**ABSTRACT**

Brassica juncea and Brassica oleracea were easily available and commonly used in our day-to-day life. Brassica juncea is extensively used as Anthelmintic and in treatment of alopecia, epilepsy, snakebite, toothache and hiccough. Brassica oleracea is extensively used as Anthelmintic, healing of peptic ulcers, slowing down growing cancer cells. The present study aimed at the in-vitro comparative study of anthelmintic activity of aqueous and hydroalcoholic extracts of seeds of Brassica juncea and flower of Brassica oleracea. The percentage yield of the extract (1.33% and 1.00% respectively) were screened for their anthelmintic activity using Pheritima posthuma. The activities were comparable with the standard drug Albendazole. When the doses of the extract is increased, a gradual increase in anthelmintic activity is observed. The study involved the determination of time of paralysis (P) and time of death (D) of the worms. In conclusion, the use of seeds of Brassica juncea and flower of Brassica oleracea as an anthelmintic have been confirmed. Hydroalcoholic extract of Brassica juncea and Brassica oleracea showed more prominent anthelmintic activity. Of these two species Brassica juncea was more efficient. Further studies are suggested to isolate the active principles responsible for the activity.

**Key words:** Brassica juncea, Brassica oleracea, Anthelmintic, Albendazole.

**INTRODUCTION**

Helminth is a broad categorical term referring to various types of parasitic worms that reside in the body. Helminthiasis is a macroparasitic worm disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. Helminthiasis can have immuno modulatory effects on the host. Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminths or development forms that invade organs and tissues. Most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea. Anthelmintic from the natural sources may play a key role in the treatment of these parasite infections. Brassica juncea (Brassicaceae) is also commonly called as Indian Mustard (English). Old herbals suggested mustard for alopecia, epilepsy, snakebite, and toothache. However, anthelmintic activity of Brassica juncea and Brassica oleracea has not so far been scientifically proved, so the present study was carried out to assess the anthelmintic activity by using their aqueous and hydroalcoholic extracts.

**MATERIALS AND METHODS**

**Plant material**

The seeds of Brassica juncea and flower of Brassica oleracea belonging to family Brassicaceae were collected from local area of Anantapur district (India) and was identified and authenticated by Dr.B.Ravi Prasad Rao M.sc.,Ph.D, Department of Botany, Sri Krishnadevaraya University, Anantapur.

**Preparation of aqueous extract of Brassica juncea**

The seeds of Brassica juncea were collected, weighed and 500 gm was taken. It was minced using mixer grinder and finely macerated. After homogenization, it was extracted in 500 ml of distilled water for 7 days in the room temperature with intermittent shaking. After incubation, the whole extracts were filtered through filter paper. The filtrate is maintained in dark.

**Preparation of hydroalcoholic extract of Brassica juncea**

The seeds of Brassica juncea were extracted by maceration process by using 1000 ml of distilled water. In the maceration procedure a total amount of 500 gm powdered seeds were macerated over night. The extract was filtered and then it was concentrated. Then it was dried by rotary evaporator. The percentage yield of aqueous extract of Brassica juncea was found to be 4.14%.

**Preparation of aqueous extract of Brassica oleracea**

Fresh flower of Brassica oleracea was collected, weighed and 500 gm was taken. It was minced using mixer grinder and finely macerated. After homogenization, it was extracted in 500 ml of hydroalcoholic solvent (50% distilled water and 50% ethanol) for 7 days in the room temperature with intermittent shaking. After incubation, the whole extracts were filtered through filter paper. The filtrate is maintained in dark. To the marc 300 ml fresh solvent was added and refluxed for 90 min. The Whole extract was again filtered and both the filtrates were mixed together and concentrated. Then it was stored in dessicator for maximum of 3 days, later preserved in a deep freezer (-20°C) for further analysis. The percentage yield of hydroalcoholic extract of Brassica oleracea was found to be 3.32%.

