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Phytochemical Study, Evaluation of Antihelminthic Potential and *In silico* Screening for Breast Cancer Using *Mimosa pudica* Linn.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Breast Cancer is a major health concern in India, where it is highly lethal for women because of unchecked cell proliferation and metastasis, which are frequently caused by interactions between the Estrogen Receptor Alpha and other receptors. Even though they work well, synthetic Anthelmintics come with dangers to the health of both humans and animals. Therefore, creating effective and safe Anthelmintics from plant sources is our main goal. We extracted *Mimosa pudica* leaves with ethanol using a Soxhlet system, and after concentration, we examined the extract using

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GC-MS, FTIR, and UV techniques. Estrogen Receptor structure and flavonoid compound databases from PubChem and Protein Data Bank were used for *in silico* testing against Breast Cancer and anthelmintic action, respectively. Using molecular docking and drug similarity investigations, the effectiveness of natural compounds against Breast Cancer was evaluated.

Keywords: Breast cancer; estrogen receptor alpha; anthelmintics; mimosa pudica leaves; soxhlet extraction; GC-MS, FTIR; UV analysis.

1. INTRODUCTION

Helminth parasites are among the most common infectious agents in the world today, affecting nearly one-third of the worldwide population and causing a number of crippling illnesses and syndromes. Helminths cause long-term, chronic infections in both human and animal hosts that are significantly correlated with host immune response downregulation. Due to a lack of reliable vaccinations, pharmacological efficaciousness limitations, growing treatment resistance, and fast reinfection in settings where transmission cannot be stopped, the elimination of helminth diseases is still an elusive aim.

Drudge et al. [1] provided an accurate account of the first instance of anthelmintic resistance. Many studies indicating a decline in anthelmintic efficacy have since been reported. Plant-based anthelmintics could be a substitute for other parasite infection treatments [2]. Knowledge about the possible effects of plant extracts on specific illnesses and pests can be obtained through research on therapeutic plants. Because of this, research in this field has made significant strides in improving both human and animal health. [3].

Globally, breast tumors are the second most common cause of mortality. One woman out of every nine in the UK will have this illness at some point in her life. A number of variables, including gender, food, alcohol use, physical activity, family history, way of life, and endocrine factors (both endogenous and exogenous), are linked to breast tumors. In addition, mammography density and prior benign breast cancer are significant risk factors for breast cancer. Yet the most significant element in the pathophysiology of breast cancer remains unclear [4]. Breast cancer has thus become the second most common cause of death for women [5,6].

The World Health Organization estimates that 80% of people still use herbal medicine, particularly those in Asia, Latin America, and Africa [7–12]. It makes sense that the majority of native, frequently exotic plants are used for screening natural sources for various forms of biological activity and therapeutic potency.

Mimosa pudica Linn. known as “Chue Mue” belongs to taxonomic group Magnoliopsida and Family Leguminosae. It is a Shrubby plant, the compounds on the leaves are sensitive on touching [13]. Many helminths undertaken extensive mitigations through body tissues, which both damages tissues directly and initiate hyper-sensitivity reaction which may causes cancer [14]. By using sensitivity of *Mimosa pudica*, we may be used to control the sensitivity of helminths. The anthelminthic property of *Mimosa pudica* is used to treat the helminthic infections [15]. The leaves of the plants have amino acids and Mimosine [16]. The leaves of *Mimosa pudica* is also used for Anti-ulcer, Anti-Diarrhea, Anti-Convulsant properties [17].

Considering the advances made in this research in recent years, this study aimed to evaluate the antihelminth activity and insilico docking studies for breast cancer using *Mimosa pudica* plant extracts.

2. MATERIALS AND METHODS

2.1 Plant Materials

The Plant *Mimosa pudica* grows nearly throughout the tropical and subtropical regions of India. They were grown in moist and warm climate. The leaves of plants were collected from district of Cuddalore. It was identified and confirmed by Dr.K. Nirmalkumar, Head of Department, Department of Botany, Periyar arts college, Cuddalore.

