

Antifungal activity of *Mimosa pudica*, Isolation and NMR Characterization of Bioactive Components.

Abstract:

In this study, the whole plant of *Mimosa pudica* was extracted using absolute ethanol. The crude ethanolic extract and its isolated triterpenoid glycoside were tested for antifungal activity towards *Aspergillus flavus* and *Trycophyton ruburum* using Well Diffusion Method. At concentrations of 25 – 100 mg/mL, the extract possesses antifungal activity (from being partially active to very active) against *Aspergillus flavus* and *Trycophyton ruburum*. The TLC profile of the crude extract indicated many compounds with R_F values of 0.41, 0.43, 0.56, 0.68, 0.89, and 0.90. The isolation and purification were established using extensive thin layer and column chromatographic processes by employing various solvents with varied polarity. The results of thin layer and column chromatography gave rise to the isolation of a triterpenoid-glycoside with R_F value of 0.41. The spectral analyses involving ^1H -NMR, ^{13}C -NMR, ^{13}C -DEPT, $^{135}\text{COSY}$, HMBC, HMQC and infrared were useful in proposing the structure of the compound.

Keywords: Chromatography, Antifungal activity, Zone of inhibition, *Mimosa pudica*, Secondary metabolites.

INTRODUCTION

The harmful side effects, high cost of other forms of treatment and their non availability to the poor populations who live in the remote areas are some of the reasons for the demand for herbal medicine. The increasing prevalence of multidrug-resistant strains of bacteria and the recent

34 emergence of strains with reduced susceptibility to antibiotics raise the
35 spectre of untreatable bacterial and viral infections. Thus, adding to the
36 urgency to search for new infection fighting strategies. (Sieradzki *et al.*,
37 1999). Contrary to the synthetic drugs, antimicrobial substances of plant
38 origin are not associated with many side effects and have an enormous
39 therapeutic potential to heal many infectious disease. (Iwu *et al.*, 1999).

40 *Mimosa pudica* Linn is a creeping annual or perennial herb often grown for its
41 curiosity value, as the compound leaves fold inward and drop when touched and
42 reopens within minutes. It belongs to the *Fabaceae* family. The generic name
43 *Mimosa* is derived from the Greek *mimos* , meaning “mimic”, alluding to the
44 fact that the leaves move in response to something moving against them. The
45 specific epithet is taken from the Latin word *pudica*, meaning bashful or
46 shrinking to contact (Barneby, 1991). *Mimosa* is a genus of about 400 species of
47 herbs and shrubs, in the subfamily *mimosoideae* of the legume family
48 *Fabaceae*. The plant is native to Brazil, but is now a pantropical weed. The
49 species is known by numerous common name including: sensitive plant,
50 humble plant, shameful plant, touch-me-not, chuimui, and ant-plant. The
51 vernacular names in the three major languages of Nigeria include: agbogho
52 mechie ukwu (Igbo language), kama walkinka (Hausa language) and ewe
53 padimo or patomo (Yoruba language).

54 In Portugal, Africa, Rio de Janeiro, Sao Paulo City and other southern
55 capitals, the name is nao-me-toque (touch-me-not). In Brazil, the name is
56 dormideira (roughly “sleeper”). In Spanish, the name varies as mori-vivi or
57 morivivi (roughly “I died, I lived”) in the Dominican Republic, Puerto Rico
58 and other Spanish-speaking Caribbean islands (Union County College Biology
59 Department, 2008). In Costa Rica, it is dormilona. In the Philippines, it is
60 called “makahiya” which means “quite shy”. It is putri-malu (shy princess) in
61 Indonesia; pokok semalu (shy plant) in Malaysia; nidi kumba (sleeping plant)
62 in Sri Lanka; mateloi (false death) in Tonga (Churchward, 1959),

63
64 In south Asia many unrelated names are also common. In Hindi, it is known as
65 chhui-mui (that which dies upon touch). In Bengali, the shrub is called lojjaboti
66 (that bashful girl). In Malayalam, it is called thottavaadi (wilts by touch). In
67 Marathi, it is called lazalu (shy). In Tamil, it is called thottasiningi (acts when
68 touched) and in Kannada, it is known as muttidare muni (angered by touch). In
69 Burmese (Myanmar) it is called hti-ka-yoan (crumbles when touched). In
70 Liberia, it is known as the picker weed (Union county College Biology
71 Department, 2008).

