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**In vitro anthelmintic activity of *Passiflora foetida* L. hydroalcoholic and ethyl acetate extracts**Sudhakar Kommu<sup>♦</sup>, M. Chinnaeswaraiiah, P. Meghana, A. Leela, M. Sravani and SK. Moin Baba

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## Article Info

## Article history

Received 11 May 2023

Revised 17 June 2023

Accepted 18 June 2023

Published Online 30 June-2023

## Keywords

*Passiflora foetida* (L.)

Anthelmintic activity

*Pheretima posthuma*

Albendazole

Phytochemicals

## Abstract

India is blessed with a wealth of medicinal plants, *Passiflora foetida* (L.) being one among them. It is popularly known as stinking passion flower, is an herbaceous climber. Several illnesses are treated using the secondary metabolites discovered in medicinal plants. It has numerous phytochemical components in *P. foetida* extracts. The very existence of phytochemicals suggests that plants play a major part in the treatment of diseases. *P. foetida* hydroalcoholic and ethyl acetate extracts were evaluated in *Pheretima posthuma* test worms at three distinct doses (25, 50, and 100 mg/ml) for their anthelmintic effectiveness. Results were analysed in terms of worm death and paralysis times and activity levels were compared with that of albendazole, which served as the gold standard. It was noted that the paralyzing and death times were dose dependently reduced. The hydroalcoholic and ethyl acetate extracts exhibited significant anthelmintic activity but hydroalcoholic extract showed more anthelmintic activity than ethyl acetate extract. In accordance with the research results, *P. foetida* may be used as an anthelmintic agent.

## 1. Introduction

The WHO estimates that traditional medicines are used by 88% of the population in 198 countries worldwide (Urvesh *et al.*, 2019). In the world, there has been a noticeable increase of interest in natural products as a potential source for new medications in recent years. Many of the current prescribed drugs are derived from plants. Natural products obtained from plant, animal and minerals can be used for the treatment of human diseases. India is enriched with the natural sources. Plants are used for the treatment of various ailments from ancient times itself. Nature provides the drugs in the form of herbs. Almost all flowering plants have medicinal properties and they are around 400,000 species. Nowadays, 80% of people obtain their basic medical needs for a number of diseases *via* traditional medicines. Nowadays, medicinal plants are used commonly due to the fact that green medicine is safe and easily available, whereas the synthetic drugs which are not safe and adverse effects are more common. Therefore, herbal remedies are considered to be the best for the prevention and treatment of some ailments (Sreesha *et al.*, 2017).

One among these plants for healing is *P. foetida*. Several illnesses can be treated using the secondary metabolites found in medicinal plants. These phytochemicals have the ability to reduce diseases occurrence (Jagadish *et al.*, 2015). *P. foetida* is a herbaceous climber, more or less viscous, densely hirsute, tendrils axillary, simple, leaves up to 10 x 9 cm, usually 3-lobed, silky hairy, with glandular hairs on margins, petioles glandular-ciliate but without true petiolar glands, stipules pectinate, corolla white, corona violet and white or purple and white,

berries globose, 2 cm across, orange when ripe, flowers occur during october to march.

The plant is referred to by a variety of names across languages, including Assamese - Koth-bel, junka phool, mewa, lota bel, jumka lata; Bengali - Jumka lata; English - Stinking passion flower, love in a mist; Hindi - Jumka lata, raaki phul, krishna kamal; Irula - Varingodi; Kannada - Kukkiballi; Malayalam - Poochapazham, amoomapazham; Manipuri - Lam radhikanachom; Marathi - Vel ghani; Tamil - Mossukattan, sirupunnaikalli; Telugu - Thelajumuki; Sanskrit - Mupparisavalli, mukkoopera, lomaphala, swaduphala. *P. foetida* occur around the globe as a weed and it is applied to the management of hysteria, asthma, sore head, giddiness, skin disease, diarrhoea, digestive problems, for poisonous bites (Shruthi *et al.*, 2022).

Helminthes or parasitic worms infection affect about 2 billion people world-wide. Anthelmintics are medications that kill or stupefy parasitic worms so that they can be eliminated from the body. They are also known as vermifuges or vermicides, which are also horrifying terms. The present helminth medications; however, are ineffective against gastrointestinal helminthes. Nowadays, the use of some anthelmintics results in toxicity to human beings. Examples of plants with anthelmintic activity are *Ocimum sanctum*, *Carica papaya*, *Centratherum anthelminticum*.

## 2. Materials and Methods

## 2.1 Collection and authentication

The *P. foetida* whole plants were collected from surroundings of Kodad town, Suryapet district, Telangana and India. The plant material was authenticated by Botanist, Dr. K. Srinivasa Reddy, Assistant Professor, Department of Botany, Govt. Degree College for Woman, Nalgonda, Telangana. The entire plant was shade dried, crushed and kept in an airtight container. The powdered material was employed in the extraction procedure.

