

Original Research Article

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

Abstract:

Crude Ethanolic extract and isolated triterpenoid-glycoside from the crude extract of *Mimosa pudica* was used for antifungal activity towards *Aspergillus flavus* and *Trycophyton rubrum* using Well diffusion method at different concentrations of 25 mg, 50 mg and 100 mg/disc and the results have been illustrated. In this study, the entire plant parts of *Mimos pudica* were extracted using absolute ethanol. The TLC of the crude extract indicated many compounds with R_F values 0.41, 0.43, 0.56, 0.68, 0.89, and 0.90. The isolation and purification was 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 0.41. The spectral analyses involving $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, $^{13}\text{C-DEPT}$, $^{135}\text{COSY}$, 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 emergence of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial and viral infections, adding to the urgency to search for new infection fighting strategies. (Sieradzki *et al.*,

1999). Contrary to the synthetic drugs, antimicrobial substances of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious disease. (Iwu *et al.*, 1999). *Mimosa pudica* Linn 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 (Barneby, 1991). *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 name including

I. Sensitive plant

II. Humble plant

III. Shameful plant

IV. Touch-me-not

V. Chuimui

VI. Ant-plant

Vernacular- names:

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

54 Igbo Language: Agbogho mechie ukwu.

55 Hausa Language: Kama walkinka.

56 Yoruba Language: Ewe padimo/ Patomo

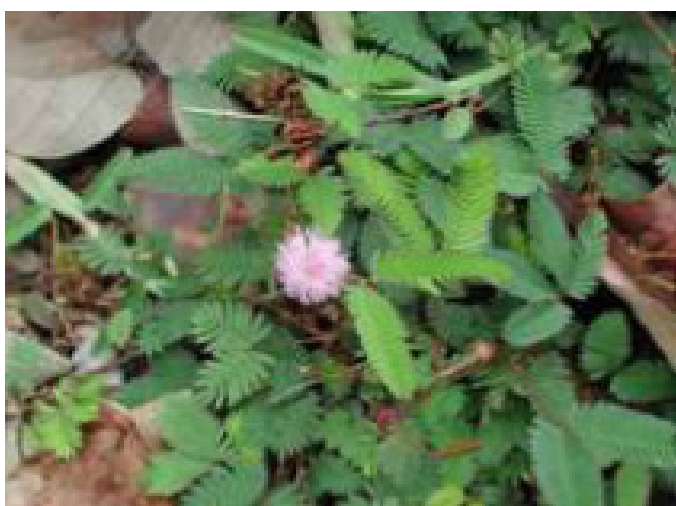
57 In European language/ culture areas we have nao- me- toque (touch- me- not),
58 sensitive or dormideira (roughly sleeper”) in the Portuguese (with the former
59 being more common in Portugal. Africa and Rio, dejaneiro, the middle in Sao
60 Paulo city and the Southern capitals and the latter elsewhere in Brazil), while in
61 Spanish, it varies in names such as mori- vivi or morivivi (DOMINICAN
62 REPUBLIC, PUERTO RICO and other Spanish- speaking Caribbean islands,
63 roughly translating to “I died, I lived”) (Union county College Biology
64 Department, 2008) and Dormilona (Costa Rica).

65 In Austronesia names vary more: in the philippines it is called makahiya, with
66 maka-meaning “quite” or tendency to be”, and –hiya meaning “ shy” or
67 “shyness”,while in Tonga for example it is known as Mateloi (false death)
68 (Churchward, 1959), being Putri- malu (shy princess) in Indonesia and Pokok
69 Semalu (shy plant), in Malaysia. In Sinhala (Sri Lanka) it is called Nidi kumba
70 (sleeping plant).

71 In south Asia many unrelated names are also common. In Hindi it is known as
 72 chhui-mui (that which dies upon touch). In Bengali, the shrub is called lojjaboti
 73 (“that bashful girl”). In Malayalam it is called thottavaadi (“wilts by touch). In
 74 marathi it is called lazalu (“shy”). In Tamil, it is called thottasiningi (“acts when
 75 touched”) and in kannada, it is known as muttidare muni (“angered by touch”).
 76 In Burmese (Myanmar) it is called hti ka yoan, which means “(crumbles when
 77 touched)”. In Liberia, it is known as the picker weed (Union county College
 78 Biology Department, 2008).

