individuals have the right to health. This right means that everyone has the right to obtain the highest attainable standard of physical and mental health, which includes access to all medical services and high-quality medicines. However, the Philippines as a member of the third world economy is having difficulties in providing all these. The people are resorting to other means such as herbal medicines available. Also, there is limited research-based information on herbal medicines in the country.

Plants are excellent sources of food, chemicals, and herbal medicines. Many important drugs have been directly or indirectly derived from them [1]. The medicinal plants are useful for curing of various human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables, and roots that have defense mechanism and protect from various diseases. One of the well-known medicinal plant is the Black Mulberry Tree.

In the Philippines, the Black Mulberry Tree (*Morus nigra*) leaves are harvested when the plant reaches six months and harvests take place every three months. Japan and China can only grow leaves of Black Mulberry Tree (*Morus nigra*) every six months because these countries experience long winters [2]. The *Morus nigra* is commonly grown to produce leaves which are usually fed to silkworms for the manufacture of silk threads. Silkworm Farming in La Union and Negros Occidental in the Philippines had been a great source of income to the community. However, the cutting of hectares of planted *Morus nigra* over the last ten years slumped the production of Silkworm.

It is necessary to know the probable uses of the Black Mulberry Tree (*Morus nigra*) from its roots, its branches, its leaves, and its fruits. The determination, therefore, of phytochemical constituents of Black Mulberry (*Morus nigra)* would be of great help to utilize programs and products that would enhance the use of Mulberry. It could also increase its marketability, thus, creating job opportunities to our locals. This study was conducted primarily to describe the Black Mulberry Tree that is grown in Pangasinan, Philippines; to identify the phytochemical constituents present on this tree; and, to describe the medicinal purposes of the phytochemical constituents of *Morus nigra*.

The parts of the Black Mulberry Tree (*Morus nigra)* samples were taken from San Nicolas, Alcala, Pangasinan. The preparation of samples for analysis were done in Canarvacanan, Alcala, Pangasinan. The samples were limited only in the Black Mulberry Tree since the White and Red Mulberry Trees are not propagated in the Philippines.

The physical description of the Black Mulberry Tree (*Morus nigra)* is performed in the University of the Philippines, Diliman, Quezon City. The phytochemical analysis of present phytochemical constituents was done by Industrial Technology Development Institute in Bicutan, Taguig. There were no isolation and purification of the plant constituents in this study.

OBJECTIVES OF THE STUDY

This research sought to determine the physical characteristics of the Black Mulberry Tree (*Morus nigra)* in terms of color, height, habitat, propagation, size, and shape. It also sought to determine the amount of phytochemicals that could be found in the different parts of Black Mulberry Tree (*Morus nigra)* and their medicinal purposes.

MATERIALS AND METHODS

This study described the characteristics of Black Mulberry Tree (Morus nigra) and determined the present phytochemicals in its four different parts. The presence of phytochemicals was measured qualitatively and were analyzed and described using Laboratory Method Analysis. The samples of the study were subjected to extraction and were subjected to different laboratory tests to identify the phytochemicals present. The medicinal uses of the phytochemicals found were discussed about pharmaceutical and medicinal findings.

Collection of Samples

The researcher secured the plant samples of Black Mulberry Tree from San Nicolas, Alcala, Pangasinan. The samples were the parts of Black Mulberry Tree such as roots, branches, leaves and fruits.

Preparation of Samples

The samples were washed two times with tap water and a final rinse using distilled water. It was then placed in a confined room in the researcher's residence in Canarvacanan, Alcala, Pangasinan.

The samples were carefully placed inside plastic containers. Some of the fresh samples were submitted to the University of the Philippines in Diliman, Quezon City for taxonomical classification and physical description.

The samples were air dried for three weeks. They were weighed, sealed and labeled. The researcher brought the dried samples to the Industrial Technology Development Institute for phytochemical analysis. The scientists and laboratory experts in Industrial Technology Development Institute at Bicutan performed the phytochemical analysis on leaves, roots, branches, and fruits of the Black Mulberry Tree.

Phytochemical Analysis

1. Tests for Alkaloids

Materials: Hydrochloric Acid
Mayer's / Wagner's Reagent

A 4.0 g sample of dried roots, leaves, branches, and fruits were weighted with methanol. The aqueous solution of the acid-soluble portion of the methanol fractions were extracted with 1 % HCl and 2 drops of Mayer's or Wagner's reagent which were added to the filtered acid extract. A cream coloured precipitate was observed in the case of Mayer's reagent while a reddish-brown precipitate was observed in the case of Wagner's test.

