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Secondary Metabolites from Mimosa Pudica: Isolation, Purification and NMR Characterization

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Abstract: *Aim: isolation, purification and NMR characterization of secondary metabolites in Mimosa pudica. Study design: extraction, isolation and identification of the secondary metabolties from the petroleum ether and metabolites extracts from Mimosa pudica.*
Methodology: *In the course of our study on Mimosa pudica, a compound was isolated and purified using different chromatographic techniques and the structure of the compound determined on the basis of their spectroscopic data (IR, HNMR, COSY, DEPT, MS).*
Place and Duration of Study: *this work was carried out in the chemistry Department Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria within the month June 2016 to December 2016.*
Result: *The entire plant parts of Mimosa was extracted using absolute ethanol, the TLC of the crude extract indicate the many compounds with Rf values 0.41,0.43,0.56,0.68,0.89 and 0.90. Purification: The results of several chromatographic techniques on the isolated gave rise to a triterpenoid- glycoside with Rf value 0.55. The spectral analyses involving HNMR, NMR, DEPT, ^{135} COSY, HMBC and IR, MS were useful in proposing the structure of the compound MP11.*
Keywords: *Mimosa pudica, Mimosine, secondary metabolites, chromatography, characterization.*

I. Introduction

According to World Health Organization (W.H.O), more than 80% of the world's population relies on traditional medicine for their primary health-care needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredient that can be used to treat chronic as well as infectious diseases. Several screening studies have been carried out in different parts of the world (Jina *et al.,* 2005).

There are several reports of the antimicrobial activity of different herbal extracts in different regions of the world. Because of the side effects and the resistance that pathogenic micro-organisms build against conventional antibiotics. Recently much attention has been paid to extracts and biologically active compounds isolated from different plant species used in herbal medicine (Jina *et al.,* 2005).

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. 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:
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") (Union county College Biology Department, 2008) 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) (Churchward, 1959), 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 (Union county College Biology Department, 2008).

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 (Gandhiraja *et al.,* 2009). 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 (Genest *et al.,* 2008). Reported major pharmacological activities are; antiviral properties, aphrodisiac properties, antimicrobial properties, anti-venom activities, anti-hepatotoxic and antioxidant effects, diuretic effect, hyperglycemic effect, wound healing effect etc. (Amalraj and Ignacimuthu, 2002). Some of the isolated secondary metabolites are bufadienolide, D-pinitol, norepinephrine, P-coumaric acid, mimopudine, mimosine, potassium-5-O-β-gluco-(Clark-Lewis and Porter, 1972).pyranosygentisate etc. (Yadava and Yadav, 2001), (Ueda *et al.*, 2000), (Sallaud *et al.,* 1995), (Fromm and Lautner, 2007; Nair *et al.,* 2007 and Genest *et al.,* 1999). 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 (Ueda *et al.,* 1999) .

Mimosa pudica leaves and flowers

II. Materials and Methods

Sample collection and preparation:

Fresh and wholesome parts of *Mimosa pudica* were collected during the month of August 2014, 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. Dried plant materials were powdered using Mechanical grinder. Then the powdered material was preserved in an air tight container; ready for extraction.

Extraction and Isolation of Plant Material:

2kg of the milled sample was percolated in 98% ethanol for 48 hours. Thereafter, it was filtered through Whatman Filter Paper (No 42). The filtrate was concentrated using the Digital Heidolph Rotary-evaporator (4000 series) at crude extract (48.9g). the crude extract was partitioned between $CHCl_3$ and water and a $CHCl_3$ – soluble fraction (16.3g) was obtained. 10.0g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60 – 80^{o}C) and aqueous methanol. 5.0g of the $CHCl_3$ fraction was then subjected to column chromatography over silica gel (200 mesh) and eluted gradually with 100ml petroleum ether, then petroleum ether: $CHCl_3$ (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 100ml $CHCl_3$. Then $CHCl_3$: Methanol (90:01; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90) and 100ml methanol, followed by methanol: Ethyl acetate (80:20; 60:40; 40:60; 20:80) and 100ml Ethyl acetate to yield twelve major fractions. Chromatographic (partition chromatography, column chromatography and TLC) and spectroscopic (^{1}H-NMR, ^{13}CNMR, COSY, DEPT, HMBC, HMQC, IR) techniques were employed to isolate, purify, characterize and identify active constituents from $CHCl_3$ extracts of the sample.

III. Results and Discussion

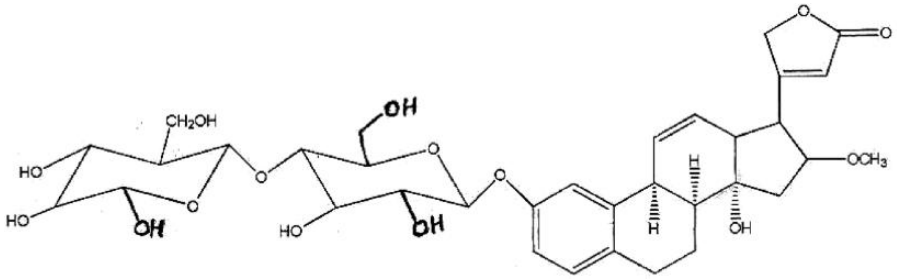

Fig 1: Structure of MP-11

Compound [1] labelled MP-11 was eluted with chloroform and petroleum ether at the ratio of 60:40. Thin layer chromatography carried on MP-11 showed one spot (R_F 0.55). Analysis of IR is shown in Table 1. The IR revealed Vmax 2980cm^{-1}, 1700cm^{-1}, 1600cm-1 and 1250cm^{-1} for aliphatic, carbonyl, olefinic, aromatic and ether respectively. Analysis of ^{1}H-NMR revealed the presence of olefinic protons at ɠH 5.20, cluster of peaks between ɠH0.8 to ɠH2.35 clearly indicated the triterpenoid structure, and three proton singlet at ɠH3.5 indicates the presence of a methoxy group in the molecule. Then the presence of two anomeric protons at ɠH5.45 and ɠH5.25 confirmed the presence of two sugars.

^{13}C-NMR spectrum revealed the presence of six aromatic carbons at ɠC 123.25, ɠ124.00, ɠ126.89, ɠC 127.01, ɠC 129.04 and ɠC 133.88. Methylene carbons chemical shift were seen at ɠC 21.60 – 8C 22.41, methane carbon peaks were observed at C ɠ26.96 to ɠC 33.39, while the signal at ɠC 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-11 was proposed as shown in figure [1] with molecular formula $C_{34}H_{44}O_5$ which is a triterpenoid-glycoside.

Table 1: Infra-red Analysis of MP-11

IR Absorption (cm^{-1})	Functional Group	Compound Type
2980	-CH_2	Aliphatic
1700	C=O	Carbonyl
1670	C=C	Olefin
1600	C=C	Aromatic
1250	C – O	Ether

IV. Conclusion

The result of this analysis revealed that the plant *Mimosa pudica* has many of its secondary metabolites responsible for its numerous biological activities yet to be isolated and documented. Thus the isolated compound contributes to the scientific evidence for the use of this plant in traditional medicine for the treatment of diseases in Nigeria

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