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Phytochemical investigation and enzyme inhibitory activity of *Mimosa pudica* Linn.

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ABSTRACT

The phytochemical studies on the plant of Mimosa pudica resulted in isolation of Mimosine (5 α amino 3 hydroxy 4 oxo 1 H (H) pyridine propianic acid), α- spinasterol, Phenyl ethylamine derivatives are being reported for the first time from this plants. These compounds have been characterized on the basis of spectral and other data. The isolated compounds (JS I, JS II, JS III and JS IV) were found out in amylase and urease enzyme. The isolated compounds JS IV only showed high enzyme inhibitory activity.

Keywords :Phyto chemical, *Mimosa pudica*, Enzyme inhibitory activity , IR, NMR.

INTRODUCTION

Mimosa pudica, (Family : Mimosaceae). A diffuse under shrub, 50-90cm high cultivated through out the tropical and subtropical parts of India. Stem and rachis clothed with pickles. Leaves bipinnate digitatively arranged, with 10-20 pairs of leaflets; flowers in pinkish globoseheads. Properties and uses ascribe in Traditional medicine[1,2,3,4]. A decoction of the root of the plant is considered useful in gravel and other urinary complaints. A paste of the leaves is applied to glandular swellings. The juice of leaves is used in dressings for sinus and also an application for sores and piles. The juice of leaves is used in the treatment of diabetes mellitus. The roots have contraceptive properties. Preliminary investigations on the diuretic activity of *Mimosa pudica* Linn. Leaf decoction showed moderate diuretic response in albino rats and dogs. The aqueous extract of *Mimosa pudica* Linn. Seeds showed nematicidal activity against the second stage juveniles of meloidogyne incognita chitwood [5,6]. Anti depressant activity of *Mimosa pudica* Linn. was studied by Molina et al[7] the root

powder of *Mimosa pudica* Linn. On estrous cycle and ovulation in cycling female albino rat was studied by Valsala[8]. Neutralization of lethality, myotoxicity and toxic enzymes of Naja kaouthia venom by *Mimosa pudica* Linn. Wa studied by Mahnt [9].The quantitative examination o fdifferent sugars indicates that the mucilage is composed of D-xylose and D-gluconic acid R.K.Hulyalkr[10]. Seeds were reported to yield sitosterol. The leaves contained alkaloids, tannins were found absent in the 50 percent ethanolic extract of the plant mucilage of *Mimosa pudica* Linn. was found to be a neutral polysaccharide. Mimosa Himalayana Gamble roots collected from jarwa forest (Madhya Pradesh) showed the presence of saponins, alkaloids and flavonoids in a preliminary chemical study [11,12,13]. However, root samples collected from the Sohellwa forest range in the same state, contained flavonoids but were found devoid of saponins and alkaloids. Other parts of the plant) i.e., the whole plant (excluding roots) was found to be devoid of tannins[14]

EXPERIMENTAL SECTION

Plant materials

Fresh aerial parts of *Mimosa pudica* Linn were collected during the month of April 2007, from Thamaraipatti village, Madurai District, Tamilnadu. The plant was identified and authenticated by Dr.Stephen Botanist Professor in American college, Madurai and herbarium specimen deposited at K.M.College of Pharmacy, Madurai. The fresh plant material was then dried under shade. Dried plant material was powdered using mechanical grinder and passed through 60 # sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Plant collection and extract preparation

About 1kg of dry coarse powder of the whole plant of *Mimosa pudica* linn was extracted first with petroleum ether (40-60°C) by hot continuous percolation using Soxhlet apparatus. The extractions were continued for 72 hours. The petroleum ether extract was filtered and concentrated to a dry mass by using vacuum distillation. A greenish black residue was obtained.

The marc left after the petroleum ether extract was taken and subsequently extracted with chloroform up to 72 hours. The chloroform extract was then filtered and concentrated to a dry mass. A dark green residue was obtained.

The marc left after the chloroform extraction was dried and extracted with methanol. The extraction was continued up to 72 hours. The methanol extract was filtered and concentrated by vacuum distillation. A dark green residue was obtained.

Preparation of isolated compounds from chromatography

After screening the various extracts obtained from 1kg of coarse powder, the petroleum ether extract and chloroform extract were found to be promising. The petroleum ether extract (62gms) was a dark gummy residue. The chloroform extract was green viscous residue (21gms) were mixed and chromatographed over silica gel (100-200mesh). The column was then developed with a series of solvent starting with n-hexane, petroleum ether, benzene, chloroform, ethyl acetate and methanol. Fraction of 50ml were collected upto ethyl acetate-methanol system.

