



## Assessment of Anthelmintic Activity of *Pisum sativum*

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### ABSTRACT

In Indian medicinal plant literature, plant of *Pisum sativum* have been traditionally reported medicinal value as astringent, anticancer activity. Diuretic activity, Hepatoprotective activity, Antispermato-genic activity, Antioxidant activity, Antidiabetic activity. The extract of Whole plant of *Pisum sativum* was screened for anthelmintic activity on Indian earth worm in comparison to standard drug Albendazole. The concentrations *Pisum sativum* extracts and Albendazole were kept same for comparative activity. Saline water was kept as control. Determination of anthelmintic activity was done by recording the paralysis time and death time. Phytochemical test on plant extracts were carried out. The result showed that the anthelmintic activity of plant extracts was comparable to that of the reference drug Albendazole.

**Keywords:** *Pisum sativum*, Helminthiasis, Anthelmintic activity, Albendazole.

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### INTRODUCTION

Helminths is a Greek word meaning “worm”. It was supposed to be used only for intestinal worms but now includes tissue parasite free living species and mainly other worms<sup>1</sup>. Helminth infection are common parasitic infection affecting large number of populations, especially children. Helminths could be classified into three classes as Nematodea, Cestoidea and Trematodea. A wide range of worms are observed varying size from less than 1 mm – 1 m. People living in Australia, south-east Asia, India, Mexico, Sri Lanka, and Thailand are most affected by this infection. African people living in Sub-Saharan region are one of the most affected peoples<sup>2</sup>. From the current assessment 12-13% of world population have been infected by helminths which causes to severe morbidity it occurs due to persistence shortage, reduction in efficiency and poor socio-economic growth. AIDS, Malaria and Tuberculosis are more prone to be infected by helminthic. Inappropriate Sanitization is the primary reason of the Helminths infections in peoples. They mainly enter through contaminated drinking water or raw meats from infected animals. They may also get enter in body through skin by insect bites or walking and swimming in contaminated soil and water<sup>3</sup>. Helminths are host dependent macro-parasite. They require living host for survival, reproduction causing physical, nutritional,

cognitive impairment in young children<sup>4</sup>. The life cycle of Helminths is complicated which requires several hosts. The primary host for helminths infection is humans, in which worms reproduce sexually which leads to form eggs or larvae which could affect secondary host.

### Common symptoms of helminths infections are:

- Abdominal pain
- Hypoproteinemia
- Nausea
- Diarrhea
- Cough
- Slow down the mental growth and physical development

Anthelmintic drugs are drugs which are used for treatment of helminthic infections caused by various worms. These are the drugs which act locally to emit the worms from GIT or may also act systematically to eliminate adult helminths and prevent tissue and organs from developmental forms<sup>5</sup>. Anthelmintic drugs usually kill worms by either starving them to death or paralyzing them as worms don't store the energy. Anthelmintic drugs show paralytic action due to this they don't have ability to uphold their position in gut<sup>6</sup>. One of the most common synthetic anthelmintic drug used is Albendazole. It acts by blocking the glucose uptake of larvae; thus, it decreases the level of glycogen in adult which leads to decrease in formation of ATP, due to this the worms get immobilized and leads to death<sup>7</sup>.

### Need of Work

The synthetic antihelminth drugs are primarily used for treatment of helminthic infection caused by the various



