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ANTIMICROBIAL PROPERTIES OF MIMOSA PUDICA EXTRACTS AGAINST SELECTIVE PATHOGENIC MICROBES

Jagessar R.*, C. Erskine K. and Oladimeji V.

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***Corresponding Author**

Dr. Jagessar R.

ABSTRACTS

The antimicrobial properties of *Mimosa pudica* against human pathogens: *E. coli*, *S. aureus*, C. *albicans* and V. *cholera* was investigated by the disc diffusion assay, using three different concentrations, under asceptic conditions. The highest AZOI of 132.7 mm^2 was induced by the ethanolic extract against *E.coli*. Negligible zone of inhibition was induced by the hexane extracts against *S .aureus*, *C. albicans*. The order of potency of extract, against the human pathogens was solvent dependent. For example, the ethanolic extract, induced an AZOI of 86.55 mm^2 against *S.aureus*, whereas the ethanolic extract at a concentration of 0.42g/ml, induced AZOI of 117.8 mm^2. The significant zone of inhibition induced by the ethanolic extract.

KEYWORDS: *Mimosa pudica*, human pathogens, AZOI, asceptic conditions.

INTRODUCTION

Research in the design and synthesis of antimicrobials will continue to be problematic on our planet, considering that bacteria and fungi develop resistance to antimicrobials over a period of time.[1-5] This results from indiscriminate use of commercial antimicrobial drugs for the treatment of infectious diseases and the current global antibiotic resistance.[1-5] Many synthetic drugs have several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavor.[1-5] Plants have a long therapeutic history over thousands of years and still considered to be promising source of medicine in the traditional health care system.[6] Plants also have a wide variety of secondary metabolites some of which are antimicrobial.[7-9] Crude plants extracts have also demonstrated antimicrobial activity.[10-12] Guyana flora is richly biodiversified and it's organic and aqueous extracts have been shown to possess potent and selective antimicrobial activity to date, compared with standard antibiotics: penicillin, nystatin and ampicillin.[13-17] This paper reports the antimicrobial properties of *Mimosa pudica* (*Fabaceae*), a leguminosea against *Escherichia. coli*, *Staphylococcus. aureus* and *Vibro. Cholera and Candida. albican*

Mimosa pudica, also called sensitive plant, sleepy plant, action plant, touch-me-not, shameplant. It is a creeping annual or perennial flowering plant of the pea/legume family *Fabaceae* and *Magnoliopsida* taxon, often grown for its curiosity value. It has compound leaves which fold inward and droop when touched or shaken, defending themselves from harm, and re-open a few minutes later. It is native to South and Central America, and can be found in Southern United States, South Asia, East Asia and South Africa as well. It is usually found on soils with low nutrient concentrations. *Mimosa pudica* is well known for its rapid plant movement. Like a number of other plant species, it undergoes changes in leaf orientation termed "sleep" or nyctinastic movement during darkness and reopens in light (seisomatic movement).[18-20]

It propagate from seed, seeds germinate between 14-21days at 70^0F. The *Mimosa pudica* has over 300 species that belongs to the bean pea family leguminosea.

Fig. 1.0: ***Mimosa pudica.***

Medicinally, the roots and leaves are commonly used as bitter, astringent, acrid, cooling vulnerary, alexipharmic, diuretic antispasmodic, emetic, constipating and febrifuge. The present study intends to investigate the phytochemical constituents and antimicrobial activity of the plant extracts of *Mimosa pudica* against pathogenic microbes.