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## 2.2 Extract preparation

The entire plant powder was extracted using the ultrasonication process with ethyl acetate and hydroalcoholic solvents. The extraction of *P. foetida* powder (whole plant) conducted by using ACZET Pvt. Limited, ultrasonic cleaner, model CUB 2.5, Sr.no 2019 10003 with electronic timer, quick degassing and heating. In this, a volumetric flask containing the plant powder sample and the solvent (first with ethyl acetate, then with 70 % ethanol in water and a solvent to sample ratio of 10:1) are placed in a temperature controlled ultrasonic bath and sonicated for four hours at a temperature below 100°C (typically 60°C). In order to maintain the sample to solvent ratio during the extraction, this mixture was covered with aluminium foil to prevent evaporation. Following extraction, the extract is concentrated and separated from plant debris using muslin cloth that has been folded twelve times. The percentage yield, colour and consistency were determined. The extracted product was used for phytochemical screening, quantitative estimation of phytochemicals and anthelmintic activity (Sudhakar *et al.*, 2022).

## 2.3 Screening for phytochemicals

*P. foetida* extracts were subjected to a qualitative phytochemical screening process using standard methods (Khandelwal, 2017; Kokate, 2011; Arpita, 2018).

## 2.4 Quantitative estimation of total flavonoids

The following method was used to carry out a quantitative analysis of total flavonoids present in hydroalcoholic extract of *P. foetida*. In a 250 ml beaker, 2.50 g of the sample were added to precisely 50 ml of 80 % aqueous methanol, which was then capped and let to stand for 24 h at room temperature. After removing the supernatant, the residue was extracted three more times using the same amount of ethanol. The sample went through a filter made of Whatman filter paper number 42 (125 mm). Subsequently, the filtrate was put into a crucible and dried over a water bath. A desiccator was used to cool the material inside the crucible before being weighed to ensure its constant weight. Calculated the flavonoid percentage with following formula (Poongothai, 2019):

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

## 2.5 Evaluation of anthelmintic activity

The effectiveness of *P. foetida* ethylacetate and hydroalcoholic extracts as anthelmintics against *Pheretima posthuma* was examined. By using a bioassay to measure the time of the worm's paralysis and their time of death, different concentrations (25, 50, and 100 mg/ml) of each extract were evaluated. We used saline water as the control and albendazole as the standard reference. On adult Indian earthworms, the test was carried out. Earthworms were employed for anthelmintic investigation after being removed from moist soil and thoroughly cleaned with normal saline. The earthworms were separated into four groups, each with six earthworms. Different extracts were added to DMSO (dimethyl sulfoxide) in small amounts, and the volume was then adjusted with saline water to 10 ml. Petri plates were filled with different extracts and standard drug solutions.

All of the earthworms were put into a 10 ml formulation containing three various concentrations of albendazole, a hydroalcoholic extract and an ethyl acetate extract. It was noted how long it took for

individual worms to become paralysed and death. When there was no movement visible other than when the worms were shaken ferociously, this was known as a time for paralysis. After losing their ability to move when submerged in warm water (50°C), the worm's body colours began to fade, signaling that they had died (Sudhakar *et al.*, 2019).

## 3. Results

### 3.1 Organoleptic results of extract

*P. foetida* whole plant powder was extracted with ethyl acetate and hydroalcoholic solvents by using ultrasonication extraction method. Colour, consistency and percentage yield were recorded in Table 1. Among them, hydroalcoholic extract showed highest yield compared to other extract shown in Table 1.



Figure 1: Ethyl acetate extract.

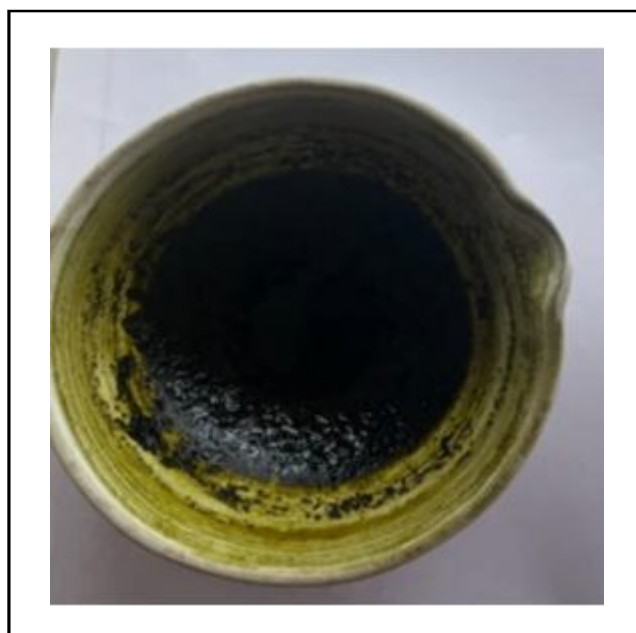


Figure 2: Hydroalcoholic extract.