**Preparation of hydroalcoholic extract of Brassica oleracea**

Fresh flower of Brassica oleracea was collected, weighed and 500 gm was taken. It was minced using mixer grinder and finely macerated. After homogenization, it was extracted in 500 ml of hydroalcoholic solvent (50% distilled water and 50% ethanol) for 7 days in the room temperature with intermittent shaking. After incubation, the whole extracts were filtered through filter paper. The filtrate is maintained in dark. To the marc 300 ml fresh solvent was added and refluxed for 90 min. The Whole extract was again filtered and both the filtrates were mixed together and concentrated. Then it was stored in dessicator for maximum of 3 days, later preserved in a deep freezer (-20°C) for further analysis.

**Drugs and chemicals**

Albendazole (Micro Lab.Ltd., Goa), normal saline and ethanol are used.
Phytochemical analysis

Preliminary phytochemical screening of *Brassica juncea* and *Brassica oleracea* extract revealed the presence of carbohydrates, proteins, amino acids, glycosides, alkaloids, flavonoids, tannins, and polyphenols. Table 1.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Brassica juncea</th>
<th>Brassica oleracea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diterpenoids</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Steroids</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Saponins</td>
<td>–</td>
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</tr>
</tbody>
</table>

+ = Present
- = Absent

Anthelmintic assay

The anthelmintic activity was evaluated on adult Indian earthworm using the reported methods with slight modification.[11,12] 50 ml formulations containing *Brassica juncea* and *Brassica oleracea* in five different concentrations of aqueous extract of its various fractions (100, 150, 200, 300, and 500 mg/ml) and hydroalcoholic extract of its various fractions (100, 150, 200, 300, and 500 mg/ml) were prepared and six worms (same type) were placed in it. The wide range of dose was taken to establish the relationship between dose and pharmacological activity and also to find out the minimum and maximum dose that can be better therapeutically effective in comparison to standard drug. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) followed with fading away of their body colour. Albendazole (20 mg/ml) was used as reference standard.

RESULTS AND DISCUSSION

Earthworms are invertebrates composed of many segments. They donot have bones and move by contracting and relaxing the body segments in sequence. Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy, enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer.[13] All anthelmintics essentially kill worms by either starving them to death or paralyzing them because worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs. Any disruption in this process results in energy depletion. Interfering with feeding for 24 hours or less is sufficient to kill most adult parasites. Parasites will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut.[13] Another possible mechanism of action is that they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and causes death.[14] Preliminary phytochemical screening of *Brassica juncea* and *Brassica oleracea* extract revealed the presence of carbohydrates, proteins, amino acids, glycosides, alkaloids, flavonoids, tannins, and polyphenols.

The possible mechanism of action of tannins may be:

a. Interfere with energy generation by uncoupling oxidative phosphorylation
b. May interfere with glycoprotein of cell surface
c. They can bind to free proteins in the gastrointestinal tract of host animal or to glycoprotein on the cuticle of the parasite and cause death.[14]

Alkaloids may act on central nervous system and cause paralysis of the earthworm. The effect would be due to presence of alkaloids which may suppress the transfer of sucrose from the stomach to the small intestine together with its antioxidant effect which is capable of reducing the nitrate generation which can interfere in local homeostasis which is essential for the development of helminths.[17]

Albendazole binds to free β-tubulin, inhibiting polymerisation and thus interfering with microtubule dependent glucose uptake by the worms.[18]

Both the aqueous and hydroalcoholic extracts showed anthelmintic activity in dose dependent manner as shown in (Table 2).

From the above results it was concluded that hydroalcoholic extract of *Brassica juncea* and *Brassica oleracea* showed more potent anthelmintic activity than aqueous extract of *Brassica juncea* and *Brassica oleracea*. Further work will emphasize the isolation and characterization of active principles responsible for anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of *Brassica juncea* and *Brassica oleracea* as an anthelmintic drug.
ACKNOWLEDGEMENTS

The authors thankful to Dr. P.Ramalingam, Division of Medicinal chemistry, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur for providing necessary facilities and Dr.B.Ravi prasad Rao, Deparatment of Botany,Sri Krishnadevaraya University,Anantapur for authentification of plant specimens.

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Source of support: Nil, Conflict of interest: None Declared