There are few articles that report the antimicrobial properties of *Pudica mimosa*. Four articles were reviewed, using the Google Search Engine. In one article, the antibacterial activity of the water and ethanol extracts of *Mimosa pudica* was evaluated via the disc diffusion method. The total flavonoid as quercetin equivalent (QE) per gram (dry weight) of these two extracts was also estimated. The *in vitro* antibacterial activity indicates that the ethanol extract showed significant activity against *E.coli*, *S.aureus*, *B.subtilis* and *S.typhi* with the zone of inhibition: 11mm, 19 mm, 17mm and 16 mm respectively. The water extract only inhibited the growth of *S.aureus* (14mm) and *B. subtilis* (15mm) and there was no resistance against *E.coli* and *S.typhi*. The analysis of total flavonoid content found that the ethanol extract contains higher amount of flavonoid than water extract and flavonoid is responsible mainly for the antibacterial activity of *Mimosa pudica L.*[21]

The antimicrobial activity of ethanolic and aqueous extracts of *Mimosa pudica* thorns were evaluated against bacteria and fungi by agar well diffusion method. The microorganisms used were *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus* and *Candida albicans*. The plant extracts were taken in various concentrations of 25, 50, 75 and100 µg/well. The standards used for antibacterial and antifungal activity were

Streptomycin and Clotrimazole respectively. *E.coli* showed maximum zone of inhibition in both the ethanolic and aqueous thorns extracts of *Mimosa pudica* at higher concentrations. Amongst the two extracts, the results clearly indicate that the aqueous thorn extracts of *Mimosa pudica* exhibited highest zone of inhibition of 24.2 ± 0.34 mm against the bacteria *Escherichia coli* and 18.1 ± 0.17mm against the fungi *Candida albicans* at 100µg/well.[22]

Mimosa pudica herb has been found to have antiasthmatic, aphrodisiac, analgesic, anti-inflammatory and antidepressant activities. In one study, the active phytocomponents of *M. pudica* were revealed using phytochemical analysis. The antimicrobial activity was investigated using disk diffusion method. The activity was tested against *Straphyloccus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* at different concentrations of 25, 30 and 35 mg/ml. At 35mg/L, AZOI of 23.23 ±0.23 mg/L, 23.9 ±0.29 mg/L, 22.4 ±0.176 mg/L, 19.9 ±0.30 mg/L were obtained for *S. aureus*, *E.coli*, *P. aeruginosa* and *C. albicans* respectively.[23]

2.0 MATERIALS AND METHODOLOGY

➢ 2.1 Collection of plant

Plant materials were collected from the University of Guyana compound, Turkeyen, at the back of CSBD (Centre for the Study of Biological Diversity) and the Chemistry annex area. The perimeter of the collection location is approximately 3200yards. The plant materials were collected between the first to the fourth week in March. After the collection, plant materials were properly sun-dried. Following this, there was a reduction in the plant biomass via the removal of water. After plant was properly dried, the plant materials were milled using a grinding mill. The weight for the milled plant materials recorded 535.8g.

➢ 2.2 Solvent extraction

After milling, plant materials were transferred into a cylindrical jar, where solvent for the extraction was added. Three different solvent was used for the extraction process. These includes hexane, dichloromethane and ethanol. The extraction process is as follow, solvent were added to the plant materials, and then left for 48 to 72 hrs. After this period, the solvent was then filtered into an empty conical flask. Hexane and ethanol extractions were both carried out three times while dichloromethane was only done once. Hexane extraction was first carried out, followed by dichloromethane, then ethanol. All three solvent extractions were filtered into different conical flask.

After filtration, $NaSO_4$ salt was used to remove available water from the extracts. And finally the extract filtrate was removed in *vacuo*, using rotary evaporator to yield viscous oil. Following the completion of the evaporation process, specific concentrations of the extract were prepared and then extracts were ready for anti-microbial work.

Prior to the microbial test, extracts were stored in an open condition. A marker is placed on the extracts container to know the quantity of the content. Thus in some situation when the quantity is reduced via rotator evaporation, the respected solvent is added to refill the extract.

➢ 2.3 Anti-Microbial test

Four strains of microbes were used as test organisms. The susceptibility of these microbes were tested using the three previously mentioned plant solvent extracts. The bacteria includes: *Escherichia. coli, Staphylococcus. aureus* and *Vibro. Cholera and Candida. albican*. A new culture of each species were produced, from the starter culture. It is important to produce or propagate bacteria culture before any laboratory exercise.

