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PHYTOCHEMICAL SCREENING AND ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF *MIMOSA PUDICA*

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ABSTRACT

The side effects and the increasing microbial resistance to orthodox/synthetic drugs in the management of infections remain an issue in modern medicine, hence the increasing research exploration in phytomedicine. Therefore, this study was aimed at assessing the antimicrobial characteristics of *Mimosa pudica* leaves and to screen for its bioactive components using standard microbiological and physic-chemical methods. Aqueous, ethanol and methanol extracts of the leave sample were tested against some clinical bacterial and fungal isolates at different concentrations of 100mg/ml, 50mg/ml and 25mg/ml. Methanol extract was seen to have exhibited a better antimicrobial activity over ethanol extract, while aqueous extract recorded the least antimicrobial activity against all the test organisms. The minimum inhibitory concentration of the various extracts against the test organisms were recorded at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml, with ethanol and methanol extracts recording a significant MIC values against many of the test organisms. Phytochemical screening of the *Mimosa pudica* plant extracts revealed presence of bioactive constituents: alkaloids, glycosides, flavonoid, saponin, steroids and tannin. While the study have scientifically validates the use of plants in traditional and modern medicine, extracts of *Mimosa pudica* leave showed to be a better and more promising antibacterial than antifungal agent.

Keywords: Antimicrobial, extract, phytochemical, methanol, ethanol.

INTRODUCTION

While synthetic antibiotics has undoubtedly recorded significant successes in the management of diseases and infections through their static and cidal effects (but not without limitations like side effect and microbial resistance), nature as it were has been a source of medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to treat diseases all over the world (Nair *et. al.*, 2005). Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness (Balakumar and Rajan, 2011). The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds (Kiruba *et. al.*, 2011; Rajan *et. al.*, 2011; Mohana *et. al.*, 2009).

Contrary to synthetic drugs, antimicrobial substances of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. Antimicrobial properties of certain Indian medicinal plants were reported based on

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folklore information and only few reports are available on inhibitory activity against certain pathogenic bacteria and fungi. Higher plants produce a large number of diverse chemical compounds with different biological activities (Harborne and Willans, 2000).

Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory study (Satish *et. al*., 2007; Okigbo and Ogbonnaya, 2006.). While natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders (Newman, 2007), about 25% of prescribed drugs in the world originate from plants (Rates, 2001). In developing countries and particularly in Bangladesh about 80% of the population relies on traditional plant based medicines (folk medicine) to treat serious diseases including infections, cancers and different types of inflammation (FA0, 2004).

Mimosa pudica L. belongs to the family Mimosaceae. It is a creeping annual or perennial herb often grown for its curiosity value, as the compound leaves fold inward and droops when touches and reopen within a few minutes. *Mimosa pudica* is native to Brazil, but is now a pan tropical weed (Doss *et. al.,* 2011). With its pharmacological activities, *Mimosa pudica* has been reported to contain alkaloid, glycoside, flavonoid and tannis. Therefore, this study was aimed at investigating the antimicrobial characteristics of extracts of *Mimosa pudica* leaves, and to screen for its phytochemical compositions responsible for its antimicrobial activity.

MATERIALS AND METHODS

Sample Collection and Processing

Fresh leaves of *Mimosa pudica* was harvested within the neighbourhood of Wellspring University, Irhirhi, Benin City, Edo State, Nigeria. The harvested plant leaves was sun dried and later pulverized into coarse particles using clean mortar and pestle and then stored in a clean sterile dry container.

Test Microorganism

All clinical isolates used in this work were obtained from University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The bacterial cultures were *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*; while the fungal cultures used were *Candida albican*, *Penicillun notatum*, *Aspergillus niger*.

Standardisation Of The Test Organisms

A loopful of the stock culture of each test organisms was inoculated into 5ml each of sterile nutrient broth and incubated for 24 hours. Exactly 0.2ml of overnight culture of each test organisms was inoculated into 20ml of sterile nutrient broth and incubated for 3-5 hours. The turbidity of the culture was compare with that of 0.5 Mac-Farland to standardize the culture to 10^6cfu/ml.

