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EVALUATION OF ANTHELMINTIC PROPERTY OF *CARICA PAPAYA* SEEDS

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ABSTRACT

Background: For inflicting life-threatening infection in humans and different live stalks, the simplest action for controlling the calamitous situation is to apply potent anthelminthic remedy, but there are few anthelminthic medications on the market and those which might be to be had create resistance in the course of remedy in most of the species of helminths. Consequently, we are going to evaluate the anthelminthic assets of *Carica Papaya* seeds for regulating the life- threatening infection in humans and different live stalk. **Methods:** The seeds of *Carica Papaya* were kept for extraction, and the hot maceration method was adapted for extraction. The obtained extract was subjected to phytochemical screening and the tests for alkaloids, saponins, tannins, anthraquinone glycosides, reducing sugar for glycosides, and flavonoids were carried out by standard procedure. For the in vitro anthelminthic study, suspension of our crude extract was prepared at a dose of 200 mg/ml by using HPMC as a thickening agent, and for standard, albendazole was used. *Phertima posthuma* was used for anthelminthic studies because of its anatomical and physiological uniformity with the roundworms which are present in the intestine. The seeds of *Carica Papaya* were kept for extraction, and the hot maceration method was adapted for extraction. **Results:** Out of the seven samples of different concentration ratios, we got a 99% yield from the sample of pure water (powder), 82% from the sample of 70:30 HA and 0% from the sample of pure ethanol. Phytochemical screening was carried out by standard procedure. We found alkaloids, tannins, saponins, reducing sugars of glycosides, and flavonoids in our crude extract, and we got a negative result for anthraquinone. The sample of pure water (powder) shows the least time of paralysis and death, and as compared to reference standard albendazole, it shows only a difference of 12 sec for paralysis and 20 sec for the death of worms. **Conclusion:** As the average time of paralysis and death for extract of pure water (powder) was minimum as compared to other extracts and as compared to albendazole, it showed a difference of 12 secs for paralysis and 20 secs for the death of earthworms so we concluded that our evaluation was successful.

KEYWORDS: *Carica Papaya*, *Phertima Postuma*, Albendazole, Pure Water, Anthelminthic, Helminth.

INTRODUCTION

The word helminth is a Greek word that means worms. It has a plague history. The helminth egg was discovered in mummified human feces thousands of years ago, and we recognized many clinical features of helminth infection from Hippocrates' writings, Egyptian medical papyri, and the Bible.[1] Helminth has a low mortality rate, but its morbidity is high enough to increase the burden of noncommunicable disease in a number of developing countries. Helminth infection is common in children.[2]

They consist of two groups: flatworms, or Platyhelminthes, and nematodes, or roundworms. The bodies of flatworms consist of a plasma membrane, while the bodies of roundworms consist of a tough cuticle. The characteristic features of all helminths include relatively and very large body structures, and all are termed active feeders. Most flatworms are hermaphroditic or bisexual, whereas roundworms consist of separate sexes. The exposure of helminthic infections depends upon the climatic conditions, food preferences, hygiene, and contact with vectors.[3] In the case of nematodes, especially male nematodes, they consist of accessory sex organs along with the alimentary canal and respiratory system. But the tapeworm does not have an alimentary canal and, due to this, it absorbs its nutrients from the tegument.[4]

Intestinal helminths which are responsible for causing infection in humans include roundworms and flatworms, and their larval stage takes place in an intermediate host, whereas the adult worms survive in a mammalian host. The other nematodes like hookworms and whipworms do not have the pulmonary phase of a life cycle. There is no need for an intermediate host – eggs pass through the intestinal lumen, and due to fecal contamination of food, ingestion of infectious eggs takes place and a new cycle for infection begins, e.g., a naturally occurring *Heligosomoides polygyrus*, it is limited to the intestine of the host.[5]

Different types of helminths infection which are occurred in humans are as follows.

