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Invitro Anthelmintic Activity of Bark of *Azadirachta indica* against *Ascardi galli* and *Eudrilus eugeniae*

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Abstract

The present study was undertaken to evaluate the anthelmintic activity of aqueous and ethanolic extracts of the bark of *Azadirachta indica* (Family–Meliaceae) and its different extracts (*viz.* ethanol and aqueous) against *Ascardi galli* and *Eudrilus eugeniae*. Various concentrations (50&100 mg/ml) of ethanolic and aqueous extracts and its various fractions were evaluated in the bioassay involving determination of time of Paralysis (P) and time of Death (D) of both types of the worms. Piperazine citrate was used as standard anthelmintic drug and distilled water was used as control. The results of the present study indicated that the alcoholic ethanol and aqueous extracts of the bark significantly exhibited paralysis ($P < 0.05$) in worms in lower dose 50 mg/ml and also caused death of worms especially at higher concentration of 100 mg/ml, as compared to standard drug. Further studies are in process to isolate the active principle/s responsible for the activity.

Keywords: *Azadirachta indica* bark, anthelmintic activity, *Ascardi galli* and *Eudrilus eugeniae,* piperazine citrate

1. Introduction

Azadirachta indica A. Juss, known as Neem in vernacular, belongs to the family Meliaceae and is widely distributed in Asia, Africa and other tropical parts of the world [1]. In Nepal, neem plants are distributed in the Terai (tropical) and the foothills (subtropical) of the country. Neem is a versatile medicinal plant, almost every part of which is being used in folklore and traditional systems of medicine for the treatment of a variety of human ailments. Traditionally, most of the Nepali people clean their teeth with neem twigs, take its juice as a tonic to increase appetite and use it in fever or to remove intestinal worms. Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections [2]. However, apart from these uses, there are several reports on the biological activities and pharmacological actions based on modern scientific investigations, such as antiviral [3], antibacterial [4], antifungal [5] and Chaurasia, anti–inflammatory and antipyretic [6], antiseptic [7], antiparalitic, antioxidant [8], etc. The present study has been undertaken to evaluate the in–vitro anthelmintic activity of crude extract of *Azadirachta indica* bark–ethanolic and aqueous extracts against *Ascardi galli* and *Eudrilus eugeniae*.

2. Materials and Methods

2.1 Plant Collection and Authentication

The fruits of *Azadirachta indica* were collected from Ramling Mudgad District, Latur (Maharashtra); and

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authenticated by Botanical Survey of India, Pune (Maharashtra). A voucher specimen has been deposited at the herbarium of CHECHA1.

2.2 Plant Extraction

The plant material (bark) was dried at room temperature (25–35 °C) and powdered with the help of an electric grinder. The course material was extracted successively with ethanol and the plant mark was finally macerated with distilled water. The extracts were dried at 50°C in a water bath. The percentage yields obtained of the different successive extracts were 15.10% and 11.31% respectively.

2.3 Worms Collection and Authentication

African species earthworms *Eudrilus eugeniae* (Annelida) were collected from the water logged areas of soil and *Ascardia galli* (Nematode) worms were obtained from freshly slaughtered fowls (Gallus gallus). Both worm type were identified at the Agriculture Research Station, Aland road, Gulbarga.

2.4 Preparation of Test Sample

Samples for in–vitro study were prepared by dissolving and suspending 2.5 g of each extract (petroleum ether, alcoholic and aqueous) in 25 ml of distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 25 and 50 mg/ml[9].

2.5 Anthelmintic Assay

The anthelmintic assay was carried out as per the method of Ajayieoba et al. [19] with minor modifications. The assay was performed on adult African earthworm *Eudrilus eugeniae* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Eudrilus eugeniae* worms are easily available and used as a suitable model for screening of anthelmintic drug [10]. *Ascardia galli* worms are easily available in slaughter houses and are suitable model for screening of anthelmintic drugs [11]. The 50 ml formulations containing four different concentrations, of each ethanolic and aqueous extract (50 and 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. This was done for both worms. Time for paralysis was noted when no movement of any sort could be observed except the worms which were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously, nor, when dipped in warm water at 50 °C [12, 13] Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control.