79 This plant has a history of use for treatment of various ailments and the most
 80 commonly used plant part for this purpose is the root; but flowers, bark and
 81 fruit can also be utilized. Several research works have been carried out to study
 82 about the phytochemical components of *Mimosa pudica* and also about the
 83 antimicrobial activity of the plant (Gandhiraja *et al.*, 2009). Phytochemical
 84 studies on *M. pudica* have revealed the presence of alkaloids, fatty acids, non-
 85 protein amino acid (mimosine), flavonoids, C-glycosides, sterols, terpenoids,
 86 and tannins (Genest *et al.*, 2008). Reported major pharmacological activities
 87 are; antiviral properties, aphrodisiac properties, antimicrobial properties, anti-
 88 venom activities, anti-hepatotoxic and antioxidant effects, diuretic effect,
 89 hyperglycemic effect, wound healing effect etc. (Amalraj and Ignacimuthu, 2002).
 90 Some of the isolated secondary metabolites are bufadienolide, D-pinitol,
 91 norepinephrine, P-coumaric acid, mimopudine, mimosine, potassium-5-O- β -
 92 gluco-(Clark-Lewis and Porter, 1972).pyranosygentisate etc. (Yadava and Yadav, 2001),

93 (Ueda *et al.*, 2000), (Sallaud *et al.*, 1995), (Fromm and Lautner, 2007; Nair *et al.*, 2007 and
 94 Genest *et al.*, 1999). Two well-known movements are observed in *M. pudica*: one
 95 is the very rapid movement of the leaves when it is stimulated by touch, heat
 96 etc, and the other is the very slow, periodical movement of the leaves called
 97 nyctinastic movement which is controlled by a biological clock (Ueda *et al.*,
 98 1999) .



99

100 *Mimosa pudica* leaves and flowers

101 **Materials and Methods**

102 **Sample collection and preparation:**

103 Fresh and wholesome parts of *Mimosa pudica* were collected during the month
 104 of October 2015, from Ndi-Ojigwe compound in Okoko Item, Bende Local
 105 Government Area of Abia State, Nigeria. The plant was identified and
 106 authenticated by Mr. I. Ndukwe in plant taxonomy section, forestry Department
 107 of Michael Okpara University of Agriculture Umudike, Nigeria. The fresh plant

108 materials were dried under shade to prevent interference of uv-radiation from
109 the sun. Dried plant materials were powdered using Mechanical grinder. Then
110 the powdered material was preserved in an air tight container; ready for
111 extraction.

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

113 2kg of the milled sample was percolated in 98% ethanol for 48 hours.
114 Thereafter, it was filtered through Whatmann Filter Paper (No.42). The
115 filtrate was concentrated using the Digital Heidolph Rotary-evaporator
116 (4000 series) at crude extract (48.9g). The crude extract was partitioned
117 between CHCl_3 and water and a CHCl_3 – soluble fraction (16.3g) was
118 obtained. 10.0g of the CHCl_3 fraction was then partitioned between
119 petroleum ether (60 – 80°C) and aqueous methanol. 5.0g of the CHCl_3
120 fraction was then subjected to column chromatography over silica gel
121 (200 mesh) and eluted gradually with 100ml petroleum ether, then
122 petroleum ether: CHCl_3 (95:5; 90:10; 85:15; 80:20; 75:25; 70:30; 65:35;
123 60:40; 55:45; 50:50; 40:60; 30:70; 20:80; 10:90), and 100ml CHCl_3 .
124 Then CHCl_3 : Methanol (90:01; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70;
125 20:80; 10:90) and 100ml methanol, followed by methanol: Ethyl acetate
126 (80:20; 60:40; 40:60; 20:80) and 100ml Ethyl acetate to yield twelve (12)
127 major fractions .Chromatographic (partition chromatography, column
128 chromatography and TLC) and spectroscopic (^1H -NMR, ^{13}C NMR,
129 COSY, DEPT, HMBC, HMQC, IR) techniques were employed to isolate,
130 purify, characterise and identify active constituents from CHCl_3 extracts
131 of the sample.

132

133 **Antifungal activity of extracts from *Mimosa pudica*:**

134 **Preparation of extract stock solution**

135 A stock of extract was prepared by dissolving 0.2 g (200 mg) of the plant
136 extract in 2.0 ml of dimethylsulphoxide (DMSO) to get a concentration of 100

137 mg/ml of the stock solution. This stock solution was diluted with sterile distilled
138 water to give concentration of 50 mg/ml and 25 mg/ml.

139 **Fungal Media (Potato dextrose Agar):**

140 200 g of Potato slices were boiled with distilled water. The potato infusion was
141 used as water source of media preparation. 20 g of dextrose was mixed with
142 potato infusion. 20 g of agar was added as a solidifying agent. These
143 constituents were mixed and autoclaved. The solidified plates were bored with
144 6mm diameter cork borer. The plates with Wells were used for antifungal
145 studies

146 **Preparation of discs**

147 Filter paper discs (Whatmann No. 1) were sterilized in hot air oven inside glass
148 Petri dishes at 160°C for 2 hours. Each disc was impregnated with 20 µl of the
149 plant extract solution at the various concentrations of 25 mg/ml 50 mg/ml and
150 100 mg/ml and labeled accordingly. The discs were placed in an incubator at 40
151 °C and left for 2 hours to dry. They were used immediately and the remaining
152 was stored at 4 °C.

