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Phytochemical Screening and In Vitro Anthelmintic Activity of Ethanolic 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 to 10ml of respective formulation. To observe anthelmintic activity, all the investigations were carried out by ethanolic extract with different concentrations of 10, 20, 50mg/ml, significant activity like time of paralysis and time of death were noted. At highest concentrations of 50mg/ml, a significant anthelmintic activity was observed and compared with piperazine citrate (10, 20, 50mg/ml as standard reference and distilled water as control). Herbal drugs and synthetic drugs were equally effective in helminthic infestations but ethanolic extract exhibits potentiality and have significant anthelmintic activity.

INTRODUCTION

Globally, medicinal plants are extensively used for the treatment of various diseases (Gover et al., 2002). According to the World Health Organization (WHO), medicinal plants are the great source to offer a diverse range of potential therapeutic drugs and these drugs can be relatively safe and economical as compared to the synthetic medicines (Mekuria et al., 2017; Ekor, 2014; Bahmani et al., 2014). Since the last many years, herbal drugs are increasingly focused by researchers and several plants are being monitored for their prospective therapeutic effects (Uprety et al., 2010). Based on the therapeutic value of medicinal plants the current study provides an updated insight of Bryophyllum pinnatum which is extensively used in folk therapeutics. Bryophyllum pinnatum (family: Crassulaceae) is also known as Kalanchoe pinnatum or Bryophyllum calycinum (Sadhana et al., 2017). It is 3 to 5 meters high perennial herb and has opposed glabrous leaves (Afzal et al., 2012; Kamoj and Saluja, 2017). It has a sour taste, hot strength and sugary post-digestive effect. The herb contains a wide range of valuable chemicals that could be responsible for its various pharmacological effects.

Around the globe, it is consuming for the treatment and management of various pathologies such as conjunctivitis, edema, piles, cuts, eczema, constipation, epilepsy, cholera, asthma, chest colds, menstrual disorders, chicken pox and fever (Quazi et al., 2011). The plant parts are frequently applied for the cure of burns, rheumatoid arthritis, anti- septic, blisters, cough suppression, insect bites, psychiatric disorders and abdominal discomforts (Sadhana et al., 2017). It is well-known for its anti-inflammatory, wound healing, analgesic and hemostatic qualities (Ferreira et al., 2014). Leaves extracts are useful for the remedy of jaundice, hypertension, renal stones and diabetes. Slightly heated leaves are applied on superficial skin infections and also used for the dropping of placenta in Southeast Nigeria, hence it act as a tocolytic agent to prevent the premature labor (Gupta et al., 2016; Mouhssin et al., 2015). The plant is also used for the cure of leg edema, fever, gout, abscesses, otitis and palpitations (Afzal et al., 2012). Bryophyllum pinnatum is widely utilized in ayurvedic medicines for the treatment of numerous conditions such as menorrhagia, hemorrhoids, vomiting, corns, ophthalmia and hematemesis. Root extract is being used for its hepatoprotective, laxative, diuretic and anti-psychotic effects (Afzal et al., 2013). Paste of the crushed leaves is applied on skin for the treatment of boils and abscesses (Saikia et al., 2006). In Germany, anthroposophic physicians prescribed Bryophyllum pinnatum preparations for tocolysis and behavioral disorders.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Bryophyllum Pinnatum plant leaves were collected from the same trees present in the

sathupalli forest area.

Preparation of Bryophyllum pinnatum leaves powder:

Plant leaves are collected and air-dried to prevent it from direct sunlight impact to minimize

undesirable chemical reactions of plant metabolites. Dry conditions are crucial to prevent the

formation of artifacts as a result of microbial fermentation and subsequent degradation of the

plant metabolites. Hence in the present study, leaves are dried in shade and then powder with

a mechanical grinder. The powder was passing through sieve number 44 and stored in an

airtight container for further studies.

Extraction procedure:

Ethanolic Extraction:

Fresh leaves of bryophyllum were washed with distilled water and air dried for 5 min chop

the leaves into small pieces and soak in ethanol and water in 70:30 ratio. In an air tight

container & macerate with frequent agitation for 3 days. Then filter the solution& obtain

alcoholic and hydro alcoholic extract. Dry the contents using a rotatory evaporator. Store the

contents in airtight container.

Phytochemical Screening:

The extracts of different plant materials were subjected to phytochemical studies using the

Standard method described by Trease & Evans (1989).

