

The Anthelmintic activity of leaves of *Trachyspermum ammi*

Mr. Mukul Tambe, Mrs. Shubhashree Mahadik

Abstract— *Trachyspermum ammi* Linn. (family: Umbellifereae) is an important medicinal seed spice. Leaves of *Trachyspermum ammi* are used widely in the Ayurvedic medicines for the treatment of helminthiasis and gastric colic. Previous studies revealed the anthelmintic activity of roots and fruits. However, no studies were carried out to delineate anthelmintic activity of leaves. Hence the present study was carried out to evaluate anthelmintic activity of leaves of *Trachyspermum ammi*. In-vitro anthelmintic activity of leaves extracts in ethyl acetate, chloroform, petroleum ether on Indian earthworms (*Phertima prosthuma*) was tested. The earthworms were exposed to series of concentrations ranging from 20 to 100 mg/ml of different leaf extracts and standard drug, Albendazole. Paralysis time and the death time were noted after the exposure. Results revealed that a dose dependent decrease of paralysis time and death time was observed in all the extract and Albendazole treated groups. The mean paralysis time observed at highest concentration i.e. 100 mg/ml of ethyl acetate, chloroform and petroleum ether extracts were found to 2.07, 4.65, and 9.47 min, respectively. The mean death time observed at highest concentration i.e. 100 mg/ml of ethyl acetate, chloroform and petroleum ether extracts were found to 6.17, 9.8 and 9.8 min, respectively. Ethyl acetate extract was found to have more potency than other extracts and albendazole. Hence *Trachyspermum ammi* leaf extracts were found to have anthelmintic activity. The study validates the traditional use of leaves of *Trachyspermum ammi* on helminthiasis.

Keywords— Ajwain, Albendazole, Anthelmintic activity, Helminthiasis, Leaves, *Phertima prosthuma*, *Trachyspermum ammi*

1 INTRODUCTION

Helminthiasis is an infectious disease caused by parasitic worms called helminths. These parasites are classified into tapeworms, roundworms and flukes. These worms live in gastrointestinal tract or sometimes in other organs inducing physiological damage [1]. Prevalence of various helminthic infection is more than 50% [2]. Present treatments for helminthiasis include drugs like albendazole, mebendazole, piperazine citrate, levamisole etc. [3]. However, these treatments exhibit side effects like dizziness, diarrhea [4], undesirable neurological side effects [5] etc. Therefore, herbal drugs are preferred over chemotherapy. There are various traditional medicines available for helminthiasis Tobacco, Walnut, Wormwood, Clove, Kalonji seeds, Garlic, Malefern, Pineapple, Diatomaceous earth, Soya and other legumes [6].

Trachyspermum ammi L. belonging to family Umbellifereae is a highly valued medicinally important seed spice [7]. It is known as Yavaani in Ayurveda, Ajwain in Hindi, Omum in English [8]. Ova in Marathi, Ajamoda in Sanskrit, Yavano in Gujrati, Ajmoola in Kannada etc. *Trachyspermum ammi* is a native of Egypt and is cultivated in Iraq, Iran, Afghanistan, Pakistan, and India. In India, it is cultivated in Madhya-Pradesh, Uttar-Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal [9].

(38.6%), tannins, glycosides, saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron and nicotinic acid. *T. ammi* seeds contain 2.5 - 5% essential oil and the principal constituents of essential oil are phenols- thymol (35 - 60%), carvacrol (11%). The remainder of the oil is called thymene which contains p-cymene (50 - 55%), beta-pinene (4 - 5%), limonene with gamma-and betaterpinenes (30 - 35%) [8],[9]. *T. ammi* with its characteristic aromatic smell and pungent taste is widely used as a spice in curries. Traditionally it is used as a carminative and gastro intestinal regulator [10]. Seeds are used in small quantities as flavouring agent, preservatives, in medicine and for the manufacture of essential oil in perfumery [11]. In Indian system of medicine, *T. ammi* is administered in household remedy as a paste of crushed fruits and applied externally for relieving colic pains & for asthma [9]. *T. ammi* has been shown to possess anti-aggregatory effects [12]; anthelmintic [13]; antihyperlipidaemic [14]; antifilarial [15]; insecticidal [16]; kidney stone inhibitory [17]; molluscicidal [18]; mosquito repellent [19]; and nematicidal & antifungal activities [20]; *T. ammi* oil exhibited a remarkable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibrio cholera* [9],[21],[22],[23]. Extracts prepared in different solvents exhibited variable activities suggesting their centuries old usage in the treatment of gastrointestinal disorders. This historical use of *T. ammi* seeds to cure various gastrointestinal disorders has also been scientifically validated [8]. Methanol extracts of fruits *T. ammi* showed significant in-vitro inhibitory effect on hepatitis C virus (HCV) protease at a concentration 100 µg/ml [24].

