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In Vitro Anthelmintic Activity and Phytochemical Characterization of Fruit of *Carissa carandas*

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Pravin Vasantrao Gomase

Department of Pharmacognosy, J.E.S's College of Pharmacy, Nandurbar. MS.425412 India.

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ABSTRACT

Helminthiasis, or worm infection, is one of the world's most common diseases. Helminthiasis affects over half of the world's population, and the disease is on the rise. The aim of the present study was to evaluate the anthelmintic activity of petroleum ether, chloroform and methanol extracts of fruit of *Carissa carandas* using *Eudrilus eugeniae* and *Haemonchus contortus*as test worms. The time of paralysis and time of death were studied and the activity was compared with Albendazole as reference standard. The methanol extract of fruit of *Carissa carandas* exhibited significant anthelmintic activity as evidenced by decreased paralyzing time and death time. The results thus support the use of *Carissa carandas* as an anthelmintic agent.

1. INTRODUCTION

Helminthiasis, or worm infection, is one of the world's most common diseases. Helminthiasis affects over half of the world's population, and the disease is on the rise. Because of poor sanitation, poor family hygiene, hunger, and crowded living situations, it is not just limited to tropical and subtropical countries, but is also endemic in many locations.[1]

Intestinal nematodes infect an estimated 2 billion individuals worldwide. Anthelmintic medications are used to treat parasitic infections caused by helminthes. The demand for novel and effective anthelmintic is enormous, as the chemical medications currently used to control helminthes are costly, and most of them lose their potency within 20 years due to resistance. Because of the strong link between these diseases and poverty, eradicating Helminthiasis is particularly difficult.[2]

Carissa carandas is an evergreen thorny shrub belongs to Apocynaceae family, which is commonly known as karonda. It has small berry-shaped fruits, used as additive inmany pickles or as a spice in northern India. It is drought resistant plant that can be grown in a wide range of different types of soils. Approximately more than 25 species of genus *Carissa* are known, out of which five species are native to India. It is commonly used to make ledge for orchards. It has been found that the fruit is the richest source of iron, vitamin C and pectin. Even it is used as an ingredient in most of the edible preparations such as jam, jelly, squash and syrup.[3] Therefore, the present work aims to study the in vitro anthelmintic activity and phytochemical characterization of *Carissa carandas* and their extracts using different organic solvents.

2. MATERIAL AND METHODS

2.1.Plant material

Carissa carandas (Apocynaceae) plant and fruits were collected from the Satpuda region, Dhadagaon village in Nandurbar District, Maharashtra in the month of July 2022 (Fig. 1). The plants and fruits of healthy and disease-free plants were used to test the medicinal properties of the plant.

2.2.Extraction of Powdered Plant Material

The fruits of *Carissa carandas* (Apocynaceae) was collected and dried in the shade and then pulverized in a grinder. Material was passed through 120 meshes to remove fine powders and

coarse powder was used for extraction. The powdered was utilized for successive extraction by prescribed in standard reference using Petroleum ether, chloroform and methanol as solvent for extraction of powdered plant.[4]

2.3.Preliminary Phytochemical screening

2.3.1. Qualitative phytochemical analysis

Phytochemical tests were performed using Petroleum ether, chloroform and methanol extracts to determine the presence of different phytochemicals following established standard protocol. The plant extracts were subjected for the test of alkaloid, carbohydrates, fixed oils and fats, glycosides (Cardiac, Anthraquinone, and Saponin), phenolic compounds and tannins, proteins and amino acids, flavonoids, lignin, terpenoids, and diterpenes. Qualitative phytochemical examinations were carried out for all extracts of fruit as per the standard methods.[4, 5]

2.4.Selection of animals

Adult Indian earthworms (*Eudrilus eugeniae*) and tapeworms (*Haemonchus contortus*) were used to test the activity. Earthworms were bought from local suppliers, while tapeworms were taken from the bowel of a recently slaughtered goat's intestine which had been cleansed to eliminate all faeces. The worms were authenticated by Dr. Zuber Shaikh, HOD, Zoology Department, RFNS Senior Science College, Akkalkuwa. The worms were placed in regular saline to offer them with the usual leaving circumstances they require. Earthworms were chosen to examine anthelmintic activity because they were morphologically and physiologically similar to intestinal roundworms.

