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# Phytochemical and Therapeutic Evaluation of an Ethnomedicinal Plant of Tripura: *Mimosa pudica*

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The methanolic extract of whole plant of *Mimosa pudica*, an ethnomedicinal plant of Tripura, India showed spermicidal and anticoagulant activity and separated compounds were also evaluated. The structure of the most active component is reported.

**Key Words: Spermicidal activity, Anticoagulant activity, *Mimosa pudica*, Structure.**

## INTRODUCTION

*Mimosa pudica* commonly known as 'Touch Me Not' is widely found in Tripura, India and is generally used for number of diseases which include burning sensation, piles, constipation, fever, diabetes *etc*. It also gives good results in gynecological disorders[1]. Tribal people of Tripura are also traditionally utilizing the plant for certain purposes such as anticoagulant, anti-amoebic and also as a spermicidal agent. Keeping in mind the traditional application, phytochemical investigation, of methanolic extract of whole plant of *Mimosa pudica* growing in Tripura, India and its spermicidal, anticoagulant activity are reported in this compilation. Further same activities were also evaluated for separated compounds and the structure of the most active component was elucidated.

## EXPERIMENTAL

The whole plant of *Mimosa pudica* were collected in the month of November, 2007, from the area of Regional Institute of Pharmaceutical Science And Technology, Agartala, Tripura, India, identified by the expert of Pharmacognosy department of the Institute. The whole plant was cut and shed dried for 7 days, powdered, passed through a 40 mesh sieve and then subjected for methanolic extraction in a Soxhlet Apparatus. TLC was performed for the extract using various solvent systems *viz*., benzene, butanol:water:dioxane, butanol:acetic acid:water and the $R_f$ values[2-4] were reported in Table-1. The separation of component from the extract was carried out by column chromatography using ethyl acetate as a mobile phase. The solvents from the separated fractions were removed and the dried residues were collected. The dried crude extract and separated components were dissolved in DMF for spermicidal studies and in case of anticoagulant study distilled water was used as the vehicle. The structure of the most active component was elucidated with the help of different spectra such as IR, NMR, LC-MS and the melting point was determined by open capillary method (melting point apparatus used: INDO M-AB-92) and is not corrected. The methanolic extract of *Mimosa pudica* (MEMP) was also subjected for the phytochemical investigation by adopting standard procedure.

TABLE-1
$R_f$ VALUE DETERMINATION OF *Mimosa pudica*

| Solvent system | Spot number | Distance traveled by components (cm) | Distance traveled by solvent (cm) | $R_f$ values |
|---|---|---|---|---|
| BWD | 1 | 0.9 | 5.3 | 0.04 |
| | 2 | 1.7 | | 0.38 |
| | 3 | 2.7 | | 0.62 |
| | 4 | 3.1 | | 0.80 |
| | 5 | 4.7 | | 0.98 |
| BAW | 1 | 0.4 | 5.8 | 0.06 |
| | 2 | 0.9 | | 0.15 |
| | 3 | 2.7 | | 0.46 |
| | 4 | 4.0 | | 0.68 |
| | 5 | 4.6 | | 0.79 |
| | 6 | 5.0 | | 0.86 |
| B | 1 | 0.7 | 5.3 | 0.13 |
| | 2 | 1.8 | | 0.33 |
| | 3 | 2.9 | | 0.54 |
| | 4 | 3.2 | | 0.60 |
| | 5 | 3.9 | | 0.73 |
| | 6 | 4.7 | | 0.88 |

BAW = Butanol:acetic acid:water (4:2:1), BWD = Butanol:water: dioxane (4:2:1), B = Benzene.

**Spermicidal activity[5]:** Spermicidal activity of the extract and separated component were carried out by using healthy human sperm (100-150 million spermatozoa/mL, ≥ 80 % motility, 2.1 mL/ejaculate, pH = 7.9). 1 mL of the sperm volume was subjected in the study for each concentration. Sperm volume and extract volume ratio was 10:1 for each case. After the treatment of sperm with crude extracts and separated components the motility of the sperm were recorded after 10, 20 and 30 min. The effect of the vehicle (DMF) was also observed and recorded in Table-2. Total 6 samples of sperm were subjected for tests.

