



STUDY FOR ANTHELMINTIC ACTIVITY OF CASHEW APPLE (*ANACARDIUM OCCIDENTALE*) EXTRACT.

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ABSTRACT

Alcohol and aqueous extracts from the whole plant of *Anacardium occidentale* were investigated for their anthelmintic activity. Various concentrations (250 and 500mg / ml) of each extract were tested in the bioassay, which involved the determination of time of paralysis and time of death time of the worms. Both extracts exhibited significant anthelmintic activity at highest concentration of 500mg/ml. Albendazole (20 mg/ml) was included as standard reference and distilled water as control. The Preliminary phytochemical analysis indicated the presence of various phytoconstituents in all the tested extracts

Keywords: *Anacardium occidentale*, Cashew apple extract, Anthelmintic assay, Phytochemical screening, triterpenoids.

INTRODUCTION

Medicinal plants used in India for centuries as an important therapeutic source for treating a variety of ailments and has been found to be immense global importance.

There is a reluctance observed in accepting herbal remedies by modern system because of the lack of documentation regarding these called scientific validity and quality.¹

India is perhaps the largest producer of medicinal herbs and rightly called the 'Botanical garden of the world'. Medicinal herbs have been in use for thousands of years in one form or in the Indigenous system of medicines like Ayurveda, Siddha and Unani.²

The Anacardiaceae family consisting of several plants with immense pharmacological activity. Out of the plant *Anacardium occidentale* has been reported to have immense pharmacological and therapeutic activity. Various research work carried out has proved it to be used in various diseases like dermatitis, hyperglycemia, antiviral, anti inflammatory activity. It is traditionally accepted in Ayurveda to have anthelmintic activity.

Anacardium occidentale (Family: Anacardiaceae) is a native of tropical America, naturalized and cultivated throughout India especially near the coastal area, like Kerala from which the plant of interest is collected.

Anacardium occidentale is used medicinally wherever it is found growing. The fruit and pericarp are officinal in Portugal. All parts of the plant like leaves, false fruit and bark have been traditionally used to relieve variety of ailments. *Anacardium occidentale* is a small tree with a short thick crooked trunk. Branches terete glabrous. Leaves coriaceous; 10-15 by 3, 8-7.5 cm, obviate or elliptic.

The fruit is acrid sweet, hot, digestible, aphrodisiac, anthelmintic; cures "vata" and "kapha" tumours, ascites,

fever, ulcer, leucoderma and skin disease, dysentery, piles, loss of appetite as mentioned in Ayurveda.

The bark is said to have alternative properties. The root is considered purgative and the fruit is mainly used as antidiarrheal.

The tar from the bark is used as a counter irritant. As an external application it has been recommended in leprosy, ring worm, and obstinate ulcers, it is powerfully rubefacient and vesicant and requires to be used with caution.³

Keeping in mind about the adverse effect and toxicity of synthetic drugs, in the present study anthelmintic potential of alcoholic and aqueous extracts of false fruit of *Anacardium occidentale* have been evaluated along with preliminary phytochemical analysis.

MATERIALS AND METHODS

The false fruit was collected from our campus in the month of March-April. The false fruits were identified and authenticated by Department of Botany, Payyannur College, Payyannur, Kerala. And a specimen voucher was kept in the department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram for further reference. The fruits were cleaned, reduced the size by cutting to small parts and then it is dried under shade. It is coarsely powdered with the help of a blender. The coarse powder of fruit was then exhaustively extracted in a Soxhlet apparatus.

In this extraction process 250 grams of dried powder was extracted by using soxhlet extraction process with 500ml of Ethyl alcohol and distilled water and chloroform (99:1) as solvent separately. The extractive values are given in the Table no: 1. The extracts were concentrated by distilling the solvent and preserved under refrigeration for further studies. The dry extracts obtained were subjected to various chemical tests to detect the presence of different phytoconstituents^{4,5}



Preliminary Phytochemical Analysis of the extracts was done to identify the chemical constituents present in the extracts prepared specifically as follows in Table No 2.

- Test for carbohydrates was done using Molisch reagent and Fehling's reagent.
- Test for glycoside was done by following Tollens test, Legal's test, Born Tragger's test.
- Test for triterpenoids was performed by Hirschorn test. Libermann storch mora sky test and test for Tannins and Phenolic compounds were also performed.