3. Results and Discussion

As shown in Table 1 & (Fig. 1 & 2), the results showed that ethanolic and aqueous extracts of *Azadirachta indica* bark and its different fractions exhibited anthelmintic

Table 1: Anthelmintic activity of bark extract of *Azadirachta indica*

Test sub.	Concentration mg/ml	Time taken for Paralysis (P) and for Death of worms (D) in min.			
		Eudrilus eugeniae		*Ascardia galli*	
		P	D	P	D
Vehicle	–	–	–	–	–
Piperazine citrate	10	11.06 ± 0.23	16.47 ± 0.19	10.47 ± 0.18	15.67 ± 0.17
Ethanolic extract(EE)	50	13.25 ± 0.46*	28.05 ± 0.55*	12.25 ± 0.42*	26.57 ± 0.42*
	75	12.95 ± 0.49*	19.18 ± 0.40*	11.85 ± 0.48*	18.17 ± 0.52*
	100	7.91 ± 0.52**	13.50 ± 0.76**	6.58 ± 0.53**	12.02 ± 0.66**
Aqueous extract(AQE)	50	31.40 ± 0.48	33.25 ± 0.60	28.60 ± 0.36	32.42 ± 0.80
	75	25.74 ± 0.45	25.77 ± 0.49	24.72 ± 0.43	24.77 ± 0.40
	100	19.03 ± 0.68*	21.12 ± 0.53*	18.55 ± 0.43*	16.97 ± 0.67*

Values are expressed as MEAN ± SEM

One way ANOVA followed by Dunnets 't' test.

Note: n=6 in each group. *P<0.01, **P<0.001.

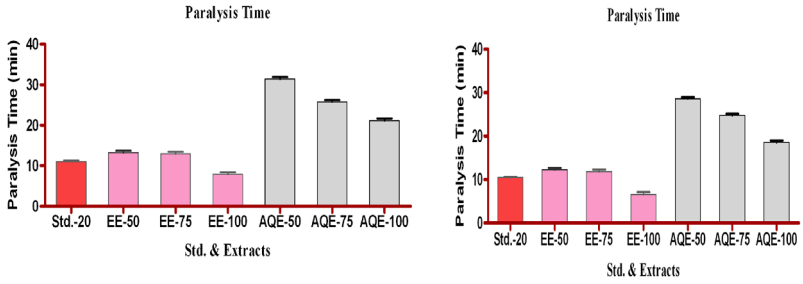

Fig. 1. Graph showing the paralysis time of ethanolic & aqueous extracts.

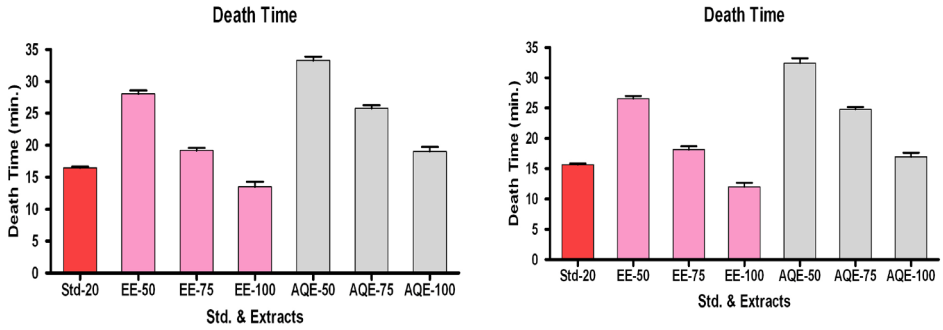

Fig. 2. Graph showing the death time of ethanolic & aqueous extracts.

activity using *Eudrilus eugeniae* worms in dose–dependant manner giving shortest time of Paralysis (P) and Death (D) with 100 mg/ml concentration. The ethanolic extract caused paralysis at 7.91 ± 0.52 min. and time of death at 13.50 ± 0.76 min., while aqueous fractions revealed paralysis at 19.03 ± 0.68 min. and time of death at 21.12 ± 0.53 min., respectively against the earthworm *Eudrilus eugeniae*. The standard drug, Piperazine Citrate showed the same at 11.06 ± 0.23 and 16.47 ± 0.19 min, respectively.

Ascardia galli worms also showed sensitivity against ethanolic and aqueous extracts of *Azadirachta indica* bark. The ethanolic extract caused paralysis time at 6.58 ± 0.53 min. and death time at 12.02 ± 0.66 min., while aqueous fractions revealed paralysis at 16.97 ± 0.67 min. and time of death at 18.55 ± 0.43 min., respectively, against the *Ascardia galli* worms. At high concentration of 50 mg/ml. Piperazine Citrate exhibited the same at 10.47 ± 0.18 and 15.67 ± 0.17 min.

Piperazine Citrate, by increasing chloride ion conductance of worm muscle membrane, produced hyperpolarization and reduced excitability that led to muscle relaxation and flaccid paralysis [14] The bark extract of *Azadirachta indica* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to standard drug Piperazine Citrate. Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents contained within them. Tannins were shown to produce anthelmintic activities [15] Chemically tannins are polyphenolic compounds [16]. It is possible that tannins contained in the extracts of *Azadirachta indica* produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death [17, 18].

4. Conclusion

The study has shown that ethanolic and aqueous bark extracts of *Azadirachta indica* have significantly determined anthelmintic activity. But ethanolic bark extract of *Azadirachta indica* showed most significant anthelmintic activity as compared to the aqueous extracts. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

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