Preparation Of Plant Extracts

Ten grams (10g) of the processed *Mimosa pudica* leave sample were measured in 90ml each of aqueous, ethanol, and methanol in three different conical flasks separately, covered with

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cotton wool wrapped with aluminium foil and content was allowed to stay for 24 hours before filtration using Whatmann No.1 filter paper. Filtrates were concentrated to paste-like form using the steam bath at 60^0C. Concentrates were dissolved with dimethyl sulfoxide and stored in sterile container and kept in the refrigerator at 4^0C until needed for use.

Reconstitution Of Extracts

For the dilution of crude extract for antimicrobial assay, the extract was reconstituted using distilled water to obtain 100mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml concentrations. These were obtained by dissolving 0.5g of the extract in 5ml of the distilled water to obtain 100 mg/ml which was doubly serially diluted to obtain 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. The reconstituted extracts were then stored at 4^oC in sample bottles until required.

Antimicrobial Activity

Antimicrobial activity was performed to evaluate the antimicrobial properties of each extracts employing the agar well diffusion method as described by Ahmad and Beg, (2001). Nutrient agar plates were prepared for all extracts, 0.5ml inoculum of each selected bacterium was uniformly spreaded on agar plates with the help of glass spreader. After five minutes, three wells of 4mm in diameter were bored with the help of borer and the wells were filled with each extracts. The plates were incubated at 37°C for 24 hours and observed zone of inhibition. Similarly, potato dextrose agar plates were prepared for all extracts, 0.5ml inoculum of each selected fungus was uniformly spreaded on the PDA plates with the help of glass spreader. After five minutes, three wells of 4mm diameter were bored with the help of borer and the wells were filled with each extracts. The plates were incubated at room temperature for 24-48 hours and observed zone of inhibition.

Minimum Inhibitory Concentration (MIC)

The broth dilution method as earlier described by Bailey and Evelyn (1970) was employed in the determination of the minimum inhibitory concentration (MIC) of the extracts. To each test tube, 1.5ml of nutrient broth was dispensed. To the first test tube, was added 1.5ml of extract and serially diluted out to various concentrations ranging from 50 - 3.125mg/ml. A loop full of the test bacteria culture was inoculated into each of the test tubes and incubated at 37^0C for 24 hours and observed for growth in the form of turbidity. Similarly, this was also done for all fungal isolates using potato dextrose broth and incubated at room temperature for 24 – 48 hours.

Minimum Bactericidal/Fungicidal Concentration (MBC and MFC)

The bactericidal and fungicidal concentrations of the extracts were determined with the absence of growth of bacteria and fungi colonies on plates. For MBC 20µl of MIC content were cultured on nutrient agar plates and incubate at 370C for 24 hours. Thereafter, plates were observed for bacteria colonies. For MFC 20µl of MIC tube content were cultured on potato dextrose agar plates and incubate at room temperature for 24 - 48 hours. Thereafter, plates were observed for fungi colonies.

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Phytochemical Analysis

Alkaloid: Crude sample extract was mixed with 2ml of 1% HCl and heated gently. Mayer' and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Steroids: 1ml of sample filtrate was added to 10% concentrated H2SO4 and was observed for green colour.

Tannins: About 0.5g each of dried powder sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Glycosides*:* 4ml of sample extract was mixed with 2ml of lacial acetic acid containing 1-2 drops of 2% solution of FeCl2. Mixture was then poured into another test tube containing 2ml of concentrated H2SO4. A brown ring at the interphase indicated the presence of glycoside.

Saponins: 2g of the powder sample was boiled in 20ml of distilled water. The formation of the stable foam was as an indication for the presence of saponin.