Rechromatography

The T.L.C. guided identification of the fractions 5-12 which was brownish semisolid, was re-chromatographed in a column of neutral alumina and eluted with benzene-chloroform systems. A total of 14 fractions of 25ml each were pooled, concentrated and dried, which is named compound JS-I.

The T.L.C. guided identification of the fractions 23-41 which was yellowish semisolid, was re-chromatographed in a column of neutral alumina and eluted with chloroform systems. A total of 14 fractions of 25ml each were pooled, concentrated and dried, which is named compound JS-II.

The T.L.C. guided identification of the fractions 42-71 which was brownish semisolid, was re-chromatographed in a column of neutral alumina and eluted with chloroform systems. A total of 14 fractions of 25ml each were pooled, concentrated and dried, which is named compound JS-III.

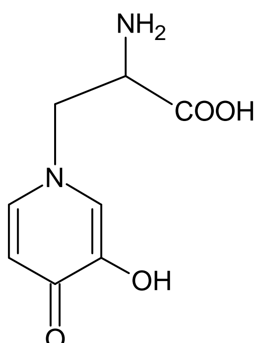
Preliminary phytochemical investigation

The qualitative chemical test of various extracts of *Mimosa pudica* was carried out using standard procedure[15,16,17,18] .Carbohydrate, sterols, Coumarins, Flavonoids and Alkaloids are present in the extracts.

Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on a Perkin –Elmer FTIR using KBr discs. PMR on Bruker spectrospeir 200MHz NMR instrument using $CDCl_3$ as solvent and TMS as internal reference (Chemical shifts in δ, ppm) Elemental analysis of all the synthesized compounds were performed on a Perkin Elmer 2400. Series – II Elemental CHNS analyzer.

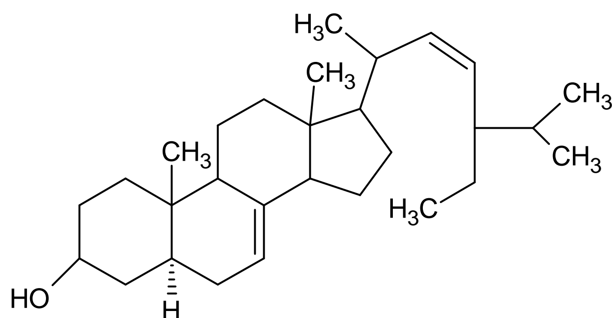
Phytochemical investigation[19,20,21]

Compound –A. (JS-I)



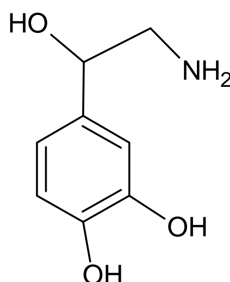
It is solid light brown colour. The solvent system used for TLC is chloroform: pet ether (9:1).The Rf value is 0.43. Melting point 147-149°C .It is soluble in benzene, chloroform, insoluble in n- hexane, alcohol and water. IR spectral data 3382cm^{-1} N-H stretching, 2921cm^{-1} C-H stretching, 1664, 1550cm^{-1} C=C stretching, 769cm^{-1} C-H out of plane Bending, 1463cm^{-1} C-H bending. NMR spectral data 0.9-1.7δ side chain protons of methyl and methylene groups(CH_3 and CH_2), 2.05 δ methylene group adjacent to C=C, 2.35-2.6 δ methylene proton adjacent to C=N, 7.25-8.2 δ aromatic proton. UV spectral data λ max 330nm

Compound-B(JS-II)



It is solid light yellow colour. The solvent system used for TLC is chloroform: pet ether (1:9).The R_f value is 0.89. Melting point 76-78°C .It is soluble in benzene, chloroform, insoluble in n- hexane, alcohol and water. IR spectral data 2921-2854cm^{-1},C-H stretching, 1733cm^{-1} C=O stretching, 1461cm^{-1} C-H bending. NMR spectral data, 0. 8δ methyl proton groups(CH_3 and CH_2), 1.25 δ methylene proton,1.65 δ methylene proton adjacent to C=C, 2.1δ methylene proton adjacent to carbonyl group.

