



**IN VITRO ANTHELMINTIC ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS.**

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**ABSTRACT**

The present study is aimed at investigating the anthelmintic activity of methanol extract of *Macaranga peltata* stem bark and hydroalcohol extracts of *Anacardium occidentale* leaves and *Anacardium occidentale* seed testa on African earthworm, *Eudrilus eugenia*. Three different concentrations: 30 mg/ml, 50 mg/ml and 100 mg/ml of drug extracts were prepared by suspending the extracts in 1% CMC solution. Albendazole was taken as the reference standard in concentration of 30 mg/ml. Anthelmintic activity was studied by determining the time taken for paralysis (vermifuge) and death (vermicide) of earthworms when placed in a petridish containing 15 ml of three different concentrations of drug solution (30 mg/ml, 50 mg/ml and 100 mg/ml). The study revealed marked anthelmintic activity exhibited by the three extracts.

**KEYWORDS:** *Macaranga peltata*, *Anacardium occidentale*, *Eudrilus eugenia*, Anthelmintic activity.

**INTRODUCTION**

Infections caused by parasitic helminthes are rife all over the world, with the tropical and subtropical developing nations being at major risk. About half of the world's population is estimated to be infected with gastrointestinal helminthes. These infections have a wide range of undesirable effects on the human as well as animal health. The effects include chronic illnesses, malnutrition, cognitive changes, anemia etc. causing substantial discomfort to the individual. Some of these infections, like schistosomiasis and hookworm disease are associated with serious morbidity.<sup>[1]</sup> Because of the pervasiveness of these infections and the debilitating effect they have on the health, their treatment is of great moment.

Attempts to treat these infections have been made since the days of yore. Extracts of herbs and plants are traditionally used to treat helminthiasis. Anthelmintic activity of extracts of some plants like *Chenopodium album*, *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana* and some tannin containing plants has been reported.<sup>[2]</sup> There are several benefits associated with making a traditional approach in treatment of helminthiasis. Since these infections are predominantly widespread in the poor and developing nations, plant anthelmintics provide a cheaper and reliable option to the users as compared to the imported drugs. Also, since the herbal remedies do not contain any fixatives or preservatives, they are presumably more biodegradable and lead less to bioaccumulation in patient's bodies and environment.<sup>[2]</sup>

Though treatment of helminthiasis with synthetic class of drugs like benzimidazoles has been successful, it has brought along with it, the concomitant plight of resistance developed by the helminthes to these drugs. Thus, use of traditional medicines provides yet another edge over synthetic drugs as it has been suggested that the diversity of herbal anthelmintics has precluded the occurrence of resistance despite many years of use.<sup>[2]</sup> The present study intends to investigate the anthelmintic activity of methanol extract of *Macaranga peltata* stem bark and hydro alcohol extracts of *Anacardium occidentale* leaves and seed testae.

*Macaranga peltata* belongs to the family *Euphorbiaceae* and has a long history of use in traditional medicine for treatment of cuts, swellings, sores, bruises etc. Over 190 metabolites have been isolated mainly from the leaf extracts and different species of this genus. They exhibit a wide range of pharmacological activities including anti-cancer, anti-plasmodial, anti-oxidant etc.<sup>[3]</sup> *Macaranga peltata* is a dioecious woody species of tree found widely in India, Sri Lanka and Thailand. In India, it is locally called as Chandada and in Sri Lanka as Kenda. Kenda leaves are used for the purpose of flavoring in Sri Lanka. The major use of *Macaranga peltata* is in making wooden pencils and plywood industry.<sup>[4]</sup>

*Anacardium occidentale* or the Cashew tree is a tropical evergreen tree. The seed of the tree, the cashew, is widely consumed while the leaves of these trees are known to possess antioxidant, antimicrobial activity.<sup>[5]</sup>

## MATERIAL AND METHODS

### Plant materials

Stem bark of *Macaranga peltata* was procured from Nicholas Piramal Limited, Mumbai.

*Anacardium occidentale* testa was obtained from small scale cashew manufacturing unit in Sawantwadi region of Sindhudurg, Maharashtra, India.

