

Phytochemical and antibacterial study of *Lagerstroemia speciosa* (L.) Pers. and its ethnomedicinal importance to indigenous communities of Benguet Province, Philippines

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The methanolic extract of *Lagerstroemia speciosa* (L.) Pers. leaves, locally known as *Banaba*, collected from Sablan, Benguet Province, Philippines was subjected to phytochemical analysis for secondary metabolites and antibacterial screening against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using a modified Kirby-Bauer method. Phytochemical analysis revealed the presence of anthraquinones, flavonoids, saponins, and tannins. The extract exhibited high antibacterial activity against three of the bacteria in order of sensitivity as *Escherichia coli* > *Staphylococcus aureus* > *Pseudomonas aeruginosa*, but had no activity against *Salmonella typhimurium*. The medicinal value of this plant could be attributed to the presence of one or more of the detected metabolites. Antibacterial activity possibly shows a basis for traditional use of the plant as a local health remedy to the indigenous communities of Benguet Province, Philippines.

Keywords: *Lagerstroemia speciosa* (L.) Pers., Antibacterial, Phytochemistry, *Banaba*, Indigenous use

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The bane that diseases bring to mankind has fueled the incessant quest for effective, yet affordable, therapeutic substances, leading even the most sophisticated drug companies to continue exploring the possibilities that the plant kingdom can offer. Throughout history, plant materials have served as a reservoir of potential new drugs. Yet, only a small portion of the approximately 270,000 known plants thus far have been investigated for medicinal activity¹. Herbal knowledge from local indigenous communities has long been the basis for investigating the further potential of plants as therapeutic agents. From supplements to curative agents, many plants and their constituents have made it to commercial markets, some gaining widespread recognition by both the public and by scientific organizations for their safety and effectiveness as drugs.

Many diseases, particularly those caused by pathogenic microbes, have been successfully treated to this day with a variety of available antibacterial drugs. However, prices of drugs also are increasing, and the search for alternative medicinal sources still persists. Even with an ever increasing number of synthetic drugs, it is still impossible to disregard the contributions of plants to modern medicine. Studies on plant remedies not only serve to make the public more conscious regarding sources of alternative medicines, but also serve to open more avenues for future drug discovery. The objectives of this study were: (1) To review the medicinal uses of the *banaba* plant in the province of Benguet; (2) To establish its antibacterial property; and (3) To determine the secondary metabolites present through phytochemical screening methods, presenting the possible constituents responsible for its medicinal action.

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The *L. speciosa* tree, locally known in the Philippines as *banaba*, is found in various areas in the country such as in Northern Luzon to Palawan, Mindanao, and the Sulu Archipelago, in most or all islands and provinces, and chiefly in secondary forests². Being found naturally in the Philippine Cordillera Region, it serves as an accessible remedy to various diseases experienced by the locals of Benguet.

Methodology

Banaba leaves were collected from Bayabas, Sablan in Benguet Province, Philippines. These leaves were subjected to extraction, then to phytochemical screening and antibacterial testing.

Preparation of extracts

The *banaba* leaves were washed, dried, then comminuted. Twenty five gm was weighed, then subjected to maceration for 24 hrs in 250 ml of methanol. The liquid portion was collected then subjected to slow evaporation in a water bath in temperatures not exceeding 40⁰ C.

Phytochemical screening

Phytochemical screening method was carried out following the method set by Guevara, Aguinaldo, Espeso and Nonato³. Metabolites such as sugars, glycosides, tannins and proteins were found to be present.

Antibacterial screening

One of the standard assay methods for testing antimicrobial activity is the Kirby-Bauer method, also referred to as the disc diffusion method. A culture medium is prepared in the assay plates and subsequently streaked uniformly with the test microorganisms.

A modified Kirby-Bauer technique was used to screen for the *banaba* extract's antibacterial activity. Four microbes, namely, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Escherichia coli*, were used to test the antibacterial activity. All bacteria used in the study were acquired from the University of the Philippines Diliman Natural Science Research Institute.