species of helminthes. Ideally the anthelmintic agent should have large scale of action and should have effective curing ability with single therapeutic dose, without causing any harm to host<sup>8</sup>. The requirement for new and effective anthelmintic drug is massive, as the synthetic drugs used in the control of helminth is costly and the majority of them lose their efficiency in 15-20 years due to the problem of resistance<sup>9</sup>. Various synthetic medicine have been used for the treatment of helminthic infection like Albendazole, Benzimidazole, Thiabendazole, Levamisole, Butamisole, Pyrantel, Morantel, Oxantel, Bephenium and Thenium. Anthelmintic drugs have various adverse drug reactions like toxocariasis, severe renal failure, sickness, vomiting, abdominal pain<sup>10-13</sup>. These side effects caused by synthetic anthelmintic drugs can be overcome by using herbal formulation, herbal formulations are safe to use and cost effective, In ancient times near to 3000 years ago in India when there are no synthetic medicines was developed then people used Ayurvedic plants<sup>14-16</sup>. The herbal drugs contain chemicals as alkaloids, glycoside, flavonoid, glycoside, carbohydrate and protein<sup>17</sup>. In phytochemical screening, *Pisum sativum* extract indicated the presence of therapeutically active chemical constituent's flavonoids polyphenols alkaloids terpenoids and absence of saponin<sup>[15]</sup>. *Pisum sativum* contains various chemical constituents such as flavonoids, iso-flavonoids, anthocyanin and phenolic acids<sup>18-19</sup>. It also contains various nutrients like Protein, carbohydrate, starch, folate, vitamins as well as minerals<sup>20,21</sup>. *Pisum sativum* shows various pharmacological activities like Anticancer activity<sup>22,23</sup>. Anti-hyperglycaemic and anti-diabetic activity, Antioxidant activity, ACE inhibitors activity<sup>24-29</sup>.

## MATERIALS AND METHODS

### Plant Material

The plant material was gathered from nearby region of Pune, and was confirmed from branch of pharmacology AISSMS College of pharmacy, Pune. The pods of *Pisum sativum* were gathered in month of April. Collected plant pods were washed with water to eliminate soil and different contaminants. The pods were dried before preparation of extract.

### Drugs and Chemicals

**Table 1:** Drugs and chemicals

Sr.no	Reagent
1	Deionized water
2	Albendazole tablet

## METHODS

### Preparation of aqueous extract of *Pisum sativum*<sup>30</sup>

Green peas (*Pisum sativum L*) were bought from a nearby shop in Pune, and straight away taken to the laboratory for in addition processing. The peas were rinsed very well with faucet water, dried with tissue paper and the seeds

were isolate from the seed coat. The seed coats (the outer peel), which include nonedible component, were then cut into smaller portions and seed were triturated with mortar and pestle. Approximately 25 g of the outer peels was dipped in a 100 ml of deionized water in a 250 ml conical flask and heated for 10-15 minutes with non-stop stirring. This was then allowed to chill to room temperature and filtered via Whatman No.1 filter paper. The filtrate of *Pisum sativum* extract transferred in a neat and clear bottle and saved at 4 °c till addition use.

### Selection of the Experimental Model

Indian adult earthworms obtained from a neighborhood vendor were washed with normal saline to remove fecal matter. As per the experiment protocol earthworms of 8-10 cm length and 0.3-0.4 cm in width were used. Easy availability, anatomical and physiological similarities of earthworm with human intestinal round worm parasite was helpful to be used initially for in vitro evaluation (assay) of anthelmintic activity.

### Experimental Design<sup>31</sup>

Test extract of *pisum sativum* was examined for anthelmintic activity using earthworms. Numerous concentrations of extract were tested using bioassay; determinations for time of paralysis and time of death were included in testing. The standard reference drug was Albendazole while saline water was taken as control. Prepared extracts and standard drug solution was placed in different petri plates in which later the earthworms were released as per group and different concentrations. Earthworms were grouped into five and all the solutions were freshly prepared in normal saline before commencement of experiment. Time taken for paralysis and death of worms were the two notable observations in which time of paralysis was interpreted when no motility was seen except when the worms were vigorously disturbed. Interpretation of death was done when earthworms lost their mobility followed by fading away of their body colour.

### Preliminary Phytochemical Analysis:<sup>32</sup>

The aqueous extract of *Pisum sativum* was obtained from the above procedure and then subjected to qualitative tests for the identification of various plant constituents like alkaloids, flavonoids, carbohydrates, glycosides, saponin, proteins, and steroids.

#### 1. Detection of alkaloids

a) **Mayer's Test:** 1-2 drops of Mayer's reagent (Potassium mercuric iodide) treated with few ml filtrates. A creamy white/yellow precipitate formation indicates the presence of alkaloids<sup>31-33</sup>.

b) **Wagner's Test:** 1-2 drops of Wagner's reagent (Iodine in potassium iodide) treated with few ml filtrate. A brown/reddish precipitate formation indicates the presence of alkaloids.



c) **Dragendroff's Test:** 1-2 ml of Dragendroff's reagent (Solution of potassium bismuth iodide) treated with few ml filtrate. A reddish-brown precipitate formation indicates the presence of alkaloids.

d) **Hager's Test:** 1-2 ml of Hager's reagent (Saturated picric acid solution) treated with few ml filtrate. A creamy white precipitate formation indicates the presence of alkaloids.

