

BIOCHEMICAL ANALYSIS OF MELIA AZEDARACH (CHINABERRY) LEAF EXTRACT COLLECTED FROM LAOANG, NORTHERN SAMAR, PHILIPPINES

ABSTRACT

Melia azedarach belongs to the *Meliaceae* family and is known by various names, such as Chinaberry, ride of India, Bead-tree, Cape lilac, Persian lilac. It is commonly found in warmer and tropical regions, including Southeast Asia and various other parts of the world. The leaves, bark, stem and roots have a medicinal application and effective against the herpes simplex virus. This study focuses on the characterization of bioactive potential of *Melia azedarach* (Chinaberry) leaf extract. Physical properties were determined in terms of color, odor, solubility, boiling point, density, and pH. Furthermore, the secondary metabolites were determined in terms of alkaloids, phenols, tannin, saponin, and phenolic compound. Likewise, Fourier-Transform Infrared Spectroscopy is used to determine the functional group present on the leaf extract. Lastly, to determine the bioactive potential of *Melia azedarach* (Chinaberry) leaf extract Ultraviolet Visible (UV-vis) Spectrophotometer was used. Result showed that *Melia azedarach* (Chinaberry) has a yellow green color with a pleasant odor, miscible with ethanol and water, immiscible with benzene, lower boiling point than water, less dense than water and a pH which is moderately acidic. Furthermore, a positive result showed in phytochemical screening for alkaloids, phenols, tannin, saponin and phenolic compound, which implies that the plant sample can be used as antioxidant, analgesic, and anti-inflammatory. Subsequently, FTIR and UV-vis analysis showed a positive result for the determination of functional group and bioactive potential among secondary metabolites which means that it is possible for the application in developing therapeutic medicines and in developing natural remedies. Therefore, the findings of this study will benefits the whole scientific community since it would provide added information and evidences as possible application for the development of pharmaceutical medicine. Furthermore, this study is beneficial to the whole scientific community since it would provide added information about the characterization of bioactive potential present in these leaf extract. It also, provides alternative treatments for common health issues particularly in communities that are in far-flung areas with limited access to modern healthcare. Overall, this compound simplifies treatments, may lower healthcare costs, and significantly improves quality of life.

Keywords: chinaberry, medicinal plants, *Melia azedarach*, secondary metabolites

I. INTRODUCTION

In a world where modern medicine overshadowed traditional remedies it led to a loss of understanding and appreciation for our cultural and scientific heritage. Traditional remedies is abundant in the environments as a natural source for bioactive compounds with medicinal potential offering safer, cheaper, abundant and often more effective options compared to modern medicines. In addition, *Melia azedarach* is a deciduous tree that is derived from Greek word; *Melia* which means “a flowering ash or manna ash” and also means as a “poisonous tree” which belongs in the family of *Meliaceae* and in English as Chinaberry.

Biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. These phytochemicals are used as sources of direct medicinal agents. They served as a raw material base for elaboration of more complex semi-synthetic chemical compounds (Malhotra, 2015). In fact, the whole plant parts (leaves, stem, barks and roots) are known to have a medicinal property and a well-documented medical plant (Awadh *et al.*, 2001) and have been used by the indigenous and tribal people in India in a long period of time, and used in ayurvedic medicine and Unani medicine in India and Arab countries as an antioxidant, analgesic, anti-inflammatory, insecticidal, rodenticidal, antidiarrheal, antidiabetic, antirheumatic, antihypertensive (Deepika *et al.*, 2013).

Furthermore, leaves are naturally known to help with both internal and external diseases such as anemia, jaundice, eczema, measles, skin diseases, scabies and diabetes and have a potential as anticancer agent (Ervina *et al.*, 2021). In addition, the bark is used as a diuretic, deobstruent, and anti-diarrhea (Shekhavat *et al.*, 2014). According to the study of (Deepika *et al.* 2013) bark decoction is used in fever to relieve nausea, vomiting, thirst, loss of appetite and stomachaches. Meanwhile, roots contain limonoids, terpenoids, flavonoids, and phenolic compound which mainly serve as antioxidant activity of the plant (Aoudia *et al.*, 2012) meanwhile; root bark is used as an anti-malarial before the discovery of quinine (Asnake *et al.*, 2016). Furthermore, the fruit of *Melia azedarach* tree tastes sweet but is poisonous. Although it is poisonous, it is used in making a tonic that acts as a strong laxative and also used to treat leprosy and scrofula. In fact, when the fruit is dried, it is effective against parasites and is considered a helpful herbal treatment for diabetes.