Preparation of the inoculum

To prepare the bacterial inoculums from each of the microorganisms, a loopful of each test organism was taken from each respective agar slant and

subsequently sub-cultured into test-tubes containing nutrient agar broth. The tubes were then subjected to incubation for 24 hrs at 37⁰ C, after which, the obtained broth with microorganisms was standardized based on a 0.5 MacFarland turbidity standard by dilution with normal saline solution. This was done for all of the tubes to have a uniform population density of microorganisms.

Preparation of culture media and Assay plates

Two preparations of Mueller-Hinton agar differing in concentration were employed for the modified Kirby-Bauer technique utilized in the study. The first preparation uses the original concentration of Mueller-Hinton agar found on its label. This is referred to as the "base agar." The second preparation of Mueller-Hinton uses only half of the original concentration of agar dissolved in water. This is referred to as "soft agar," to which was added the prepared bacterial inoculum.

To prepare the plates for antibacterial screening, base agar is first poured onto the plates then allowed to solidify. Afterward, the soft agar containing the bacterial inoculum was poured over the solidified base agar and also allowed to congeal.

Sterile filter paper disks impregnated with the *banaba* extract were then placed on the prepared culture medium. The plates were then incubated at 37⁰ C for 24 hrs.

Separate plates were also prepared in the same manner for the negative control (methanol) and the positive controls (Kanamycin, Chloramphenicol, Streptomycin, Vancomycin).

Observation of the Zone of Inhibition

After 24 hrs, the zones of inhibition of the *banaba* extract, positive and negative controls were measured using a transparent ruler. Antibacterial screening was reproduced in triplicates.

Results and discussion

Ethnomedical importance of *Banaba* in Benguet Province

In recent times, ethnomedical and traditional pharmacological approaches are achieving great appreciation in modern medicine, because the search for new potential medicinal plants is often based on an ethnomedicinal origin⁴. Knowledge of the plants used medicinally by indigenous people could lead to further research and new drug discoveries.

Banaba grows widely in the Cordillera region and in other parts of Northern Luzon and Mindanao. Its various parts, from its leaves to its fruits, have been used by indigenous people for various medicinal purposes.

Table 1 presents various uses for the different parts of the *L. speciosa* plant. The plant is popular as a febrifuge and cleansing agent. Its leaves are boiled and taken as tea, which is said to detoxify and remove impurities from the body. Many studies have established its anti-diabetic property⁵⁻⁸, presenting data that the *banaba* plant possesses an “insulin-like” principle⁹, specified in some studies as corosolic acid¹⁰. Among its previously reported medicinal properties are its anti-oxidant¹¹⁻¹², antihypertension¹³ and anti-inflammatory¹³⁻¹⁴ features.

It can be noted that the plant is used by the community for treating urinary tract infection, a condition commonly brought about by bacteria, and is also used to relieve signs and symptoms of infection such as fever. This led the researchers to investigate its potential antimicrobial property against four common pathogens.

Table 1—Ethnomedical uses of *L. speciosa*

Plant parts	Uses
Leaves	Cleansing agent, febrifuge (Relief of fever), diuretic, treatment for urinary tract infections, treatment for diabetes
Bark	Stimulant, febrifuge (Relief of fever), Anti-diarrheal agent
Fruits (mature, dried)	Treatment for diabetes
Roots	Treatment of mouth ulcers

Phytochemical constituents

Phytochemical screening for plant constituents was done using the method described by Aguinaldo *et al.*³. Table 2 shows that a number of both primary and secondary metabolites are present in the crude methanolic extract of *L. speciosa* leaves. Primary metabolites such as glycosides and tannins exist abundantly in the leaves, while sugars and proteins can also be found but in lesser concentrations. Secondary metabolites that were found to be present were steroids, anthraquinone glycosides, flavonoids, saponins, and tannins. Several of these constituents may possibly be responsible for the plant’s antimicrobial activity. Flavonoids, for instance, have been of interest to the scientific community because of recent reports on their antiviral, antifungal, anti-inflammatory and cytotoxic properties³. Recent reports have also shown that tannins have potential value as cytotoxic and antineoplastic agents,³ and some have shown antibacterial and anti-HIV property.¹⁵