2. Tests for Steroids and Triterpenes

Liebermann- Burchard Test

Materials: Acetic Acid Anhydride
Concentrated Hydro Sulfuric Acid

A small amount of alcoholic extract was dissolved in acetic acid anhydride. The soluble portion was decanted and 1-2 drops of concentrated sulfuric acid was added. A green color, either immediately or slowly turned into red and blue tones. A pink to red color is indicative of triterpenoids while a blue color is indicative of steroids.

3. Test for Flavonoids

Materials: 10% Hydrochloric Acid
Magnesium Turnings

One (1) ml of alcoholic extract or a small amount of dried alcoholic extract was treated at 1 ml 10% HCl and magnesium turnings. Formation of red color is observed as there are flavonoids.

4. Test for Saponins

Materials: Distilled Water
Test Tube

The formation of persistent foams during plant extraction or during the concentration of plant extract is a reliable evidence that saponins are present. Indeed, if large quantities of saponins occur in a plant, it was difficult to successfully concentrate aqueous alcoholic extracts even when using a rotary evaporator. A simple test for saponins is therefore, to shake up an aqueous alcoholic plant extract in a test tube.

5. Test for Tannins

Materials: Ferric Chloride
Distilled Water

The alcoholic extract was extracted with hot water and the aqueous extract was filtered. Two (2) drops of ferric chloride test solution is added. An indication is a dark colored precipitate which may either be black, dark blue, blue black-green or blue green.

6. Test for Glycosides

Materials: Fehling's Solution
10% Hydrochloric Acid
Ferric Chloride
Sodium Carbonate
Filter Paper
Distilled Water
Test Tubes

The alcoholic extract was dissolved in hot water and filtered. The filtrate was used for the test. A 2 ml sample was placed in two test tubes. To sample 1, 1 cc of dilute HCl was added. To sample 2, nothing was added. Then the two test tubes were placed in a boiling water bath for 5 minutes. The test tubes were cooled. The samples were both neutralized with anhydrous sodium carbonate until no more effervescence was produced. Then Fehling's Solution was added which was prepared by mixing 3 ml of Fehling's A with 3 ml of Fehling's B. One (1) ml of this Fehling's solution was used. The two test tubes were heated in a water bath for 2 minutes. A brick red precipitate was observed. An increase in the amount of brick red precipitate in the hydrolyzed sample (the sample to which the dilute acid is added) as compared to the other sample indicated the presence of glycosides.

Fehling's Solution A was prepared by putting 34.64 g of Copper Sulfate in a flask and mixed with 500 ml distilled water.

Fehling's Solution B was a mixture of 50 g of sodium hydroxide and 173 g of potassium sodium tartrate and 500 ml of distilled water.

Further, the data was recorded by the scientists and laboratory experts from Industrial Technology Development Institute. The researcher secured a copy of the taxonomical phytochemical analysis results.

RESULTS AND DISCUSSION

The data-findings were presented and analyzed according to the phytochemical analysis of the different parts of Black Mulberry Tree (*Morus nigra*) namely, leaves, bark, roots, and fruits.

Physical Characteristics of the Black Mulberry Tree

Morus nigra, Black Mulberry, is a slow-growing, deciduous tree. The species is known to have escaped from cultivation in Denmark and Austria, is weedy in Spain, southeastern Australian bush land, and South Africa, and has been reported as invasive in southern Brazil. Invasive traits include its longevity, rapid growth rate, tolerance for droughts, infertile and rocky soil, and resistance to cold, easy seed dispersal by biotic vectors attracted to its sweet, edible fruits, and repeated introductions for cultivation around the world. Considering current evidence, risk of introduction for this species is medium to high, although further research is needed.

Table 1. Physical Characteristics of Black Mulberry Tree *(Morus nigra)*

Physical Attribute	Physical Characteristics
Color	Bark- scaly brown or dark brown Leaves- yellow-green and green Roots- brownish to yellowish or white Fruits- green, red, dark blue or black
Height	6-9 meters
Habitat	Warm- temperate regions
Propagation	Stem cuttings and pollination
Size and shape	Leaves- 7-12.5 cm long, one or more lobes Fruits- 1.3-2.5 cm long

Table 1 shows the physical characteristics of the Black Mulberry Tree in terms of color, height, habitat, propagation, and size and shape.

Color

The bark of the Black Mulberry Tree (*Morus nigra*) has a scaly brown to dark brown color. The leaves are yellow green when young and turns to be green as it matures. The roots are brown-yellowish and sometimes white. The fruits when young are colored green and will turn into red as it ripens and dark-blue or black when fully ripe.

Height

Morus nigra is a dioecious tree and grows up to 6-9 m in height, slender but with

numerous branches; it tends to be a bush if not trained when young.

Habitat

Morus nigra thrives in warm-temperate regions with long, hot summers. It can tolerate drought, infertile soils, and cold temperatures down to -10°C but does not do well in hot tropical zones with humid summers and grows best at lower altitudes when sheltered from wind and in coastal areas. It prefers warm, well-drained soil such as deep loams and in cultivation it is recommended to avoid planting the species in shallow, chalk, or gravelly soils.