II. METHODOLOGY

Melia azedarach (Chinaberry) leaf was collected at Barangay Rawis, Laoang, Northern Samar. Collection and extraction of plant sample, determination of physical properties, phytochemical test, and FTIR analysis were conducted at the Bio-Physical Laboratory Complex, College of Science while the UV-vis analysis was done at the Technology Innovation Center both situated at the University of Eastern Philippines, University Town, Northern Samar.

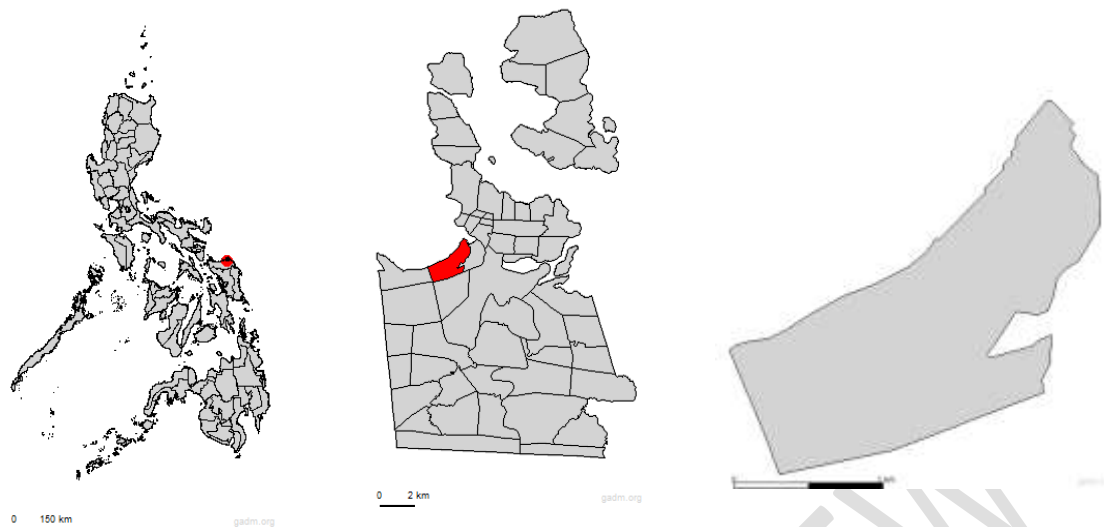


Figure 1. Map of Barangay Rawis, Laoang, Northern Samar

Plant collection and preparation

Young leaves of *Melia azedarach* (Chinaberry) were collected at Barangay Rawis, Laoang, Northern Samar. The collected plant sample leaves were placed in a plastic bag and was transported to the Chemistry Laboratory at University of Eastern Philippines, University Town, Northern Samar where the test was conducted.



Figure 2. Image of *Melia azedarach* (Chinaberry)

Preparation of plant sample

Young leaves of *Melia azedarach* (China berry) were collected, washed, and air dried for a day. After air drying, the leaf samples were dehydrated and blended into fine powder form. A mass of powdered leaf extract were macerated, and soaked in a 95% technical grade ethanol for 3 days. The regulating suspension are filtered, and suspended for simple distillation. Then the leaf extract is incubated using 60°C temperature for a period of time in order to remove the remaining alcohol present in the sample. The leaf extract of *Melia azedarach* (Chinaberry) was collected and refrigerated until use.

Determination of physical properties of *Melia azedarach* (Chinaberry) leaf extract

The methods from Espuelas *et al.* (2019) and Lim *et al.* (2020) were employed with modification. *Melia azedarach* (Chinaberry) leaf extract underwent physical properties in terms of color, odor, solubility, boiling point, density, and pH.

Test for Color

The colors of the extracts were assessed by evaluators using their sense of sight. Chinaberry leaf extract are placed in a transparent test tube. Then, using the sense of sight, the evaluators describe the color of the leaf extract.

Test for Odor

Odors were assessed by evaluators using their olfactory sense. Chinaberry leaf extract are placed in a clear odorless petri dish. Then, using the sense of smell, the evaluators can describe the odor of Chinaberry leaf extract.

Test for Solubility

Three (3) solvents were used, namely; water, ethanol and benzene. Then, each test tube was added with each solvent. The test tubes were observed to determine the solubility of the samples. The results were recorded as miscible or immiscible. Three trials were done for solubility determination.

Test for Boiling point

Chinaberry leaf extract are poured into a test tube. The test tube was placed on an oil bath and the temperature are recorded when the plant sample extract started to boil. The process is recorded. Three (3) trials are done.

