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STUDIES ON THE QUANTITATIVE ESTIMATION OF TOTAL FLAVONOID CONTENT AND POTENTIAL *IN VITRO* ANTHELMINTIC ACTIVITY OF ETHONOLIC LEAF EXTRACT OF *BRYOPHYLLUM PINNATUM*

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ABSTRACT

The present research work is about phytochemical investigation and anthelmintic activity of *Bryophyllum pinnatum* leaves. Standardization of crude drug extracted from plant plays an important role in identifying the quality and purity of drugs. Phytochemical constituents are extracted by aqueous and ethanolic extracts from *Bryophyllum pinnatum* leaves belonging to the family Moraceae. This research find highlights that aqueous extracts of *Bryophyllum pinnatum* leaves had the highest number of phytochemicals. The aqueous extract of *Bryophyllum pinnatum* leaves holds the greatest potential to treat various human diseases and has profound medical applicability. *In-vitro* anthelmintic activity was evaluated by taking adult Indian earthworms, Pheretima posthuma having anatomical and physiological resemblance with intestinal roundworms. The earthworms were washed in normal saline solution before they were placed in of respective formulation.

Keywords: *Bryophyllum pinnatum* leaves, Phytochemical screening, Pheretima posthuma, Piperazine citrate

1. INTRODUCTION:

The use of medicinal plant is growing worldwide because of the increasing toxicity and allergic manifestations of the synthetic drugs. Helminth infections are among the most common infections in man, affecting a large proportion of the world's population **[1]**. Plants are more important in human life and commonly used in treating or preventing specific diseases and are considered to play a salubrious role in health care. Various experimental studies indicated that peptide, unsaturated long chain fatty acids, alkaloids, essential oils, phenols, flavonoids, tannin, and water or ethanol soluble compounds of medicinal plants are potentially significant against different diseases. This infection can affect most populations in endemic areas with major economic and social consequences **[2]**. A range of medicinal plants with anthelmintic properties is widely used by traditional healers, but still there is need for research into medicinal plant claim to be effective in the management of Helminth infection. The plant, *Bryophyllum pinnatum* (Crassulaceae) Synonym: Kalanchoe pinnata, Pers, Bryophyllum Calycium Salisb; Common names: air plant, love plant, miracle leaf, life plant, Zakham-e-hyat, panfutti, Ghayamari **[3]**, it has been accepted as an herbal remedy in almost all parts of the world **[4-5]**. Vernacular Name in different launguages are Sanskrit: Pashanabheda -English: Air plant - Hindi: Zakhmhaiyat, Patharchoor - Kannada:Gandukalinga - Tamil: Malaikalli, Ranakalli -Telugu: Ranapaluka -Marathi: Gayamari - Bengali: Koppatha, Patharkuch

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals: Each chemical employed in the investigation is of analytical quality such as piperazine citrate (SD Fine Chemicals Ltd., Mumbai) and Ethanol (Merck, India).

2.2 Collection and processing of plant sample:

The fresh aerial parts (leaves) for the planned work of *Bryophyllum pinnatum* were gathered from Ananthagiri Village, Telangana, India, and It was then rinsed with water and desiccated in the shelter and blended into a fine powder. It was then sieved at 20 mesh and stored. Subsequently verified by Dr. K. Srinivasa Reddy, Assistant Professor, Department of Botany, Govt. Degree College for Woman, Nalgonda, Telangana. A voucher specimen was kept in herbarium voucher No. 06/HB/ANRP/2023.

2.3 Extraction procedure:

2.3 Preparation of extract:

Plant Material Extraction Using a soxhlet apparatus and ethonol as the solvent, about 25 g of powdered crude medicine made from *Bryophyllum pinnatum* leaves were extracted over the course of 16 hours. Following extraction, the solvent was collected, and the extracts were concentrated using a rotary evaporator set to a temperature of 40 to 45°C. After that, gather the extract and use Whatman filter paper (No. 1) to filter it. A different calculation was made for percentage yield. The crude extracts were then refrigerated at 4-5ºC in screw-capped little glass vials with appropriate labeling. The extracts were put to additional use **[6-9]**.

2.4 Phytochemical Screening:

According to protocol, preliminary phytochemical analyses were carried out to identify the class of secondary metabolites found in both extracted leaf samples **[10]**.

2.5 Animals:

At the Department of Zoology, SR& BGNR Govt, Degree College, Khammam, adult Indian earthworms *(Pheritima posthuma)* were gathered from places flooded by water, identified, and thoroughly washed with water to eliminate any dirt particles. After that, the worms were employed in every experiment and methodology for the in vitro anthelmintic research.