2.2 Preparation of Plant Extract

The plant material was gathered in the form of leaves, which were then dried in the shade and ground into a coarse powder using a machine. The powdered substance that resulted was put to use in additional research. Each sample, which

weighed 25 g, was extracted individually with 250 ml of ethanol using the Soxhlet equipment, and the extract was then collected and dried. After allowing for five cycles, the condensed extract was then diluted in ethanol to a concentration of 100 mg/ml. Next, the sample solution and extracted solution were combined in a beaker, covered with a foil sheet, and paper holes were punched for evaporation [18]. The dried plant extracts were redissolved in dimethyl sulfoxide to produce a solution of 10 mg/10 ml for each extract, which was then tested for phytochemicals [19]. To observe the powder's microscopic characteristics, dried leaf powder was employed. In order to identify the presence of lignified cells, calcium oxalate crystals, and starch grains using quantitative microscopy, the powder medicine was individually treated with phloroglucinol-Hcl solution, glycerin, and iodine solution.

"Total ash, acid insoluble ash was also determined. Extractive values were determined. Preliminary Phytochemical Studies the powder of dried leaf was subjected to continuous soxhlet extraction with s organic solvents such as ethanol" [20]. "After concentration and drying of each extract in vacuum desicator identification of phytoconstituents was carried out using thin layer chromatography method by detecting reagent. (i.e) Dragendroff reagent" [21].

For analysis of anti-helminth studies, worms are collected from microbiology lab around Pondicherry.

2.3 Phytochemical Screening

"The preliminary qualitative phytochemical screening test were utilized to find out phytoconsitituents present in the plant extract. Advanced techniques like Gas Chromatography (GC), Liquid Chromatography (LC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC) etc. are very helpful for detection of phytoconstituents both qualitatively as well as quantitatively" [19].

2.4 FTIR Analysis

"Dried powder of *Mimosa pudica* was used for FTIR analysis. 1 mg of the dried extract powder was encapsulated in 10 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of the extract was loaded in FTIR spectroscope, with a Scan range from 4000 to 650 cm^{-1} with a resolution of 4 cm^{-1}" [20].

2.5 UV analysis

"The extract was centrifuged at 3000 rpm for 10 minutes to collect the supernatant or remove the debris from the homogenate. The supernatant liquid was then diluted to 1:10 with the same solvent. Dilutions were done in a 10 ml volumetric flask. The extract was scanned in wavelength ranging from 200 to 800 nm. The distinctive peaks of the UV-Visible were detected, and their values were recorded" [22].

3. ANTHELMINTIC ASSAY

3.1 Preparation of Plant Extract

"The dried leaf powder (100 g) of *Mimosa pudica* leaf was extracted separately in sterile distilled water and ethanol by keeping them in respective solvents for 24 hours and was then filtered using Whatman filter paper. The pH of the extracts was adjusted to 7 and these extracts were further diluted to 5, 10, 15, 20 mg/ml in normal saline. The activity was carried out using Mathew et.al method" [23]. **Worm collection:** *Pheretima posthuma* (Indian earthworm) were collected form the Sivas Nursery Garden Kattukuppam, Puducherry. **Preparation of standard drug:** Albendazole, the standard drug was prepared with different concentrations 5, 10, 15 and 20 mg/ml using normal saline [24].

3.2 Assay of Anthelmintic

"The earthworm *Pheretima posthuma* was divided into five groups consisting of two equal sized earthworms in each group was released into 30 ml of the experimental formulation kept in a petri dish. The first group served as normal control which is treated only with normal saline. The second group served as standard drug, containing albendazole at 5, 10, 15 and 20 mg/ml in normal saline. The polar solvent and intermediate polar solvent (ethanol and acetone) at different concentrations (5, 10, 15 and 20 mg/ml). All the test solutions and standard solutions were prepared freshly before starting the experiment. Observation was made for the time taken for paralysis and death time of individual worms" [25]. "The paralysis time was noted when there are no movement earthworms. Death time of the worms were confirmed when the worms were unable to move and the

appearance of a white secretion and fading of their body colour around its body" [26].