TABLE-2
EFFECT OF THE EXTRACT AND SEPERATED COMPONENTS OF *Mimosa pudica* ON SPERM

| Extract/component (1 mg/mL) | Percentage decrease in motility (average result ± SEM) | | |
|---|---|---|---|
| | 10 min | 20 min | 30 min |
| Crude Extract | 20 ± 0.013 | 31 ± 0.026 | 38 ± 0.032 |
| MP-1 | 0 | 15 ± 0.041 | 27 ± 0.084 |
| MP-2 | 10 ± 0.034 | 2 ± 0.072 | 28 ± 0.025 |
| MP-3 | 15 ± 0.032 | 21 ± 0.071 | 26 ± 0.043 |
| MP-4 | 20 ± 0.015 | 30 ± 0.024 | 42 ± 0.086 |

*MP-1 to MP-4 are the separated components of the extract *Mimosa pudica* by column chromatography using ethyl acetate as mobile phase.

**Anticoagulant activity[6]:** The anticoagulant activity was studied both *in vitro* and *in vivo*. The *in vitro* test was carried out by taking human blood and *in vivo* test was carried out in rabbit.

In the *in vitro* anticoagulant study, blood was collected from six healthy volunteer and the clotting time was recorded from the crude extract, control (distilled water) and compared with the standard anticoagulant EDTA (mixture 1:1 ratio). Results are shown in Table-3.

TABLE-3
*IN VITRO* ANTICOAGULANT STUDY OF THE EXTRACT OF *Mimosa pudica*

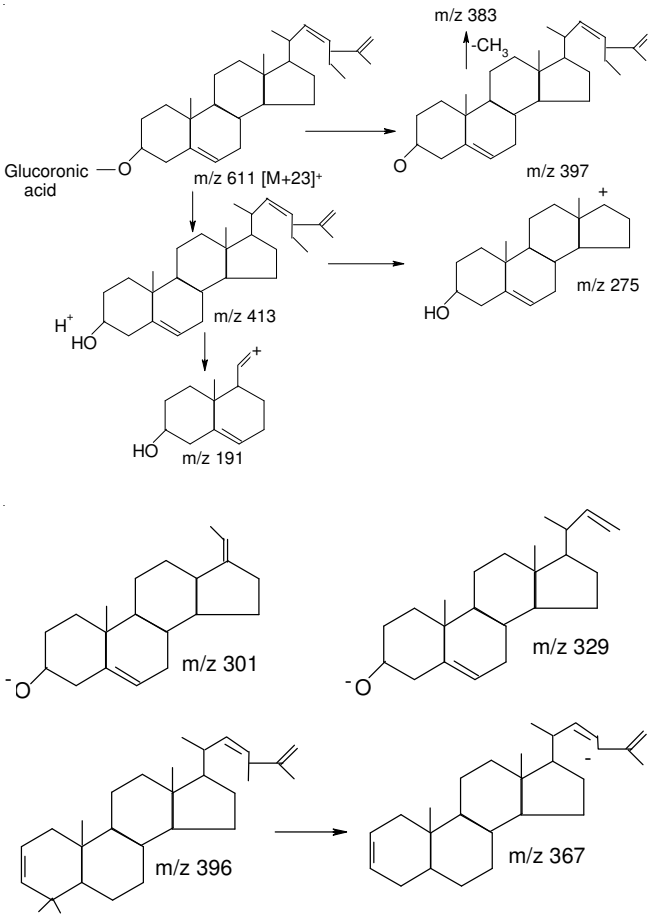
| Volunteer No. | Blood withdrawn (mL) | Normal clotting time (s) | Clotting time of control (s) | Clotting time with extract (s) | Clotting time of standard (EDTA) (s) |
|---|---|---|---|---|---|
| 1 | 3 | 280 | 282 | 640 | 720 |
| 2 | 3 | 270 | 270 | 630 | 710 |
| 3 | 3 | 260 | 261 | 620 | 700 |
| 4 | 3 | 250 | 251 | 610 | 695 |
| 5 | 3 | 265 | 264 | 625 | 710 |
| 6 | 3 | 245 | 246 | 615 | 690 |

