INHIBITORY ACTIVITY OF MAKAHIYA (*Mimosa pudica Linn*) LEAF EXTRACT TO THREE TEST ORGANISMS

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ABSTRACT: With the reported phytochemical analysis of Mimosa pudica Linn, the study determined its leaf extracts' inhibitory activity to three test organisms through methods of extraction and microbial test. The leave of Mimosa pudica were collected from Lussoc Ilocos Sur and was evaluated against three multi drug resistant human pathogens .The antimicrobial activities are important diagnostic laboratories to analyse the resistance of microorganisms to an antimicrobial agent. This study is in support of the Philippine Department of Health's continuous research for traditional alternative herbal medicine. The leaf extract of Mimosa pudica strongly inhibited the growth of Bacillus subtilis and Staphylococcus aureus The researcher recommends the establishing of Mimosa pudica as traditional, alternative medicine for diseases/ illnesses caused by the above mentioned bacteria. Hence the MIC of the secondary metabolites as well as the isolation and characterization of the primary metabolites of the plant should also be done as it has yielded a promising potential as an antimicrobial.

Keywords: Mimosa pudica Linn, Makahiya, Inhibitory Activity Test/Antimicrobial Test, Traditional/Alternative Medicine

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1.1 INTRODUCTION

Historically, the use of herbal medicine has gone a long way. In today's world, there are emerging national and global scenarios with input on medicinal plants. The ASEAN and BIMSTEC countries have a strong bondage to review the status of medicinal plants to be used sustainably for supporting livelihood and conserve prosperity and perpetuity. (International Conclave on Medicinal Plants for ASEAN and BIMSTEC Countries, 2008).

In the Philippines, the approval of Republic Act 8423, otherwise known as Traditional Alternative Medicine Act (TAMA) of 1997 is so timely. This act mandates the creation of Alternative Health Care Development to promote and advocate the use of traditional, alternative, preventive, curative health care proven safe, effective and cost-effective and consistent with government standard on medical practices.

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoid which

have been found in vitro to have microbial properties (M. Cowan, year).

A common plant, makahiya, scientifically known as *Mimosa pudica* is the subject of the study. It is widely known as Bain-bain in Iloko and Makahiya in Tagalog. Makahiya is very common and abundant in open waste places throughout the Philippines. This sensitive plant, *Mimosa pudica Linn,* is a creeping or perennial herb that often grows in any kind of soil. At present, it is now being propagated for its unique feature that is its sensitivity. The compound leaves fold inward and droop when touched, re-opening within minutes. Other names given to this curious plant are Humble planted, Shame plant, Sleeping grass, Prayer plant, Touch Me Not and makahiya (Dr. Godofredo Umali Stuart).

In as much *as Mimosa pudica Linn* has been reported to have a proactive constituents like alkaloids, glycosides, flavonoids and tannins and that no reported of its antimicrobial property (N. Gandhiraja, et al, 2009). This study, then focuses on such. With the reported phytochemical substances present in makahiya,, this study determines the leaf extract's on antimicrobial activity to three test organisms, namely, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans*, This study specifically looked into the extract's total mean zone of inhibition and percent inhibition activity . The knowledge of the extent and mode of inhibition of specific compound which are present in leaf extracts may contribute to the successful application of natural products s source of medicine.

1.2 FRAMEWORK OF THE STUDY

The Philippine Council for Health Research and Development, the Agency that coordinates and helps fund studies on alternative medicine among many others. Executive Director Jaime Montoya has a list of eight new on-going research projects, four involve plants in the old test list (Sambong Ulisimang beta, Tsaang-gubat and Bayabas, Golden Shower tree, Saluyot, Makahiya and Guyabano) (Inquirer.net.PhilippineNewsforFilipinos).

This study is related to the one conducted by Pena (1999), "Microbiological and Phytochemical Screening of Selected Medicinal Plants Belonging to Families Leguminosae Euphorbiacae and Compositae" (1997-1999). This study was carried against the following test organisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Tricophyton metagrophytes*. She showed

that out of the twelve plants tested seven (7) were found to exhibit moderate to strong antimicrobial activity at doses of the alcohol extract ranging from 100-2,500 µg/ml. It was concluded that plant acids, tannins reducing substance and flavonoids were present in plants, and as a potential source of antibiotics (http://opinion.inquirer.net/inquirer).Makahiya plant was analyzed and concluded that the plant contain active phytochemical components. The plant was also tested against *Aspergillus fumigants, Citrobacteria* and *Klebsiella pneumonia (*N. Gandihiraja et al, 2009*).*

While reports showed that the plant extracts showed wound healing activity, an effect attributable to phenol constituents. The plant extract showed to be moderate diuretic, and also showed to have antidepressant activity. Studies of the root extract of *M. pudica* showed infertility effect an elongation of the estros cycle, a disturbance of the secretion of gonadotropin hormones in albino mice (www.stuartxchange.org/makahiya.html).

