WOUND HEALING EFFECT OF VARIOUS EXTRACTS OF MIMOSA PUDICA

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Summary

In the Present study methanolic, chloroform and diethyl ether extracts of Mimosa pudica Plants were evaluated for its wound healing activity in the form of Ointment dosage form in excision wound model in albino rats. The methanolic extract ointment of Mimosa pudica showed a significant effect in excision wound model as comparable to standard drug and other two extracts of ointment, by calculating the parameters, percentage closure of excision wound model. The Isolated compounds were characterized by instrumental analysis and IR.

Key words: Mimosa pudica, Wound healing, excision model

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Introduction

Mimosa pudica [1,2] Linn is a sensitive plant belonging to the family Leguminaceae, distributed in Brazil, India. Mimosa pudica is well known for its rapid plant movement. The plant shows a peculiar movement NYCTYNASTIC movement i.e. the leaflets fold together in the evening and the whole leaves droops downward. It then reopens at sunshine. The leaves also close up under various other stimuli such as touching, warming or shaking. The shrubs can also be transmitted to neighboring leaves. This type of movement is called SEISMONASTIC movement. The treatment of diseases in early days has begun by using various medicinal plants. They served as a good tool in altering different clinical conditions. Our land is having a vast heritage of knowledge and expertise in herbal medicine from different cultures and civilization. The purpose of the plant work is to identify the active ingredient through scientific methods and to study the pharmacological activities of the plant in shoot and root extracts of mimosa pudica.

Materials & methods

Plant material: The plant material was collected from Thaniparai hills, near watrap, tamilnadu. It was authenticated by Dr.Stephan, Dept of Botany, The American college, Madurai.

Extraction process [3]

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with methanol, chloroform and Diethyl ether and the residual marc was collected.

The extract was evaporated under reused pressure using a rotovac evaporator until all the solvent had been removed to give an extract sample with a yield of 18% w/w, 16 %w/w and 13% w/win relation to the dried starting material. Preliminary Phytochemical analysis was carried out to identify presence of Phytoconstituents in the crude extract.

The percentage yields of *Mimosa pudica* Extract

WEIGHT OF DRUG	EXTRACTION PATTERN	SOLVENT USED	WEIGHT OBTAINING (gms)	PERCENTAGE YIELD
500gms Mimosa pudica powder	Soxhlet apparatus	Methanol Chloroform Diethyl Ether	16gm 20gm 13gm	6.4% 8% 5.2%

Isolation and Identification
Column Chromatography: [4,5,6,7]

Chromatography is a separation technique of complex mixture. Currently there are many techniques are used. Among them "Column Chromatography" is a simple technique. The separation of components in Column Chromatography involves the principle adsorption. (i.e.) The components of mixture have different affinity towards adsorbent material hence, they gets adsorbed and migrate at different rate. So, it is possible to isolate single component by adjusting the solvent system. (i.e.) By increasing or decreasing the polarity of solvent system.

Sample Preparation:

The methanol, chloroform and Diethyl ether extracts (2gms) were dissolved in small amount of chloroform mixed thoroughly, with silica gel and dried to have free flowing nature. This mixture was taken for column study.

Preparation of Column:
Method: Wet Packing.

The absorbent material, silica gel was mixed with petroleum ether and poured gently from the top of the column to a desire length then the same solvent was run through the column for 2-3 times to prevent air entrapment and the solvent used was maintained up to 10cm above the column bed. The sample mixture was poured from the top of the column with the aid of funnel. The column was allowed to keep over night, undisturbed. On the next day, column was eluted with different solvents with gradually increasing the polarity, by changing the solvent. (Petroleum ether, Benzene, Chloroform, Ethyl Acetate and Methanol). The flow rate of solvent system was adjusted between 16 – 20 drops per minute. Each fraction was collected to maximum of 100ml and it was evaporated at low temperature. Then it is identified by TLC and chemical tests.