The *in vivo* test was carried out in rabbit and the anticoagulant activity was assayed by observing the bleeding time. The animals were divided into two groups, each consist of six animals. The first group of animals received the vehicle only and served as control. The second group of animals received the crude extract at a dose of 100 mg/kg body weight intraperitoneally. The bleeding time was recorded at 0 time, after 0.5, 1.0 and 2.0 h. The bleeding time was also assayed by pricking in the marginal ear vein[7] and the time at which the bleeding stops is considered as the bleeding time. The results are presented in Table-4.

TABLE-4
OBSERVATION OF BLEEDING TIME OF THE EXTRACT OF *Mimosa pudica* IN RABBIT

| Extract/control | Rabbit No. | Bleeding time in seconds after | | | |
|---|---|---|---|---|---|
| | | 0 h | 0.5 h | 1.0 h | 2.0 h |
| Control | 1 | 22 | 22 | 22 | 22 |
| | 2 | 20 | 20 | 20 | 20 |
| | 3 | 21 | 21 | 21 | 21 |
| | 4 | 23 | 23 | 23 | 23 |
| | 5 | 24 | 24 | 24 | 24 |
| | 6 | 21 | 21 | 21 | 21 |
| Extract | 1 | 22 | 26 | 30 | 35 |
| | 2 | 20 | 24 | 28 | 33 |
| | 3 | 21 | 25 | 29 | 34 |
| | 4 | 23 | 27 | 31 | 36 |
| | 5 | 24 | 28 | 32 | 37 |
| | 6 | 20 | 24 | 28 | 33 |

**Structure elucidation:** The MP4 component was subjected for structural elucidation by spectral analysis. In beginning the compound was subjected for LC-MS and it was found that the compound was showing a single peak in LC chromatogram at 254 nm with the absorbance 0.578, which indicating the purity of the compound. The fragmented structures of the compound are as below:




From the above fragmentation, pattern of ESI-MS positive and negative mode spectra, it was clear that the compound contains a sterol nucleus with three double bonds and attached with glucorinide at C-3. The IR spectrum shows the presence of -OH group at 3400 $cm^{-1}$ and C=O group at 1658 $cm^{-1}$. The

$^1$H NMR spectrum shows the sugar moiety protons at δ 20.9 as bond singlet a part from the other protons at δ 0.78, δ 0.81, δ 1.19.

## RESULTS AND DISCUSSION

The ethnomedicinal plant of Tripura, India, *Mimosa pudica* was extracted well in methanol and total four components were separated by column chromatography and they are designated as MP-1, MP-2, MP-3 and MP-4. The methanolic extract of *Mimosa pudica* was subjected for different phytochemical investigation and therapeutic evaluation as mentioned. TLC study for the extract in different mobile phases were supporting the presence of 5-6 components in the extract. Preliminary qualitative chemical tests were showing presence of alkaloids, reducing sugars, steroid. The extract was delaying the clotting time *in vitro* which was nearer to standard EDTA. In case of *in vivo* test it was found that the bleeding time was increased. Extract also possessed good spermicidal activity. Separated four components were also tested for the spermicidal activity. Component No. 4 that is MP-4 was showing spermicidal activity more than the extract which was recorded 42. The melting point of MP-4 was found 139 ºC. The glucoronic acid conjugated steroidal structure was also elucidated for this component.

## Conclusion

The methanolic extract of *Mimosa pudica* posses good anticoagulant and spermicidal activity and the most active component of the extract is bearing a steroidal structure.

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**ERRATUM**

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# Structural, Optical and Photoconductivity Studies on Benzoyl Glycine NLO Single Crystals

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