## 2. Detection of flavonoids

a) **Alkaline Reagent Test:** 1 ml extract treated with 2 ml of 2% NaOH solution. An intense yellow color formation indicates the presence of flavonoids

b) **Lead Acetate Test:** 1ml plant extract treated with few drops of 10% lead acetate solution. A yellow precipitate formation indicates the presence of flavonoids.

## 3. Detection of carbohydrates

In 5ml distilled water the extract was dissolved individually and filtrates. Filtrate was used to test for the presence of carbohydrate.

a) **Molisch's Test:** 2ml filtrate treated with 2 drops of alcoholic alpha naphthol and 1ml conc. H<sub>2</sub>SO<sub>4</sub> along the sides of the test tube. A violet ring formation at the junction indicates the presence of carbohydrates.

b) **Benedict's Test:** 0.5ml filtrate treated with 0.5ml Benedict's reagent and then it boiled for 2 minutes. A green/yellow/red color formation indicates the presence of reducing sugar<sup>31, 35</sup>.

## 4. Detection of glycosides

Dilute HCl used for hydrolysis of extract and then subjected to check for glycosides.

a) **Modified Borntrager's Test:** Few ml of extract reacted with ferric chloride solution and immersed in boiling water for approximately 5 minutes. Cooled the mixture and extract with identical volumes of benzene. Separate the benzene layer and reacted with ammonia solution. Rose pink color produce in the ammonical layer shows the presence of anthranilic glycosides.

## 5. Detection of saponins

a) **Hemolytic Test:** Place one drop of blood on glass slide to which add test extract of *Pisum sativum*, formation of hemolytic zone takes place

b) **Foam Test:** 0.5 gm of extract was shaken with 2 ml of distilled water for 15 min. If foam produced is stable it indicates the presence of saponins<sup>36-37</sup>.

## 6. Detection of proteins

a) **Biuret's Test:** To 2ml of filtrate add 1 drop of copper sulphate, 1ml of 95% ethanol and KOH pellets pink to violet color formation in ethanolic layer indicates presence of proteins.

b) **Millon's Test:** To 2ml of extract add few drops of millon's reagent and heat, formation of white precipitate

is observed indicating presence of proteins.

c) **Xanthoprotein Test:** To extract add few drops of con. Nitric acid, yellow coloration is seen indicating presence of proteins.

d) **Ninhydrin Test:** To 2ml of extract adds 2 drops of Ninhydrin solution, purple coloration is seen indicating presence of amino acids<sup>37-40</sup>.

## 7. Detection of steroid<sup>41, 42</sup>

a) **Salkowski reaction:** Extract was shaken with chloroform and then 2ml con H<sub>2</sub>O<sub>4</sub> was added along sides of test tube, reddish-brown color formation indicated presence of terpenoids

b) **Liebermann-Burchard:** Extract of *Pisum sativum* was shaken with chloroform and then few drops of acetic anhydride were to the test tube followed by boiling in water bath and rapid cooling then add con H<sub>2</sub>O<sub>4</sub> along test tube, brown ring formation at junction of layers the upper later showing green coloration shows presence of steroids

## 8. Detection of phenols<sup>43-46</sup>

**Ferric Chloride Test:** To extract of *Pisum sativum* add few drops of 5% ferric chloride solution, dark green to bluish black coloration seen indicating presence of phenols.

## RESULTS AND DISCUSSION

The observations of preliminary phytochemical screening of aqueous extract of *Pisum sativum* are showed below. *Pisum sativum* extract gives positive results for presence of alkaloid, flavonoids, carbohydrate, saponins, steroids and protein.