Table 1: List of helminth infections that occurred in humans[6]

Infection	Causative Agent
Lymphatic filariasis	*Wuchereria bancrofti; Brugia malayi*
Onchocerciasis	*Onchocerca volvu*
Ascariasis	*Ascaris lumbricoides*
Hookworm infection	*Necator americanus; Ancylostoma duodenale*
Schistosomiasis	*S. mansoni S. haematobium S. japonicum*
Food-borne trematodiases	*Clonorchis Sinensis; Opisthorchis viverrine; Fasciolopsis buski; Fasciola hepatica*
Cysticercosis	*Taenia solium*
Strongyloidiasis	*Strongyloides stercoralis*
Schistosomiasis	*Schistosoma haematobium* *Schistosoma japonicum* *Schistosoma mansoni*
Fascioliasis	*Fasciola hepatica*
Hydatid cyast	*Echinococus granulosus*
Trichinosis	*Trichinella spiralis*
Trichuriasis	*Trichuris Trichur*

Classification of helminths

Classification of helminths according to their shapes are classified as follows; Table 2.

Table 2: Classification of Helminth according to shape and size[6]

Species	Shape	Groups
Schistosoma mansoni	Elongated	Helminth
Trichuris trichiura	Barrel Shape	Helminth
Enterobius vermicularis	Oval	Helminth
Hymenolepis diminuta	Round to oval	Helminth
Hymenolepis nana	Oval	Helminth
Ancylostomatidae	Oval	Helminth
Strongyloides stercoralis	Rhabditiform	Helminth
Ascaris lumbricoids	Oval	Helminth

As new synthetic anthelmintic medications or vaccines are unlikely to become accessible in the near future, alternative measures for parasite infection management are urgently needed. Several medicinal herbs have been employed (anthelmintic) in the treatment of GI nematode and other helminth infections. [7] Vernonia amygdalina and Annona senegalensis, for example, are two plants used by local farmers and live stalk holders in Nigeria.[8] In a recent experimental investigation, papaya was found to have an anthelmintic effect against *Ascaridia galli* and other parasites. It is the plant which is most commonly used from the earliest periods that contains proteolytic enzymes.[7] Therefore, the present study was carried out on *Carica Papaya* for evaluation of anthelminthic property.

MATERIALS AND METHODS

Materials

Ethanol, Methanol, Acetone, saline solution, Drangendroff's reagent, 10% ammonia solution, Felhing's reagent, 1% gelatin solution, Sodium chloride solution, were procured from Rankem Pvt. Ltd Mumbai of local market and distilled water from scientific shop of local market and Albendazole from local medical store

Plant material collection and authentication

For the evaluation, the seeds of *Carica Papaya* were used. These seeds were collected from ripe fresh papaya which were purchased from the local market of Amravati and authenticated at P.R. Pote Patil College of Agriculture, Amravati (281/2021). Then the seeds were washed with a saline solution to make them free from unwanted material, and these were kept for drying at room temperature. On drying, these materials were put through extraction (maceration).

Plant Profile

- Botanical name – *Carica Papaya*
- Kingdom – Plantae
- Sub Kingdom – Tracheobionta
- Class – Magnoliopsida
- Subclass – Dilleniidae
- Superdivision – Spermatophyta
- Phylum – Steptophyta

- Order – Brassicales
- Domain – Flower Plant
- Family – Caricaceae[9]

Figure 1: Carica Papaya[9]

Figure 2: Carica Papaya[10]

METHODS

Processing of plant material

The seeds of *Carica Papaya* are washed with saline water to remove debris and other foreign materials. After that, the collected seeds were air dried for 7 to 8 days. On drying, these seeds were weighed and stored in a clean, airtight zip lock pouch.

Extraction Process

The sample containing seeds was kept for extraction after drying. The extraction was carried out by the hot maceration technique as per standard procedure.[11,12] First, the samples were divided into seven groups for extraction; each contained a sample of 10 g the first was with pure water, the second was also with pure water containing powder of seeds (in this case, we formed coarse powder of seeds), the third was with pure ethanol (99.9%) and the hydroalcoholic preparations were prepared for the remaining samples in different concentration ratios: the first was 70:30 HA, which contains 70 ml of alcohol and 30 ml of water, the second was 30:70 HA, which contains 30 ml of water and 70 ml of alcohol; the third was of 50:50 HA, which contains 50 ml of water and 50 ml of alcohol; and the last one was prepared with ethanol, methanol, water and acetone.