Three test organisms, namely, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans* were used in this study to determine the antimicrobial activity of *M. pudica Linn* because of their medical importance most especially to their infectious characteristics. These were tested for their reaction in terms of growth and inhibition with the plant extract in most types of *Staphylococcus aureus* a Gram- positive, non- moving minute round shaped coccus that forms into the cluster. It is now considered as the most common cause of infections after injury or surgery (Mandal, 2012).

Candida albicans is often present in the skin, mouth, vagina, and intestinal tract of healthy persons and animals where it resides without cause diseases.

Bacillus subtilis is an endospore forming bacteria and the endospore it forms allows it to withstand extreme temperature as well a dry environment. Besides its many uses and applications, *Bacillus subtilis* has become the model agent in laboratory research of its very easy genetic manipulation.

There are many diseases caused by *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans* but medicines are limited also and there are no herbal medicine faced yet. This prompted the researcher to undertake a research study in the plant to establish the possibility of extracting its active constituent which may

serve as an alternative herbal medicine for many diseases.

1.3 MATERIALS AND METHODS

Only the leaves of *M. pudica* is used in the study. A kilogram of fresh leaves of *Mimosa pudica* was gathered from Santa Catalina, Ilocos Sur. After the leaves were washed with water and then shaken to remove excess water droplets, the leaf materials were cut into pieces. These were then placed in an Erlenmeyer flask and into which more than 300 ml sufficient absolute ethyl alcohol was added to completely submerge the materials. The flask was then stoppered and the materials were left to be soaked for 48 hours. After which it was filtered through a Buchner funnel with gentle suction. The filtrates were concentrated to about 20 ml. The extract was placed in a tightly stoppered container (properly labelled including the date of extraction) and then stored in the refrigerator (Adopted from the Guidebook to Plant Screening: Phytochemical and Biological by Guevarra, 2005).

The extraction procedures and microbiological testing were conducted at the Science Laboratory of the University of Northern Philippines, Vigan City, Ilocos Sur from August to September 2015.

For the determination of the inhibitory activity of the plant the Kirby-Bauer Disk Diffusion Method was used for the following microorganisms namely, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans.* These were obtained from the culture collection of the 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 and Biological by Guevarra (2005)

The Mueller Hinton Agar and Sabouraud Glucose Agar will be used as a medium for the bacteria and yeast. The agar plates will be seeded with inoculum by multiple streaking using sterile cotton swabs. The plates will be incubated at 27 C^0 for the yeast and 35-37 C^0 from the bacteria for 18-24 hours. Clear zones (diameters zone of growth inhibition) were measured were measured by vernier caliper. Interpretations of the activity on growth inhibition were done using the following. The following range of standard zone was adopted from Ongtengco, (1992).

Zone of Inhibition	Inhibitory Activity
>17	+++, strong
12-16	++, moderate
7-15	+, weak
6 or 0	-, negative

The antimicrobial activity testing will be in three replications.

% Inhibition= Gc- Ge / Gc * 100 where: Gc- growth of the control

Ge – growth of the extract

1.4 RESULTS AND DISCUSSIONS

The phytochemical screening had been conducted (Gandhiraja et al, 2009) recently and has been studied and viewed that *M. pudica* contains toxic phytochemical substances. Thus, there is an increasing demand of accurate knowledge of the antimicrobial testing of the plant. In as much as *M. pudica* has been reported to have bioactive components, *Mimosa pudia Linn*, invites the attention of the researcher for its pharmacological activities like antimicrobial activities against different organisms. In the present investigation, the antimicrobial activity of the plant extract was also tested against potentially pathogenic microorganism like *Bacillus subtilis, Staphylococcus aureus,* and *Candida albicans.*

Table 1 depicts the results of antimicrobial testing of the plant. The inhibitory activity of *M.pudica* refers to the action of its leaf extracts to interfere with the growth of the test organisms of this study.