4. *In silico* Docking Study of Breast Cancer

4.1 Protein Preparation

The 3-dimensional crystal structure of Human Estrogen Receptor with PDB: 2IOG in complex with the ligand was retrieved from the B-Carotene, B-D-Xylopyranose, Crocetin, D-Glucuronic Acid, L-Norepinephrine, Gallic Acid, L-Ascorbic Acid, Linolenic Acid, Mimosine, Octadeca-9, 12- Dienoic Acid, And Turgorin [27]. "The complexes bound to the receptor molecule, all the heteroatoms and the non-essential water molecules were removand finally hydrogen atoms were merged to the target receptor molecule using Argus Lab" [28,29].

4.2 Ligand Preparation

"Totally 11 Flavonoids were identified from the Pubmed literatures which shows inhibitory effects towards Breast Cancer" [30]. "The three-dimensional structure of the flavonoids was downloaded in sdf format using Pubchem and converted to PDB format using Pymol and further used for docking studies" [31].

5. RESULTS AND DISCUSSION

5.1 UV Analysis

The UV-Visible study of leaf extract of *Mimosa pudica* green color solvent shows prominent absorptions in665nm, 276 nm, 253 nm, 276 nm, 350 to 370nm So, the most absorbed color is violet, and the transmitted color is yellow-green. These absorption ranges show that the chromophores contain C_6H_5, N=O, C=C=O, N=N and C_nH_{2n-2}.

The present study revealed the presence of phytochemicals like alkaloids, tannins, flavonoids, phenols etc., the more phytochemicals and the highest percentage of extractive (8.99%) were found in ethanol extract and showed the anthelmintic activity.

In the present study, anthelmintic activity of polar extract and semipolar extract(i.e) ethanol and acetone leaf extracts of *Mimosa pudica* was tested against *Pheretima posthuma* (Indian earthworms) in their reaction to anthelmintic activity when compared to standard drug Albendazole, whereas ethanol extract shows higher anthelmintic activity.

The alkaloids were reported to act on the CNS and cause paralysis on worms. The tannins interfere with energy generation in worms by uncoupling oxidative phosphorylation then bind to free protein to GIT and leads to death of worms.

The phytochemicals separate or togetherly may block tubulin or glucose uptake and damage in the mucopolysaccharide Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color. As compared to the polar and semipolar leaf extract both show similar anthelmintic activity. The paralysis and death time is more or over same.

5.2 In silico Docking Studies

5.2.1 Breast cancer docking studies in *Mimosa pudica*

Binding energy in molecular docking refers to the strength of the interaction between a protein and a ligand. It is a measure of how tightly the ligand binds to the protein and is an important factor in determining the efficiency of the docking process. Crocetin and turgorin shows highest binding energy and possess greater anti cancer potential,when compared to albendazole which has binding energy as -7.91. Flavonoids present in the plant extract functions as selective Estrogen Receptor modulators (SERMs) [32,33] changes the endogenous activities of Estrogen Receptor, which will slow down or prevent the developments of breast cancers [31,7,34-36].

Table 1. Macroscopic studies

S.No.	STUDY	RESULT
1.	Size	10–20 pairs, 0.6–1.2-cm long, 0.3–0.4-cm broad
2.	Color	Yellowish Green.
3.	Shape	Petiolate or stipulate
4.	Odour	Pungent

Table 2. Determination of total ash and acid insoluble ash

S.NO.	Type of ash	Percentage (w/w)
1.	Total ash	7.78%W/W
2.	Acid insoluble ash	1.429%W/W