**Table 1: The results of hydroalcoholic and ethyl acetate extracts of *Passiflora foetida* (L.) whole plant**

S. No.	Property	Hydroalcoholic extract	Ethyl acetate extract
1.	Colour	Greenish black	Greenish black
2.	Odour	Characteristic	Characteristic
3.	Consistency	Sticky	Sticky
4.	% yield	4 g	2 g

### 3.2 Phytochemical screening

The bioactive substances changed depending on the type of extracts and the extraction method (Kuntal Das *et al.*, 2021). So, using the

typical procedure outlined in the methodology section, the phytochemical screening was done on the two distinct extracts. As summarised in Table 2, the findings showed that the concentration of secondary metabolites was highest in the hydroalcoholic extract.

**Table 2: Results of photochemical screening of *Passiflora foetida* (L.)**

S. No.	Chemical constituents	Hydroalcoholic extract	Ethyl acetate extract
1.	Alkaloids	+	++
2.	Glycosides	++	++
3.	Flavonoids	++++	+
4.	Tannins and phenolic compounds	++++	+
5.	Saponins	+	<b>Absent</b>
6.	Steroids	++	<b>Absent</b>
7.	Amino acids	<b>Absent</b>	+
8.	Proteins	+++	+
9.	Carbohydrates	++	<b>Absent</b>

**Note:** ++++ = Abundantly, ++= Moderately, + = Small amount present

### 3.3 Quantitative determination of phytochemicals

The group of polyphenols known as flavonoids, which are typically present in plants, is the most significant one for the human diet

(Suman *et al.*, 2022). As a result, the total flavonoid content of a hydroalcoholic extract of the entire *P. foetida* plant was determined and summarised in Table 3.

**Table 3: Quantitative determination of flavonoids of hydroalcoholic extract**

S. No.	Phytochemical	Wt. of sample (g)	Wt. of dried filtrate (g)	% yield
1	Flavonoids	5	1.98	39.6

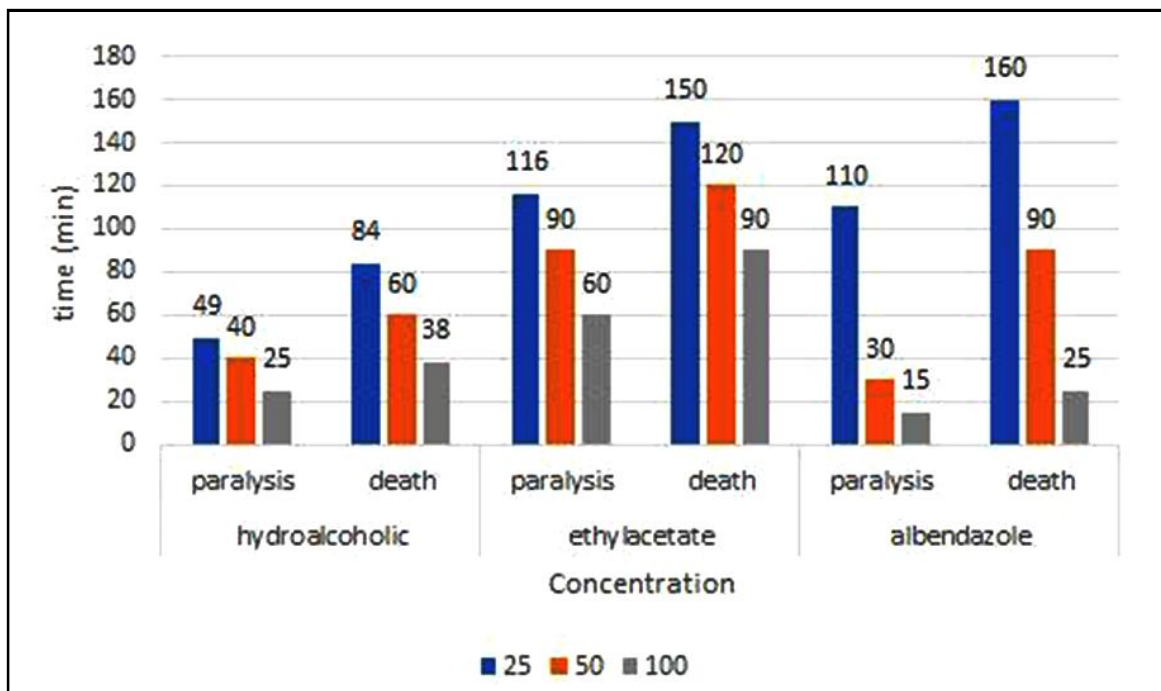
### 3.4 Anthelmintic activity

*P. foetida* ethyl acetate and hydroalcoholic extracts were chosen for testing for anthelmintic action. The outcome was seen in the organism's paralysis and death. Both extracts exhibited action,

although the hydroalcoholic extract shown greater activity than the other extract. Overall, both extracts activity indicated it was concentration dependant and the result was more noticeable when compared to the control and the standard as summarised in Table 4 and Figure 3.