However, the fresh leaves of *Trachyspermum ammi* were not much explored for its anthelmintic activity. The present study

• Mukul Tambe is currently working at RAIFR, A Unit Of Central Council For Research In Ayurvedic Sciences, Ministry of AYUSH Pune, Maharashtra India. PH- (+91) 8309112982 E-mail: rxmukul.tambe@gmail.com

• Mrs. Shubhashree Mahadik is currently working at Symbiosis International University, Lavale, Pune, Maharashtra, India. PH-(+91) 98817153079. E-mail: mahadiksu@gmail.com

Omum seed analysis has revealed it to contain moisture (8.9%), proteins (15.4%), fats (18.1%), fiber (11.9%), carbohydrates

was carried out to evaluate the potential of leaves of *Trachyspermum ammi* for its anthelmintic activity.

2 MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *Trachyspermum ammi* were collected from Local Market, Pune, Maharashtra and were authenticated by Dr. R. D. Joseph, HOD; Department of Botany Abeda Inamdar Senior College, Azam Campus, Camp, Pune. All the data obtained was analyzed by one-way ANOVA followed by Tukey's multiple comparison post hoc. test, using Prism Pad Statistics Software Version 4. The difference of $p < 0.05$ was considered significant in all the cases.

Experimental model

Indian earthworms *Phertima prosthuma* [25] were used to study anthelmintic activity. The earthworms were collected from the moist soil from near region of Pune and washed with water. The collected earthworms were identified as *Phertima prosthuma* by Mrs. Azeemunisa Maniyar, HOD, Department of Zoology Abeda Inamdar Senior College, Azam Campus, Camp, Pune. Indian earthworm *Phertima prosthuma* is 3-5 cm in length and 0.1-2 cm in width were used due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human being. Twenty-five groups of equal size earthworms consisting of six in each group were used for the study [26].

Preparation of extract

The fresh leaves of *Trachyspermum ammi* were collected & crushed. The leaves were extracted using Ethyl Acetate, Chloroform and Petroleum Ether respectively by continuous hot extraction method using Soxhlet Apparatus for 72 hours. Percentage yield of Ethyl acetate, Chloroform and Petroleum ether were 1.7%, 1.1% and 0.82% respectively. All the extracts obtained were filtered, concentrated & dried thoroughly to ensure no traces of solvent left in it that may hamper the activity.

Standard drug

Albendazole was used as standard drug. Various concentrations of Albendazole ranging from 20mg/ml to 100mg/ml concentration were prepared in 1% gum acacia in normal saline solution for the activity [27].

Preparation of extract for the activity

Series of concentrations of Ethyl Acetate Extract, Chloroform Extract and Petroleum Ether Extract were prepared ranging from 100mg/ml to 20mg/ml in 1% gum acacia in normal saline solution for the activity.

Anthelmintic investigation

Twenty-five groups of approximately equal size earthworms consisting of six earthworms in each group were used for the study. All groups were treated with Albendazole, Ethyl Acetate Extract, Chloroform Extract and Petroleum Ether Extract (100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml) respectively. 1% gum acacia in normal saline was used as a control group. Observations were made from the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when worms lost their motility followed by fading away of their body color [28].

3 RESULTS AND DISCUSSION

Anthelmintic activity of various extract of fresh leaves of *Trachyspermum ammi* is confirmed by examining the time taken for paralysis (P) and death (D) for *Phertima prosthuma* worms were reported in Table 1 and 2.