Table 3. Determination of extractive values

S.NO	Type Of Extractive Value	Percentage(W/W)
1.	Ethanol	8.99%W/W

Table 4: Preliminary Phytochemical Analysis

Phytoconstituents	Observation
Carbohydrates	+
Alkaloid	+
Proteins & Amino Acids	+
Tannins & Phenolics	+
Flavonoids	+
Triterpenoids	-
Saponins	+
Glycosides	-

- *(+) Indicates the presence of chemical constituents*
- *(-) Indicates the absence of chemical constituents*
- *The present study revealed the presence of phytochemicals like alkaloids, tannins, flavonoids, phenols, carbohydrates, proteins etc., the more phytochemicals and the highest percentage of extractive (8.99%) were found in ethanol extract*

Table 5: FTIR analysis of *Mimosa pudica*

Functional group	Wavenumber (cm^{-1})
N=O	1550 and 1350
C_nH_{2n-2}.	2150–2100
N=N	2160-2120
C_6H_5	3100–3050 (s), 900–690 (s)

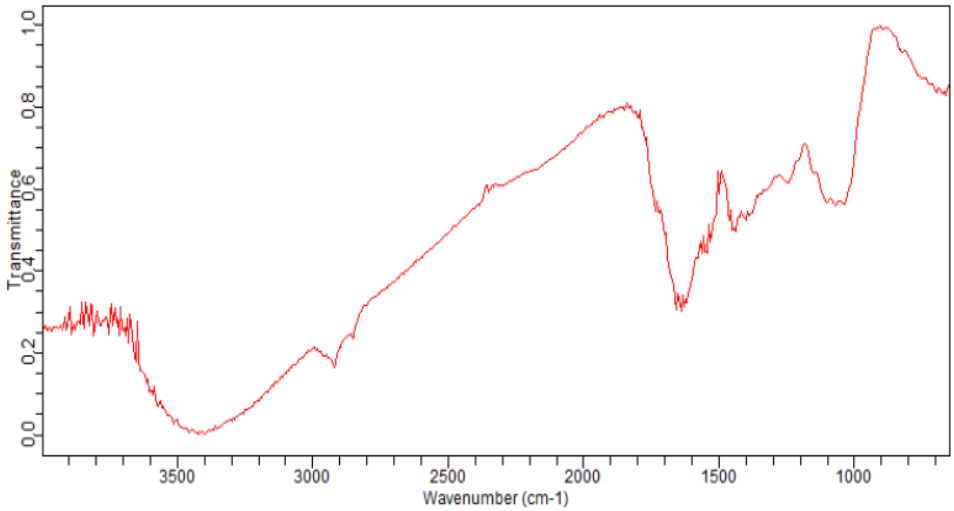

Fig. 1. FTIR spectrum of *Mimosa pudica*

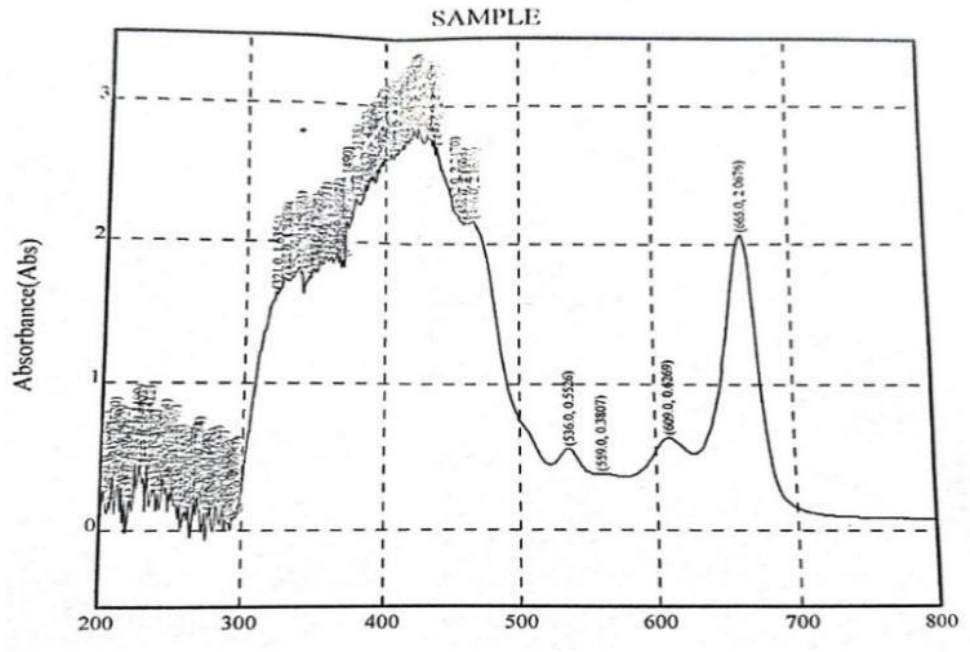

Fig. 2. UV-vis spectrum of *Mimosa pudica*

Table 6. Assay of Anthelminthic activity in *Mimosa pudica plant extract*

Extracts	Paralysis time				Death time			
	5mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	5mg/ml	10 mg/ml	15 mg/ml	20 mg/ml
Ethanolic extract	20 sec	17 sec	15 sec	10 sec	14 sec	12 sec	9 sec	7 sec
Acetone (polar extract)	15 sec	10 sec	8 sec	5 sec	8 sec	7 sec	5sec	3sec

- *Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color*

Table 7.Docking Score of various protein against flavonoids

S.NO	Compound name	Binding energy (Kcal/mol)	Inhibition constant	No. of Hydrogen bond formed	Interaction energy	Vander waal dissolution energy
1	β-Carotene	-5.98	41.67	0	-10.45	10.45
2	β-D-Xylopyranose	-5.27	137.57	3	-5.27	-5.03
3	Crocetin	-7.65	2.49	3	-12.12	-11.14
4	D-Glucuronic Acid	-5.97	41.78	3	-6.27	-4.97
5	L-Norepinephrine	-6.12	32.84	2	-6.71	-5.63
6	Gallic acid	-6.01	39.42	3	-6.31	-5.0
7	L-Ascorbic acid	-6.17	29.98	3	-6.77	-6.35
8	Linolenic acid	-5.86	50.46	1	-10.63	-10.3
9	Mimosine	-5.85	51.21	3	-6.75	-5.15