Compound –C (JS-III)



It is solid light yellow colour. The solvent system used for TLC is chloroform: pet ether (9:1).The R_f value is 0.682. Melting point 182-184°C .It is soluble in benzene, chloroform, insoluble in n- hexane, alcohol and water. Melting point 182-184°C. It is soluble in benzene, chloroform, slightly soluble in n-hexane, insoluble in alcohol and water. IR spectral data 3436cm^{-1} O-H stretching,3085cm^{-1} Sp^{2}C-H stretching, 2921cm^{-1} Sp^{3}C-H stretching, 1693cm^{-1}C=O stretching, 1627, 1542cm^{-1} C=C stretching,1286cm^{-1} C-N stretching,1187cm^{-1} C-O stretching, 808cm^{-1} N-H Bending. NMR spectral data 1.2δ alicyclic methylene proton, 1.6 δ methylene proton, 2.2δ methylene group adjacent to aromatic ring, 2.7δ methylene proton, 6.0 δ vinylic proton, 7.3δ aromatic proton, 13.3δ enolic O-H proton.

Table 1: Preliminary phytochemical investigation of *Mimosa pudica*

Name of extract	Sugar	Flavanoid	Coumarin	Alkaloid	Sterol	Glycosides
Petroleum ether	+	-	-	+	+	-
Chloroform	-	-	+	+	+	-
Methanol	-	-	+	+	-	-

Enzyme inhibitor activity[22-24]

Preparation of the enzyme
1. Salivary amylase
10ml of the saliva was collected and diluted to 100ml with cold phosphate buffer pH 7.1. The solution was centrifuged at 8000 rpm for 20mts and the clear supernatant was used.

2. Extraction of crude inhibitor
There are several reports on the extraction of enzyme inhibitors from plant materials. We have adopted the method Buonocore et.al 100mgs of dried plant powder was extracted with 2.5% of cold TCA with magnetic stirrer for 45mts. The solution was centrifuged to get a clear supernatant, which was neutralized to pH 7 with dilute sodium carbonate and used for the assay of enzyme inhibition. This is referred as the crude inhibitor.

Amylase assay
Amylase activity was determined by the method of Bernfeld 1ml of the enzyme solution was added to 2ml of phosphate buffer pH6.9 containing 2MNacl and the reaction started with the addition of 2ml 1% soluble starch solution. The tubes were incubated at 37°C fir 20mts. The reaction was arrested by the addition of 1ml for dinitrosalicylic acid colour reagent. The tubes were kept in a boiling water bath for 10mts, cooled and diluted t a final volume of 10ml with distilled water. The absorbance was measured at 530nm in an Erma photoelectric colorimeter. Amylolytic activity was calculated as maltose equivalents liberated. One unit of enzyme is defined as the milligrams of maltose liberated per minutes under the assay conditions. The standard curve was constructed using pure anhydrous maltose. The assays were run along suitable blank (without enzyme).

To measure the amylase inhibitory activity, suitable amount of 0.1% mercuric chloride was pre incubated with the enzyme, and the assay was carried out as described above. The inhibition caused by 0.5% of mercuric chloride was arbitrarily fixed as 100% inhibition. Suitable amounts of plant extract were similarly tested for the presence of amylase inhibitors in the extract. The decrease in the inhibitory activity of the crude plant extract was defined on the basis of these parameters. The crude extracts showed about 80-100% inhibition.

$$\% \text{ inhibition} \quad \frac{\text{Abs of control (no inhibitor)} - \text{Abs of sample}}{\text{Abs of control (no inhibitor)}} \times 100$$

The crude extracts inhibitory activity was not destroyed when heated to 80°C, thus revealing its likely non proteinaceous nature. From these observations it can be inferred that further purification can lead to more information on these natural enzyme inhibitors.

3. Assay of Urease
Urease enzyme was prepared from horse gram seeds. The assay of enzyme was carried out according to the method described by Malhothra using Nessler's reagent. A standard was prepared using NH_4SO_4.urease inhibition was compared with parachloromercuric benzoate (PCMB), the activity of which was set arbitrarily to 100%. Under these conditions the crude extract showed about 100% of inhibition.

Table 2: Enzyme inhibitory activity of *Mimosa pudica*

Enzyme	Standard inhibitor	Extracts (percentage on inhibition)				
		1	2	3	4	5
Amylase	Mercuric chloride	100%	Nil	Nil	trace	100%
Urease	Para chloro mercuric benzoate	100%	Nil	Nil	trace	75%

1. Standard inhibitor
2. JS-I
3. JS-II
4. JS-III
5. JS-IV

RESULTS AND DISCUSSION

The melting point of the isolated compounds were found out by open capillary tube method and the results were uncorrected. The purity of the compounds was checked by TLC using silica gel G as an adsorbent, ethyl acetate and chloroform (9:1) were used as mobile phase. The spot was visualized by iodine vapour or dinitrophenyl hydrazine solution. The structure of the isolated compounds was characterized by its IR, HNMR spectral analysis in which it complies with the normal values. The enzyme inhibitory activity in JS IV was significant when compared to other isolated compounds.

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