Qualitative Analysis on Anthelminthic Activty and *In silico* Screening of Breast Cancer by using *Mimosa pudica* Linn.

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 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 infection is most common infection which occurs in human in developing countries they pose a large threat to public. Various disease is caused by helminth infection such as Ascariasis, Taeniasis, and Cysticercosis. Our present study is about to treat a Breast Cancer by using leaves of *Mimosa pudica* Linn. [1]. Cancer has been one of the most dreaded diseases of the 20th century and is increasingly rampantly with greater intensity. There are several types of cancers. Among them Breast Cancer is the second most common cause of cancer mortality in women. Breast Cancer prognosis and treatment options are generally based on Tumor node and metastasis staging [2]. **Helminthic infections:** Helminthic diseases are brought on by helminths, which are parasitic worms. The three primary subgroups of these worms are tapeworms, roundworms, and flukes [3]. **Anthelminthic:** Anthelmintics or Anthelminthics are a group of Antiparasitic drugs that expel parasitic worms and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They may also be called Vermifuges or Vermicides [4]. **Breast Cancer:** Breast Cancer is a disease in which cells in the breast grow out of control. There are

different kinds of breast cancer. The kind of Breast Cancer depends on which cells in the breast turn into cancer [5].

2. PLANT PROFILE:

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 [6]. Many helminths undertaken extensive mitigations through body tissues, which both damages tissues directly and initiate hyper-sensitivity reaction which may causes cancer [7]. 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 [8]. The leaves of the plants have amino acids and Mimosine [9]. The leaves of *Mimosa pudica* is also used for Anti-ulcer, Anti-Diarrhea, Anti-Convulsant properties [10].

3. MATERIALS AND METHODS:

3.1 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.

3.2 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 [11]. 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 [12].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 [13]. 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 [14].

3.3 Phytochemical screening

To identify the phytochemical in leaf extract chemical test were carried out. The stock concentration of leaves extracts 10 mg/ml showed the presence of phytochemicals such as Flavonoids, Carbohydrates, Alkaloids, Proteins, Amino acids, Saponins, Tannins, and Phenol [15].

3.4 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} [16]

3.5 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 [17].

4. Anthelmintic assay:

4.1 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 [18]. **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 [19].

4.2 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 [20]. 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 [21].

5. *In silico* Docking study of Breast Cancer:

5.1 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 [21,22]. The complexes bound to the receptor molecule, all the heteroatoms and the non-essential water molecules were removed and finally hydrogen atoms were merged to the target receptor molecule using Argus Lab [23,24]

5.2 Ligand preparation:

Totally 11 Flavonoids were identified from the Pubmed literatures which shows inhibitory effects towards Breast Cancer [25]. 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 [26].

Results and Discussion:

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**

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)

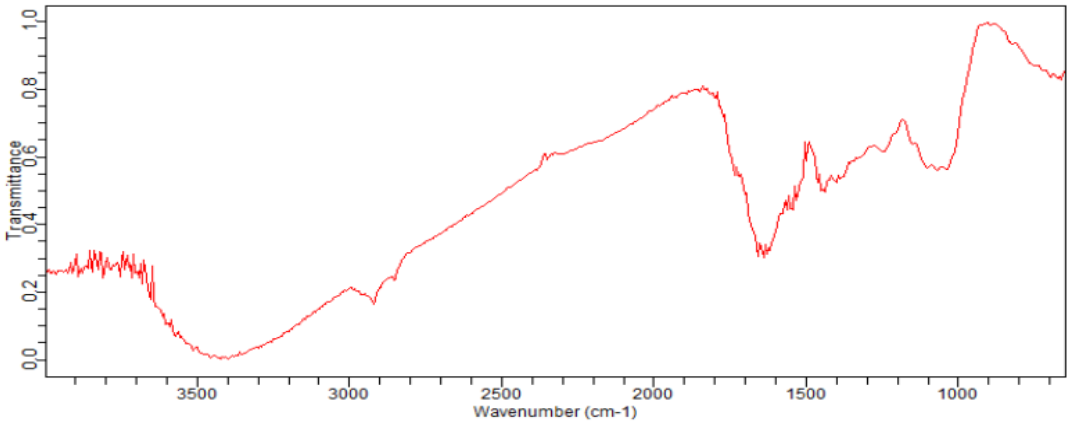

Figure.1: FTIR spectrum of *Mimosa pudica*

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}.