All seven samples were assembled for hot maceration for 24 hours and after that, the filtrate was collected and kept for evaporation. On evaporation, the solid powdered was obtained as a product.

Collection of test organisms (earthworms) and authentication.

The earthworms of the species *Phertima posthuma* were used for study as per the standard procedure[13-16] and were collected from the moist soil of the garden of Shri Shivaji College of Horticulture, Amravati and were authenticated at P.R. Pote Patil College of Agriculture, Amravati (869 (B)/2021-22). The earthworms were used for investigating anthelminthic activity because of their anatomical and physiological similarity with the roundworms that are present in the intestine.

- Common name – Earthworm
- Scientific name – Lumbricus terrestrial
- Kingdom – Animalia
- Phylum – Annelida
- Class- Citellata
- Order – Opisthopora
- Suborder – Lumnricina
- Family – Megascolecidae
- Genus – Phertima
- Species – *Phertima posthuma*[17]

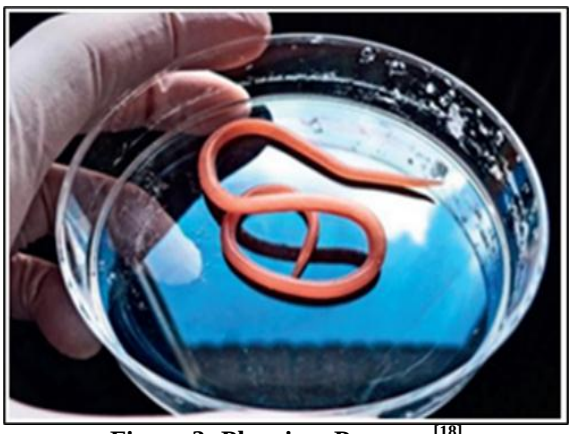

Figure 3: Phertima Postuma[18]

Phytochemical analysis of extract–

The solid powder extract, which is obtained after the extraction, was used for phytochemical screening. The phytochemical screening was carried out for secondary metabolites which are as follows: alkaloids, tannins,

saponins, reducing sugar for glycosides, flavonoids, anthraquinone glycosides as per the procedures which are mentioned in standard literature.[19-23]

***In -Vitro* anthelminthic study**

For investigation of anthelminthic activity of the obtained extract, the suspension of that crude extracts was prepared at a dose of 200 mg/ml by using HPMC as a thickening agent. As a standard drug, we used a suspension of albendazole at the same dose of 200 mg/ml.

For in vitro anthelminthic testing, we followed standard procedure by making certain modifications.[24-30] All suspensions for all extracts were freshly prepared in distilled water before starting the experimental procedure. Seven groups were prepared for in vitro testing of anthelminthic activity. Each group had three petri dishes containing the same dose of drug with one earthworm in each (*Phertima postuma*), same for albendazole. For investigation of anthelminthic activity of the obtained extract, the suspension of that crude extract was prepared at a dose of 200 mg/ml by using HPMC as a thickening agent. As a standard drug, we used a suspension of albendazole at the same dose of 200 mg/ml.

Until the paralysis and death of the individual worm, which is present in each Petri dish, the complete observations were made and the times of paralysis and the times of death of those worms were recorded.

Figure 4: Pure Water (seeds).

Figure 5: Pure Water (Powder).

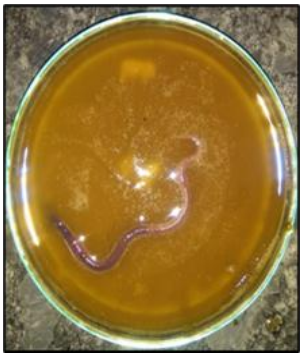

Figure 6: 70:30 HA.

Figure 7: Multi solvent.

Figure 8: 30:70 HA.

Figure 9: 50:50 HA.

Figure 10: Control.

Figure 11: Albendazole.

