ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACTS OF THREE MEDICINAL PLANTS AGAINST *ASCARIDIA GALLI*

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Abstract: The anthelmintic activity of the methanolic extracts of *Azadirachta indica, Murraya koenigii* and *Ocimum sanctum* was assessed *invitro* in *Ascaridia galli*. The worms were collected fresh from local slaughter houses and washed in normal saline. The anthelmintic activities of crude methanolic extract of the plants were determined by the adult motility assay and death in a dose and time dependent manner and were compared with ivermectin. The extracts of *M. koenigii* and *O. sanctum* killed the worms in a dose dependent manner within 5 hours whereas the extract of *A. indica* paralyzed the worms and the activity of higher dose of *Murraya koenigii* was comparable with ivermectin. The data shows that the methanolic extracts show anthelmintic activity *invitro* justifying their use in traditional medicine.

Key words: Anthelmintic, *Ascaridia galli, Murraya koenigii, Ocimum Sanctum.*

Introduction: Traditional medicine makes use of a number of plants in the treatment of various gastrointestinal (GI) disorders in animals including enteritis. One of the common causes of enteritis in livestock is endoparasitism. Nematode infections of the GI tract adversely affect the productivity in livestock. The broad spectrum anthelmintic drugs are expensive and there are several alternative measures to control parasites [1]. The control of helminthes infections demands huge investments and rapid development of anthelmintic resistance has influenced the efficacy of conventional anthelmintics for the control of GI nematodes of livestock [2]. The continuous and long term use of same group of compounds has led to the development of resistance [3]. The present study was undertaken to find a novel anthelmintic substance of natural origin from locally available medicinal plants.

Three medicinal plants *viz*, neem, tulsi and curry leaf were selected for the study. *Azadirachta indica* (Neem*)* is a member of Meliaceae family having multiple medicinal applications including use as anti-inflammatory, antipyretic, analgesic, hypoglycemic and antibacterial [4]. The effect of neem leaf and stem extracts on larvae and nematode eggs were studied to establish the anthelmintic property [4]-[5]. *Ocimum sanctum* (Tulsi) has been in use from time immemorial in Ayurvedic medicine and the eugenol and essential oils present in the plant are responsible for medicinal properties. *Murraya koenigii* (Curry leaf) is a leafy vegetable found throughout India and has got many medicinal properties including antimicrobial, analgesic, febrifuge, stomachic, anti-diarrheal [6] and the anthelmintic activity of the methanolic extract of *Murraya koenigii* in earthworms were reported [7]. The present investigation focuses on the anthelmintic activity of the methanolic extract of the leaves of neem, tulsi and curry leaf on *Ascaridia galli.*

Materials and Methods: Plant materials: Leaves of *Azadirachta indica, Ocimum sanctum and Murraya koenigii* were collected from different localities of the district of Wayanad (Kerala) during December 2012 to April 2013 and identified at the MS Swaminathan Research Foundation, Kalpetta.

Extraction of plant material: For the preparation of methanolic extract, the air dried and powdered plant materials (100g) were extracted using methanol in a soxhlet apparatus. The extract was dried using rotary vacuum evaporator and stored under refrigeration.

Preparation of the extract: 200 and 100 mg/ml of the extracts were prepared in normal saline and were used to test the anthelmintic activity.

Phytochemical analysis: Qualitative phytochemical analysis was done using color reactions and on TLC plate [8].

Evaluation of the *invitro* anthelmintic activity: *Invitro* anthelmintic activity of the plant materials were evaluated as per the method [1] with necessary modifications. The adult live and actively motile *Ascaridia galli* worms were collected from the GI tract of chicken slaughtered in the local slaughter houses and transferred to petri dishes containing the plant extracts and normal saline. For each extract, seven petri dishes were used; six for the extracts to be tested and one for control (normal saline). Each petri dish contained 2 worms and experiment was done in triplicate. Observations were made every 15 minutes till the fifth hour of the experiment and the number of worms paralyzed/ dead were counted and compared with the normal.