Various fractions of column chromatography is given in Table – 1

Various Fraction of Column Chromatography: (Table 1)

Methanol Extract

S.No.	Solvent	Ratio %	Nature of Residue
1	Petroleum Ether: Benzene	20:80	Colorless Residue
2	Petroleum Ether: Benzene	50:50	Colorless Residue
3	Benzene	100	Light Green Residue
4	Benzene: Chloroform	70:30	Light Green Residue
5	Benzene: Chloroform	20:80	Green Residue
6	Chloroform	100	Green Residue
7	Chloroform: Ethyl Acetate	80:20	Greenish Yellow Residue
8	Chloroform: Ethyl Acetate	20:80	Greenish Yellow Residue
9	Ethyl Acetate	100	Light Brown Residue
10	Ethyl Acetate: Methanol	60:40	Light Brown Residue
11	Ethyl Acetate: Methanol	20:80	Dark Brown Residue
12	Methanol	100	Brownish Residue

Chloroform Extract

S.No.	Solvent	Ratio %	Nature of Residue
1	Petroleum Ether: Benzene	50:50	Colorless Residue
2	Petroleum Ether: Benzene	20:80	Colorless Residue
3	Benzene	100	Light Green Residue
4	Benzene: Chloroform	40:60	Light Green Residue
5	Benzene: Chloroform	20:80	Green Residue
6	Chloroform	100	Green Residue
7	Chloroform: Ethyl Acetate	70:30	Greenish Yellow Residue
8	Chloroform: Ethyl Acetate	10:90	Greenish Yellow Residue
9	Ethyl Acetate	100	Light Brown Residue
10	Ethyl Acetate: Methanol	70:30	Light Brown Residue
11	Ethyl Acetate: Methanol	20:80	Dark Brown Residue
12	Methanol	100	Brownish Residue

Diethyl Ether Extract

S.No.	Solvent	Ratio %	Nature of Residue
1	Petroleum Ether: Benzene	40:60	Colorless Residue
2	Petroleum Ether: Benzene	10:90	Light Green Residue
3	Benzene	100	Light Green Residue
4	Benzene: Chloroform	30:70	Green Residue
5	Benzene: Chloroform	10:90	Green Residue
6	Chloroform	100	Green Residue
7	Chloroform: Ethyl Acetate	80:20	Greenish Yellow Residue
8	Chloroform: Ethyl Acetate	20:80	Greenish Yellow Residue
9	Ethyl Acetate	100	Light Brown Residue
10	Ethyl Acetate: Methanol	60:40	Light Brown Residue
11	Ethyl Acetate: Methanol	10:90	Dark Brown Residue
12	Methanol	100	Brownish Residue

THIN LAYER CHROMATOGRAPHY: [8,9,10,11,12,13,14]

Glass plates with silica gel-G used to perform TLC**.** Silica gel was used as stationery phase, while the mobile phase system consists of chloroform and methanol (9:1). Eluted spots were spotted on TLC and developed by using Iodine globules spots were identified and their Rf values were determined by using the formula,

Rf value = Distance Traveled by Solute / Distance Traveled by Solvent

The same Rf value portion were mixed and evaporated to dryness by using vacuum drier and proceeded for IR studies.

Thin layer chromatographic data analysis of Methanolic Extract

Detecting Reagent	Distance Run by Solvent	Number of spots	Distance Run by solute	Value	Color spot
Iodine	15cm	5	8 8.5 9.3 10.4 12.8	0.5333 0.5666 0.6200 0.6933 0.8533	Greenish Black Light Green Light Green Light Green Light Brown

Note: Solvent system (Ethyl Acetate: Methanol (20:80))

Thin layer chromatographic data analysis of Chloroform Extract

Detecting Reagent	Distance Run by Solvent	Number of spots	Distance Run by solute	Value	Color spot
Iodine	13cm	5	8.4 9 9.5 10 10.4	0.6461 0.6923 0.7307 0.7692 0.8	Light Green Light Green Light Brown Dark Brown Dark Brown

Note: Solvent system (Ethyl Acetate: Methanol (20:80))

Thin layer chromatographic data analysis of Diethyl Ether Extract

Detecting Reagent	Distance Run by Solvent	Number of spots	Distance Run by solute	Value	Color spot
Iodine	14cm	5	7.5 8 9.4 10.5 11.2	0.5357 0.5714 0.6714 0.7500 0.8000	Light Brown Light Green Dark green Brownish Orange Yellow

