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Research Article

PHYTOCHEMICAL SCREENING AND EVALUTATION OF ANTHELMENTIC ACTIVITY OF METHANOLIC EXTRACT OF *CARICA PAPAYA* LEAVES ON *PHERETIMA POSTHUMA*

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ABSTRACT

The aim of the present study was to evaluate the phyto-chemical investigation and anthelmintic activity of methanolic and aqueous extract of leaves of Carica papaya linn using Pheretima posthuma. The time of colour change, length, thickness, paralysis and time of death were studied and the activity was compared with diethyl carbamazine as reference standard. The methonol and aqueous extract of leaves of Carica papaya shows significant anthelmintic activity as evidenced by decreased paralyzing time and death time. The results concluded that the use of Carica papaya leaves possess potent anthelmintic agent.

KEYWORDS: *Anthelmintic activity, Pheretima posthuma, Diethyl carbamazine, Carica papaya leaves, Methonolic extract of Carica papaya leaves, Aqueous extract of Carica papaya leaves.*

INTRODUCTION

Helminthiasis is an infectious disease caused by nematode worm such as *Ascaris lumbricoides*, *Trichuristrichiura*, *Necator americanus*, and *Ancylostoma duodenale*. Infection occurs when ingesting contaminated food, eggs or larvae, hands or utensils or through penetration of the skin by infective hookworm larvae in contaminated soil [1]. World Health Organization estimated that more than 1.5 billion (24%) of world population are infected by parasite worm. From the ancient times, indigenous drugs have been used in the Indian medicinal system to treat different ailments and to provide therapeutic benefits. Many older systems of medicine like Ayurveda, Chinese and others are mainly supported by natural products [2]. Our traditional system of medicine has made use of the different parts of plants in different types of diseases, including anthelmintic, anti-inflammatory and antimicrobial activities. Helminthic infestations are now being recognized as a cause of chronic ill health and sluggishness amongst the children. More than half of the population in the world suffers from worm infestations of one or the other. Traditional system of medicine reports the efficacy of several natural products eliminating helminthes. During the recent years, medicinal chemistry has made great strides, especially in synthetic chemistry but, for the sake of therapeutic effect up to the level and nontoxic treatment for helminthiasis, the research of plant-derived drug therapy is mostly needed [3]. Numerous molecules have been synthesized by plants but difficult to synthesize within the laboratory due to their complex nature. Moreover, it is believed that natural products are less toxic than synthetic medicines because of plant origin. Presently, research attention is more required on the value of medicinal plants and on the remedies based on them for quality, safety, efficacy, and suitability as food for effective energy.

Carica papaya Linn. (Caricaceae), a medicinally important plant species, belongs to the genus Carica, also named as papaya. The plant was traditionally used for malaria treatment and regarded as nutraceuticals used. According to The Plant List, genus Carica has accepted 23 scientific species and 02 are unplaced in the major group of Angiosperms. The papaya tree is a naturally tropical, hollow, cylindrical, fast-growing herbaceous plant species, widely distributed in nature [4]. Papaya plant originated in Central America and is now grown in tropical areas worldwide, most particularly in Africa and Asia [5]. The C. papaya serves as a food, cooking aid, and used as an ethno-medicine to prevent and treat wide-range of diseases and disorders. Papaya plant has several pharmacological activities, such as vermifuge, laxative, hypo-tensive, stomachic, febrifuge, amoebicide, analgesic, digestive, cardio-tonic, antibacterial, cholagogue, emenagogue, wound healing and more [6, 7]. Leaf extract of CP are used in the treatment of asthma, diarrohea. [8], anthelmentic [9], anti inflammatory [10] activities have been reported. Papain present in all parts of the plant except roots [11]. The effectiveness of treatments with C. papaya relies on the amount of various chemical substances present therein. The amount of chemical substances varies within different plant parts and depends on the extraction methodology, age of plant part, cultivation, and tree sex factor. Every part of papaya plant possesses the economic value once the plant has grown on

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commercial scale. Different concentrations of papaya leaves extracts are significantly involved in ruminal bio-hydrogenation and ruminal methanogenesis procedure to decrease the production of methane and also to decrease bio-hydrogenation percentage of polyunsaturated fatty acid in vitro condition. Papaya leaves are a beneficial feed material for ruminants and helpful to minimize the environmental issues like global warming [12]. Earlier studies on pharmacological activity of leaves are hypoglycaemic, hypolipidaemic. Unripe mature fruits and seeds of C*arica papaya* also having anti-hyperglycaemic activity [13, 14]. In the present study anthelmentic activity of methonolic and aqueous extract of leaves of *Carica papaya* have been evaluated.