72
73 This plant has a history of use for treatment of various ailments and the most
74 commonly used plant part for this purpose is the root; but flowers, back and
75 fruit can also be utilized. Several research works have been carried out to study

76 about the phytochemical components of *Mimosa pudica* and also about the
77 antimicrobial activity of the plant (Gandhiraja *et al.*, 2009). Phytochemical
78 studies on *M. pudica* have revealed the presence of alkaloids, fatty acids, non-
79 protein amino acid (mimosine), flavonoids, C-glycosides, sterols, terpenoids,
80 and tannins (Genest *et al.*, 2008). Reported major pharmacological activities
81 **are:** antiviral properties, aphrodisiac properties, antimicrobial properties, anti-
82 venom activities, anti-hepatotoxic and antioxidant effects, diuretic effect,
83 hyperglycemic effect, wound healing effect **etc.** (Amalraj and Ignacimuthu, 2002).
84 Some of the isolated secondary metabolites are bufadienolide, D-pinitol,
85 norepinephrine, P-coumaric acid, mimopudine, mimosine, potassium-5-O- β -
86 gluco-(Clark-Lewis and Porter, 1972), **pyranosygentisate etc.** (Yadava and Yadav,
87 2001), (Ueda *et al.*, 2000), (Sallaud *et al.*, 1995), (Fromm and Lautner, 2007; Nair *et al.*,
88 2007 and Genest *et al.*, 1999).

89

90 **Two well-known movements are observed in *M. pudica*: one is the very rapid**
91 **movement of the leaves when it is stimulated by touch, heat and other external**
92 **factors, and the second is the very slow, periodical movement of the leaves**
93 **called nyctinastic movement which is controlled by a biological clock (Ueda *et***
94 ***al.*, 1999) .**



95

96 *Mimosa pudica* leaves and flower

97 **Materials and Methods**

98 **Sample collection and preparation:**

99 Fresh and wholesome parts of *Mimosa pudica* were collected during the month
100 of October 2015, from Ndi-Ojigwe compound in Okoko Item, Bende Local
101 Government Area of Abia State, Nigeria. The plant was identified and
102 authenticated by Mr. I. Ndukwe in Plant Taxonomy Section of the Forestry
103 Department of Michael Okpara University of Agriculture, Umudike, Nigeria.
104 The fresh plant materials were dried under shade to prevent interference of uv-
105 radiation from the sun. Dried plant materials were powdered using mechanical
106 grinder. Then the powdered material was preserved in an air-tight container;
107 ready for extraction.

108 **Extraction and Isolation of Plant Material:**

Two (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); collecting 48.9 g of crude extract. The crude extract was partitioned between CHCl_3 and water and a CHCl_3 -soluble fraction (16.3g) was obtained. The CHCl_3 fraction (10.0 g) was then partitioned between petroleum ether (at $60-80^\circ\text{C}$) and aqueous methanol. The CHCl_3 fraction (5.0 g) was then subjected to column chromatography over silica gel (200 mesh) and eluted gradually with 100 mL of petroleum ether, then petroleum ether: CHCl_3 solvent system (95:5; 90:10; 85:15; 80:20; 75:25; 70:30; 65:35; 60:40; 55:45; 50:50; 40:60; 30:70; 20:80; 10:90), and 100 mL CHCl_3 . Then CHCl_3 :Methanol (90:01; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90) and 100 mL methanol, followed by methanol:ethyl acetate (80:20; 60:40; 40:60; 20:80) and 100 mL ethyl acetate to yield twelve (12) major fractions. Chromatographic processes (partition chromatography, column chromatography and TLC) and spectroscopic techniques (^1H -NMR, ^{13}C NMR, COSY, DEPT, HMBC, HMQC, IR) were employed to isolate, purify, characterise and identify active constituents from the CHCl_3 extracts of the sample.

Antifungal activity of extracts from *Mimosa pudica*:

A. Preparation of extract stock solution

A stock of extract was prepared by dissolving 0.2 g 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.

B. Fungal Media (Potato dextrose Agar):

Potato slices (200 g) were boiled with distilled water. The potato infusion was used as water source of media preparation. Dextrose (20 g) was mixed with the potato infusion. Agar (20 g) was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6-mm diameter cork borer. The plates with wells were used for the antifungal studies.

C. Preparation of discs

Filter paper discs (Whatmann No. 1) were sterilized in hot air oven inside glass Petri dishes at 160°C for 2 hours. Each disc was impregnated with 20 µl of the plant extract solution at three concentrations: 25 mg/mL, 50 mg/mL and 100 mg/mL. The discs were placed in an incubator at 40°C and left for 2 hours to dry. They were used immediately and the remaining discs were stored at 4°C.

D. 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 inoculum size of 5x10⁸ CFU/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.

Antifungal activity of the plant extract:

The crude ethanolic extract and the isolated compound labeled MP-19 were tested against the fungal pathogens *Aspergillus flavus* and *Trycophyton rubrum* at concentrations of 25 mg/mL, 50 mg/mL and 100 mg/mL. The experiment was replicated three times for each extract and antibiotic.

Results and Discussion:

A. Antimicrobial activity of extract of *Mimosa pudica*

The results of the antifungal assay of the crude ethanolic extract of *M. pudica* (MP) indicated that the extract exhibited (from being partially

active to very active) antifungal activity against the tested microorganisms at all three different concentrations of 25 mg/mL, 50 mg/mL and 100 mg/mL. At 100 mg/mL the extract was very active against *Aspergillus flavus* . The zone of inhibition was recorded and presented in Table 1.