RESULTS AND DISCUSSION

Extraction yield

The extraction yield of all extracts of *Carica Papaya* seeds in the different solvents was different, as we carried out extraction in different solvents in different concentration ratios, like 70:30 HA, 30:70 HA, 50:50 HA, Pure water (seeds), Pure water (Seed Powder), Pure

ethanol, and multi-solvent (25% ethanol, 25% methanol, 25% water, 25% acetone).

The resultant extraction yield of all the different solvents was in grams, and it was converted into percentage and is shown in Table 3.

From the sample of pure ethanol, we got 0% yield, and from the sample of 70:30 HA, pure water (seeds), we got a higher amount of yield, i.e., 99%, 82%.

Table 3: Extraction Yield of different solvent extracts of *Carica Papaya*.

Extracts	Percentage Yield
Pure water (seeds)	82%
Pure water (Powder)	66%
Pure ethanol	0%
70:30 HA	99%
30:70 HA	49%
50:50 HA	58%
(Multi solvent Water, acetone, ethanol, methanol. 25:25:25:25)	60%

Phytochemical screening

The result of phytochemical screening is given in Table 4. We performed phytochemical screening by standard procedures and we found the presence of some secondary metabolites like alkaloids, tannins, saponins, reducing sugars of glycosides, and flavonoids in our plant material, and we got a negative result for anthraquinone glycosides.

Table 4: Result of Phytochemical Analysis of different solvent extracts of *Carica Papaya.*

Extracts	Alkaloids	Tannins	Saponins	Anthraquinone	Reducing Sugars of glycosides	Flavonoids
Pure water (seeds)	+	+	+	-	+	+
Pure water (Powder)	+	+	+	-	+	+
70:30 HA	+	+	+	-	+	+
30:70 HA	+	+	+	-	+	+
50:50 HA	+	+	+	-	+	+
Multi solvent	+	+	+	-	+	+

[(+) Present, (-) Absent]

***In vitro* anthelminthic study**

In an in vitro anthelminthic study, the average time of paralysis and death for each sample of a different concentration ratio of different solvent extracts was different. The result of this in vitro anthelminthic study is mentioned in Table 5. The graphical representation of the result of in vitro anthelminthic is shown in Fig 11 and 12.

For standard or for positive control, suspension of albendazole at a dose of 200mg was used, and for negative control, the normal saline solution was used.

Table 5: Average time of Paralysis and Death of *Phertima posthuma* against various extracts and reference drugs.

Concentration ratio (200 mg/ml)	Result			
	Time of Paralysis (mins)	Mean (mins)	Time of death (mins)	Mean (mins)
Control	-	-	-	-
Pure water (seeds)	a) 5.04 b) 5.45 c) 1.45	3.98	a) 1.00 b) 6.50 c) 3.17	3.55
Pure water (Powder)	a) 3.58 b) 5.38 c) 3.50	3.48	a) 1.10 b) 1.48 c) 2.50	1.70
70:30 HA	a) 4.00 b) 4.10 c) 4.15	4.08	a) 3.12 b) 3.03 c) 3.20	3.11
30:70 HA	a) 6.27 b) 7.40	6.34	a) 2.35 b) 3.43	3.00

	c) 5.35		c) 3.18	
50:50 HA	a) 3.42 b) 3.53 c) 5.57	3.50	a) 2.48 b) 2.45 c) 2.45	1.46
Multi Solvent (Water, ethanol, acetone, methanol) (25:25:25:25)	a) 11.00 b) 10.55 c) 11.00	10.08	a) 11.54 b) 10.37 c) 12.03	11.31
Albendazole (Positive control)	a) 3.35 b) 3.37 c) 3.36	3.36	a) 0.52 b) 1.10	0.90 sec or 1.50