153 **Disc diffusion test**

154 This was performed following the Kirby- Bauer method. Plates of Mueller
155 Hinton Agar (MHA, Hardy Diagnostics, USA) were prepared according to
156 manufacturer's instructions. The plates were dried in an incubator at 40 °C for
157 30 min. using a sterile swab stick, standardized cells suspension containing an
158 inoculums size of 5×10^8 CFU/ml was aseptically spread on the agar surface. The

159 discs of the extracts and antibiotics were placed on the inoculated plates of each
 160 test organisms. The plates were incubated at 35-37 °C for 16-18 hours. The
 161 diameter of any clear zone of inhibition obtained around the discs was measured
 162 manually using a transparent ruler.

163 **Antifungal activity of the plant extract:** The Crude ethanolic extract and the
 164 isolated compound labeled MP-19 were tested against fungal pathogens
 165 *Aspergillus flavus* and *Trycophyton rubrum* at concentrations of 25 mg, 50 mg
 166 and 100 mg. The experiment was replicated three times for each extract and
 167 antibiotic.

168 Results and Discussion:

169 Antimicrobial activity of extract of *Mimosa pudica*

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

S.No	Organisms	Concentration of (mg)/Zone of inhibition (mm)		
		25mg	50mg	100mg
1	<i>Aspergillus flavus</i>	13	16	22
2	<i>Trycophyton ruburum</i>	11	14	17

172

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

S.No	Organisms	Concentration of (mg)/Zone of inhibition (mm)		
		25 mg	50mg	100mg
1.	<i>Aspergillus flavus</i>	0.0	0.0	0.0
2.	<i>Trycophyton ruburum</i>	0.0	0.0	0.0

178
179 The results of the antifungal assay of the crude ethanolic extract of
180 *Mimosa pudica* labelled **MP** indicated that the plant exhibited antifungal
181 activity against the tested microorganisms at three different
182 concentrations of 25mg, 50mg and 100mg/disc. The zone of inhibition
183 was recorded and presented in table 1. While Table 2 Showed the effect
184 of the isolated compound labelled MP-19 on the micro-organisms. The
185 results of the experiment indicated that *Aspergillus flavus* and
186 *Trycophyton ruburum* showed resistance against the isolated compound
187 MP-19 from the crude ethanol extract of *Mimosa pudica*. These results
188 can show that the isolated compound may not be responsible for the
189 antifungal activity recorded in table 1.

190
191 Compound [2] labelled MP-19 was eluted with chloroform and petroleum
192 ether at the ratio of 50:50. Thin layer chromatography carried on MP-19
193 showed one spot (R_F 0.41).

194
195 Analysis of IR is shown in Table 3. The IR revealed V_{max} 2910cm^{-1} ,
196 1720cm^{-1} , 1600cm^{-1} and 1250cm^{-1} for aliphatic, carbonyl, olefinic,
197 aromatic and ether respectively.

198
199 Analysis of $^1\text{H-NMR}$ revealed the presence of olefinic protons at $\delta\text{H}5.20$,
200 cluster of peaks between $\delta\text{H}0.8$ to $\delta\text{H}2.35$ clearly indicated the
201 triterpenoid structure, and two methoxy protons signals at $\delta\text{H}4.058$ and
202 $\delta\text{H}4.239$. Then the presence of two anomeric protons at $\delta\text{H}5.45$ and

δ H5.25 confirmed the presence of two sugars. The spectrum also showed the presence of aromatic protons at δ H5.043- δ H5.315.

^{13} C-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 $C_{35}H_{46}O_{16}$ m/z 722 calculated, which is a triterpenoid-glycoside.

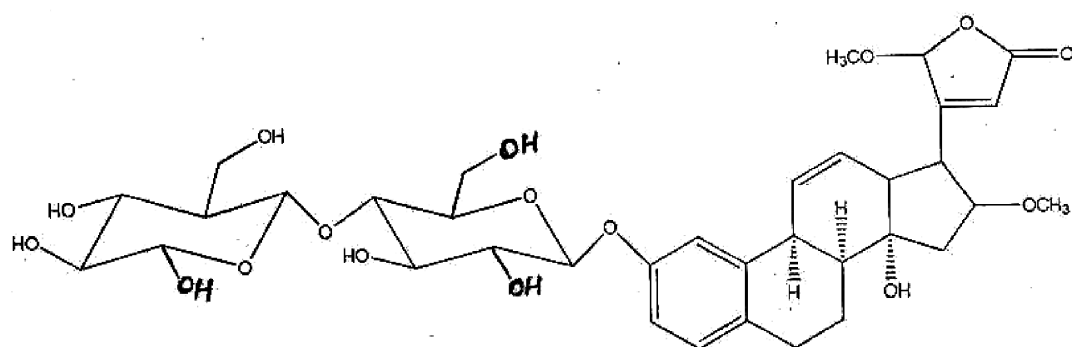


Figure 1: MP-19

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

CONCLUSION

In the present study, a triterpenoid-glycoside was isolated from *Mimosa pudica* ethanol crude extracts. The extracts possess strong antifungal activity 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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