Table 1: Zone of Inhibition (mm) produced by Crude Ethanol extracts MP against the Test Organisms

Organisms	Zone of inhibition (mm) at Indicated Concentration		
	25 mg/mL	50 mg/mL	100 mg/mL
<i>Aspergillus flavus</i>	13	16	22
<i>Trycophyton ruburum</i>	11	14	17

Legend: ZI < 10 mm (Inactive); 10 < ZI < 13 mm (Partially active);

14 < ZI < 19 mm (Active); ZI > 19 mm (Very Active)

Table 2 shows the effect of the isolated compound labelled MP-19 on the microorganisms. The results of the experiment indicated that *Aspergillus flavus* and *Trycophyton ruburum* showed resistance against the isolated compound MP-19 from the crude ethanol extract of *M. pudica*. These results can show that the isolated compound may not be responsible for the antifungal activity recorded in Table 1.

Table 2: Zone of Inhibition (mm) produced by fraction MP-19 against the Test Organisms

Organisms	Zone of inhibition (mm) at Indicated Concentration		
	25 mg/mL	50 mg/mL	100 mg/mL
<i>Aspergillus flavus</i>	0.0	0.0	0.0
<i>Trycophyton ruburum</i>	0.0	0.0	0.0

Compound MP-19 was eluted with chloroform and petroleum ether at the ratio of 50:50. Thin layer chromatography carried on MP-19 showed one spot (R_F 0.41).

Analysis of IR is shown in Table 3. The IR revealed V_{max} 2910cm^{-1} , 1720cm^{-1} , 1600cm^{-1} and 1250cm^{-1} for aliphatic, carbonyl, olefinic, aromatic and ether respectively.

Table 3: Infra-red Analysis of MP-19

IR Absorption (cm^{-1})	Functional Group	Compound Type
2910	-CH ₂	Aliphatic
1720	C=O	Carbonyl
1600	C=C	Aromatic
1250	C – O	Ether

Analysis of ^1H -NMR revealed the presence of olefinic protons at $\delta\text{H}5.20$, cluster of peaks between $\delta\text{H}0.8$ to $\delta\text{H}2.35$ clearly indicated the triterpenoid structure, and two methoxy protons signals at $\delta\text{H}4.058$ and $\delta\text{H}4.239$. Then the presence of two anomeric protons at $\delta\text{H}5.45$ and $\delta\text{H}5.25$ confirmed the presence of two sugars. The spectrum also showed the presence of aromatic protons at $\delta\text{H}5.043$ - $\delta\text{H}5.315$.

¹³C-NMR spectrum revealed the presence of six aromatic carbons at δ C 124.00, δ C 126.08, δ C 126.72, δ C 126.87, δ C 127.04 and δ C 127.22. Methylene carbons chemical shift were seen at δ C 21.55 – δ C 22.60, methane carbon peaks were observed at δ C 25.38 – δ C 33.17, while the signal at δ C 172.93 indicates the presence of carbonyl carbon, which is an integral part of the triterpenoid. Based on the chromatographic data, IR, NMR, COSY, DEPT, HMBC and HMQC, the structure of MP-19 was proposed as shown in Figure 1 with molecular formula $C_{35}H_{46}O_{16}$ of 722 and calculated for a triterpenoid glycoside.

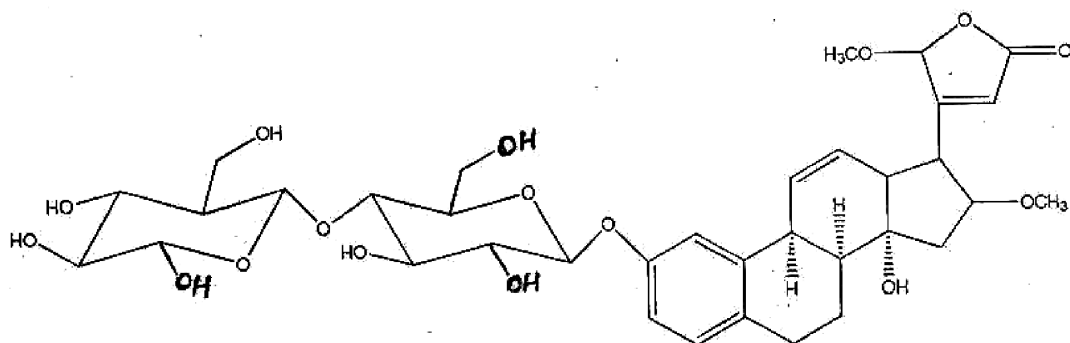


Figure 1: MP-19

CONCLUSION

In the present study, a triterpenoid glycoside was isolated from *Mimosa pudica* ethanol crude extracts. At concentrations of 25 – 100 mg/mL, the extract possesses antifungal activity (from being partially active to very active)

against *Aspergillus flavus* and *Trycophyton ruburum*, indicating that the plant contains potential antimicrobial components for the therapy of infections. Further investigation should be done to purify and isolate more bioactive components responsible for most of its documented pharmacological activities in order to promote the use of the plant in drug discovery.

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