2.5.Preparation of samples

To obtain different concentrations, required quantities of different plant extracts and a standard medication (Albendazole) were dissolved in DMSO and then diluted with distilled water. (50 mg/ml, 75 mg/ml, and 100 mg/ml)

2.6.In-vitro evaluation of anthelmintic activity [6, 7, 8]

Adult Indian earthworms and tapeworms from the intestine of a goat were used to test the antihelmintic activity. Twelve groups of six worms of approximately identical size were given 10 ml of the necessary concentration (50 mg/ml, 75 mg/ml, and 100 mg/ml of total

extract and standard). Individual worms were timed to see how long it took them to become paralysed and die. When there was no movement of any kind except when the worms were shaken strongly, the time of paralysis was recorded. After determining that worms did not move when shaken vigorously or dunked in heated water (50 °C), the time it took for them to die was recorded. As a control, 1% gum acacia was employed.[6, 7, 8]The results are presented as a mean standard error of the mean (SEM). Graph Pad Prism 5.0 was used to conduct the statistical analysis, which included one-way analyses of variance (ANOVA) and Dunnett's Multiple Comparison test. The statistical significance of $P<0.05$ was determined.

3. RESULTS AND DISCUSSION

In the present study the fruits of *Carissa carandas* (Apocynaceae) was evaluated for, phytochemical analysis, In-vitro anthelmintic activity which revealed the following results.

Figure 1: Fruits of *Carissa carandas* (Apocynaceae) Plant

Taxonomy[9]

Kingdom : Plantae

Clade : Angiosperms

Order : Gentianales

Family : Apocynaceae

Genus : *Carissa*

Species : *C. carandas*

Common Name[9]

Hindi : Karonda

Malayalam : Karakka

Telugu : Peddakalavi

Marathi : karvand

Tamil : Kalakkai

Sanskrit : Karamard

3.1.Extraction of Powdered Plant Material

Dried fruits were selected for further study, phytochemical evaluation and subjected for extraction by various solvent (pet. ether, chloroform, methanol respectively). (Table 1)

Table 1: Yield of various extracts obtained from the dried fruit of *Carissa carandas*

Sr. No.	Extract	Color	Yield (%)w/w
01	Petroleum ether	Yellows brown	5.10
02	Chloroform	Dark green	8.21
03	Methanol	Dark brown	11.21

3.2.Preliminary Phytochemical screening

3.2.1. Qualitative phytochemical analysis

Qualitative phytochemical analyses were carried out on *Carissa carandas* for different extract like pet. ether, chloroform and methanol. Petroleum ether extract contain steroids, alkaloid the chloroform extract contain steroid, alkaloids, flavonoid, tannins the methanolic extract contain saponins, alkaloids, glycosides, flavonoids, tannins, carbohydrates, proteins and amino acids.(Table 2) It gives a preliminary insight into various compounds present in a plant, based on which further study towards the biological activities of the compounds can be tracked.

Table 2: Preliminary phytochemical analysis of various extracts of fruit of *Carissa carandas*

Sr. No.	Parameter	Pet. ether Extract	Chloroform Extract	Methanol Extract
1	Carbohydrate	-	-	+
2	Protein	-	-	+
3	Amino acid	-	-	+
4	Steroids	+	+	-
5	Cardiac glycosides	-	-	-
6	Anthraquinone glycosides	-	-	-
7	Saponin glycosides	-	-	+
8	Cyanogenetic glycosides	-	-	-
9	Coumarin glycosides	-	-	-
10	Alkaloids	+	+	+
11	Flavonoids	-	+	+
12	Tannins	-	+	+