2.6 Anthelm.intic Activity Evaluation

Because *Pheretima posthuma,* an adult Indian earthworm, shares morphological and physiological similarities with human intestinal roundworm parasites, the anthelmintic activity was assessed on this species **[10]**. The anthelmintic screening was conducted using standard laboratory procedures **[11–12]**. The ethanolic extract was suspended in 1% gum acacia in regular saline (vehicle) at concentrations of 10, 20, and 50 mg/ml. To compare the activity, reference standards of piperazine citrate (15 mg/ml) in 1% gum acacia in normal saline were utilized. Six groups of six Indian earthworms each, of the same size, were released into 50 milliliters of the intended formulation.

Group II received treatment with piperazine citrate (15 mg/ml), Group III received treatment with the same vehicle (group I), Group III, IV and V received treatment with 10, 20, and 50 mg/ml of ethanolic extract. The amount of time needed for each worm to become paralyzed or to die was noted. When the worms fail to resurrect in regular saline, it was claimed that paralysis had occurred. The worms' loss of motility and subsequent fading of body colour signaled their death. The outcomes are shown in **(Table 1)**.

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3. RESULTS

3.1 Quantitative estimation of total flavonoids

A quantitative study of all the flavonoids found in *Bryophyllum pinnatum* ethanolic extract was done using the following methodology. 6 g of the sample were added to precisely 50 ml of 80% aqueous methanol in a 250 ml beaker. The beaker was then sealed and allowed to remain at room temperature for 24 hours. The residue was extracted three more times with the same volume of ethanol after the supernatant was removed. The material was passed through a filter using Whatman number 42 (125 mm) filter paper. The filtrate was then placed in a crucible and allowed to dry over a water bath. To guarantee a consistent weight, the material inside the crucible was cooled using a desiccator before being weighed. Determination of the proportion of flavonoids using:

$$\% \text{ Flavonoids} = \frac{\text{Flavonoids weight}}{\text{Sample weight}} \times 100$$

Estimation of total flavonoids

Phytochemical	Wt. of sample (g)	Wt. of dried filtrate	% yield
Flavanoid Content	6	2.9	48.3335

Table 1: Anthelmintic Activity of *Bryophyllum pinnatum*

S. No.	Treatment groups	Groups	Concentration (mg/ml)	Paralysis time (min) (Mean ± S.D.)	Death time (min) (Mean ± S.D.)
1	Control	I	Control	----------	----------
2	Standard	II	Piperzine citrate (15 mg /ml)	20.64 ± 5.18	72.25 ± 2.143
3	Ethanolic leaf extract	III	10	86.20 ± 2.034	186.86 ± 3.51
4		IV	20	40.31±2.71	143.01 ± 1.987
5		V	50	30.38 ± 3.520	79.32 ± 2.915