➢ 2.4 Agar preparation

23g of nutrient agar was weighed and placed in a cylindrical jar, after which a liter of distilled water was then added to it. It was mixed well and dissolved by heat with frequent agitation. The nutrient agar was heated until its content became transparent. The pH of the nutrient agar was taken, its register 6.7 at 35.4 degree Celsius. Agar was then sterilized in an autoclave at 121 degree Celsius. The pH was again taken, its registered 6.93 at 32.5 degree Celsius. Agar was left to cool

➢ 2.5 Agar diffusion test

This test is use to determine the minimal inhibitory concentration of an antimicrobial agent on its test organism. After the process of sterilization (autoclave of Mueller Hinton agar) and the sub-culturing of the bacteria on a Petri dish, paper discs saturated with extracts and air dried were impregnated randomly on the well in the plate. A total of four disks were impregnated per plate. To finalize this test, the plates were inverted and place in the incubator for 24hr at 37^0C. At this stage the antimicrobial agent would be given much time to diffuse to the area of low concentration from the area of high concentration. Antibiotics disk was used for positive control. After the inoculation of the bacteria strain on the Mueller Hinton agar surface, a reference antibiotics was used as a positive control. A comparison assay will be conducted. The positive control, consists of a plate of solidifying agar, streak with the

respective microorganisms, to which were applied four discs soaked in distilled water. The entire antimicrobial experiment that involves inoculation of bacteria on the agar plates, and impregnation of extracts was all carried out in the laminar flow cabinet.

3.0 RESULTS

The antimicrobial activity was evaluated using the Disc Diffusion assay and results are tabulated below.

Table 1.0: Showing the antimicrobial activities of hexane, CH_2Cl_2 and ethanol extract of *Mimosa pudica* against *S. aureus.*

Extracts type & Weight	Concentration of Extracts g/ml	Diameter of inhibition (mm)	Mean average & SD (mm)	Area of diameter of inhibition (mm^2)
CH_2Cl_2	0.001	6	6	28.3
CH_2Cl_2	0.0614	6,7	6.5±0.7	31.7
C2H50H	0.06	7,14,8,9	9.5+3.1	70.85
C_2H_50H	0.142	13,9,10,10	10.5±1.7	86.55
C2H50H	0.42	8,11,11,10	10+1.4	78.5
C_6H_{14}	0.0011	7,7,13	9±3.5	63.6
C_6H_{14}	0.06	--------	-------	-------

Table 2.0: Showing the antimicrobial activities of hexane, CH_2Cl_2 and ethanol extract of *Mimosa pudica* against *E. coli.*

Extracts type & Weight	Concentration of Extracts (g/ml)	Diameter of inhibition	Mean average & SD (mm)	Area of diameter of inhibition, mm^2
CH_2Cl_2	0.001	--------	------	-------
CH_2Cl_2 (3.195g)	0.0614	7	7	38.5
C_2H_50H	0.06	13,8,6,9	9±2.9	63.6
C_2H_50H (35.4g)	0.142	15,13,7,12	11.74+3.4	108.2
C_2H_5OH	0.42	15,14,12,11	13+1.8	132.7
C_6H_{14}	0.0011	---------	--------	-------
C_6H_{14} (7.6116g)	0.06	6,7	6.5+0.7	33.2

Table 3.0: Showing the antimicrobial activities of hexane, CH_2Cl_2 and ethanol extract of *Mimosa pudica* against *C. albicans.*

Extracts type & weight	Concentration of extracts (g/ml)	Diameter of inhibition	Mean average & SD (mm)	Area of diameter of inhibition (mm^2)
CH_2Cl_2	0.001	7	7	38.5
CH_2Cl_2	0.0614	---------	------	------
C_2H_5OH	0.06	10, 14, 8, 8	10 + 2.8	78.5
C_2H_5OH	0.142	9, 13, 9, 7	9.5 + 2.5	70.85

