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Analysis of Phytochemical Constituents and Anthelmintic Activity of Leaf Extracts of *Mimosa pudica* L.

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Abstract

***Mimosa pudica* L. is commonly called as touch me not plant, a prostrate or semi-erect sub shrub of tropical America and Australia, also found in India. The phytochemical screening of the crude extract showed the presence of carbohydrates, alkaloids, cardiac glycosides, coumarin glycosides, saponins, flavonoids, phytosterols, phenols and tannins, proteins and terpenoids. The methanol extract showed the highest percentage extractive of 11.6. The anthelmintic activity was tested on Indian adult earthworm Pheretima posthuma. The leaf was extracted separately with methanol and distilled water using cold extraction method. Different concentrations (20, 40, 60 and 80 mg/ml) of each extract were tested for anthelmintic activity by determining the time of paralysis and time of death of the worms. The aqueous and methanol extracts showed anthelmintic activity at all concentrations and its activities are well comparable with the standard drug, albendazole (positive control). The methanol extracts showed the highest anthelmintic activity than albendazole as well as the aqueous extract. Tween 20 (1%) with saline was used as negative control did not show any anthelmintic activity.**

Keywords: Albendazole, Anthelmintic, Extract, *Mimosa pudica*, Phytochemical.

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Introduction

Herbal medicines have become a subject of increasing global attention, for their health benefits and economic considerations. Medicinal plants are a rich source of secondary metabolites and are exploited from time immemorial as powerful drugs in traditional or alternative healthcare systems [1]. The demand for plant based medicines and other herbal healthcare products, including pharmaceuticals, food supplements (functional foods), cosmetics (cosmeceuticals), etc. are increasing steadily in developing and developed countries. During the last two or three decades, rapid increase in the rate of infections, antibiotic resistances in microorganisms and side effects of synthetic antibiotics are on the rise. The advancement in phytochemistry and identification of new bioactive compounds from plants has renewed the popularity of herbal medicines [2].

Helminths are the most common infectious agents of humans in developing countries and less developed countries because the helminthes predispose humans and cattle to bacterial and fungal infections. Helminth infections pose a major challenge to public health and it is mainly faced by millions of school children. Helminths are worm-like organisms which lives and receive nourishment from the living host and disturbs the hosts' nutrient absorption mechanism. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract and develop into well differentiated adult worms. Anthelmintics are a group of antiparasitic drugs which act locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminths or development forms without causing significant damage to the host [3]. Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections [4].

Mimosa pudica L. (Mimosaceae) is a creeping annual or perennial flowering plant found in Asian countries. *M. pudica* has been identified as Lajjalu in Ayurveda and has been found to have hepatoprotective, hypolipidemic, antifertility, antiasthmatic, aphrodisiac, analgesic, sedative, emetic, tonic properties and antidepressant properties [5]. Phytochemicals like alkaloids, saponins, coumarin, non-protein amino acid (mimosine), flavonoids, cardiac glycosides, sterols, terpenoids, tannins, and fatty acids were reported to be present in *M. pudica* [5].

The screening phytochemicals present in leaf extracts of *M. pudica* showed the presence of phytochemicals such as

steroids, terpenoids, carotenoids and flavanoids which are found to exhibit antimicrobial, heamolytic and foaming activity [6]. The environmental factors such as soil conditions, climate, altitude and rainfall may affect growth of plants and in turn influence the production of phytochemicals in them [7]. The aim of this study was to explore the presence of phytochemical constituents present in seven different solvent extracts and anthelmintic activity of aqueous and methanol extracts of leaf samples of *M. pudica* collected from Alappuzha, Kerala State, India.

Materials and Methods

Collection and identification of plant

The test plant, *Mimosa pudica* was collected from S.D.V. College of Arts and Applied Science College campus, Alappuzha, Kerala, India and Dr. Shaji P.K., Scientist, Environmental Resources Research Centre (ERRC), Thiruvananthapuram, Kerala State, India, authenticated this plant. The shade dried leaf samples were cleaned, washed, dried and pulverized to coarse powder using an electric grinder.

Chemicals and drugs

The solvents for the extraction process were of analytical grade and purchased from SD Fine Chemicals, Mumbai, India and the standard drug albendazole was purchased from Cipla Limited. Chemicals such as sulphuric acid, NaOH, ferric chloride, ninhydrin, Tween 20, HCl and mercuric chloride were purchased from HI Media Laboratories Pvt. Limited, Mumbai, India.