*Anacardium occidentale* leaves were procured from Yehoor forest, Thane district.

All the plant materials mentioned above were authenticated by Agharkar Institute, Pune.

### Drugs and chemicals used

Albendazole, Polysorbate 80, Xanthan gum and Carboxy methyl cellulose (CMC) were used during the experimental protocol. All the chemicals used were of laboratory and analytical grade.

### Experimental worms

The earthworms *Eudrilus eugenia* were used to carry out the experiment. They were obtained from a local supplier and were washed with normal saline to remove all the fecal matter. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used due to their anatomical and physiological resemblance with the intestinal roundworm parasites.<sup>[6]</sup>

## METHODOLOGY

### Preparation of extracts

Authenticated plant materials were further dried, powdered and stored in air tight containers. Powdered plant material was further used for extraction. Extraction was carried out using different solvents of different polarities such as methanol and water depending on the nature of various phyto-constituents present in the plant material. The extracts were concentrated in a rotary evaporator under pressure, were kept in desiccators and used for further studies.

For determination of extractive value, 10 gm of powdered material was extracted with 100 ml solvent using Soxhlet extraction technique.

### Anthelmintic Assay

The anthelmintic assay was carried out according to the procedure mentioned in *Mohammed et al* with minor modifications.<sup>[7]</sup>

The earthworms were divided into different groups with each group consisting of 6 earthworms. Each petridish was placed with 2 earthworms of nearly equal size and was observed for paralysis and death. Each type of dried extract was suspended in 1% w/v carboxy methyl cellulose (CMC) solution prepared in normal saline and three different concentrations (30 mg/ml, 50 mg/ml, 100 mg/ml) of extracts were prepared. Albendazole served as the reference standard and a suspension was prepared

using Polysorbate 80 and Xanthan gum to obtain concentration of 30 mg/ml.<sup>[8]</sup> Normal saline with 1% CMC was taken as a control.

Worms were placed in a petridish containing 15 ml of sample (drug) solution at room temperature. The time taken for complete paralysis (vermifuge) and death (vermicide) were recorded. Paralysis was said to occur when no movement could be observed except when worms were shaken vigorously or when dipped in warm water (50°C). Death of worms was concluded when the worms lost their motility followed by white secretions and fading away of their body color.

## RESULT AND DISCUSSION

The observations revealed that all the extracts had a dose dependent anthelmintic activity, with increase in the concentration of the drug, reducing the time taken for vermifuge and vermucidal activity.

The peak anthelmintic activity was exhibited by the MeOH extract of *M. peltata* stem bark at concentration of 100 mg/ml. It took (1:03 ± 0:73 min) for paralysis and (1:89 ± 0:93 min) for death.

The other test concentrations of MeOH extract of *M. peltata* stem bark and hydro alcoholic extracts of Cashew leaves and Cashew testa showed a marked anthelmintic activity in a dose dependent fashion by significantly reducing the time for paralysis (vermifuge) and time for death (vermucidal) on increasing the concentrations. The present study indicates maximum anthelmintic activity by MeOH extract of *M. peltata* stem bark. When compared to the standard Albendazole (30mg/ml), the MeOH extract of *M. peltata* had an activity superior to that of Albendazole at the concentration of 30 mg/ml.

The *Anacardium occidentale* leaves extract show the activity of (P =25:67 ± 4:91 & D= 37:49 ± 4:83) when compared to the standard Albendazole suspension (P=23:66 ± 2:04 & D=32:08 ± 3:99) whereas the activity of *Anacardium occidentale* seed testa extract shows an activity of (P = 30:00 ± 4:46 and D =40:13 ± 3:78) at concentration 30 mg/ml.

In plants, the secondary metabolites are responsible for protection of the plants from pathogens and these secondary metabolites can be classified into three broad categories: phenolic compounds (phenols, phenolic acids, flavonoids, coumarins, tannins etc); terpenes (carotenes, steroids, saponins, triterpenes etc); and alkaloids (nitrogenous compounds). The phenolic compounds are considered to be primarily responsible for the pharmacological activity.<sup>[9]</sup>

The total phenolic content in the extracts was measured using Folin-Ciocalteu based on procedures described by Singleton et al. (1999),<sup>[10]</sup> with some modifications. Total phenolic content for *Macaranga peltata* stem bark was found to be highest for methanol extract > hydro

alcoholic extract > aqueous extract > chloroform extract > petroleum extract.

