



## Phytochemical and Microbiological Testing of Makahiya (*Mimosapudica* Linn.) Leaf Extract

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### Abstract

*This study was conceptualized to identify the chemical substances present in Makahiya and test its microbiological potentials. It is with hope that this study may provide bench mark information for further studies of makahiya as alternative medicine.*

*This study primarily aimed to analyze phytochemically and microbiologically the leaves of Makahiya plant (*Mimosapudica* Linn).*

*Results of the phytochemical screening indicate that makahiya leaves contained flavonoids as shown by the formation of magenta red solution, triterpenes as indicated by the presence of red color in solution in Liebermann-Burchard Test and glycosides manifested by the formation of brick red precipitate when the extract was subjected to Fehling's Test.*

*The Kirby-Bauer Disk Diffusion Method showed that makahiya leaf extract possesses a very strong antimicrobial activity against *Staphylococcus aureus* on and *Bacillus subtilis*. However, the makahiya leaf extract showed negative inhibitions activity against *Candida albicans*.*

*It was also found out that a significant difference exists between and among the zones of inhibition of the three test organisms subjected to makahiya leaf extract.*

*It is therefore recommended that phytochemical substances present in makahiya leaves be isolated and purified to obtain their maximum therapeutic potentials; the sensitivity of other bacterial and fungal strains to the plants must be tested and pharmacological assay on the plant should be done.*

## Introduction

### Background of the Study

Herbal remedies have been used for decades and centuries. Before the discovery and availability of modern synthetic drugs, humans were completely dependent on medicinal herbs for prevention and treatment of diseases. In the history of ancient civilizations, the use of medicinal herbs for curing diseases has been documented. The drugs were used in crude forms like decoction, infusion, tincture and poultice.

People who are below the poverty line and who cannot afford the high cost of commercial synthetic medicines from drugstores opt to the use of local *plants* within their reach. These plants can be found around their house yards, rice fields, gardens, roadsides and rivers. These can be obtained readily, cheaply and entail easy preparation and application.

A common plant, makahiya, scientifically known as *Mimosa pudica* Linn. is the subject of this study. It is locally known as *bain-bain* in Iloko, and *makahiya* in Tagalog. Makahiya is very common and abundant in open waste places throughout the Philippines. The stem is erect in young plants, but becomes creeping or trailing with age. The stem is slender, branching, and sparsely to densely prickly, growing to a length of 1.5 m ( 95 ft.). The leaves are bipinnately compound, with one or two pinnae pairs, and 10-26 leaflets per pinna. The petioles are also prickly. Pedunculate (stalked) pale pink or purple flower heads arise from the leaf axils. The globose to ovoid heads are 8-10 mm in diameter (excluding the stamens). On close examination, it is seen that the floret petals are red in their upper part and the filaments are pink to lavender. The fruit consists of clusters of 2-8 pods from 1-2 cm long each, these prickly on the margins. The pods break into 2-5 segments and contain pale brown seeds some 2.5 mm long. The flowers are pollinated by the wind and insects. This plant is common in the researcher's area and local folks used the decoction of the plant against dysentery and dysmenorrhea and also as anti-asthmatic. The leaves, made into paste are also applied to swells. Hence, this study was conceptualized to identify the chemical substances present in makahiya and test its microbiological potentials. It is with hope that this study may provide bench mark information for further studies of makahiya as alternative medicine.

## Objectives

This study primarily aimed to analyze phytochemically and microbiologically the leaves of Makahiya plant (*Mimosa pudica* Linn). Specifically, it sought to determine the following:

1. What are the phytochemical substances present in the leaves of Makahiya?
2. What is the anti-microbial activity of makahiya (*Mimosa pudica* Linn) leaf extract in terms of the diameters of growth inhibition of the following test organisms, namely:
  - a) *Staphylococcus aureus*,
  - b) *Bacillus subtilis*, and
  - c) *Candida albicans*? and
3. Is there a significant difference between and among the diameters of growth inhibition of the above-mentioned organisms subjected to Makahiya (*Mimosa pudica* Linn) leaf extract?