RESULTS AND DISCUSSION:

Preliminary phytochemical screening of plants was predominant to the detection of bioactive

principles which is a new source of therapeutically and industrially valuable compounds that

may lead to the discovery of new drugs. In the present study, the presence of phytochemicals

was screened with the ethanol, aqueous extracts of the Bryophyllum pinnatum leaves and the

results are shown in Table 1. Crude extracts and medicines are manufactured based on the

principles of natural compounds even by pharmaceutical companies, may lead to large scale

exposure of humans to natural products. Presence or absence of important bioactive compounds in an extracts was identified by color reactions with specific chemicals, this procedure is simple for preliminary pre-requisite before going to phytochemical investigation. Hence, in the present work, the crude extracts obtained by aqueous, ethanol solvents were screened for the presence of phytochemicals. The ethanol extract shows the presence of saponins, flavonoids, proteins, glycosides and terpenoids, alkaloids, anthraquinones, and carbohydrates. Saponins have health benefits such as lower cholesterol, antimicrobial, anti-inflammatory and anticancer properties [15]. The aqueous extract shows the presence of steroids, flavonoids, tannins, glycosides, terpenoids, alkaloids, anthraquinones, starch, reducing sugars.

Table No. 1: Preliminary phytochemical screening of *Bryophyllum pinnatum* leaves

Test	Ethanol	Aqueous
Alkaloids	+	+
Steroids	- :	+
Tannins		+
Anthraquinones) terri	+
Resins		-
Phenols	HUMAN	+
Flavonoids	+	+
Terpenoids	+	+
Glycosides	+	+
Reducing sugars	+	+

⁺ indicates the presence of the phytochemical;

Phenolic compounds have biological and pharmacological properties such as anti-inflammatory, antioxidant, and antimutagenic and anticarcinogenic activities. Flavonoids are secondary metabolite having various pharmacological properties such as anti-oxidative, antifungal, anti-inflammatory and diuretic actions [16-18]. This research finding highlights that aqueous extracts of *Bryophyllum pinnatum* leave had the highest number of phytochemicals compared to ethanolic extract.

⁻ indicates the absence of the phytochemical

The mechanism for anthelmintic activity of plant extracts are due to the presence of secondary metabolites bind to free proteins in the gastrointestinal tract of host animal and glycoprotein on the cuticle of the parasite. The result of anthelmintic activity on earthworm *Pheretima posthuma* was shown in Table-2 reveals that, different concentrations used have shown paralysis and death of worms and it was compared in the same concentration with Piperazine citrate. This standard drug may cause hyperpolarization of worms muscle by GABA agonistic action opening Chloride ion channels that cause relaxation and depresses responsiveness to contractile action of Acetylcholine. By increasing, chloride ion conductance of worm muscle membrane initiates hyperpolarization and reduced excitability that led to muscle relaxation and flaccid paralysis.

Table No. 2. Anthelmintic activity of Ethanolic extract of Bryophyllum pinnatum leaves

Groups	Dose in Concentration (mg/ml)	Time of paralysis (min)	Time of death(min)
Control	-	-	-
Ethanolic extract	10	19.23±4.13	23.15 ± 2.35
	20	15.15± 2.58	20.06±2.28
	50	11.29± 2.19	12.48 ± 2.37
Standard drug	10	11.16 ± 1.05	13.12 ± 2.59
	20	9±2.35	11.22 ± 1.34
	50	6 ± 0.27	8.49 ± 0.28

The ethanolic extract of *Bryophyllum pinnatum* leaves and standard drug solution not only illustrate paralysis, but also causes death of worms especially at higher concentrations of 50 mg/ml, in very less time was shown in Fig 1, 2.

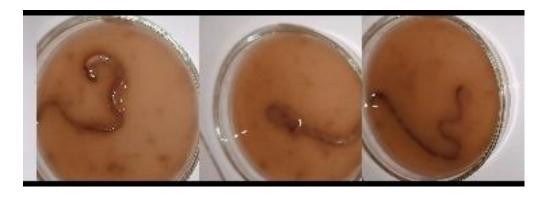


Figure No. 1: Anthelmintic activity of Ethanolic extract



Figure No. 2: Anthelmintic activity of standard drug

In addition, Tannins or their metabolites have an undeviating effect on the possibility of the pre-parasitic stages of helminthes and other phytochemical constituents might be responsible for an anthelmintic activity include flavonoids and terpenoids. This present research work shows the presence of different phytochemical constituents like triterpenoids, glycosides, anthraquinone, and flavonoids with biological activity that can be valuable therapeutic index. The plant extracts can be used for further isolation of compounds for their anthelmintic activity.

CONCLUSION

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.

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