Results are expressed as Mean ±standard deviation

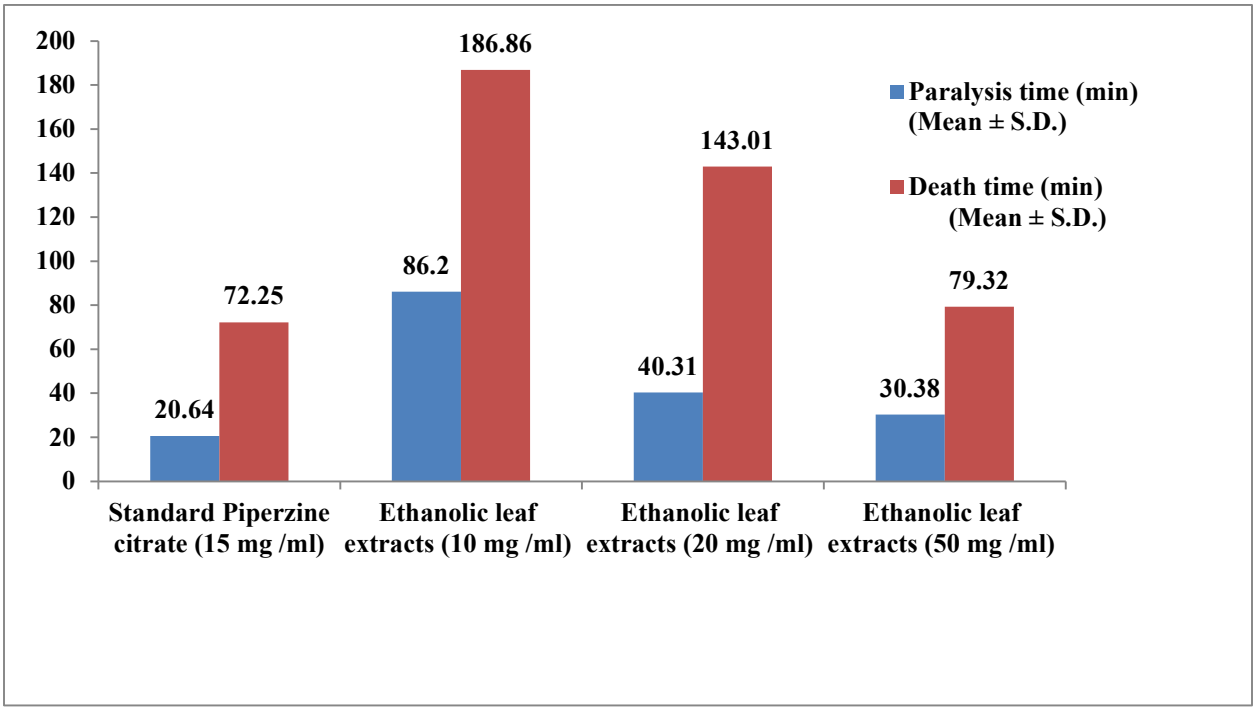

Figure 1: Anthelmintic Activity of *Bryophyllum pinnatum L.*

4. DISCUSSION

An example of an anthelmintic drug that kills worms by paralyzing them and compelling their removal from both animal and human feces is piperizine citrate. Ethanolic extract contains carbohydrates, alkaloids, glycosides, steroids, terpenoids, tannins, and phenolic substances, according to preliminary phytochemical screening. These secondary metabolites have the potential to produce artificial antihelminthic action. People can become infected with parasite infections. When immature forms penetrate humans through the skin or gastrointestinal tract (GIT), they mature into well-differentiated worms with tissue distribution characteristics. Anti-hermitic medications act either systemically to eradicate fully developed helminthes or their development forms that injure organs and tissues, or locally to eradicate worms from the gastrointestinal tract. At concentrations of 10, 20, and 50

mg/ml, the ethanolic extract of the *Bryophyllum pinnatum* plant demonstrated paralysis at 86.20, 40.31, and 30.38 minutes and death at 186.86, 143.01, and 79.32 minutes. According to **Table 1**, piperazine citrate showed signs of paralysis at 20.64 minutes and mortality at 72.25 minutes at 15 mg/ml. When compared to the usual anthelmintic medication depicted in **Figure 1**, the foregoing results demonstrate the ethanolic extracts of *Bryophyllum pinnatum* plant to have anthelmintic activity in a dose-dependent manner. Earthworms use their cilia to move. Complex polysaccharides make up the earthworm's mucilaginous outer coat. Because of this layer's sliminess, the earthworm can travel about freely. If the mucopolysaccharide membrane is broken, the outer layer will be exposed, which would restrict its range of motion and may cause paralysis **[13]**. By harming the mucopolysaccharide layer, this tactic might kill the worm. This causes stress on the body, which leads to paralysis. Worms are killed by starvation or paralysis by all anthelmintics. Because they cannot store energy, worms need to consume practically constantly to meet their metabolic needs. Energy is lost each time this process stops. Most adult parasites can be eliminated by starving them for a shorter period of time. Because paralyzed parasites are unable to hold onto their place in the stomach, they will also eventually die. Alkaloids, tannins, glycosides, phenols, and saponins were discovered during an initial phytochemical screening. It has been shown that tannins prevent the production of energy by disabling oxidative phosphorylation in conjunction with other events. Tannin can also bind to free proteins in the digestive tract of the host animal or to a glycoprotein on the worms' cuticle, which kills the worms. Sources claim that the tannins in the plant can enhance a person's capacity to absorb protein **[14]**. Increased protein absorption led to lower rates of nematode worm infection in the host animal **[15]**, although tannins directly affect the nematode cuticle through hydrogen bonding. The nematodes are killed and the skin becomes paralyzed as a result of this reaction **[16]**. The alkaloids and steroids in the extracts have the ability to reduce worm glucose support by inhibiting the transport of sucrose to the small intestine **[17]**. These benefits, along with the antioxidant qualities of flavonoids, can reduce the quantity of nitrate generated for use in protein synthesis Damage to the mucopolysaccharide barrier by saponins and tannins can also cause worm paralysis and death. This mucilaginous constructor, formed like a mucoid, protects the surface muscles.

The rupturing of the membrane will reveal the outer layer, allowing the worm's body to take in the extract's chemical composition **[18]**. These results implied the potential use of leaf extract from *Bryophyllum pinnatum L* as an alternative anthelmintic.

The ethanolic extract was found to possess anthelmintic properties. At the assessed concentration levels, it was discovered that the tested substances were fatal to the worms. It was also discovered that the activity depended on concentration for every sample that was examined. It was discovered that the potency of the test samples was inversely correlated with the worms' time to paralysis or death. The actions were similar to those of the piperazine citrate reference medications. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity.