Determination of percentage extractive

Leaf powder (5 gm) was macerated with 50 ml of respective seven solvents in closed flasks for 24 hr and was frequently shaken with 6 hr time intervals and was allowed to stand for 24 hr. After filtration, 25 ml of the filtrate was evaporated to dryness and dried at 105 °C and weighed. Percentage of soluble extractive was calculated with reference to the air dried drug. The percent extractive of each solvent extract of *M. pudica* was calculated by

$$Percent\ \ extractive = \frac{Weight\ \ of\ \ dried\ \ extract}{Weight\ \ of\ \ dried\ \ plant\ \ material} \times 100$$

Phytochemical analysis

The phytochemical constituents present in *M. pudica* leaf were carried out with seven different solvent extracts (i.e. hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) as mentioned above using standard methods [8,9].

Anthelmintic assay

Preparation of plant extract: The dried leaf powder (100 g) of *M. 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 No 1. The pH of the extracts was adjusted to 7 and these extracts were further diluted to 20, 40, 60, 80 mg/ml in normal saline containing 1% Tween 20.

Worms collection: Pheretima posthuma (Indian earthworm) were procured from The Little Flower Nursery and Organic Manures, Kalavoor, Alappuzha, Kerala, India and the faecal matter present the worms were washed with normal saline.

Preparation of standard drug: Albendazole, the standard drug was prepared with different concentrations 20, 40, 60 and 80 mg/ml using normal saline and diluted with 1 % Tween 20.

Anthelmintic assay: The earthworm P. posthuma was divided into five groups consisting of two equal sized earthworms in each group (in triplicates) 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, second group was treated with tween 20 along with normal saline served as negative control. The third group served as standard drug, containing albendazole at 20, 40, 60 and 80 mg/ml in tween 20 (1%) diluted with normal saline. The methanol and water extracts at different concentrations (20, 40, 60 and 80 mg/ml) constituted the fourth and fifth group. All the test solutions and standard solutions were prepared freshly before starting the experiment. The mean paralysis time was noted when no movement of earthworm could be observed and the death time was recorded in minutes after confirming that worms neither moved when shaken nor when an external stimuli given by putting the motionless worms in 50^{o}C hot water [10]. Deaths 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. The paralysis and death time were expressed as mean ± standard error for mean (± SEM). $P < 0.05$-0.01 considered as statistically significant.

Results

The preliminary qualitative phytochemical analysis was performed for different solvent leaf extracts of *M. pudica*. Carbohydrate was present in all the different solvent extracts. Alkaloids were present in methanol, water, chloroform, ethyl acetate and acetone extracts. Fats and oils were absent in all the tested extracts. Phenols and tannins were present only in methanol extract and it showed the presence of maximum number of different phytochemicals. The presence of other

phytochemicals present in different solvent extracts is given in Table 1.

Table 1: *Phytochemical constituents of leaf extracts.*

	Solvent extracts						
Phytochemicals	**Hexane**	**Chloroform**	**Dichloro methane**	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
Carbohydrate	+	+	+	+	+	+	+
Alkaloids	-	+	-	+	+	+	+
Cardiac Glycoside	-	-	-	-	-	+	+
Coumarin Glycoside	-	-	+	+	+	+	+
Saponins	+	+	-	-	-	-	-
Flavonoids	-	+	-	+	-	+	-
Phytosterols	-	-	+	-	+	+	-
Fats and oils	-	-	-	-	-	-	-
Phenols & Tannins	-	-	-	-	-	+	-
Proteins	-	-	-	-	-	+	+
Terpenoids	-	-	_	-	+	-	+

The percentage extractives of hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and aqueous extracts of *M. pudica* are given in the Table 2. Methanol extract showed the highest percentage extractive value of 11.6 and hexane showed the lowest percentage extractive value of 5.4.

Table 2: *Percentage extractive of leaf extracts.*

Solvents	**Hexane (%)**	**Chloroform (%)**	**Dichloro methane (%)**	**Ethyl acetate (%)**	**Acetone (%)**	**Methanol (%)**	**Distilled Water (%)**
Percentage extractive	5.4	6.2	8.4	7.6	8.9	11.6	7.5

The anthelmintic activity of aqueous and methanol extracts of *M. pudica* are given in Table 3. The aqueous extract showed considerable activity and the time taken for the paralysis and death vary with different concentrations. The earthworms exposed to 80% aqueous extract showed paralysis at 71 minutes and death at 97 minutes. Whereas the control drug at 80% concentration exhibited 16 minutes for paralysis and 48 minutes for death. Lesser concentrations took a long time for paralysis and death (Table 3) and the anthelmintic activity of aqueous extracts increased with increase in concentration. The aqueous extract exhibited poor anthelmintic activity when compared to the standard drug.