Flavonoids: Sample extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

RESULT

The microbiological and phytochemical screening results of this research work are shown in different tables below. In table 1, 2 and 3 are shown the antimicrobial activity of plant extracts in 100mg/ml, 50mg/ml and 25mg/ml concentrations respectively. In table 1, the maximum antimicrobial activity of aqueous extract was obtained against *Escherichia coli* (6.3±1.5), followed by *Staphylococcus aureus* (3.3±0.9), while the least (2.3±0.3) was recorded against *Klebsiella pneumonia* at 100mg/ml concentration. For ethanol extract, *Staphylococcus aureus* had the highest (15±0.6), followed by *Escherichia coli* (14±0.6), while the least (2.3±0.3) was recorded against *Pseudomonas aeruginosa*. For methanol extract, *Bacillus subtilis* had the highest (15.7±1.9), followed by *Klebsiella pneumonia* (15.3±0.7), while the least (14.3±0.9) was recorded against *Escherichia coli.* In table 2 at 50mg/ml concentration, the antimicrobial activity of aqueous extract was obtained against *Escherichia coli* (3±0.6) only. For ethanol extract, *Streptococcus pyogenes* had the maximum antimicrobial activity (11.3±0.9), followed by *Escherichia coli* (10.7±0.9), and while the least (3.7±0.7) was recorded against *Bacillus subtilis*. For methanol, the same maximum antimicrobial activity was obtained against *Bacillus subtilis* and *Klebsiella pneumonia* (12.7±0.9), while the least (5.7±0.9) was recorded against *Candida albicans*. In table 3 at 25mg/ml concentration, the aqueous extract showed no antimicrobial activity against the test isolates. For ethanol, the maximum antimicrobial activity was obtained against *Streptococcus pyogenes* (7.7±0.9), followed by *Escherichia coli* (5.3±0.9), and while the least (2.3±0.3) was recorded against *Bacillus subtilis.* For methanol, the maximum antimicrobial activity obtained was against *Bacillus subtilis* (10.0±0.6), followed by *Streptococcus pyogenes* (7.7±0.7), and while the least (4.0±0.6) was recorded against *Staphylococcus aureus.* In table 4, 5 and 6 are presented the minimum inhibitory concentration (MIC) of aqueous, ethanol and

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methanol extract at 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml concentrations. In table 7 is shown the minimum bactericidal concentration (MBC) of aqueous, ethanol and methanol extracts, while in table 8 is shown the minimum fungicidal concentration (MFC) of aqueous, ethanol and methanol extracts against the tested isolates. In table 9 is shown the qualitative phytochemical composition of the various plant extracts.

Table 1: Antimicrobial Activity Of Plant Extracts At 100mg/ml Concentration

ISOLATES	Zone of inhibition in mm		
	Aqueous	**Ethanol**	**Methanol**
Pseudomonas aeruginosa	0.0 ± 0.0	2.3 ± 0.3	3.4 ± 0.3
Klebsiella pneumonia	2.3 ± 0.3	13.0 ± 1.0	15.3 ± 0.7
Escherichia coli	6.3 ± 1.5	14.0 ± 0.6	14.3 ± 0.9
Bacillus subtilis	0.0 ± 0.0	10.3 ± 2.4	15.7 ± 1.9
Staphylococcus aureus	3.3 ± 0.9	10.0 ± 0.6	10.3 ± 0.3
Streptococcus pyogenes	0.0 ± 0.0	15.0 ± 0.6	12.7 ± 1.7
Penicillium notatum	0.0 ±0.0	0.0 ± 0.0	0.0 ± 0.0
Aspergillus niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Candida albicans	0.0 ± 0.0	11.2 ± 2.5	12.3 ± 1.2

Table 2: Antimicrobial Activity Of Plant Extracts At 50mg/ml Concentration

	Zone of inhibition in mm		
	Aqueous	**Ethanol**	**Methanol**
Pseudomonas aeruginosa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Klebsiella pneumonia	0.0 ± 0.0	7.3 ± 1.2	12.7 ± 0.9
Escherichia coli	3.0 ± 0.6	10.7 ± 0.9	11 ± 0.6
Bacillus subtilis	0.0 ± 0.0	3.7 ± 0.7	12.7 ± 0.9
Staphylococcus aureus	0.0 ± 0.0	7.0 ± 0.6	7.3 ± 0.9
Streptococcus pyogenes	0.0 ± 0.0	11.3 ± 0.9	11.7 ± 1.2
Penicillium notatum	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Aspergillus niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Candida albicans	0.0 ± 0.0	0.0 ± 0.0	5.7 ± 0.9

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Table 3: Antimicrobial Activity Of Plant Extracts At 25mg/ml Concentration