+ Positive - Negative

3.3.In-vitro evaluation of anthelmintic activity

The anthelmintic activity was carried out by taking twelve groups (three concentrations 50 mg/ml, 75 mg/ml, and 100 mg/ml of all extract and standard) each containing six worms of approximately equal size were subjected to 10 ml of desired concentration. The results of this investigation show that all *Carissa carandas*extracts (petroleum ether, chloroform, and methanolic) have anthelmintic action against both worms, which is proportionate to the concentration. In comparison to other extracts, the methanolic extract has high anthelmintic action, according to the findings. (See Figure. 2 & 3) (Table 3)

Table 3: Paralysis and death time of Indian earthworm and tape worm for Different extracts.

Test Sample	Concentration (mg/ml)	Indian Earth worm		Tape Worm	
		For Paralysis	For Death	For Paralysis	For Death
Petroleum ether	50	76.25 ±0.81	130.25 ± 0.25	73.58 ±1.25	122.48±0.15
	75	65.15 ± 1.58	104.26 ±0.36	63.01 ±1.36	97.48 ±0.34
	100	49.25 ± 0.26	81.68 ±1.25	42.15 ±1.48	75.28±0.94
Chloroform	50	64.28 ±0.87	110.26 ±1.38	62.48 ±0.68	78.25 ±0.26
	75	53.15 ±0.94	81.27 ±0.98	48.68 ±0.48	76.49 ±0.18
	100	46.15 ±1.02	71.68 ±1.26	37.09 ±0.26	63.34 ±0.34
Methanolic	50	51.78 ±1.64	73.94 ±0.71	41.29 ±0.34	71.64 ±1.27
	75	36.15 ±0.29	52.15 ±0.36	34.15 ±1.26	63.84 ±1.39
	100	21.54 ±0.89	40.18 ±1.56	20.15±0.34	40.26 ±1.56
Albedazole	50	34.26 ±1.32	79.63 ±0.46	30.29±0.48	68.16 ±0.87
	75	26.84 ±1.25	45.94 ±0.65	27.94 ±0.64	55.48 ±0.94
	100	20.72 ± 0.98	35.15 ±0.36	16.18 ±0.84	37.29 ±1.03

Data are mean of 6 replicates ± SEM and are significantly different at $P< 0.05$.