Table 1.Inhibitory activity of *M.pudica Linn* in leaf extracts to three test organisms

Test Organism	Replication	Trial	Zone of Inhibition (mm)	Mean	Grand Mean	% Inhibition	Inhibitory Activity
		1	**24**				
	1	**2**	**22**	**22.7**			
		3	**22**		**21.8**	**4.61%**	**+ + +**
		C	**10**				**strong**
		1	**19**				
Staphylococcus aureus	**2**	**2**	**22**	**21.7**			
		3	**24**				

		C	**8**				
		1	**20**				
	3	**2**	**21**	**21**			
		3	**22**				
		C	**8**				
		1	**22**				
		2	**20**	**21**			
		3	**21**				
		C	**6**				
		1	**24**				
Bacillus subtilis	**2**	**2**	**28**	**25.7**			**+ + +**
		3	**25**		**23.7**	**4.5%**	**strong**
		C	**7**				
		1	**24**				
	3	**2**	**23**	**23.7**			
		3	**24**				
		C	**6**				
		1	**7**				
	1	**2**	**6**	**6.3**			**-**
		3	**6**				**Negative**
		C	**6**				
		1	**6**				
Candida albicans	**2**	**2**	**6**	**6**			
		3	**6**		**6.1**	**-1.96%**	
		C	**6**				
		1	**6**				
	3	**2**	**6**	**6**			
		3	**6**				
		C	**6**				

Legend: +++= strong (-)= negative

The leaf extract total mean zone inhibition of *Staphylococcus aureus* is 21.8 (4.61%) and those of *Bacillus subtilis* was 23.7 (9.56%) and 6.1 (-1.96%)for *Candida albicans.* The antimicrobial activity of *M. pudica* leaf extract which inhibits the growth of inhibition in each of the test organisms are interpreted as strong (+++) for *Staphylococcus aureus*, strong (+++) for *Bacillus subtilis* and negative (-) for *Candida albicans.*

Treatment of +++ or strong inhibitory activity of Makahiya to *Bacillus subtilis*, and *Staphylococcus aureus*, a (-) or negative inhibitory activity for *Candida albicans.*

.Table 2Summary of ANOVA on the Difference Among and Between the Diameters of Inhibition of the Three Test Organisms

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	*p-value*	*F-critical*
Between groups	1560.07407	2	7800.03703	88.6789474	$8.22177x10^{12}$	*3.402826105*
Within groups	211.111111	24	8.796296296			
Total	1771.18519	26				

Table 2B:

Summary of Scheffe Test on Diameters of Inhibition of *M.pudica* Leaf Extract

Between	F’	F critical	Interpretation
Staphylo and Bacillus	1.41	6.8	Not significant
Staphylo and Candida	7.43	6.8	Significant
Bacillus and Candida	8.84	6.8	Significant

As shown in the table , *M.pudica* has a strong antimicrobial activity with *Staphylococcus aureus* ($\bar{x} = 20.67$) *and Bacillus subtilis* ($\bar{x} = 23.44$) did not significantly differs. This implies that the inhibitory activity between the two organisms is more or less the same. On the other hand, *Staphylococcus aureus* ($\bar{x} = 20.67$) and *Candida albicans*($\bar{x} = 6.11$) significantly differed and *Bacillus subtilis* ($\bar{x} = 23.44$) and *Candida albicans*($\bar{x} = 6.11$) significantly differed. This suggests differences in the inhibitory effects of the said organism.

1.5 CONCLUSION

Mimosa pudica Linn leaf extract can inhibit the growth of *Staphylococcus aureus* and *Bacillus subtilis* . These findings strongly support the researcher's theory *that M.pudica* leaf extract has very high antimicrobial activity. The general data have provided the basis for its wide use as therapeutic both in traditional and folk medicine

1.6 RECOMMENDATIONS

Based on the results of the study, the researcher recommends that *M. pudica* leaf extract could be used as a traditional alternative herbal medicine for diseases/ ailments caused by *Staphylo coccus aureus and Bacillus subtillis*. To draw out the full potential of the plant extracts, the researcher further recommended for the MIC'S of the plant. A further study on the isolation and characterization of the plant's leaf should be conducted as these have a promising potentials as antimicrobials not *only to Staphylococcus* and *Bacillus subtilis* but also to *Pseudomonas aeruginosa* , a very hard to eradicate.

1.7 REFERENCES

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