C_2H_5OH	0.42	16, 11, 9.13	12.25 ± 3	117.8
C_6H_{14}	0.0011	-----	-----	-----
C_6H_{14}	0.06	-----	------	------

Table 4.0: Showing the antimicrobial activities of hexane, CH_2Cl_2 and ethanol extract of *Mimosa pudica* against *V. cholera.*

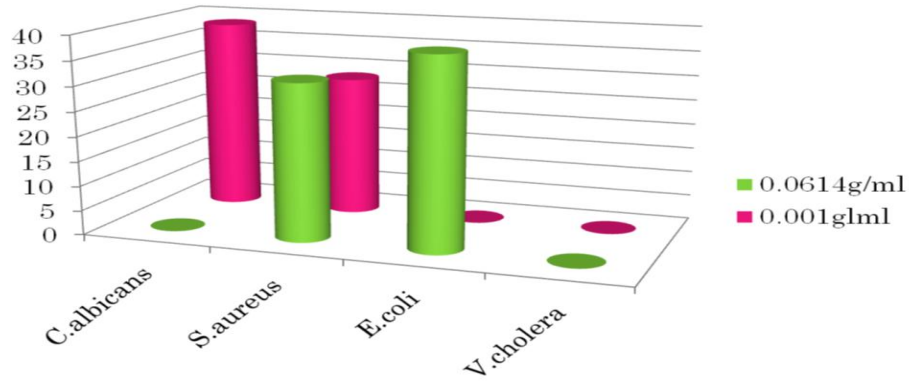
Extracts type & Weight	Concentration of Extracts (g/ml)	Diameter of inhibition (mm)	Mean average & SD (mm)	Area of diameter of inhibition (mm^2)
CH_2Cl_2	0.001	-------	------	-------
CH_2Cl_2	0.061	-------	------	-------
C_2H_50H	0.06	7,10,11,8	9 ± 1.8	63.6
C_2H_50H	0.14	12,7	9.5 ±3.5	70.85
C_2H_50H	0.42	6, 11	8.5 +3.5	56.72
C_6H_{14}	0.001	-------	-----	-----
C_6H_{14}	0.06	6, 6	6±0	28.3

Table 5.0: Showing antimicrobial activity of antibiotics (control).

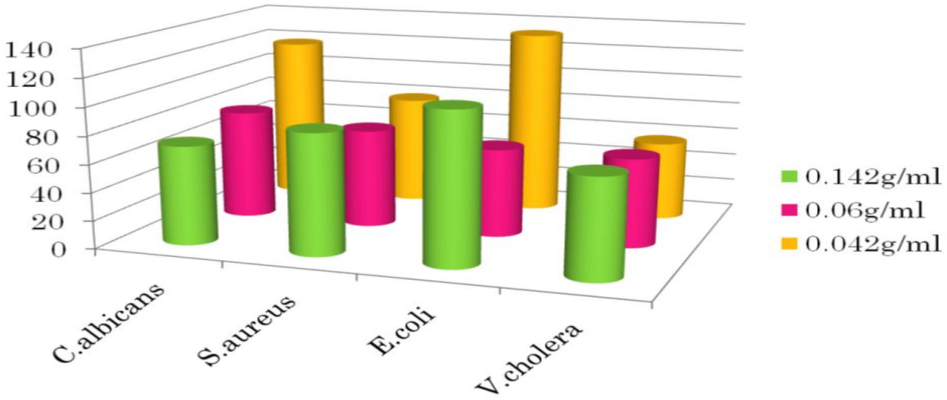
Microorganism	(Control antibiotics)	Diameter of inhibition	Area of zone of inhibition, mm^2
E.coli	Gentamicin	17, 13	78.5
S.aureus	Oxacillin	------	-----
C. ablicans	Tetracycline	------	-----
V. cholera	Tetracyline	------	-----

Table 6.0: Antimicrobial activity of control (Ethanol and Water).