Propagation

Some mulberries both requires male and female trees to produce fruit. Trees will sometimes change sex and do not bear much fruit for the first 15 years. High temperatures, strong light and long days favor maleness in mulberries, with their opposites, as well as high humidity, favoring the production of female flowers. The species is wind pollinated, and some cultivars will set fruit without any pollination, for example in California, USA. The self-fertile trees commonly produce two crops a year. *Morus nigra* occurs in coastal and warm, arid places, and is cultivated in agricultural and garden settings. It has also been reported to escape from cultivation. In addition to its use as a crop, roadside, and home garden species, *Morus nigra* has also been used in agroforestry as a windbreak, live fence, and shelter/shade tree. In the Philippines, the Black Mulberry Tree (*Morus nigra*) can bear fruit six times a year and can start bearing fruit eight months after planting. It can easily be propagated by cuttings and pollination.

Size and Shape

The tree has scaly bark and is usually kept pruned to a smaller, open, spreading shape. It can produce quite a dense and shady canopy. Leaves are rough on upper surfaces and pubescent underneath, 7-12.5 cm long, often producing leaves of several different shapes, with 1 or more lobes, multilobed leaves often appearing on the same branches as lobeless ones; abnormally shaped leaves usually produced from stem shoots or sucker growths, and frequently by very vigorous young branches. Flowers held on short, green, pendulous, nondescript catkins that appear in the axils of the current season's growth and on spurs on older wood. The flowers appear in 1.3 cm scaly clusters, female flowers ripening quickly into 1.3-2.5 cm blackberry-shaped edible fruits [3].

Phytochemical Analysis of the Black Mulberry Tree

Table 2 shows the results of the qualitative amounts of phytochemical constituents present in the different parts of Black Mulberry Tree.

About 200 grams of beige small pieces plant material in a plastic container marked as Mulberry (*Morus nigra*) Roots; About 200 grams of brown small pieces plant material in a plastic container marked as Mulberry *(Morus nigra)* Bark; About 100 grams dried ground green plant material in a plastic container marked as Mulberry *(Morus nigra)* Leaves; About 100 grams dried black plant material in a plastic container marked as Mulberry (*Morus nigra*) Fruit were submitted to Industrial Technology Development Institute at Bicutan, Taguig for phytochemical analysis.

Table 2. Phytochemical Analysis of Black Mulberry Tree *(Morus nigra)*

Phytochemical Constituents	**Leaves**	**Bark**	**Roots**	**Fruits**
Sterols	(+++)	(+)	(+)	(++)
Triterpenes	(+)	(+++)	(+++)	(+)
Flavonoids	(++)	(-)	(-)	(+++)
Alkaloids	(+)	(+)	(+)	(+)
Saponins	(++)	(+++)	(++)	(++)
Glycosides	(+)	(+)	(++)	(+)
Tannins	(++)	(++)	(++)	(+)

Legend:

(+)	Traces
(++)	Moderate
(+++)	Abundant
(-)	Absence of constituent

Sterols

An abundant amount of this constituent can be found in the leaves and moderate amount is attributed to the fruits. The bark and roots have traces of sterols in it.

Triterpenes

The leaves and fruits of Black Mulberry Tree (*Morus nigra)* have traces of triterpenes. The bark and roots have abundant amount of this phytochemical.

Flavonoids

Abundant amount of Flavonoids are found in the fruits and moderate on the leaves of Black Mulberry Tree (*Morus nigra)*. The roots and bark of the said tree has no flavonoids present. Flavonoids are pigments found in the leaves, petals, and fruits of higher plants.

Alkaloids

All of the four parts on the Black Mulberry Tree (*Morus nigra)* has only traces of Alkaloids found. This phytochemical is a bitter organic base in plants that usually contains nitrogen and oxygen.

Saponins

This phytochemical that is characterized by soapy lather has moderate amount found in the leaves, roots and bark while an abundant amount of this constituent is found in the bark.

Glycosides

This sugar like phytochemical constituent was found in all of the parts of the Black Mulberry Tree (*Morus nigra)* has traces of Glycosides except for the roots who have moderate amounts.

Tannins

All of the parts of the Black Mulberry Tree (*Morus nigra*) has moderate amount except for the fruits which has only traces of Tannins.

Medicinal Purposes of the Black Mulberry Tree

Sterols

The leaves and fruits of the Black Mulberry Tree has relatively abundant amount of sterols that play certain roles in the development of reproductive tract in humans and in the production and control of our reproductive hormones such as progesterone and testosterone [4].