## Scope and Delimitation

The study was delimited to the identification of active constituents (phytochemical) and microbiological screening of the leaves of makahiya using ethyl alcohol as the extractant. Comparison of the microbiological screening of the leaf extract with standard antibiotics was not conducted due to the unavailability of the specific antibiotics to be used.

The microbiological screening focused on the effectiveness of the extract in inhibiting the growth of certain strains of bacteria and yeast. Pure cultures of the bacteria and yeast were used.

The extraction process, confirmatory tests and microbiological screening were conducted at the Science Laboratory of the University of Northern Philippines, Vigan City, Ilocos Sur from January to February 2008.

## Review of Related Literature

At the Philippine Council for Health Research and Development, the agency that coordinates and helps fund studies in alternative medicines among many others, Executive Director Jaime Montoya has a list of eight new on-going research projects. Four involve plants on the old ten lists (*sambong*, *ulasimang bato*, *tsayang gubat* and *bayabas*) but for new uses. The other four are the golden shower tree (*Kanyafistula*), *saluyot*, *makahiya* and *guyabano*. (Inquirer.net, Philippine News for Filipinos).

Extracts of the makahiya plant were found to be a moderate diuretic, depress duodenal contractions (similar to atrophine), promotes nerve regeneration and reduce menorrhagia. It was also shown to have antidepressant activity. The strong emetic effects of extracts attributed to mimosine (<http://www.stuartxchange.com/Makahiya.html>).

This study is related to the one conducted by Pefta (1999) entitled "Microbiological and Phytochemical Screening of Selected Medicinal Plants Belonging to Families Leguminosae, Euphorbiaceae and Compositae" from 1997-1999. The study was carried out against the following test organisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Trichophyton mentagrophytes*. Four plants from each family were investigated. She found out that of the twelve plants tested, seven (7) were found to exhibit moderate to strong antimicrobial activity at doses of the alcoholic extract ranging from 100 to 2,500 mg/ml. It was also concluded that plant acids, tannins, reducing substances and flavanoids were present in the seven (7) potential sources of antibiotics. [http://opinion.inquirer.net/inquirer\\_opinion/talk\\_of\\_the\\_tow/viewarticle.php?article\\_id=93006](http://opinion.inquirer.net/inquirer_opinion/talk_of_the_tow/viewarticle.php?article_id=93006).

## **Methodology**

Only the leaves of *Mimosa pudica* Linn. were used in the study. The leaves were gathered in Lussoc, Sto. Domingo, Ilocos Sur.

Two hundred (200) grams of fresh plant material was extracted with ethanol and concentrated to 20 ml for the phytochemical and antimicrobial screening.

### **Phytochemical Analysis**

The *Mimosa pudica* Linn. extract was subjected to phytochemical screening to determine the presence of phytochemicals following the standard procedures from the Chemistry and Pharmacological Division, Department of Science and Technology, Bicutan, Taguig City, Metro Manila.

### **Screening for Antimicrobial Activity**

The Kirby-Bauer Disk Diffusion Method was used for this specific part.

For antimicrobial screening, the following micro-organisms were used: *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. These were obtained from the culture collection of Natural Science Research Institute (NSRI), University of the Philippines, Diliman, Quezon City.

The procedures used were adapted from the Manual on Extraction Procedures and Microbial Assay of Medicinal Plants by Capal et al (1998) and the Guide book to Plant screening: Phytochemical and Biological by Guevarra (2005).

The Mueller Hinton Agar and Sabouraud Glucose Agar were used as medium for the bacteria and yeast. The agar plates were seeded with inoculum by multiple streaking using sterile cotton swabs. The plates were incubated at 27 °C for the yeast and 35-37 °C for the bacteria for 18-24 hours, after which the diameter of the zone of inhibition were measured by using vernier caliper. The following interpretative range of standard zone was adopted from Ongtengco (1992).