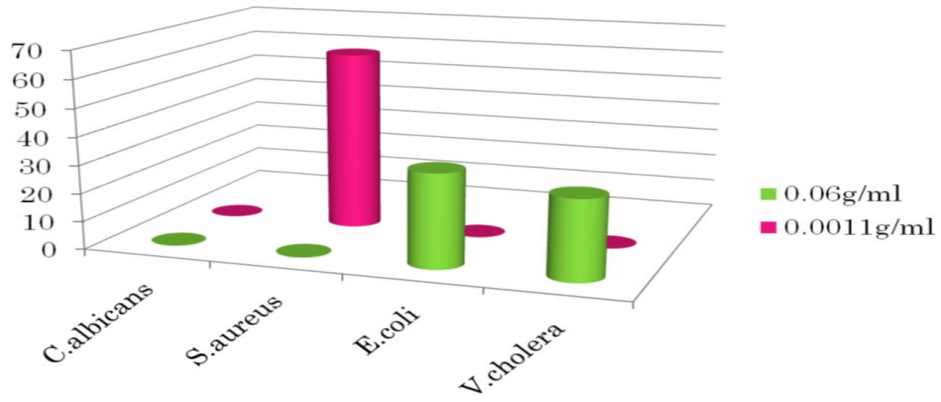
Microorganism	(Control antibiotics)	Diameter of inhibition (mm)	Area of zone of inhibition, mm^2
E.coli	Gentamicin	< 5	Too insignificant to be calculated
S.aureus	Oxacillin	< 5	Too insignificant to be calculated
C. ablicans	Tetracycline	< 5	Too insignificant to be calculated
V. cholera	Tetracyline	< 5	Too insignificant to be calculated



Graph 1: Antimicrobial activities of CH_2Cl_2 plant extract against the tested microorganisms.



Graph 2: Antimicrobial activities of ethanol plant extracts against the tested microorganisms.



Graph 3: Antimicrobial activity of hexane plant extracts against the tested microorganism.

DISCUSSION

The hexane, dichloromethane and ethanol extract of *Mimosa pudica* against human pathogenic organisms *S.aureus*, *E.coli* and *V. cholera* and *C. albicans* were investigated, using the disk diffusion assay, at concentration that range from 0.0011g/ml to 0.0614g/ml. Here, the AZOI was used as an indicator against plant's extract antimicrobial activity. The largest AZOI of 132.7 mm^2 was induced by the ethanolic extract of *Mimosa pudica* against *E.coli*, whereas negligible ZOA, was induced by the hexane extract of M. pudica against *E.coli*, against *S.aureus* at a concentration of 0.06g/ml, against *E.coli* at a concentration of 0.0011g/ml, against *C. albicans*, at a concentration of 0.06g/ml and 0.0011g/ml and against *V. cholera* at a concentration of 0.001$gmol^{-1}$.

In general, there seems to be an increase in antimicrobial activity, as the concentration of the plant extract increased. For example, the ethanolic extract, at a concentration of 0.06g/ml, 0.142g/ml and 0.42 g/ml, showed AZOI of 63.6 mm^2, 108.2 mm^2 & 132.7 mm^2, against *E.coli.* There are exceptions to this. The ethanolic extract of *M. pudisa* showed AZOI of 78.5 mm^2, 70.85 mm^2 & 117.3 mm^2 at a concentration of 0.06g/ml, 0.142g/ml and 0.42 g/ml against *C. albicans.*

The ethanolic extract showed the highest zone of inhibition for the three solvent types extract. For ethanol, this range from 56.72 mm^2 to 132.7 mm^2. For CH_2Cl_2, this range from negligible to 38.5 mm^2. For the hexane extract, this range from negligible to 63.6 mm^2. The order of potency of ethanolic extract against the microorganisms investigated follows the sequence:
E.coli > *C. albicans* > *S.aureus* > *V. cholera*