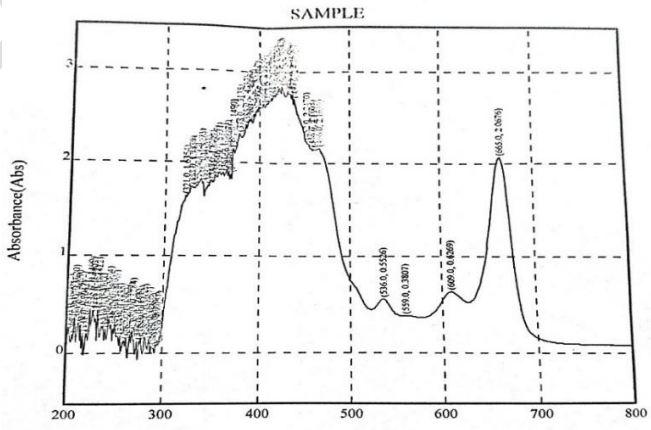

Figure2: UV-vis spectrum of *Mimosa pudica*

Table 6: Assay of Anthelminthic:

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.

Each and every different plant would have different chemical constituents and composition and may vary from one part to another plant or within the same plant. Thers are various solvent that can be dissolved according to their phytochemicals present in the plant. There is various solvent like hexane, methanol, ethanol etc., 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. The phytochemicals present in *Mimosa pudica* leaf will be useful for the activity of this plant in medicine and also it used in other system of medicine.

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.

***IN SILICO* DOCKING STUDIES:**

BREAST CANCERDOCKING STUDIES:

Table.7: Docking Score

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

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 ethanol extract exhibited anthelmintic activity in a dose-dependent manner and outperformed the conventional medication in terms of efficacy.

We conclude that two compounds have anti-Breast Cancer action after examining their drug-like features. Crocetin and Turgorin revealed as particularly promising among these

chemicals, with the most favourable biological activity data when compared to the standard drug. As a result, we believe that these two molecules are more effective as active medications for human intake.

Furthermore, *Mimosa pudica* leaf extract revealed anthelmintic efficacy (Assay) as well as AntiBreast Cancer activity (*in-silico* research). We intend to perform cell line studies and investigate the production of innovative chemicals in our future study.

REFERENCES:

1. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. The Journal of clinical investigation. 2008;118(4):1311-21. doi: 10.1172/JCI34261
2. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. Cancers. 2021;13(17):4287. doi: 10.3390/cancers13174287
3. Wakelin D. Helminths. Current Opinion in Infectious Diseases. 2000;13(5):465-9. DOI: 10.1097/00001432-200010000-00006
4. Holden-Dye L, Walker R. Anthelmintic drugs and nematocides: studies in Caenorhabditis elegans. WormBook: the online review of C. elegans biology. 2014:1-29. DOI: 10.1895/wormbook.1.143.2
5. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, Ji X, Liu W, Huang B, Luo W, Liu B. Breast Cancerdevelopment and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & diseases. 2018;5(2):77-106. doi: 10.1016/j.gendis.2018.05.001
6. Joseph B, George J, Mohan J. Pharmacology and traditional uses of *Mimosa pudica*. International journal of pharmaceutical sciences and drug research. 2013;5(2):41-4.
7. Yap GS, Gause WC. Helminth infections induce tissue tolerance mitigating immunopathology but enhancing microbial pathogen susceptibility. Frontiers in immunology. 2018;9:411888.
8. Bendgude RD, Maniyar MG, Kondawar MS, Patil SB, Hirave RV. Anthelmintic activity of leaves of *Mimosa pudica*. Int J Inst Pharm Life Sci. 2012;2(1):120-5.