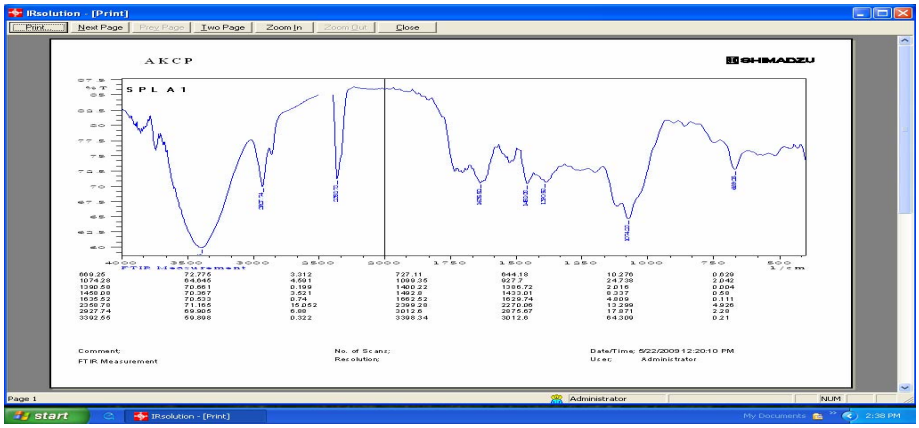
Note: Solvent system (Ethyl Acetate: Methanol (10:90))

Spectral Evidence for Isolated Compound

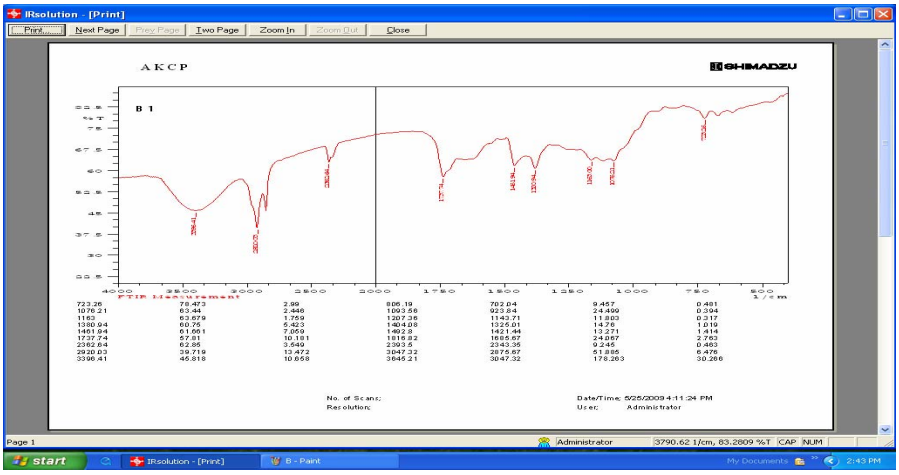
FTIR Spectral Analysis:

The structure of isolated compound was elucidated by Shimadzu – 8400 Series Fourier Transformer - Infrared Spectrophotometer in KBr pallet method. IR results are shown below:

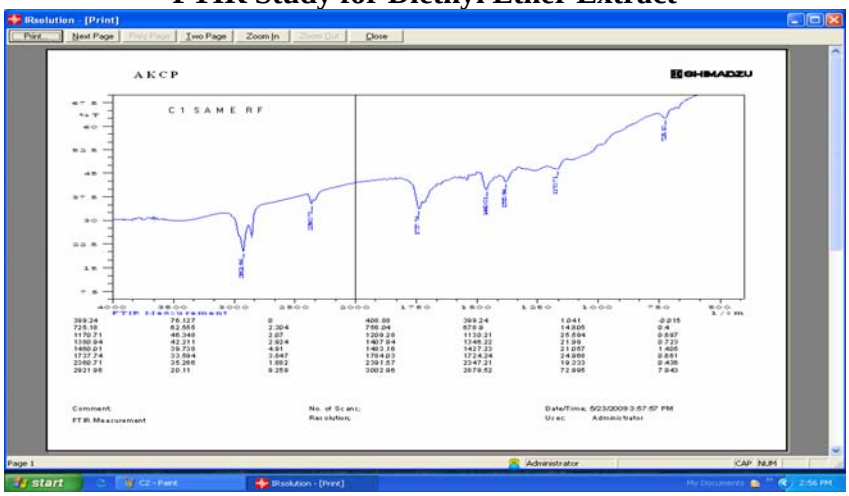
FTIR Study for Methanol Extract



FTIR Study for Chloroform Extract



FTIR Study for Diethyl Ether Extract



Qualitative Chemical Evaluation [15]

All the extracts obtained were subjected to qualitative tests for various plant constituents and observed that presence of glycosides, Phytosterol and alkaloids as major active constituents are confirmed by suitable chemical tests.

Formulation of Ointment:[16,17,18]

Ointment Formulation: shown in Table

Type: Water miscible base.