ISOLATES	Zone of inhibition in mm		
	Aqueous	Ethanol	Methanol
Pseudomonas aeruginosa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Klebsiella pneumonia	0.0 ± 0.0	3.3 ± 0.9	7.3 ± 0.9
Escherichia coli	0.0 ± 0.0	5.3 ± 0.9	5.3 ± 0.9
Bacillus subtilis	0.0 ± 0.0	2.3 ± 0.3	10.0 ± 0.6
Staphylococcus aureus	0.0 ± 0.0	3.3 ± 0.9	4.0 ± 0.6
Streptococcus pyogenes	0.0 ± 0.0	7.7 ± 0.9	7.7 ± 0.7
Penicillium notatum	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Aspergillus niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Candida albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 4: Minimum Inhibitory Concentration Of Aqueous Extract In mg/ml

Isolates	100 mg/ml	50 mg/ml	25.0 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	MIC (mg/ml)
Pseudomonas aeruginosa	+	+	+	+	+	+	Nil
Klebsiella pneumoniae	-	+	+	+	+	+	100
Escherichia coli	-	-	+	+	+	+	50
Bacillus subtilis	+	+	+	+	+	+	Nil
Staphylococcus aureus	-	+	+	+	+	+	100
Streptococcus pyogenes	+	+	+	+	+	+	Nil
Penicillium notatum	+	+	+	+	+	+	Nil
Aspergillus niger	+	+	+	+	+	+	Nil
Candida albicans	+	+	+	+	+	+	Nil

KEY: + = Growth - = No Growth

Table 5: Minimum Inhibitory Concentration Of Ethanol Extract In mg/ml

Isolates	100 mg/ml	50 mg/ml	25.0 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	MIC (mg/ml)
Pseudomonas aeruginosa	-	+	+	+	+	+	100
Klebsiella pneumonia	-	-	-	+	+	+	25

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Escherichia coli	-	-	-	-	+	+	12.5
Bacillus subtilis	-	-	-	+	+	+	25
Staphylococcus aureus	-	-	-	+	+	+	25
Streptococcus pyogenes	-	-	-	-	+	+	12.5
Penicillium notatum	+	+	+	+	+	+	Nil
Aspergillus niger	+	+	+	+	+	+	Nil
Candida albicans	-	+	+	+	+	+	100

KEY: + = Growth - = No Growth

Table 6: Minimum Inhibitory Concentration Of Methanol Extract In mg/ml

Isolates	**100 mg/ml**	**50 mg/ml**	**25.0 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**	**MIC (mg/ml)**
Pseudomonas aeruginosa	-	+	+	+	+	+	100
Klebsiella pneumoniae	-	-	-	+	+	+	25
Escherichia coli	-	-	-	-	+	+	12.5
Bacillus subtilis	-	-	-	-	+	+	12.5
Staphylococcus aureus	-	-	-	+	+	+	25
Streptococcus pyogenes	-	-	-	-	+	+	12.5
Penicillium notatum	+	+	+	+	+	+	Nil
Aspergillus niger	+	+	+	+	+	+	Nil
Candida albicans	-	+	+	+	+	+	100

KEY: + = Growth - = No Growth

Table 7: Minimum Bactericidal Concentration For Aqueous, Ethanol And Methanol extracts

Isolates	Aqueous (mg/ml)	Ethanol (mg/ml)	Methanol (mg/ml)
Pseudomonas aeruginosa	Nil	100	100
Klebsiella pneumonia	100	50	50
Escherichia coli	100	25	25
Bacillus subtilis	Nil	50	25
Staphylococcus aureus	100	50	50

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Streptococcus pyogenes	Nil	25	25

Table 8: Minimum Fungicidal Concentration For Aqueous, Ethanol And Methanol Extracts

Isolates	Aqueous (mg/ml)	Ethanol (mg/ml)	Methanol (mg/ml)
Penicillium notatum	Nil	Nil	Nil
Aspergillus niger	Nil	Nil	Nil
Candida albicans	Nil	Nil	100

Table 9: Qualitative Phytochemical Composition Of Plant Extract

Parameters	Aqueous	Ethanol	Methanol
Alkaloids	+	+	+
Glycosides	-	+	+
Flavonoid	-	+	+
Saponin	+	+	-
Steroids	-	+	+
Tannin	+	+	-

KEY: + = Present - = Absent

DISCUSSION

Plants have been the traditional sources of raw materials for medicine. The trend of using natural products has increased and the active plant extracts are frequently used for new drug discoveries and for the presence of antimicrobials (Das *et. al.*, 1999). Result findings of this research work clearly revealed the antimicrobial properties and the phytochemical compositions of the plant extracts of *Mimosa pundica*.