<b>Zone of Inhibition</b>	<b>Inhibitory activity</b>
>17	+++ , strong
12– 16	++ , moderate
7--15	+ , weak
6 or 0	negative

The antimicrobial activity testing was done in three replications.

## Results and Discussion

### Phytochemical Analysis

Results of the phytochemical screening of makahiya leaf extract as shown in Table 1 indicates that makahiya leaves contained a trace of alkaloids since slight opaqueness was produced. Flavonoids are also present as shown by the formation of magenta red solution. The extract also yielded positive with triterpenes as indicated by the presence of red color in solution in Liebermann-Burchard Test and positive with glycosides as manifested by the formation of brick red precipitate when the extract was subjected to Fehling's Test

The presence of these phytochemicals in makahiya leaf extract is an indication that makahiya has curative effects and therefore can be used as alternative medicine.

Table 1. Phytochemical Analysis of Makahiya Leaf Extract

Plant Constituent	Chemical Test	Result	Descriptive Result
Alkaloids	Mayer's test	+	Formation of slight opaqueness
Flavanoids	Bate-smith and Metcalf test	+	Formation of magenta red
Glycosides	Fehling's test	+	Formation of brick red precipitate
Saponins	Froth test	+	Formation of froth
Tanins	Ferric chloride test		No blue black and brownish-green color
Sterols	Liebennann Burchard test		No formation of blue color
<i>Triterpenes</i>	Liebennann Burchard test	+	Formation of red color

### Antimicrobial Activity

Using the Kirby Bauer *Disk* Diffusion Method, Table 2 shows that makahiya leaf extract possesses a strong antimicrobial activity against *Staphylococcus aureus* as shown by the 21.8 mm zone of inhibition and *Bacillus subtilis* with 23.5 mm zone of inhibition. However, the 6mm zone of inhibition manifested by the makahiya leaf extract indicated that it has negative inhibitory activity against *Candida albicans*.

It was also found out that a significant difference exists between and among the zones of inhibition of the three test organisms subjected to makahiya leaf extract.

Table 2. Antimicrobial Activity of Makahiya (*Mimosa pudica* Linn.) Leaf Extract Against Three Test Organisms

Test Organism	Replication	Trial	Diameter of Inhibition mm	Mean	Grand Mean	Antimicrobial Activity
<i>Staphylococcus aureus</i>	1	1	24	22.7	21.8	+++ strong
		2	22			
		3	22			
		C	10			
	2	1	19	21.7		
		2	22			
		3	24			
		C	8			
	3	1	20	21		
		2	21			
		3	22			
		C	8			
<i>Bacillus subtilis</i>	1	1	22	21	23.5	+++ strong
		2	20			
		3	21			
		C	6			
	2	1	24	25.7		
		2	28			
		3	25			
		C	7			
	3	1	24	23.7		
		2	23			
		3	24			
		C	6			
<i>Candida albicans</i>	1	1	7	6.3	6.1	Negative
		2	6			
		3	6			
		C	6			
	2	1	6	6		
		2	6			
		3	6			
		C	6			
	3	1	6	6		
		2	6			
		3	6			
		C	6			

**Legend:**

C=control (+++)=strong (++)=moderate (+)=weak



## Conclusion

1. Makahiya leaves contain a trace of alkaloids, flavonoids, saponins, triterpenes and glycosides.
2. Makahiya leaf extract possesses a strong antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. However, it has negative inhibitory activity against *Candida albicans*.
3. A significant difference exists between and among the zones of inhibition of the three test organisms subjected to makahiya leaf extract.

## Recommendations

Based on the results of the study, the following recommendations are hereby advanced:

1. The phytochemical substances present in makahiya leaves should be isolated and purified to obtain their maximum therapeutic potentials.
2. The sensitivity of other bacterial strains to makahiya extract must be tested.
3. Other pharmacological effects such as antipyretic and hypoglycemic effects of makahiya leaf extract must be tested.

## References

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