### Observation Table

**Table 2:** Preliminary phytochemical screening

Sr no.	Phytochemical test	Result
1	Test for alkaloids	Positive
2	Test for flavonoid	Positive
3	Test for carbohydrates	Positive
4	Test for glycoside	Negative
5	Test for saponin	Positive
6	Test for protein	Positive
7	Test for steroids	Positive
8	Test for phenols	Negative

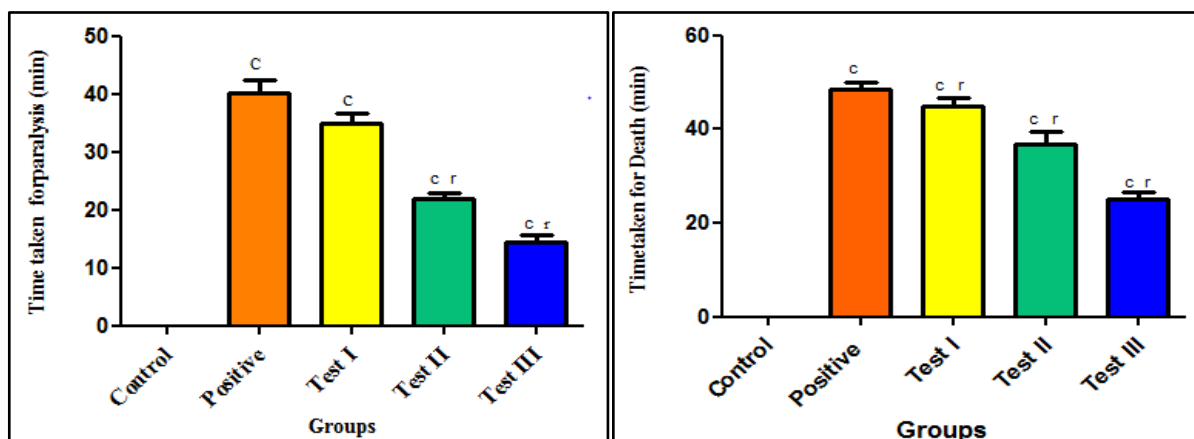
### Anthelmintic activity of aqueous extract of *Pisum sativum* analysis

Aqueous extract of *Pisum sativum* was given, which shows significant activity on earthworm. It was seen that When Control Group was compared with Positive Group and Test Group- I, II, III it showed significant ( $p < 0.001$ ) in paralytic condition and death. When Positive Group was compared with Test Group-III and Group-III it showed significant ( $p < 0.001$ ) in paralytic condition and death.



**Table 3:** Anthelmintic activity of aqueous extract of *Pisum sativum* analysis

Treatment	Concentration for (mg/ml)	Paralysis time min	Death time min
Group I -Control	-	No paralysis	No paralysis
Group II -Albendazole	(50mg/ml-Albendazole)	40.17±2.30	48.50 ± 1.60
Group III - Test I	(5ml extract + 4ml Albendazole-50mg/ml)	35.00±1.65	38.00 ± 1.71
Group IV - Test 2	(10ml extract + 4ml Albendazole-200mg)	21.83 ±1.13	28.00 ± 2.63
Group V - Test 3	(15ml extract + 4ml Albendazole-200mg)	14.33 ±1.30	19.00 ± 1.48



Values represent mean ± SEM; n=6; Analysis was performed using one way ANOVA Followed by Tukey's multiple comparison tests. A p value less than 0.05 was considering as statistically significant. p value: a<0.05, b< 0.01, c< 0.001 when compared with control. p< 0.05, q<0.01, r<0.001 when compared with positive control. x< 0.05, y< 0.01, z< 0.001 when compared with standard group

## CONCLUSION

Phytochemical analysis of the extracted revealed presence of phytoconstituents such as alkaloid, flavonoids, carbohydrates, glycoside, and protein. The present data indicate that aqueous extract of *Pisum sativum* is to be a safe anthelmintic effect and could be used as a part of therapy to treat parasitic infections of humans. Based on the findings of the present study it is concluded that, the aqueous extract of *Pisum sativum* found to have confirm their anthelmintic activity. We can conclude that aqueous extract of *Pisum sativum* exhibited most significant anthelmintic activity among the other Group. During study this plant showed very significant anthelmintic activity at Group- I, II, III measured by time taken for paralyse / death of the earth worms. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

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