9. Parmar FE, Kushawaha NI, Highland HY, George LB. In vitro antioxidant and anticancer activity of *Mimosa pudica* Linn extract and L-Mimosine on lymphoma daudi cells. Cancer cell. 2015;1:100-4.
10. Ayissi Mbomo R, Gartside S, Ngo Bum E, Njikam N, Okello E, McQuade R. Effect of *Mimosa pudica* (Linn.) extract on anxiety behaviour and GABAergic regulation of 5-HT neuronal activity in the mouse. Journal of Psychopharmacology. 2012;26(4):575-83. doi: 10.1177/0269881111398686.
11. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. Journal of microbiology & biology education. 2014;15(1):45-6. doi: 10.1128/jmbe.v15i1.656
12. Gandhiraja N, Sriram S, Meenaa V, Srilakshmi JK, Sasikumar C, Rajeswari R. Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. Ethnobotanical leaflets. 2009;2009(5):8.
13. Kumar D, Singh H, Bhatt U, Sharma J, Sharma S, Soni V. Physiological performance of *Mimosa pudica* L. under different light quality and photoperiods. Physiologia. 2022;2(4):132-53. doi.org/10.3390/physiologia2040012
14. Pal P, Datta S, Basnett H, Shrestha B, Mohanty JP. Phytochemical analysis of the whole plant of *Mimosa pudica* (Linn.). UJPSR. 2015;1(1):1-9.
15. Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. BioMed research international. 2014;2014. 6. doi.org/10.1155/2014/497606
16. Sivarajan VV, Balachandran I. Ayurvedic drugs and their plant sources. Oxford and IBH publishing; 1994.
17. Dhivya K, Kalaichelvi K. Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of Micrococca mercurialis (L.) Benth. International Journal of Herbal Medicine. 2017;5(6):40-4.
18. Jamkhande PG, Barde SR. Evaluation of anthelmintic activity and *in silico* PASS assisted prediction of Cordia dichotoma (Forst.) root extract. Ancient science of life. 2014;34(1):39-43. doi: 10.4103/0257-7941.150779
19. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of *Cayratia auriculata* (in vitro). Maedica. 2019;14(4):350. doi: 10.26574/maedica.2019.14.4.350

20. Mali RG, Wadekar RR. In vitro anthelmintic activity of *Baliospermum montanum* muell. arg roots. Indian journal of pharmaceutical sciences. 2008;70(1):131. doi: 10.4103/0250-474X.40352
21. Wurtz JM, Egner U, Heinrich N, Moras D, Mueller-Fahrnow A. Three-dimensional models of estrogen receptor ligand binding domain complexes, based on related crystal structures and mutational and structure− activity relationship data. Journal of medicinal chemistry. 1998;41(11):1803-14. doi: 10.1021/jm970406v.
22. Muhammad SA, Suganya SU, Ravi SU, Venkatachalapathi SE. Synthesis of novel cyclohexanone derivatives as Bcr-Abl T1351 Inhibitors. Int J Pharm Pharm Sci. 2015;7(2015):12.
23. Al-Jumaili MH, Al Hdeethi MK. Study of selected flavonoid structures and their potential activity as breast anticancer agents. Cancer Informatics. 2021;20:11769351211055160. DOI: 10.1177/11769351211055160
24. Suganya J, Radha M, Naorem DL, Nishandhini M. In silico docking studies of selected flavonoids-natural healing agents against breast cancer. Asian Pacific Journal of Cancer Prevention. 2014;15(19):8155-9. DOI: 10.7314/apjcp.2014.15.19.8155
25. Vijayalakshmi M, Dhanapradeeba V, Kunjiappan S, Sundar K, Pandian SR. Targeting TLRs with the Derivatives of Mimosa Pudica: An In Silico Approach. Biointerface Res. Appl. Chem. 2023;13(3). doi.org/10.33263/BRIAC133.237
26. Palanichamy C, Pavadai P, Panneerselvam T, Arunachalam S, Babkiewicz E, Ram Kumar Pandian S, Shanmugampillai Jeyarajaguru K, Nayak Ammunje D, Kannan S, Chandrasekaran J, Sundar K. Aphrodisiac performance of bioactive compounds from *Mimosa pudica* Linn.: In silico molecular docking and dynamics simulation approach. Molecules. 2022;27(12):3799. doi: 10.3390/molecules27123799.