Table 3: *Anthelmintic activity of extracts.*

Group	**Extract**	**Dose mg/ml**	**Response**	
			Paralysis (minutes)	Death (min)
I	Normal Control	- - - - - -	- - - - - -	- - - - - -
II	Negative Control	- - - - - -	- - - - - -	- - - - - -
III	Standard (Albendazole)	20 40 60 80	163 ± 0.58 151 ± 1 19 ± 0.58 16 ± 0	212 ± 1 198 ± 0.58 57 ± 0 48 ± 1.2
IV	Water	20 40 60 80	195 ± 1.52 159 ± 110 2 ± 1 71 ± 0.5	234 ± 1 188 ± 1 136 ± 1 97 ± 0.57
V	Methanol	20 40 60 80	22 ± 1 18 ± 0.57 14 ± 1 15 ± 1.52	30 ± 1 27 ± 0.57 23 ± 1.5 17 ± 1

All Values represent Mean ± SD; n=6 in each group. Comparisons made between standard versus treated groups, $P < 0.05$ was considered significant. The methanol extract showed higher activity than the standard drug. The time taken

for death and paralysis was very low. The methanol extract of 80% took only 15 minutes for paralysis and 17 minutes for death. The lowest concentration of 20% showed 22 minutes for paralysis and 30 minutes for death. The time taken for the death and paralysis of the methanol extract is lower than that of the standard drug.

Discussion

Different plants would have different chemical constitution and the composition of these chemicals may vary from one part to another part within the same plant. The phytochemicals present in the plant can be dissolved in various solvents depending on its solubility. Therefore seven solvents such as hexane, chloroform, dichloromethane, acetone, ethyl acetate, methanol and distilled water were selected to prepare different extracts to determine the soluble phytochemical constituents. The present investigation revealed the presence of phytochemicals such as carbohydrate, alkaloids, cardiac glycoside, coumarin glycoside, saponins, flavonoids, phytosterols, phenols, tannins, proteins and terpenoids in different solvent extracts of *M. pudica* leaf and a similar observation was reported by Rajendran and Sundararajan [11]. More phytochemicals and the highest percentage extractive (11.6%) were found in methanol extract and it showed the highest anthelmintic activity. Phytochemicals or secondary metabolites usually occur in complex mixtures that differ among plant organs and stages of development [12]. Knowledge of the phytochemical constituents present in *M. pudica* leaf will be very useful for the maximum exploitation of this plant in medicine. It has been reported that phytochemicals present in plants are the vital sources of antiviral, antitumor and antimicrobial agents, so they are used as constituents in allopathic medicine [13] and also in other systems of medicine.

In the present study, anthelmintic activity of aqueous and methanol leaf extracts of *M. pudica* was tested against P. posthuma which resembles intestinal worms in their reaction to anthelmintic agents and are easily available. The aqueous extract exhibited poor anthelmintic activity when compared to the standard drug albendazole, whereas the methanol extract showed the highest activity than the standard drug. The presence of alkaloids, phenols and tannins in methanol extract may be accountable for the highest anthelmintic activity. The aqueous extract contained alkaloids and this was responsible for the anthelmintic activity. Phenols and tannins were absent in aqueous extract. The anthelmintic activity of aqueous and methanol extracts increased with increase in the concentrations. Bendgude et al. [14] also reported that *M. pudica* extracts (aqueous and ethanol) significantly reduced the paralysis and death time of worms in a dose dependent manner when compared to the standard reference drug, albendazole. Phytochemicals such as alkaloids, tannins, phenols etc. are reported to have significant anthelmintic activity [15] and alkaloids were reported to act on the central nervous system and cause paralysis of the earthworm. Tannins were reported to interfere with energy generation of worms by uncoupling oxidative phosphorylation or they bind to the free protein of the gastrointestinal tract and leads to the death of the worms [16]. Phytochemicals together or separately may act by inhibition of tubulin polymerization and block glucose uptake [17] and damage the mucopolysaccharide membrane of worms and this will expose the outer layer and restrict their movement of earthworms which finally may cause paralysis and ultimately death [18].

Conclusions

The phytochemical screening of *M. pudica* in hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and aqueous leaf extracts revealed the presence of phytochemicals such as carbohydrate, alkaloids, cardiac glycoside, coumarin glycoside, saponins, flavonoids, phytosterols, phenols, tannins, and proteins in different extracts. Most of the phytochemicals were present in methanol extract and most of them were absent in hexane extract. Aqueous and methanol leaf extract of the *M. pudica* showed anthelmintic activity in a dosage dependent manner. Methanol extract showed the highest anthelmintic activity at all concentrations tested than the standard drug.

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