For *Anacardium occidentale* leaves, the total phenolic content was found to be highest for hydro alcoholic extract > methanol extract > chloroform extract > water > petroleum ether extract.

The total phenolic content for cashew testa was found to be highest for hydro alcoholic extract > methanol > water > petroleum ether (60- 80°C) > chloroform extract.

The available literature on phytochemical constituents of *Macaranga peltata* bark MeOH extract indicate the presence of carbohydrates, tannins and saponins in very high amount, flavonoids, alkaloids and sterols in moderate amount and glycosides & amino acids in fewer amounts. It is also revealed that the plant methanolic

extract does not contain phenols and anthraquinones.<sup>[11][12]</sup>

From the literature, the secondary metabolites identified in the *Anacardium occidentale* seed testa are: tannins, phenolics, flavonoids like catechin, epicatechin and epigallocatechin.<sup>[9]</sup>

The constituents observed in the *Anacardium occidentale* leaves are carbohydrates, proteins, saponins, saponin glycosides, alkaloids, tannins and other phenolic compounds.<sup>[13]</sup>

Thus, the anthelmintic activity can assumedly be attributed to the phenolic contents of the plant extracts, since all the extracts show presence of phenolic compounds like tannins and flavonoids.

**Table 1: Anthelmintic activity of Plant extracts**

SR No.	Drug	Concentration (mg/ml)	Time (in minutes)	
			Paralysis (P)	Death (D)
1	Control	-	No paralysis	No death
2	Albendazole	30 mg/ml	23:66 ± 2:04	32:08 ± 3:99
3	<i>Macaranga peltata</i> Stem Bark (Methanol extract)	30 mg/ml	8:58 ± 0:64	10:33 ± 1:82
		50 mg/ml	3:21 ± 0:91	6:04 ± 2:97
		100 mg/ml	1:03 ± 0:73	1:89 ± 0:93
4	<i>Anacardium occidentale</i> Testa (Hydroalcohol extract)	30 mg/ml	30:00 ± 4:46	40:13 ± 3:78
		50 mg/ml	25:93 ± 4:92	39:08 ± 5:73
		100 mg/ml	14:49 ± 1:09	18:87 ± 2:14
5	<i>Anacardium occidentale</i> Leaves (Hydroalcohol extract)	30 mg/ml	25:67 ± 4:91	37:49 ± 4:83
		50 mg/ml	19:33 ± 1:53	21:63 ± 2:41
		100 mg/ml	12:06 ± 2:42	12:80 ± 1:72

All Values represent Mean ± SD; n=6 in each group.

## CONCLUSION

The results of the study clearly indicate the potent anthelmintic activity of the crude methanol extract of *M. peltata* stem bark against the earthworms *Eudrilus eugenia*. The results show superior efficacy of *M. peltata* extract (P = 8:58 ± 0:64, D= 10:33 ± 1:82) when compared to the standard Albendazole suspension at 30 mg/ml concentration. *M. peltata* is abundantly found in the Indian forest areas.<sup>[14]</sup> Due to its ready availability and potent anthelmintic activity exhibited in the study, methanolic extract of *M. peltata* stem bark can serve as a promising substitute in treatment of helminthiasis.

Also, the hydro alcoholic extracts of *Anacardium occidentale* leaves and seed testa have shown an anthelmintic activity worthy of taking into account. Though the potency of these hydro alcoholic extracts has been shown to be lesser than that of methanolic extract of *M. peltata*, the activity of *Anacardium occidentale* leaves extract is almost equipotent, when compared to that of standard and hence, has potential of supplanting the conventional synthetic anthelmintics used. The *Anacardium occidentale* seed testa extract can provide as an economical substitute in anthelmintic treatment, since

the testa of *Anacardium occidentale* seed is a byproduct of the Cashew industry.<sup>[15]</sup> and shows a considerable anthelmintic activity at the concentration of 30 mg/ml, when compared to the standard.

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