

# Quantitative Determination of Secondary Metabolites and Antibacterial Activity of Mimosa *Pudica*

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**Abstract:** The side effects and the increasing microbial resistance to synthetic drugs in the management and treatment of infections remain an issue in modern medicine, hence the increasing research in phytomedicine. Therefore in the present study, the active phytocomponents of Mimosa pudica's wholesome parts were revealed using quantitative phytochemical analysis. The antibacterial activity of the Ethanolic extract was studied using Well diffusion method. The activity was tested against Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeroginosa, Escherichia coli and Mycobacterium smegmatis at different concentrations of 25, 50 and 100 mg/disc and the results have been illustrated.

**Keywords:** Mimosine, Secondary metabolites, Antibacterial, Zone of inhibition, Mimosa pudica, phytochemicals

## **1. INTRODUCTION**

Medicinal properties of plants are the most precious gift of Mother Nature to Mankind. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment. Various medicinal plants have been used for years in daily life to treat diseases all over the World [1]. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness [2]. The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds [3-5].

*Mimosa pudica* is one of such important medicinal plants. It is a creeping annual or perennial herb often grown for its curiosity value, as the compound leaves fold inward and drop when touched and reopens within minutes. It belongs to the *Fabaceae* family. The generic name *Mimosa* is derived from the Greek mimos (meaning mimic) alluding to the fact that the leaves moves in response to something moving against them. The specific epithet is taken from the Latin word *pudica*, meaning bashful or shrinking to contact [6]. *Mimosa* is a genus of about 400 species of herbs and shrubs, in the subfamily *mimosoideae* of the Legume family *Fabaceae*. The plant is native to Brazil, but is now a pan tropical weed, The species is known by numerous common names including Sensitive plant, Humble plant, Shameful plant, Touch-me-not, Chuimui, Ant-plant.



Mimosa pudica leaves and Flower

#### **1.1. Vernacular- Names**

Non-English common names in three major languages in Nigeria, European language, culture areas include;

Igbo Language: Agbogho mechie ukwu.

Hausa Language: Kama walkinka.

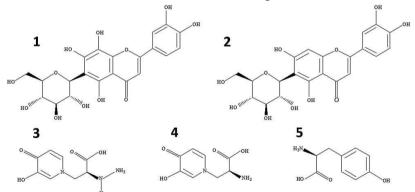
Yoruba Language: Ewe padimo/ Patomo

In European language/ culture areas we have nao- me- toque (touch- me- not), sensitive or dormideira (roughly sleeper") in the Portuguese (with the former being more common in Portugal. Africa and Rio, dejaneiro, the middle in Sao Paulo city and the Southern capitals and the latter elsewhere in Brazil), while in Spanish, it varies in names such as mori- vivi or morivivi (DOMINICAN REPUBLIC, PUERTO RICO and other Spanish- speaking Caribbean islands, roughly translating to "I died, I lived") [7] and Dormilona (Costa Rica).

In Austronesia names vary more: in the philippines it is called makahiya, with maka-meaning "quite" or tendency to be", and –hiya meaning "shy" or "shyness", while in Tonga for example it is known as Mateloi (false death) [8], being Putri- malu (shy princess) in Indonesia and Pokok Semalu (shy plant), in Malaysia. In Sinhala (Sri Lanka) it is called Nidi kumba (sleeping plant).

In south Asia many unrelated names are also common. In Hindi it is known as chhui-mui (that which dies upon touch). In Bengali, the shrub is called lojjaboti ("that bashful girl"). In Malayalam it is called thottavaadi ("wilts by touch). In marathi it is called lazalu ("shy"). In Tamil, it is called thottasiningi ("acts when touched") and in kannada, it is known as muttidare muni ("angered by touch"). In Burmese (Myanmar) it is called hti ka yoan, which means "(crumbles when touched)". In Liberia, it is known as the picker weed [7].