Sterols also play a very important role in strengthening the body's immune system, thereby increasing the number of specialized cells (T-cells) which fight off bacteria and viruses in our defenses against infections. It can effect a response to diseases like breast, colon and prostate cancer, arthritis, hepatitis and HIV. On the other hand, bark and roots have only minimal potentials because there are only traces of sterols in it.

Triterpenes

The leaves and fruits have very little wax coatings which can serve as protective functions in repelling insects and microbial attacks.

A study from *Artocarpus Altilis,* a common Indian Tree also demonstrated an insecticidal and hypertensive activities of the plant because of triterpenes present. Pure triterpenes were also found to be very effective in the treatment of wound healing defects. It was also found out that the extracts of triterpenes from two species of *Maprounea Africana* act as anti-HIV agents.

Flavonoids

Since the fruits and leaves has considerable amount of flavonoids, they do have the potential to treat capillary bleeding and fragility because they strengthened capillary walls in which Vitamin C could not [5].

They have diuretic properties and have the potential to treat common colds. The flavonoids present in these plants are like carotenoids only that carotenoids are oil soluble while flavonoids are water soluble. They function as antioxidants that is protecting us from aging and cancer. Flavonoids in foods can also protect us from heart disease

Alkaloids

Alkaloids have the potential to be used or processed into an herbal drug as analgesic, narcotics, stimulants, miotics, mydriatics, antimalarial, and antispasmodics and in the

treatment of mental disorders, hypertension and tumors. The Black Mulberry Tree (*Morus nigra)* will only have a low potential since there are only traces of alkaloids in it.

Saponins

Saponins have detrimental effect on one's health because it can cause sneezing and can irritate the mucuous membrane. They can also destroy red blood corpuscles by hemolysis or the liberation of hemoglobin. Saponins have a bitter taste and reduce the food intake of livestock animals. Saponins were employed as fish toxicants [6].

Glycosides

Glycosides showed significant antioxidant activity, anticancer and antitumor activity, hepato-protective activity, anti-inflammatory activity, anti-diabetes activity, antiviral activity, antibacterial and antifungal activity, and other biological effects.

Tannins

Tannins is beneficial in the treatment of burns because it can precipitate the protein of the exposed tissue and will provide an antiseptic protective coat thus preventing external infection. It is also very useful in the vegetable tanning industry since it can precipitate the protein. But prolonged utilization of tannin rich plant such as the drinking of ordinary tea is hazardous due to its carcinogenic potential. All the tannins are relatively resistant to digestion and fermentation thus decreased ability of the animal to consume the plant and act as astringents thus shrinking and contracting structural proteins in the skin, mucosa and the gastrointestinal tract.

CONCLUSIONS AND RECOMMENDATIONS

Based on these findings, the researcher concludes the following in this study:

1. The *Morus nigra* is a dioecious tree and grows up to 6-9 m in height, classifying it as a medium sized tree. It thrives in warm-temperate regions with long, hot summers. Leaves are rough on upper surfaces and pubescent underneath, 7-12.5 cm long, often producing leaves of several different shapes, with 1 or more lobes. The flowers appear in 1.3 cm scaly clusters, female flowers ripening quickly into 1.3-2.5 cm blackberry-shaped edible fruits. Black Mulberry Tree (*Morus nigra*) can bear fruit six times a year and can start bearing fruit eight months after planting. It can easily be propagated by cuttings and pollination.
2. In terms of phytochemical analysis, the roots and bark are abundant in triterpenes and lacks flavonoids. The bark is also abundant in saponins that causes destructive consequences to the body. The leaves have abundant sterols and the fruits are rich in flavonoids.
3. The beneficial phytochemical constituents are the sterols that are abundant in the leaves of Black Mulberry Tree *(Morus nigra*); flavonoids that are abundant in the fruits and alkaloids that are found in traces in all of the parts of Black Mulberry Tree (*Morus nigra*). The hazardous phytochemical constituent is the tannins which were found in traces and moderate amounts in all of the parts of Black Mulberry Tree (*Morus nigra*).Saponins, a hazardous phytochemical is found in moderate amounts in all the parts of Black Mulberry Tree (*Morus nigra*) except for the bark which contains abundant amount of this constituent.

Based on these conclusions, the following recommendations are provided in this study:

1. As to the phytochemical constituents, all of the parts of the Black Mulberry Tree (*Morus nigra*) has beneficial constituents that could be isolated for further testing.
2. The relatively abundant amount of saponins and tannins in the different parts of *Morus nigra* could be isolated and should not be used for human consumption.
3. Proximate Analysis on moisture, crude fat, crude protein and crude fiber content must be done to determine if the dried samples were all fit to be processed as dried herbal drugs.
4. Black Mulberry Tree (*Morus nigra*) to be cultivated and to be propagated on a larger scale because of its medicinal value.

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