As shown in table 1, the maximum zone of inhibition at 100mg/ml was 6.3±1.5 against *Escherichia coli* for aqueous extract, 15 ± 0.6 against *Streptococcus pyogenes* for ethanol extract, and 15.7±1.9 against *Bacillus subtilis* for methanol extract. Similarly at a reduced concentration of 50mg/ml as shown intable 2, the maximum zone of inhibition was 3.0±0.6 against *Escherichia coli* for aqueous extract, 11.3 ± 0.9 against *Streptococcus pyogenes* for ethanol extract, and 12.7±0.9 against *Bacillus subtilis* and *Klebsiella pneumonia* for methanol extract. On further reduction in concentration of the extract to 25mg/ml as shown in table 3, none of the test isolates showed any level of sensitivity with aqueous extract, while the maximum zone of inhibition of ethanol extract was 7.7±0.9 for *Streptococcus pyogenes*; and for methanol extract, it was 10.0±0.6 for *Bacillus subtilis*. Meanwhile, only *Candida albicans* among the tested fungal isolates showed significant susceptibility to ethanol and methanol extracts with the maximum zone of inhibitions of 11.2±2.5 and 12.3±1.2 at 100mg/ml concentration. According to Rekha *et. al.*, (2009), methanolic fraction of *Mimosa pudica* leaves have antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeuroginosa and Salmonella typhi*. While this research findings has clearly demonstrated extracts of *Mimosa pundica* as a better antibacterial agent (for both gram positive and gram negative bacteria infections) to antifungal agent, it could be said from

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the results also that ethanol and methanol were better extracting solvent of bioactive phytochemical compounds compared to aqueous which is a polar solvent.
The minimum inhibitory concentration (MIC) of the different extracts against the test isolates as carried out at various concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml showed varied results. For aqueous extract, *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus* were minimally inhibited at 100mg/ml, 50mg/ml and 100mg/ml respectively. While both *Streptococcus pyogenes* and *Escherichia coli* had the highest minimum inhibitory effects at 12.5mg/ml concentration of the ethanol extract, *Klebsiella pneumonia*, *Bacillus subtilis* and *Staphylococcus aureus* had a minimum inhibitory concentration of 25mg/ml; and *Pseudomonas aeruginosa* and *Candida albicans* had 100mg/ml MIC. Similarly for methanol extract, *Escherichia coli*, *Bacillus subtilis* and *Streptococcus pyogenes* had the minimum inhibitory concentration of 12.5mg/ml while *Klebsiella pneumoniae* and *Staphylococcus aureus* had 25mg/ml, with *Pseudomonas aeruginosa* and *Candida albicans* having the least at 100mg/ml.

While the minimum bactericidal concentration (MBC) of aqueous extract against few bacteria isolates were 100mg/ml, the MBC for ethanol and methanol extracts ranged from 100 – 25mg/ml, However, only methanol extract was seen to have a minimum fungicidal concentration (MFC) of 100mg/ml for *Candida albicans* only. This is in agreement with the work by Palwinder *et. al.*, (2011) as previously reported. It has been widely observed and accepted that the medicinal value of plants lies in the bioactive phytochemicals present in the plants (Veermuthu *et. al.*, 2006). Results of the qualitative phytochemical screening showed that all the phytochemicals (alkaloids, glycosides, flavonoid, saponin, steroids and tannin) screened for were all present, with some variation of detectable presence or absence among the different extracting solvents used. These phytochemical compounds are believed to be the bioactive ingredients of *Mimosa pudica* responsible for its antimicrobial activity.

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