Results:

Table 1: Percent yield of methanolic extract from different plants			
Plant	Part tested	Type of extract	% yield
Azadirachta indica	Leaf	Methanolic	15.17
Ocimum sanctum	Leaf	Methanolic	10.23
Murraya koenigii	Leaf	Methanolic	12.53

Table 2: Phytochemical constituents from different methanolic extracts			
Phytochemical constituent	*Azadirachta indica*	*Ocimum sanctum*	*Murraya koenigii*
Steroid	-	-	-
Alkaloid	+	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Phenolics	+	+	+
Diterpenes	+	-	+
Triterpenes	+	+	_
Saponins	-	+	-

Tannins, flavonoids, glycosides, and phenolics were present in all the extracts and none of the extracts showed the presence of steroids.

Table 3: *Invitro* anthelmintic activity of crude methanolic extracts of neem, tulsi, and curry leaf on *Ascaridia galli*							
Treatment	Dose (mg/ml)	% of motile worms (hrs)					
		0	1	2	3	4	5
A.Indica	200	100	100	100	80	20	10
	100	100	100	100	80	20	20
O. sanctum	200	100	100	100	50	0	0
	100	100	100	100	60	20	0
M. koenigii	200	100	20	0	0	0	0
	100	100	80	50	20	0	0
Ivermectin	0.1	100	0	0	0	0	0
	0.05	100	60	40	0	0	0
Normal Saline/ Control		100	100	100	100	80	60

The movement of the treated and control nematodes were observed for a period of 5 hours and the % motility chart was prepared (Table 3). The control flukes were active (80%) till 4 hrs after which their motility decreased. The worms treated with ivermectin @ 100 mcg/ml were dead by the first hour itself where as those treated with 50 mcg/ml were dead by 3rd hour. The activity of worms treated with the extract of *M. koenigii* @ 200 mg/ml decreased by 1st hour itself with only 20% active whereas all of them were dead by second hour. The results indicated that the extract of *M. koenigii* was far more superior to the other two in inhibiting the motility of *Ascaridia galli.*

Discussion: The present study shows that the methanolic extract of *Murraya koenigii* and *Ocimum sanctum* is able to kill the intestinal nematode of poultry *viz*, *Ascaridia galli* within 5hrs of exposure whereas the methanolic extract of *Azadirachta indica* paralyzed the worms *in vitro*. These findings confirm the traditional claims regarding the anthelmintic

activity of these plants against human and animal gastrointestinal nematodes. There were reports on the anthelmintic activity of aqueous extracts of neem leaf, stem and root barks against the hatching of eggs and survival of larvae of strongylid nematodes of goats [5]. The methanolic extract of neem killed the third stage larvae of *Haemonchus contortus* after 24 hrs of treatment [4]. There are also several other studies in the anthelmintic activity of *A. indica* against nematodes of ruminants [9]-[10]. The anthelmintic activity of essential oils of tulsi and

lemon grass was evaluated *invitro* against liver fluke and it was shown that 1% lemon grass oil showed an anthelmintic effect [11].

Anthelmintic activity of the aqueous and alcoholic extracts of *Scleropyrum pentandrum* (Dennst.) Mab. against Indian earth worms were reported (12). There are several studies on the anthelmintic activity of phytochemicals against *Ascaridia galli* which include *Brassica nigra* seeds [13], *Wrightia tinctoria* [14], *Eupatorium triplenerve* and *Alpiniag alanga* [15]. Several commercial anthelmintic molecules contain phenolic groups and reports suggest that phenolic compounds possess good anthelmintic property. The ability of phenols to interfere with the energy generation is considered to be the main cause of the anthelmintic activity. Flavonoids contained in the plant also add to the anthelmintic activity [15]. Presence of tannins in the extract may affect the glycoprotein cuticle of the helminthes causing paralysis and death [16]-[17]. The phytochemical analysis of all the three extracts revealed the presence of flavonoids, tannins and phenolic compounds. Identification and separation of the active principles can lead to the development of a novel anthelmintic with a new mechanism of action and thus provide a solution to the ever emerging threat of anthelmintic resistance.

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