10	Octadeca-9, 12-dienoic acid	-6.19	29.1	0	-10.96	-9.84
11	Turgorin	-9.42	124.39	4	-11.21	-10.2
12	Cyclophosphamide	-5.99	40.85	1	- 7.48	-7.42

6. CONCLUSION

Mimosa pudica leaf ethanol extracts phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, and proteins in different extracts employing both polar and semi-polar solvents. The ethanol extract contained higher quantities of the majority of these phytochemicals than the acetone extract.

The efficaciousness of the ethanol extract was superior to that of the traditional drug, and it demonstrated dose-dependent anthelmintic activity. The extract includes alkaloids, which are said to act on the central nervous system and paralyze worms. The tannins cause oxidative phosphorylation to be uncoupled, which hinders worms from producing energy. This free protein then binds to the GIT and kills the worms. Separately or in combination, the phytochemicals may prevent the uptake of tubulin or glucose and cause harm to the mucopolysaccharide. When plant extracts' drug-like characteristics are examined, in-silico docking experiments reveal that the extracts have anti-breast cancer properties. With the best biological activity data when compared to the reference medication, crocetin and turgorin emerged as the two most promising of these compounds. Flavonoids present in the extracts possess the anti cancer activity.As a result, we believe that these are more effective as active medications for human intake.

Although several currently available treatment plans and options are employed to cure cancer their immense adverse effects make scientists intend to seek those active agents from natural sources. The present study therefore designed to make a medicinal plant *Mimosa pudica* linn *a* suitable source of active compounds for treating cancer and helminth infection.

Thus, We intend to perform cell line studies and investigate the production of innovative chemicals in our future study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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