MATERIALS AND METHODS

Plant Collection and Authentication and extraction:

Fresh leaves of *Carica papaya* Linn were collected from local area of Vijayawada and authenticated by Dr. M. Raghuram (Assistant Professor, Dept of botany and microbiology), Achariya Nagarjuna University, Nambur.

After authentication, fresh leaves of *Carica papaya* were collected in bulk, washed under running tap water, dried under shade for a period of 7 days and then pulverized in mechanical grinder to obtain coarse powder. The dried powder was stored in airtight bottles. The leaves was dried in shade and powdered to get a coarse powder separately. About 500gm of dry coarse powder was extracted with hydro-alcoholic solution of methanol and with dis.water separately (methanol: water:: 2:1) (40-60^{0}C) by continuous hot-percolation using soxhlet apparatus. The extraction was continued for 72 hours. The Powdered drug was again extracted with methanol for 72 hours. The methonolic extract as well as the aqueous extract was filtered and concentrated and a greenish black residue was obtained and it is dried in a rotary evaporator [14, 15].

Screening of primary and secondary metabolite: The extract obtained from *Carica papaya* leaves subjected to qualitative tests for identification of the chemical constituents by using simple and standard methods described by TREASE and EVANS [16].

Extraction Methodology:

Aqueous Extract of *Carica papaya* Linn leaves:

The coarse powdered material (20 gm) was macerated in distilled water for 72 hours. After maceration, the solutions were filtered and the concentrate was evaporated on water bath until syrupy consistency is left and then evaporated in a rotary evaporator. The dried extracts recovered were placed in sterilized screw-capped bottles and stored at refrigeration temperature.

Methanolic Extract of *Carica papaya* Linn leaves:

Plant powder (20 g) was successively extracted with methanol in a Soxhlet apparatus for 72 hrs. Extracts were filtered & concentrated by using rotary evaporator until dry mass is obtained [17].

Anthelmintic Screening:

For the anthelmentic activity adult *Pheretima posthuma* with approximately equal size (12 cm) taken. The earth worms collected from moist soil and cleaned with distilled water. The earth worms acclimated in saline solution for 1 hr. The animals was divided into five groups each group consisted of two earthworms the earthworms placed in petri dish containing 10 ml of The methanolic and aqueous extracts (10, 20, 30, 40 & 50 mg/ml conc.) The extract and standard drug (Diehtyl carbamazine) solution were poured in different Petri dishes. Observation were made for time taken to change the colour, thickness, diameter, paralyze (paralysis was said to occur when earthworms didn't revive in normal saline) and death (death was concluded when earthworms lost their motility and followed with their body colors fading away). All the results were expressed as a mean ± SEM of two earthworms in each group.

RESULTS AND DISCUSSION

Anthelmentic activity of C*arica papaya* leaves conformed by examining the time for paralysis and death for *Pheretima posthuma* in table.2. C*arica papaya* plant contains many active biological compounds used in the treatment of loss of apptite, alopecia, diarrohea, blindness. *Carica papaya* shows anthelmentic activity in dose dependent manner. It shows shortest time of paralysis (p=11 min) and death (D=12 min) in 50 mg/ml. While the time of paralysis and death increases in 40, 30, 20, 10 mg/ml respectively as compared to 50 mg/ml. Thus leaves of C*arica papaya* shows anthelmentic activity as compared to standard, reference and control.