This plant has a history of use for treatment of various ailments and the most commonly used plant part for this purpose is the root; but flowers, back and fruit can also be utilized. Several research works have been carried out to study about the phytochemical components of *Mimosa pudica* and also about the antimicrobial activity of the plant [8] Phytochemical studies on *M. pudica* have revealed the presence of alkaloids, fatty acids, non-protein amino acid (mimosine), flavonoids, C-glycosides, sterols, terpenoids, and tannins [9]. Reported major pharmacological activities are; antiviral properties, aphrodisiac properties, antimicrobial properties, anti-hepatotoxic, antioxidant effect, diuretic effect, hyperglycemic effect, and wound healing effect etc. [10]. Some of the principal bioactive compounds of interest which have been commonly associated with these therapeutic properties and disease conditions are shown in Figure 1.



**Figure1.** Structures of therapeutic compounds of interest in Mimosa pudica: (1) 3,4,7,8-tetrahydroxyl- $\beta$ -D-glucopyranosyl flavone, (2) 3,4,5,7-tetrahydroxyl- $\beta$ -D-glucopyranosyl flavone, (3) mimosine amine, (4) mimosine, (5) tyrosine. [11].

Other isolated secondary metabolites from *M. pudica* are bufadienolide, D-pinitol, norepinephrine, Pcoumaric acid, mimopudine, potassium-5-O- $\beta$ glucopyranosygentisate, etc. [12-18], Two well-known movements are observed in *M. pudica*: one is the very rapid movement of the leaves when it is stimulated by touch, heat etc, and the other is the very slow, periodical movement of the leaves called nyctinastic movement which is controlled by a biological clock [19]. The present study intends to study about the antibacterial activity of the Ethanolic extracts from *Mimosa pudica* against selected bacterial and as well determine quantitatively its phytochemical composition in order to support its uses in traditional medicine in South East of Nigeria.

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# 2. MATERIALS AND METHODS

## **2.1. Sample Collection and Extraction**

Fresh and wholesome parts of *Mimosa pudica* were collected during the month of October 2016, from Ndi-Ojigwe compound in Okoko Item, Bende Local Government Area of Abia State,Nigeria. The plant was identified and authenticated by Mr. I. Ndukwe in plant taxonomy section, forestry Department of Michael Okpara University of Agriculture Umudike, Nigeria. The fresh plant materials were dried under shade to prevent interference of uv-radiation from the sun. 2 kg of the milled sample was percolated in 98% ethanol for 48 hours. Thereafter, it was filtered through Whatmann Filter Paper (NO 42). The filtrate was concentrated using the Digital Heidolph Rotary-evaporator (4000 series) to a crude extract of 48.9 g.

# 2.2. Phytochemical Determination

Two grams of sample was defatted with 100ml of diethylether.

The phytochemical compounds including: alkaloids, saponins, flavonoids, tannins, phenols, anthocyanin and gyanogenic glycosides were carried out using method of Harbone.[20]

# 2.3. Antimicrobial Activity of Extracts of Mimosa Pudica

#### 2.3.1. Preparation of Extract Stock Solution

A stock of extract was prepared by dissolving 0.2 g (200 mg) of the plant extract in 2.0 ml of dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml of the stock solution. This stock solution was diluted with sterile distilled water to give concentration of 50 mg/ml and 25 mg/ml.

#### 2.3.2. Test Micro-Organisms

The strains of microorganisms used (*Staphylococcus aureus* ATCC 25923; *Enterococcus faecalis* ATCC 7080; *Pseudomonas aeruginosa* ATCC 27853; *Escherichia coli* ATCC 25922 and *Mycobacterium smegmatis* ATCC 19420) were purchased from the American Type Culture Collection (ATCC, USA). The Type Culture organisms were maintained on Nutrient agar slants after reactivation on Nutrient agar plates. The Nutrient agar medium used was from Biomark Laboratories, India. A cell suspension of each microorganism was prepared by transferring colonies from the nutrient agar plate to turbidity standard tube No. 0.5 with sterile normal saline.

## 2.3.3. Preparation of Discs

Filter paper discs (Whatman No. 1) were sterilized in hot air oven inside glass Petri dishes at 160  $^{\circ}$ C for 2 hours. Each disc was impregnated with 20 µl of the plant extract solution at the various concentrations of 25 mg/mL 50 mg/mL and 100 mg/mL and labeled accordingly. The discs were placed in an incubator at 40  $^{\circ}$ C and left for 2 hours to dry. They were used immediately and the remaining was stored at 4  $^{\circ}$ C. Similarly, discs of chloramphenicol and DMSO were also prepared as control.