**Table 4: Anthelmintic activity of *Passiflora foetida* (L.)**

S.No	Groups	Conc. (mg/ml)	Duration of paralysis (min)	Time to death (min)
1	Control	—————	—————	—————
2	Hydroalcoholic extract	2550100	494025	846038
3	Ethyl acetate extract	2550100	1169060	15012090
4	Albendazole	2550100	1103015	1609025



**Figure 3:** Graphical representation of anthelmintic activity of *Passiflora foetida* (L.) whole plant ethyl acetate and hydroalcoholic extracts.

#### 4. Discussion

In this study, different phytoconstituents have identified in the coarse powder of *P. foetida* whole plant by using ethyl acetate and hydroalcoholic solvents in ultrasonication extraction process. Colour, consistency and percentage yield were recorded in Table 1. Hydroalcoholic extract showed high extractive yield compared to ethyl acetate extract. The phytochemical studies were explored as per the procedures discussed above and phytochemicals like flavonoids, saponins, tannins, alkaloids and glycosides were found in the hydroalcoholic and ethyl acetate extracts of the *P. foetida* whole plant are given in the Table 2. The quantitative phytochemical analysis for hydroalcoholic extract was performed. The total flavonoids content was mentioned in Table 3.

The hydroalcoholic extract's anthelmintic activity was more potent than ethyl acetate and comparable to that of the albendazole, which is the standard drug. At a concentration of 100 mg/ml, the hydroalcoholic extract displayed comparable significant activity to the reference drug albendazole (100 mg/ml), with the times for paralysis and death being 25 and 38 min for the hydroalcoholic extract, respectively, and 60 and 90 min for the ethyl acetate extracts. According to (Table 4), the hydroalcoholic extract produced considerable anthelmintic action in a dose-dependent manner.

Earthworms can move by means of ciliary motion. The earthworm's mucilaginous outer layer is made up of complex polysaccharides. The earthworm is able to travel freely because this layer is slimy. The outer layer will be exposed if the mucopolysaccharide membrane is damaged, which would limit its ability to move and may even result in paralysis. By harming the mucopolysaccharide layer, this approach may result in the death of the worm. This irritates the body, which produces paralysis. By starving them to death or paralyzing them, anthelmintics all work to kill worms. Worms cannot

store energy, so they must consume food virtually constantly to maintain their metabolic needs. Energy is lost whenever there is an interruption in this process. Most adult parasites can be killed by preventing them from eating for 24 h or less. Paralysed parasites will also die because they will be unable to hold their place in the gut.

Alkaloids, tannins, glycosides, flavonoids, phenols, saponins, and steroids were all discovered during a preliminary phytochemical screening. Tannins potential mode of action may involve disrupting oxidative phosphorylation, which is a process that produces energy. Animals consume and absorb more digestible protein from plants that contain tannin. This is achieved by tannins in the rumen forming protein complexes, which later dissolve at low pH in the abdomen to release more protein for metabolism in the small intestines of ruminant animals. The earthworm was paralysed by alkaloids that may have acted on its central nervous system.

#### 5. Conclusion

The results of this investigation indicate that the hydroalcoholic and ethyl acetate extracts of *P. foetida* contain active ingredients that have anthelmintic activity. As there are a number of phytoconstituents, the plant possesses wide range of medicinal properties also. Further studies might be carried out to explore more about the plant. From the result, we found that both extracts have significant biological activity. Hydroalcoholic extract showed more anthelmintic activity than ethyl acetate extract. The active components that give *P. foetida* extracts their anthelmintic properties still need to be isolated and characterized.

#### Acknowledgements

We are grateful to the administration of Anurag Pharmacy College in Ananthagiri, Kodad, for providing us with this significant opportunity.

## Conflicts of interest

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

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## Citation

**Sudhakar Kommu, M. Chinnaeswaraiyah, P. Meghana, A. Leela, M. Sravani and SK. Moin Baba (2023).** In vitro anthelmintic activity of *Passiflora foetida* L. hydroalcoholic and ethyl acetate extracts. *Ann. Phytomed.*, **12**(1):413-417. <http://dx.doi.org/10.54085/ap.2023.12.1.99>.