Table No. 1: Phytochemical screening of *Caricca* papaya leaves

S. NO.	CHEMICAL COMPONENTS	RESULTS	
		Aqueous	Methanol
1	Carbohydrates	+ve	+ve
2	Proteins	-ve	-ve
3	Amino acids	-ve	-ve
4	Steroids	+ve	+ve
5	Glycosides	+ve	+ve
6	Anthraquinone	+ve	+ve
7	Saponin	+ve	+ve
8	Flavonoids	+ve	+ve
9	Alkaloids	+ve	+ve
10	Vitamins	-ve	-ve
11	Tannins	+ve	+ve

+ve = Present; - ve Absent

Table No. 2: Anthelmintic activity of aqueous and Methanolic extract of *Carica papaya* leaves against *Pheretima posthuma*

Test sample	Time taken by earthworms for	
TREATMENT	**Paralysis (min)**	**Death(min)**
	Mean +_ SEM	**Mean +_ SEM**
Distilled water	**ABSENT**	**ABSENT**
Test (carica papaya leaves)	**11±0.02081**	**12±0.1154**
Aqueous Extract		
10mg/ml	65±0.1	120±0.2
20mg/ml	52±0.2645	98±0.3055
30mg/ml	45±0.2	80±0.1527
40mg/ml	38±0.2886	63±0.3605
50mg/ml	24±0.1154	46±0.2081
Methanolic Extract		
10mg/ml	31±0.2886	32±0.6305
20mg/ml	28±0.5	29±0.3605
30mg/ml	20±0.2	21±0.1154
40mg/ml	16±0.1	17±0.6305
50mg/ml	11±0.2081	12±0.1154

Table No. 3: Anthelmintic activity of aqueous and methanolic extracts of standard (Diethyl carbamazine) against P*heretima posthuma*

Test sample	Time taken by earthworms for	
TREATMENT	**Paralysis (min)**	**Death(min)**
	Mean +_ SEM	**Mean +_ SEM**
Distilled water	**ABSENT**	**ABSENT**
Standard Diethylcarbamazine	**7±0.1527**	**8±0.2309**
Aqueous Extract		
10mg/ml	60±0.2309	95±0.3055
20mg/ml	55±.0.1154	83±0.1527
30mg/ml	42±0.1	75±0.2645
40mg/ml	37±0.4358	59±0.9165
50mg/ml	22±0.2645	44±0.0577
Methanolic Extract		
10mg/ml	25±0.2081	26±0.1
20mg/ml	19±0.0577	20±0.1527
30mg/ml	14±0.2516	15±0.1527
40mg/ml	11±0.0577	12±0.3214
50mg/ml	7±0.1527	8±0.2309

Table No. 4: Acute toxicity of aqueous extract of *Carrica papaya* leaves on *Pheretima postuma*

AECP concentration (mg/ml)	Colour	Length	Time of paralysis (min)	Time of death (min)
10mg/ml	No change	12 cm	60±0.2309	95±0.3055
20mg/ml	No change	12cm	55±0.1154	83±0.5527
30mg/ml	Pale yellowish	11.8cm	42±0.1	75±0.2645
40mg/ml	Moderate yellow	11.1cm	37±0.4358	59±0.9165
50mg/ml	Yellowish green	10.3cm	22±0.2645	44±0.0577

Table No. 5: Methanolic extract of *Carrica papaya* leaves on *Pheretima postuma*

MLECP Concentration in (mg/ml)	Colour	Length	Paralysis (min)	Death (min)
10mg/ml	No change	12cm	25±0.2081	26±0.1
20mg/ml	Slightly yellowish	10.2cm	19±0.0577	20±0.1527
30mg/ml	Dark green	8.7cm	14±0.2516	15±0.1527
40mg/ml	Brown	7.9cm	11±0.0577	12±0.3214
50mg/ml	black	7.3cm	7±0.1527	8±0.2309

CONCLUSION

From the above result our research is concluded that the study revealed methanolic extract of C*arica papaya* leaves having anthelmintic activity against *Pheretima posthuma*. The steroid, glycosides, and flavonoids, tannins are present in leaves may be responsible for anthelmintic activity. Our standard drug (Diehtyl carbamazine) having more potent anthelmintic activity but it has more side effects. So our natural drug having anthelmintic activity with less side effects. Our test drug shows more potent effect in methanolic extract when compare to the aqueous extract.

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