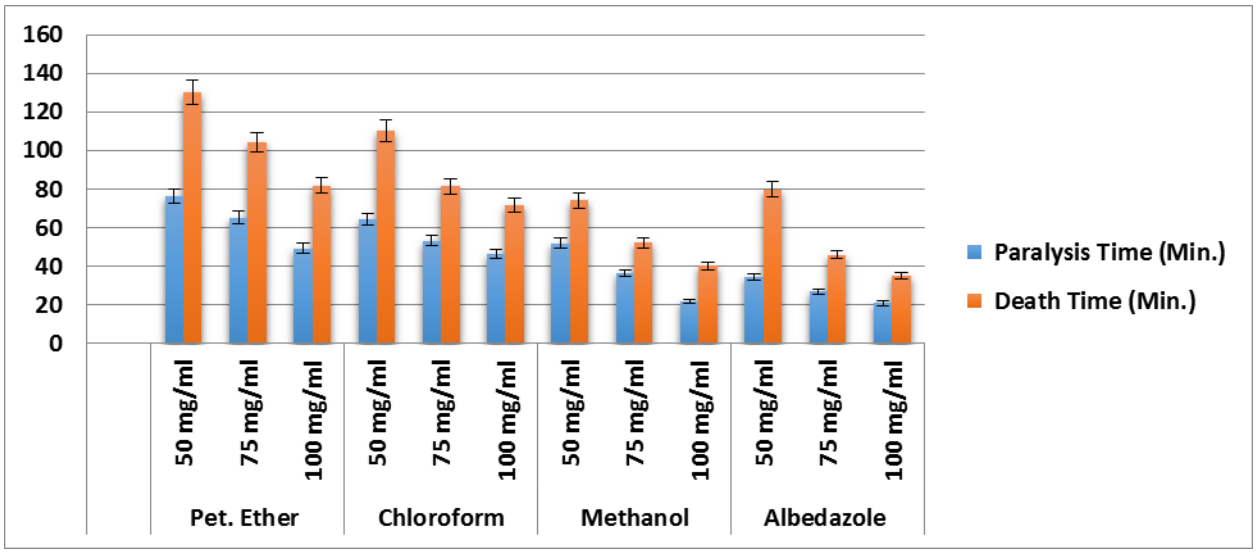

Figure 2: Paralysis and death time of Indian earthworm (*Eudrilus eugeniae)*

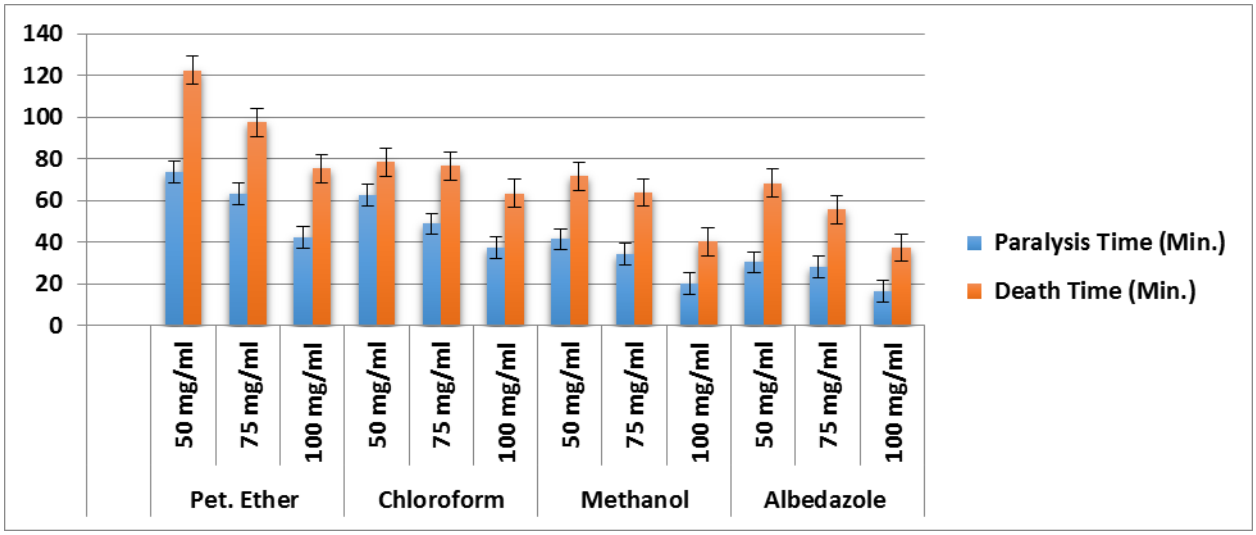

Figure 3: Paralysis and death time of tapeworm (*Haemonchus contortus*)

As a result, it is possible to deduce that the anthelmintic action of various plant extracts is related to the presence of phenolic compounds, flavonoids, tannins, and alkaloids components, which may act singly or in combination.

4. CONCLUSION

Adult Indian earthworms (*Eudrilus eugeniae*) and tapeworms were used to test the anthelmintic activity (*Haemonchus contortus*). The results of this investigation show that all *Carissa carandas* extracts (petroleum ether, chloroform, and methanolic) have anthelmintic action against both worms that is proportionate to the concentration. In comparison to other extracts, the methanolic extract has high anthelmintic action, according to the findings. In comparison to other extracts, methanolic extract has a higher concentration of alkaloids, flavonoids, tannins, and phenolic chemicals, which may explain the activity.

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