Invitro Studies:

Anthelmintic study

Pheretima posthuma (Annelida), commonly known as earthworm were collected from the water logged areas from the nearby locality and roundworms (nematode) were obtained from freshly slaughtered Fowls. The anthelmintic assay was carried as per the method of Ajaiyeoba *et al* [6] with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings⁷⁻¹⁰. Because of easy availability, earthworms have been used widely for the evaluation of anthelmintic compounds in vitro.¹¹⁻¹⁵

Round worm (*Ascaridia galli*) Worms are easily available in plenty from freshly slaughtered fowls Ten milliliter of formulation containing two different concentrations each of crude alcoholic and aqueous extract (250mg/ml, 500mg/ml in distilled water) were prepared and six worms (same type) were placed in it. The process for the study was carried out for both the worms. Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously. Thereafter the time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) followed by fading away of their body colours. Albendazole (20 mg/ml) was selected as reference standard while distilled water as a control¹⁶⁻¹⁷ for the given experiment.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of alcoholic extract revealed the presence of steroids, glycosides, tannins, phenolics and triterpenoids, while aqueous extract showed the presence of steroids, glycosides, carbohydrates, flavonoids and saponins. The results are reported in Table no 2. As shown in Table no 3 the alcoholic and aqueous extract of false fruit of *Anacardium occidentale* displayed significant anthelmintic properties at higher concentrations. Both the extracts showed anthelmintic activities in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 500mg/ml concentration, for both type of worms. The alcoholic extract of false fruit of *Anacardium occidentale* caused paralysis in 5.23 min and death in 10.00 min, while aqueous extract showed paralysis and death in 23.17 and 37.10min against the earth worm *Pheretima posthuma*

The reference drug Albendazole showed the same at 7.17 min and 30.33 min. The mean \pm SEM values (statistical analysis) were calculated for each parameter¹⁸.

Table 1: Extractive Values

Sl no	Extract	Extract w/v	Percentage yield
1	Aqueous	2.7mg/ml	1.08%
2	Alcoholic	3.2mg/ml	1.28%

Table 2: Qualitative phytochemical analysis of plant extract

Chemical Tests	Ethanol Extract	Aqueous Extract
Steroids	+	+
Glycosides	+	+
Carbohydrates	-	+
Flavonoids	-	+
Tannins and Phenolics	+	+
Triterpenoids	+	-
Saponins	-	+

(+): Shows the presence of the given chemical constituent.

(-): Shows the absence of the given chemical constituent

Table 3: Anthelmintic activity of alcohol and aqueous extract of *anacardium occidentale* false fruits

Sl No	Test	Paralysis time (min)		Death time (min)	
		Earth worm	Round worm	Earth worm	Round worm
1	Aqs 250	32.11 \pm 0.4	40.00 \pm 0.1	44.00 \pm 0.3	47.00 \pm 0.6
2	Aqs 500	23.17 \pm 0.1	30.17 \pm 0.5	37.10 \pm 0.2	37.37 \pm 0.2
3	Alc 250	10.20 \pm 0.7	11.15 \pm 0.1	25.17 \pm 0.3	27.20 \pm 0.3
4	Alc 500	5.23 \pm 0.3	6.00 \pm 0.3	10 \pm 0.3	12.57 \pm 0.4
5	Dist water	48 \pm 0.5	50 \pm 0.3	71 \pm 0.4	67 \pm 0.5
6	Standard	7.17 \pm 0.2	11.00 \pm 0.4	30.33 \pm 0.5	29.00 \pm 0.4



Ascaridia galli also showed sensitivity to the alcoholic and aqueous extract of false fruit of *Anacardium occidentale*. The alcoholic extract caused paralysis and death in 6.00 min and 12.57 min aqueous extract displayed paralysis and death in 30.17 min and 37.37 min respectively, at higher concentration of 500 mg/ml. Albendazole did the same at 11.00 and 29.00 min.

To be an effective anthelmintic, a drug must be able to penetrate the cuticle of the worm or gain access to the alimentary tract. An anthelmintic drug can act by causing paralysis of the worm, or damaging cuticle, leading to partial digestion or to rejection by immune mechanism. Anthelmintic drugs can also interfere with the metabolism of worm, and since the metabolic requirement of these parasites vary greatly from one species to another.¹⁹

The predominant effect of Albendazole on the worm is, Cytoskeletal structure of helminthes include microfilaments, microtubules and β -tubulins. Under normal conditions microtubule assembly is dependent on β -tubulin function where in β -tubulin dimmers are continually being polymerized from one end and then depolymerized at the other end of the microtubule. Benzimidazoles (Albendazole) bind to the β -tubulin and prevent their assembly, i.e. breakdown of microtubules with selective and irreversible inhibition of glucose uptake. The end result is depletion of parasites glycogen stores, reduced formation of ATP, disruption of metabolic pathways and ultimately parasitic death.²⁰