Test for Density

The densities are obtained by dividing the mass of the extract by the volume of the extract. The extracts used in determination are poured into a previously weighed graduated cylinder. Then the volumes are recorded. Then it is weighed on the triple analytical balance. The mass of the

extract is obtained by subtracting the mass of the empty graduated cylinder from the mass of the cylinder with the plant extract.

Test for pH

The pH is determined by dipping the multi-parameter into a beaker containing the sample extract. The results of pH level are recorded. The process was repeated in triplicate.

Phytochemical Screening

To test for the phytochemical profile, methods from the previous study of Dagalea *et al.* (2022) and Dianito *et al.* (2022) were used. However, specific methodology for each test were also reported utilizing the result of the previous studies.

Alkaloid Test

Picric test and Mayers test were used to detect the presence of alkaloid on *Melia azedarach* (Chinaberry) leaf extract.

Picric test. By adding a picric solution into sample extract, an orange color was formed indicating the presence of alkaloids (Dahanayake, *et al.*, (2019).

Mayer's test. Few drops of Mayer's reagent were added to the sample extract. A yellowish or cream white precipitate was formed, indicating the presence of alkaloids (Raaman, 2006).

Saponin Test

Froth test was used for the detection of saponins in *Melia azedarach* leaf extract. For froth test, the extract of Chinaberry was diluted with distilled water then the diluted extract was transferred into the test tube and then shaken for about 15 minutes. The presence of stable froth indicates that saponins are found in the extract (Valenteros, 2023).

Confirmation Test for Saponin

The capillary test was used to determine the presence of saponin. If the level of the plant extract in the capillary tube were half than in the other tube containing water, the presence of saponin can be inferred. This was done by loading a capillary tube with the leaf extract by immersing the tube to a height of ten mm in the plant extract. Likewise, another capillary tube was loaded with distilled water. Then it was kept in a vertical position to allow the liquid inside to flow out freely. After sometime, the heights of the liquids in the two tubes were compared (Vicencio, 2021).

Test for Phenolic Compounds

Potassium dichromate test was used in determining the presence of phenolic compound. Chinaberry leaf extract was added with potassium dichromate solution. The formation of dark color indicates the presence of phenolic compounds (Kumar *et al.*, 2018)

Tannin test

Ferric chloride test was used for the determination of the presence of tannins on Chinaberry leaf extract. Sample extract was diluted with distilled water and added ferric

chloride solution. A transient greenish to black color indicated the presence of tannin (Auwal *et al.*, 2014).

Confirmation test for Tannins

Braymer's test was used to confirm the presence of tannins. Sample extract was added with distilled water and 10% ferric chloride solution. A blue-green color indicates the presence of tannins (Singh *et al.*, 2017).

Test for Flavonoids

Lead acetate test was used in determining the presence of flavonoids. Chinaberry leaf extract was treated with 10 % lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids (Go *et al.*, 2017).

Determination of Functional Groups using Fourier-Transform Infrared Spectroscopy

The characterization was carried out using Perkin Elmer Spectrum IR Spectrum 2 (Version 10.7.2). Sample extract of *Melia azedarach* (Chinaberry) were placed into the FTIR machine and analyzed for their functional groups.

Determination of Bioactive Potential of *Melia azedarach* (Chinaberry) Leaf Extract using Ultraviolet-Visible Spectrophotometer

Bioactive potential was carried out using Double Beam UV Visible Spectrophotometer model BSDBU-201-B. Beer Lambert's Law was used to determine the concentration of the sample which was directly proportional to the absorbance of the light. The Beer-Lambert law is expressed as: $A = \epsilon Lc$, where A , is the absorbance, ϵ is molar absorption coefficient $M^{-1}cm$, l is the optical path length, and c is the molar concentration M .

In determining the concentration of solution is expressed the formula as:

$$c = \frac{A}{(\epsilon)(l)}$$

III. RESULTS AND DISCUSSION

As shown in Table 1, the plant extract had a yellow green color, with a pleasant odor. Additionally, it has an average boiling point of $89.67^{\circ}C$, which had lower boiling point than water which means that the sample was more volatile than water. Boiling points of sample were essential for a wide range of scientific, industrial, and everyday applications. Moreover, sample extract has a density of 0.87 g/mL which was slightly less dense than water since water has a density of 0.99983895 g/mL . Furthermore, the pH of the plant extract was moderately acidic with its pH value of 5.81, which an important quantity that reflects the chemical conditions of a solution. This pH range is relevant in many natural processes, industrial applications, and biological systems, affecting chemical reactions, health, environmental conditions, and agricultural productivity. Understanding and managing pH within this range is essential for optimizing outcomes in these various contexts. Consequently, polar solvent are liquids that can dissolve polar compounds. It is because both polar solvents and polar compounds have dipole moments and have oppositely