#### 2.3.4. Disc Diffusion Test

This was performed following the Kirby- Bauer method. Plates of Mueller Hinton Agar (MHA, Hardy Diagnostics, USA) were prepared according to manufacturer's instructions. The plates were dried in an incubator at 40 °C for 30 min. using a sterile swab stick, standardized cells suspension containing an inoculums size of 5x10<sup>8</sup> <sup>CFU</sup>/ml was aseptically spread on the agar surface. The discs of the extracts and antibiotics were placed on the inoculated plates of each test organisms. The plates were incubated at 35-37 °C for 16-18 hours. The diameter of any clear zone of inhibition obtained around the discs was measured manually using a transparent ruler. The experiment was replicated three times for each extract and antibiotic.

Table1. Percentage composition of phytochemicals in Mimosa pudica				
Constituent	(%)			
Alkaloid	9.05 ±0.098			
Flavonoid	$8.23 \pm 0.16$			
Steroid	2.49 0.021			
Saponin	8.15 ±0.09			
Phenol	$1.02 \pm 0.05$			
Tannin	0.083 0.00014			
Cyanogenic-glycoside	$0.122 \pm 0.0028$			
Anthocyanin	$1.913 \pm 0.126$			

# 3. RESULTS AND DISCUSSION

 Table1. Percentage composition of phytochemicals in Mimosa pudica

Result based on mean  $\pm$  standard deviation of triplicate sampling measurements.

The quantitative estimation of the percentage of phytochemicals contain in *Mimosa pudica* in the present study is summarized in **table 1.** The whole plant consist of alkaloid 9.05 %, flavonoid 8.32 %, steroid 2.49 %, saponin 8.15 %, phenol 1.02 %, tannin 0.083 %, cyanogenic glycoside 0.122 % and anthocyanin 1.913 %. Alkaloids are the most efficient phytochemical compound. True isolated alkaloids and the synthetic derivatives are used as the basic medicinal compounds because of their analgesic, anti-spasmodic and bacterial properties [27]. The presence of tannins and phenols in the plant can attest to its use for healing of wounds, hemorrhoids in herbal medicine [28]).

The plant also contains an appreciable amount of flavonoids. The biological functions include protection against allergies inflammation, platelets aggregation, and microbes. Flavonoids through their free radical and scavenging property tend to lower cholesterol level and reduce the risk of heart attack [29]. Flavonoids have multiple biological activities that include estrogenic effect as well as inhibiting the action of some enzymes [30].

## 3.1. Antimicrobial Activity of Extract of Mimosa Pudica

Organisms	Concentration (mg/ml)		n	Control Antibiotic (Chloramphenicol) 100mg/mL
	25	50	100	
Staphylococcus aureus (ATCC 25923)	3.5	7.0	9.8	30.0
Enterococcus faecalis (ATCC 7080)	0.0	0.0	0.0	30.6
Pseudomonas aeroginosa (ATCC 27853)	12.0	14.5	18.0	31.0
Escherichia coli (ATCC 25922)	5.5	10.6	14.0	30.0
Mycobacterium smegmatis	0.0	0.0	0.0	19.3

**Table2.** Zone of Inhibition (mm) produced by Crude Ethanol extracts against the Test Organisms

*ATCC* = *American Type Culture Collection* 

The result of the antimicrobial assay of the Ethanol extract of *Mimosa pudica* shown in table 2 indicated that the plant exhibited antibacterial activity against

Staphylococcus aureus, Pseudomonas aeroginosa and Escherichia coli at three different concentrations of 25 mg, 50 mg and 100 mg/disc. The inhibitory effect is stronger on Pseudomonas aeroginosa than on Staphylococcus aureus and Escherichia coli at low concentration of 25 mg as indicated in table 2. The extract did not show any level of sensitivity on Mycobacterium smegmatis and Enterococcus faecalis at any of the concentrations used.

## 4. CONCLUSION

This study showed that the selected *Mimosa pudica* extracts have antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Escherichia coli*. Also from the above studies, we can see that the traditional plants may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. Further investigation should be carried out and documented in other to isolate, purify and characterize the active compounds responsible for the antibacterial activity.

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