Figure 1: Paralysis time of earth worm

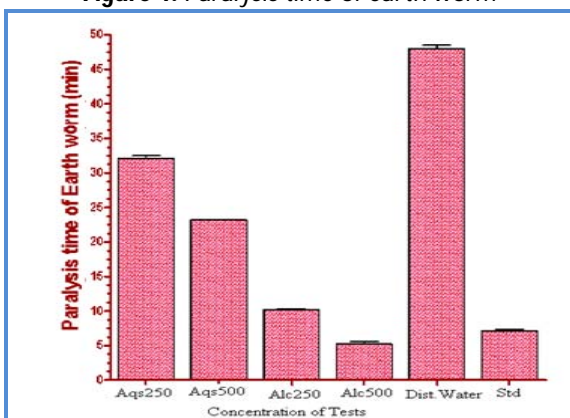


Figure 2: Paralysis time of round worm

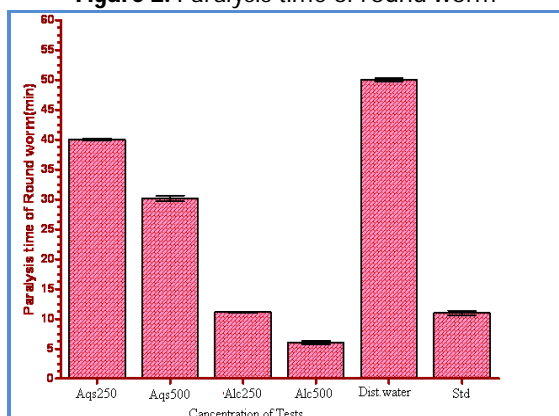


Figure 3: Death time of earth worm

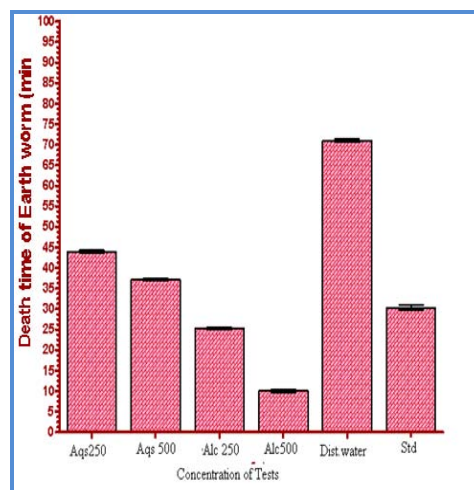
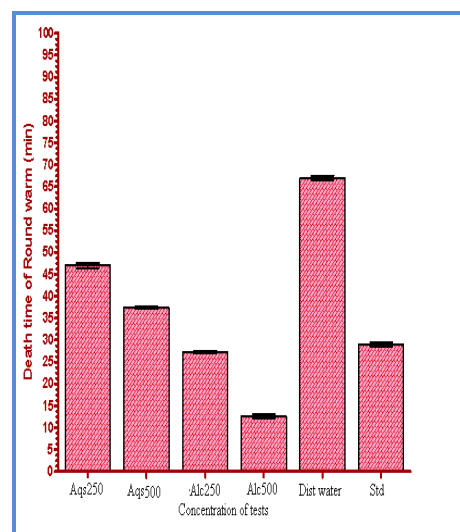


Figure 4: Death time of roundworm



The false fruit extract of *Anacardium occidentale* not only demonstrated paralysis but also caused the death of worms. Phytochemical analysis of the crude extracts revealed presence of tannins as one of the chemical constituent. Tannins were shown to produce anthelmintic activities²¹. Chemically tannins are polyphenolic compounds.²² Some synthetic phenolic anthelmintics e.g. niclosamide, oxcyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling parasite specific fumarate reductase mediated oxidative phosphorylation reaction. It has been proved there is an optimum presence of tannin in the given plant extract which is a polyphenolic compound. the given tannins is capable to produce the similar effect as that of phenols. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal²³ or glycoprotein on the cuticle of the parasite²⁴ and cause death to it.

CONCLUSION

In conclusion the traditional claim of false fruit of *Anacardium occidentale* as an anthelmintic have been confirmed as the false fruit extract displayed activity against the worm used in the study. Further studies to isolate and reveal the active compound(s) contained in the crude extract of *Anacardium occidentale* and to establish the mechanism (s) of action are required to be done in future.

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