6. CONCLUSIONS

The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing several diseases through free radical scavenging activity. Since the plant Bryophyllum pinnatum leaves has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identify bioactive compounds. The present analyses suggest that Bryophyllum pinnatum leaves contain potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising. The present results will form the basis for collection of new plant species for further investigation in the potential discovery of new bioactive compounds. Further studies are need for in-vitro model are required to find out and to establish the effectiveness and pharmacological rationale for the use of plant leaves as an anthelmintic drug. The biological parameter can be concluded that the plant Bryophyllum pinnatum leaves have significant anthelmintic activity. The plant extracts can be used for further isolation of compounds for their anthelmintic activity

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

7. REFERENCES:

[1] Bundy DA, Immunoepidemiology of intestinal helminthic infection: The global burden of intestinal nematode disease, **Trans. Royal. Soc. Trop. Med. Hyg**. 8; 1994: 259-261.

[2] Tagbota S, Townson S, Antiparasitic properties of medicinal and other naturally occurring products, **Adv. Parasitol**. 50; 2001: 199-205.

[3] Sondhi SM, Shahu R, and Archana M. **Indian Drugs.** 31(7); 1994: 317-320.

[4] Kirtikar KR and Basu BD. Indian Medicinal Plants volume IV, Lalit Mohan Basu publications; 1990: 2724-2727.

[5] Hemmati M, Rezvani A and Djahanguiri B. Prevention of Aspirin – Induced Gastric Ulceration in Rats by L-Methyldopa and Disulfiram. **Pharmacology** 9; 1973: 374-376.

[6] Muniappan M and Sundararaj T. Antiinflammatory a nd antiulcer activities of *Bambusa arundinacea.* **Journal of Ethnopharmacology** 88; 2003: 161-167.

[7] Nishina A and Uchibori T. Antimicrobial activity of 2,6 dimethoxy-p-benzoquinone, isolated from Thick stemmed Bamboo and its Analogs. **Agricultural and Biological Chemistry** 55:1991; 2395-2398.

[8] Macwan C, Patel HV and Kalia K. A comparative evaluation of *in vitro* antioxidant properties of Bamboo *Bambusa arundinacea* leave s extracts**. Journal of cell and tissue Research** 10; 2010: 2413-2418.

[9] Joshi RK, Patil PA, Mujawar MHK, Kumar.D, Kholkute SD. Hypoglycae mic activity of *Bambusa arundinacea* leaf ethanol extract in euglycaemic a nd hyperglycaemic wustar rats. Phar macologyonline. 3; 2009: 789-795.

[10] Thorn GW, Adams RD, Braunwald E., Isselbacher KJ and Petersdorf RG. Harrison's Principle of Internal Medicine, McGraw Hill Company; 1977: 1088.

[11] Mathew AS, Patel KN and Shah BK. Investigation on antifeedant and anthelmintic pote ntial of *Adhatoda vasica* Nees**, Indian Journal of Natural Products**. 14(1): 1998; 11-16.

[12] Nargund LVG, Anthelmintic activit y of 8-fluoro-9-substituted-(1, 3)-benzothiazolo-(5,1-6)-1,3,4-triazoles on *Pheretima posthuma,* **Indian Drugs**. 36(2); 1999: 137-139.

[13] B. Srilehka, K. Raveendra Babu, S.K. Naseer Jani, G. Navya Sri, Jahangir Alom and M. Chinnaeswaraiah (2023). Phytochemical analysis and in vitro anthelmintic activity of methanolic extract of Samanea saman (Jacq.) Merr. leaves.Ann. Phytomed., 12(2):1-4. http://dx.doi.org/10.54085/ap.2023.12.2.20.

[14] Suman; Parvesh Devi; Sheetal. and Sushila Singh. (2022). Phytochemical screening and determination of total phenols, flavonoids and micronutrients of floral and leafy parts of Prosopis cineraria (L.) Druce (Angiosperms: Fabaceae). Ann. Phytomed., 11(1): 523-529.

[15] Patel, J.; Kumar, G.S.; Qureshi, M.S. and Jena, P.K. (2010). Anthelmintic activity of ethanolic extract of whole plant of Eupatorium odoratum. International Journal of Phytomedicine, 11(12):127-132.

[16] Vidyadhar, S.; Saidulu, M.; Gopal, T.K.; Chamundeeswari, D.; Rao, U. and Banji, D. (2010). In vitro anthelmintic activity of the whole plant of Enicostemma littorale by using various extracts. International Journal of Applied Biology and Pharmaceutical Technology, 1(3):1119-25.

[17] Rana, S.; Chandel, S. and Thakur, S. (2021). Isolation, identification and characterization of Cytospora chrysosperma associated with canker disease of Salix alba L. Ann. Phytomed., 10(2):124-129.

[18] Mulla, W.A.; Thorat, V.S.; Patil, R.V. and Burade, K.B. (2010). Anthelmintic activity of leaves of Alocasia indica Linn. International Journal of Pharmtech Research, 2(1):26-30.