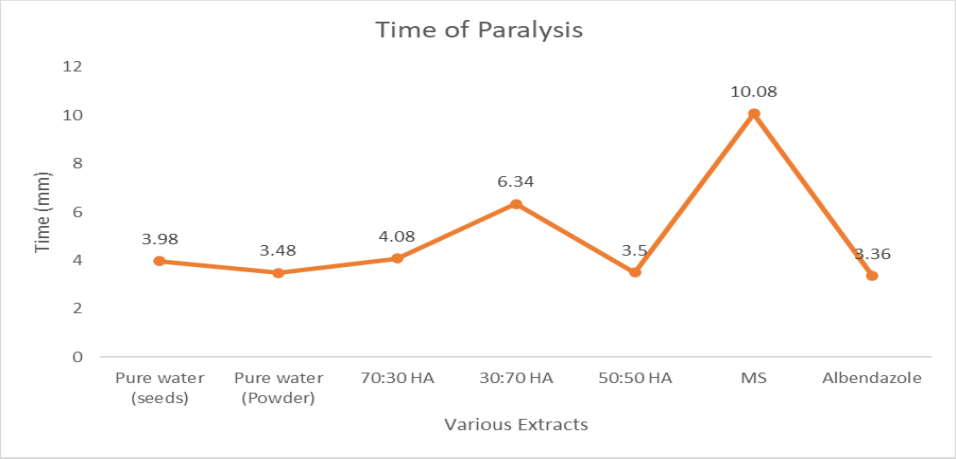

Figure 11: Graphical Representation of average of time of paralysis of worms.

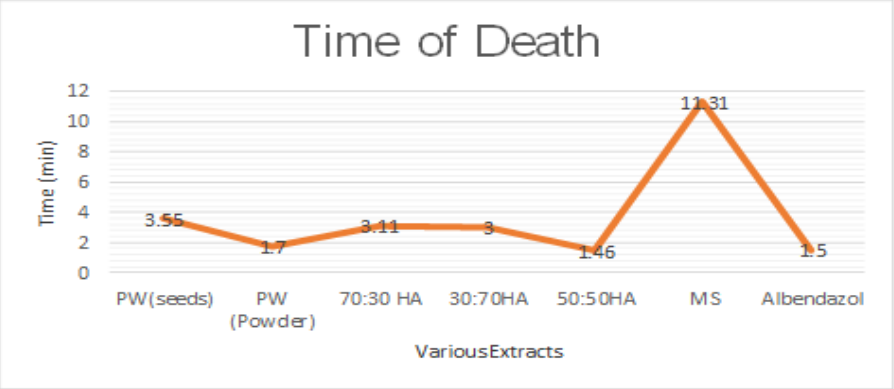

Figure 12: Graphical Representation of average of time of death of worms.

CONCLUSION

For this study, we first collected seeds from ripe fresh papaya, which was collected from the local market of Amravati. On collecting these seeds, they were washed with saline solution to remove the debris and other foreign materials. Then, after washing, the seeds were kept for drying for 7 to 8 days. After drying, the seeds were subjected to extraction. The extraction was carried out by hot maceration. After we got the extraction yield, we got the extraction yield of pure water (seeds) and the sample of 70:30 HA showed high amounts of extraction yield, which were 99% and 82% as compared to other samples, and from a sample of pure ethanol, we got 0% percentage yield.

After that, the phytochemical analysis of our obtained extract was carried out, in which all tests were carried out as per standard procedure and showed the presence of tannins, alkaloids, saponins, flavonoids, reducing sugar for glycosides, and it showed negative results with the test of anthraquinone. Then an in vitro anthelminthic study was carried out to investigate anthelminthic activity. For that purpose, we collected earthworms of species *Phertima posthuma* from the moist soil of the garden and then the in vitro study on earthworms was carried out. In the in vitro anthelminthic study, the average time for paralysis of all extracts i.e., pure water (seeds), pure water (powder), 70:30 HA, 30:30 HA, 50:50 HA, and 50:50 HA, and albendazole was 3.98, 3.48, 4.08, 6.34, 3.50, 10.08 (mins) and for death 3.55, 1.70, 3.11, 3.00, 1.46, 11.31, and 1.50 (mins).

But the least time is of Pure water (Powder) for both death and paralysis and as compared to reference

standard albendazole it shows only difference of 12 and 20 secs. Therefore, from above research we concluded that the anthelminthic activity of *Carica Papaya* seeds was evaluated successfully.

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