Method of Preparation: Mixed the ingredients, heated gently with stirring until homogenous mixture forms. Stirred to cool.

Types of Ointment prepared: There are three types of ointments are prepared. There are,

1. Base + Mimosa pudica Methanolic Extract
2. Base + Mimosa pudica Chloroform Extract
3. Base + Mimosa pudica Diethyl Ether Extract

10% concentration of ointment was prepared.

Method of preparation: In this preparation 1gm of suitable extract is mixed with 10gms of ointment base (10%). Then it is stirred well until homogenous base is obtained.

S.No.	Ingredients	Official Formula	Working Formula
1.	Emulsifying wax	30gm	3gm
2.	White soft paraffin	50gm	5gm
3.	Liquid paraffin	20gm	2gm

Pharmacological screening [19,20,21,22,23]

Excision Wound Model:

Animal:

Wister Albino rats (150-200gms).

Procedure:

Wister Albino rats (150-200gms) were selected and made into five groups of 6 animals each for the experiment. The animals were housed in the experimental room which was maintained as per IAEC guide lines. The experimental animals were anaesthetized using lignocaine 2% injections, over the local selected region. The rats were depilated over the region excision wound was infected by cutting a way of 500mm square thickness of skin from the predetermine area, the wound was left and rest to the open environment then the drugs reference standard (0.2 % w/w Nitrofurazone ointment) control (simple ointment base B.P)and Mimosa pudica diethyl ether, chloroform, alcohol leaves extracts were applied till the wound was healed. This model was used to monitor the wound contraction and wound closer time. Wound contraction was calculated as % reduction in wound area.

Effect of Methanolic, Chloroform and Diethyl ether extract ointments of Mimosa pudica on excision wound model.

Group	Avg. wt of animal	Drug /extract	Size of wound surface area day 0 (mm^2)	Day 1 (mm^2)	Day 4 (mm^2)	Day 8 (mm^2)	Day 12 (mm^2)	Day 16 (mm^2)	Percentage of wound healing.
I	150-200 gm.	Control	50.24	50.24	48.24	44.20	40.46	35.46	40.45 %
II		Nitrofurazone ointment (0.2% w/w)	50.36	50.36	28.26	12.56	3.14	0.758	98.44 %
III		Alcoholic extract (10%w/w)	51.26	51.26	38.46	28.26	12.56	3.14	93.87 %
IV		Chloroform extract(10% w/w)	50.54	50.54	28.36	30.26	20.54	18.76	80.72 %
V		Diethyl ether extract (10%w/w)	50.42	50.42	48.46	40.32	36.16	20.45	70.76 %

Values are mean ± SEM of 6 animals in each group. Numbers in Parenthesis indicate percentage of wound contraction. P<0.001Vs respective control by students Control"t" test.

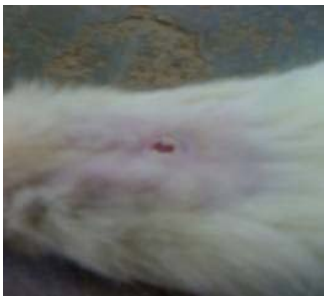
Control 0 day

Standard 0 day

Control 16 day

Standard 16 day

Mimosa Pudica (Diethyl ether Extract10%W/W)



Mimosa pudica (chloroform Extract10 %W/W)

Mimosa Pudica (Alcohol Extract 10%W/W)

Results & discussion

The present work is the pharmacological studies on the extracts of Mimosa pudica. The soxhlet extraction procedure carried out using Mimosa pudica powder with by successive solvent Methanol, Chloroform and Diethyl Ether. The preliminary chemical analysis indicates the presents of alkaloids, glycosides and phytosterols.

The wound healing activity was studied by using five groups; Contraction of the excision wound was promoted from Day 1 of the treatment till Day 16. The epithelization of wound in case of mice treated with extracts was found to be quite earlier than control. It is also comparable with the marketed preparation. It suggests that the leaves extracts of Mimosa pudica promoted wound healing activity. The excision wound model showed excellent wound healing property in alcoholic leaf extract which was well compared with standard drug. The results are shown in Table.

Conclusion

On the basis of the results obtained in the present investigation it is possible to conclude that the methanolic, chloroform and Diethyl ether extract ointment (10%w/w) of mimosa pudica has significant wound healing activity. In both extract ointment, the methanolic extract ointment (10%w/w) showed significant effect when compare to standard drug and other two extract in excision wound model.

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