charged moieties in the same chemical compound (Madhu, 2020) since the sample plant was miscible with distilled water, and ethanol which they are also polar solvents, while it was immiscible with benzene that is non-polar solvent. Solubility is one of the important rates limiting parameters to reach their desired concentration in complete circulation for pharmacological response. Poorly-water soluble drugs having slow drug absorption leads to insufficient and gastrointestinal mucosal toxicity and variable bioavailability (Bajait, *et al*, 2019).

Table 1. Summary of physical properties of *Melia azedarach* (Chinaberry) leaf extract

Physical Properties Parameters	Results
Color	Yellow green
Odor	Pleasant
Solubility	Polar
Boiling point	89.67°C
Density	0.87 g/mL
pH	5.81

Table 2 showed positive results for alkaloids, saponin, phenolic compound, tannin, and flavonoid. The formation of orange colored from the picric test and a presence of creamy white precipitate from Mayer's test on *Melia azedarach* extract indicated that there was a presence of alkaloid content on the sample. Additionally, alkaloids have a several pharmacological activities on human health such as anti-cancer, anti-inflammatory, Anti-malarial, Anti-microbial, Anti-hypertensive, Anti-diabetic, antioxidant (Rajput *et al.*, 2022). Furthermore, positive result of saponin was detected on *Melia azedarach* leaf extract using froth (honeycomb formation), and a confirmation test using the capillary tube test (extract had lower level than the distilled water). Saponin is good in decreasing blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning (Shi *et al.*, 2004). In addition to this, potassium dichromate test was used to determine the presence of phenolic compound on *Melia azedarach* leaf extract, the formation of black color indicated a positive result for phenolic compound. Phenolic compound has also demonstrated anti-inflammatory properties to treat skin diseases, rheumatoid arthritis, and inflammatory bowel disease. Plant extracts and phenolic compounds exert protective effects against oxidative stress and inflammation caused by airborne particulate matter, in addition to a range of anti-inflammatory, anticancer, anti-aging, antibacterial, and antiviral activities (Rahman. *et al*, 2021). Moreover, ferric chloride test was used to determine the presence of tannin in *Melia azedarach* leaf extract, the formation of greenish to black color indicated a positive result for tannin. For the confirmation test, braymer's test was used. This test indicates that *Melia azedarach* was positive for tannins. Tannins exert several pharmacological effects, including antidiabetic, wound healing, cardiovascular protection and antidiarrhoeics and used in applications for veterinary, food additives, biopesticides (Pizzi 2021). Flavonoids, is a class of polyphenol secondary metabolites, that are presented broadly in plants and diets. They have various bioactive effects including anti-viral, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer, anti-aging, etc. (Wang *et al.*, 2017).

Table 2. Summary of phytochemical screening of *Melia azedarach* (Chinaberry) leaf extract

Phytochemical tests	Observation		Results
Alkaloid	Picric test	Mayer's test	Positive
	Formation of Orange color	Formation of creamy white precipitate	
Saponin	Froth test		Positive
	Formation of honeycomb		
Confirmatory test for saponin	Less than the level of distilled water		
Phenolic compound	Formation of black color		Positive
Tannin	Formation of black color		Positive
	Formation of blue-green color		
Flavonoid	Formation of yellow precipitate		Positive
Confirmatory test for flavonoid	Formation of reddish black color		

As shown in the line graph for *Melia azedarach* (Chinaberry) leaf extract, it has a registered peak of 3289.60cm⁻¹, 89.93% T which corresponds to OH functional group. The functional group of C-H registered peak of 2923.61cm⁻¹, 82.33%T and 2852.96cm⁻¹, 86.71%T represented the presence of alkane. Carboxyl compound with a peak of 1744.39cm⁻¹, 86.97%T, and secondary amino compound with a peak of 1647.16 cm⁻¹,86.68%T. these functional groups present were quite similar to the functional group present on the study of Coates, (2000).