Antibacterial activity

Table 3 shows the results for the Antimicrobial Susceptibility Test for *L. speciosa* leaf extract, presenting the zones of inhibition of the *L. speciosa* leaf extract against the various species of test organisms. It can be seen that the extract exhibits the most activity against *E. coli*, and the least activity against *S. typhimurium*. Table 3 also shows the resulting zones of inhibition using the positive and negative controls. It can be seen that the values for *E. coli*, *S. aureus* and *P. aeruginosa* are higher than the negative control, thus indicating an antimicrobial activity against these three bacterial species. All of the

Table 2—Phytochemical screening results of *L. speciosa* leaves

Phytochemical constituent	Test performed	Presence in <i>L. speciosa</i> leaves
<i>Primary metabolites</i>		
Glycosides	Lead acetate test	++
Sugars	Fehling’s test	+
Tannins	Ferric chloride test	++
Proteins	Millon’s test	+
<i>Secondary metabolites</i>		
Alkaloids	Mayer’s test, Dragendorff’s test	-
Steroids	Keller-Killiani test, Lieberman-Burchard test	+
1. Anthraquinone glycosides	Borntrager’s test	+
Flavonoids	Bate-Smith-Metcalf test, Wilstater Cyanidin	+
Saponins	Froth test	+
Tannins	Gelatin test, Ferric chloride test	+
2. Cyanogenic glycosides	Guignard test	-

Table 3—Zone of Inhibition (mean in mm) of *L. speciosa* leaf extract and Positive and Negative controls

	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>
Methanol	7	7	7	7
<i>L. speciosa</i> extract	15	10	10	7
Kanamycin	14	16	12	13
Streptomycin	16	14	15	18
Vancomycin	10	9	8	8
Chloramphenicol	18	24	15	22

Table 4—Interpretation of Results for a 6mm diameter paper disc³

Zone of Inhibition	Interpretation
<10 mm	Inactive
10-13 mm	Partially active
14-19 mm	Active
>19 mm	Very active

positive controls had effects against the four species, except for Vancomycin, with the highest value being only 10, expressed as partially active, as interpreted from Table 4 which shows the basis of interpretation of the gathered data. The plant extract's zone of inhibition for *E. coli* is comparable with that of Kanamycin and Streptomycin, having values of 14 and 16, respectively, which are also interpreted as active. Based on Table 4, it can be concluded that the extract is active against *E. coli*, partially active against *S. aureus* and *P. aeruginosa*, and inactive against *S. typhimurium*.

Considering several other plants used medicinally by communities in Sablan according to studies by Balangcod, *et al.*¹⁶, *L. speciosa* leaf extract is comparable in its antimicrobial properties against various bacteria tested. *Livistona rotundifolia*, for instance, has a similar degree of antimicrobial activity against *S. aureus*; while *Ageratum conyzoides* L. and *Bidens pilosa* L. have the same strength as *L. speciosa* against *P. aeruginosa*. Compared to *Psidium guajava* L., however, *L. speciosa* is relatively stronger against *E. coli*, but weaker against *S. aureus* and *P. aeruginosa*.

Conclusion and recommendations

There are numerous alternative sources of medications nowadays, and plants, which have been used throughout history, are becoming more and more popular with millions of people worldwide. This study has shown that the use of *L. speciosa* by local communities from Benguet, Philippines, has a valid scientific basis. Its antibacterial property against certain microbes presents a potential for treating

various infectious diseases, especially with the presence of various secondary metabolites like anthraquinones, flavonoids and tannins. Further study of the pharmacologically active constituents of the *L. speciosa* plant and its parts is needed to further establish its use for treatment of other conditions and to determine and isolate the specific compound responsible for such pharmacologic activity.

In conclusion, the use of *L. speciosa* by indigenous communities of Benguet, especially against bacterial conditions like urinary tract infections (UTI) and the relief of fever, is established by the leaf extract's inhibition of bacteria such as *E. coli* that may cause several infections.

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