The order of potency of CH_2Cl_2 extract against the microorganism followed the sequence:
C. albicans = *E.coli* > *S. aureus* > *V. cholera*

For the hexane extract, the susceptibility trend is:
S. aureus > *E.coli* > *C. albicans* = *V. cholera*

Fig. 1.0 (a) shows the ZOI ( ) induced by the ethanolic extract against *E. coli*, whereas Fig. 2.0 (b) shows the ZOI ( ) induced by the ethanolic extract against *S. aureus*. The results were graphically transformed. Graph 1 shows the antimicrobial activities of CH_2Cl_2 plant extract against the tested microorganisms. Graph 2, shows the antimicrobial activities of ethanol

plant extracts against the tested microorganisms. Graph 3 shows antimicrobial activity of hexane plant extracts against the tested microorganism

Little or no antimicrobial selectivity was observed. For the ethanolic extract, little or no antimicrobial selectivity was observed. For the CH_2Cl_2 extract, selectivity was observed over *V. Cholera*. The hexane extract showed antimicrobial selectivity towards *S.aureus*. In addition, the DZOI, induced by the plant extracts were less than those induced by standard antibiotics such as ampicillin and nystatin.

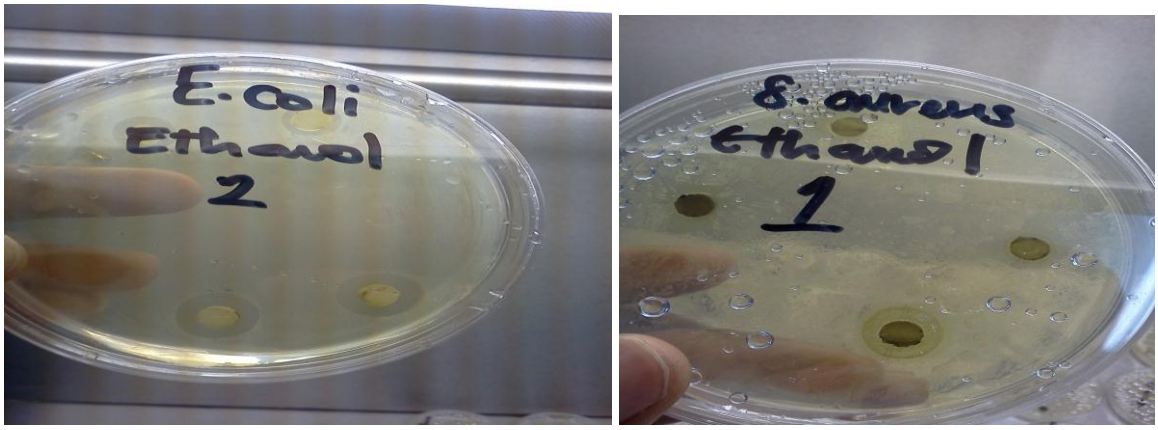


Fig. 1.0 (a) Fig. 1.0 (b).

CONCLUSION

In conclusion, the hexane, CH_2Cl_2 and C_2H_5OH extract of *M. pudica* displayed antimicrobial properties against the pathogens: *E.coli*, *S.aureus*, *K. pneumonia*, *C. albicans*, and *V. cholera*. The highest zone of inhibition was induced by the ethanolic extract of *M. pudica* and this range from 56.72 mm^2 to 132.7 mm^2, whereas negligible AZOI were obtained in some cases. Antimicrobial potency seems to be concentration dependent, with some exceptions. Little or no antimicrobial selectivity was observed. In addition, the DZOI, induced by the plant extracts were less than those induced by standard antibiotics such as ampicillin and nystatin. Thus, *M. pudica* extracts can be used to treat infections arising from *E.coli*, *S.aureus*, *K. pneumonia*, *C. albicans* and *V.cholera*, particularly those arising from *E.coli*.

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