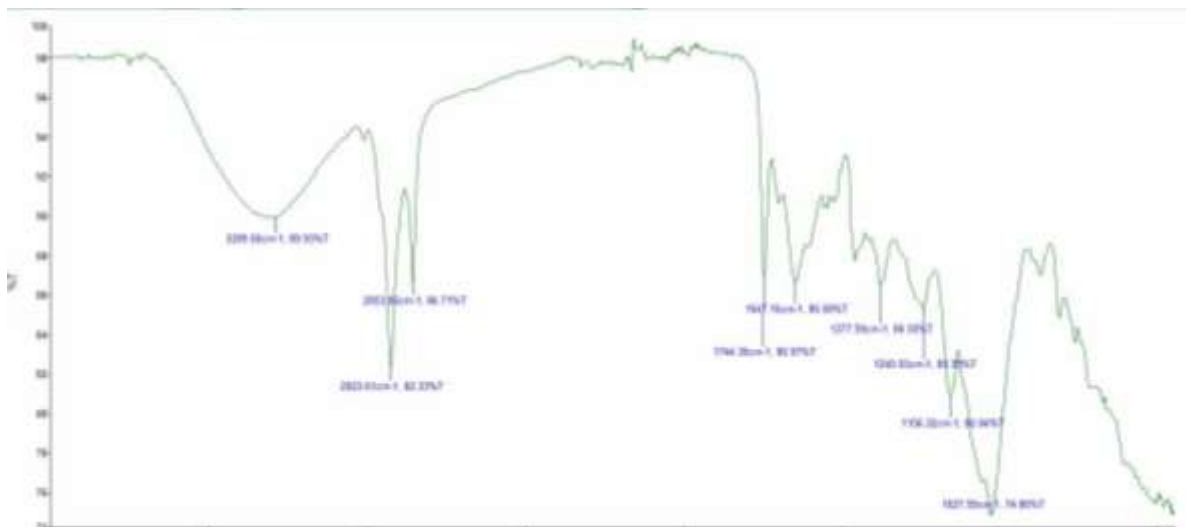


Figure 3. FTIR analysis of *Melia azedarach* (Chinaberry) leaf extract

Table 3. FTIR peak values of *Melia azedarach* (Chinaberry) leaf extract

Observed peaks (cm-1)	IR Assignment	
	Functional Group	Range (cm-1)
3289.60 cm-1	O-H stretching (Alcohol)	3200-3550
2923.61 cm-1	C-H (Alkane)	2840-3000
2852.96 cm-1	C-H (Alkane)	2840-3000
1744.39 cm-1	C=O stretching (Aldehyde)	1720-1740
1647.16 cm-1	C=C stretching(conjugated alkene)	1600-1650

The absorbance spectra of *Melia azedarach*(Chinaberry) leaf extract, has its highest wavelength of 375nm, 350nm, 319nm, 265nm, and 216.90nm, with absorption spectra of 1.134, 1.123, 1.106, 0.521, and 1.862. The concentrations using Beer-Lambert's Law revealed that there are 0.42 M, 0.74M, 0.67M and 0.76M for alkaloid, 1.81M for saponin, 9.24M, 2.09M, 3.72M, 3.34M and 3.78M for phenolic compound, 1.18 M and 1.33 M for tannin and lastly, 1.07M and 0.24M for flavonoids.

The UV-VIS profile of plant extract was analyzed at 200 to 600 nm wavelength to identify the compounds of *Melia azedarach* (Chinaberry) leaf extract. In the previous study, absorption bond occurs at 265nm, 319nm, 350nm, and 375nm has characteristics of an alkaloid. The peak

was identified as alkaloid due to its occurrence at 234nm-676nm (Patle T.K., *et al.*, 2020). The peak 350nm and 375nm were also identified as tannin due to its absorption occurrence of 350nm-500nm. Saponin due to the occurrence 203nm (Qi *et al.*, 2006), occurrence at 200nm-400nm characterize as phenolic compound and 210nm-280nm characterize as flavonoids Arshan *et al.*, (2020). In this case, all peaks occur in this range. This therefore suggests the presence of these secondary metabolites in the extract. This means that it has biological properties which play an important role in food, agriculture and pharmaceutical industries. They have also various bioactive effects including anti-viral, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer, anti-aging and others.

CONCLUSION

The findings of the study revealed that the extract of *Melia azedarach* (Chinaberry) was analyzed to determine its physical properties that are rich in composition and its bioactive potential can be used to treat various diseases and may help to produce medicine in pharmaceutical company. The functional groups that are present in this plant are alcohol, aldehydes, alkane and alkene. Furthermore, it has a various bioactive effects including anti-viral, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer, anti-aging which can recognize the presence of bioactive compounds and preventing diseases.

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