TABLE 1
PARALYSIS TIME

Treatme nt	Albendazole (Standard Drug)	Ethyl Acetate Extract	Chloroform Extract	Petroleum Ether Extract	Control (Saline solution)
Concentration					
20mg/ml	23.37±0.4787	6.96±0.6421***	17.10±0.4898**	32.05±0.4203	> 30
40mg/ml	19.87±0.2630	5.01±0.0236***	15.17±0.5852**	25.17±0.2753	> 30
60mg/ml	17.87±0.8539	3.05±0.4203***	7.65±0.5066**	16.20±0.6271*	> 30
80mg/ml	15.17±0.2363	2.42±0.0957***	6.47±0.3862**	12.45±0.4203*	> 30
100mg/ml	13.00±0.4082	2.07±0.1500***	4.65±0.5066**	9.47±0.5251*	> 30

Effect of different extracts of *Trachyspermum ammi* on Paralysis time (min.) of *Phertima prosthuma*.

Values are expressed as Mean±SEM

*** $P < 0.0001$, ** $P < 0.001$, * $P < 0.01$ when compared to Standard group.

TABLE 2
DEATH TIME

Treatme nt	Albendazole (Standard Drug)	Ethyl Acetate Extract	Chloroform Extract	Petroleum Ether Extract	Control (Saline solution)
Concentration					
20mg/ml	43.42±0.4349	12.05±0.4203***	25.00±1.4719**	41.87±1.0307	> 60
40mg/ml	40.62±0.4787	10.12±0.1258***	19.80±0.5715**	35.00±0.7071*	> 60
60mg/ml	32.87±0.8539	8.06±0.02630***	13.32±0.5377**	27.00±0.4082*	> 60

80mg/m	22.37±	7.25±	12.07±	20.37±	> 60
1	0.2986	0.2887***	0.4349**	0.4787*	
100mg/	18.85±	6.17±	9.80±	9.80±	> 60
ml	0.6292	0.2363***	0.3559*	0.3599*	

Effect of different extracts of *Trachyspermum ammi* on Death time (min.) of *Phertima prosthuma*.

Values are expressed as Mean±SEM

***P<0.0001,**P<0.001,*P<0.01 when compared to Standard group.

The assay was performed on adult Indian earthworm, *Phertima prosthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in-vitro [29].

Literatures have revealed that there are many anthelmintic drugs having different mechanism action for lethality of helminths. Albendazole and Piperazine citrate are commonly used drugs in the in-vitro anthelmintic activity. However Albendazole has found to be more efficacious than that of the Piperazine citrate, hence it was chosen as standard drug for the present study [30],[31],[33].

Albendazole, as a vermicide, causes degenerative alterations in the intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. Degenerative changes occur in the endoplasmic reticulum, the mitochondria of the germinal layer, and the subsequent release of lysosomes results in a decreased production of ATP. Due to diminished ATP production, the parasite is immobilized and eventually dies [33].

In the present study it was observed that all the extracts of leaves of *Trachyspermum ammi* showed certain degree of anthelmintic activity on selected worms, whereas Ethyl Acetate extract of plant showed highly significant activity as compared to Standard Drug.

The Ethyl Acetate extract demonstrated paralysis as well as death of worms in very less time as compared to Standard Drug even at low concentration. The conclusion can hence be made that constituent(s) responsible for the anthelmintic activity may be polar.

The results obtained with Chloroform extract were similarly significant when compared with the Standard drug but

Chloroform extract was found to be less potent than Ethyl acetate extract. The polarity of Chloroform being lesser than Ethyl Acetate, the constituent(s) extracted may be less polar, hence leading to the assumption that constituents responsible for the activity may be less polar as compared to the constituents of Ethyl acetate extract.

On the other hands, The Petroleum Ether extract also showed significant activity in comparison with that of the Standard Drug. Both of previous solvents are polar and Petroleum ether is non-polar solvent; so the components extracted in Petroleum Ether would be non-polar supporting to assumption that they also may be responsible for Anthelmintic activity to some extent.

Anthelmintic activity of *Trachyspermum ammi* leaves may be due to presence of active constituent(s) present in polar or the combination of polar and non-polar constituents. The conclusion may be drawn from the results obtained, that polar constituent(s) are having more potent activity than non-polar one, as the activity of Ethyl Acetate and Chloroform extracts is much significant than other Petroleum Ether extract. The possible mechanism of the anthelmintic activity of *Trachyspermum ammi* cannot be explained on the basis of our present results. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity. The further aim is to isolate and to characterize the exact constituent(s) responsible for Anthelmintic activity.

4 CONCLUSION

On the basis of present results and available reports, *Trachyspermum ammi* as an anthelmintic have been confirm as it displayed activity against the worm used in present study. Ethyl Acetate extract displayed profound anthelmintic activity in study. The drug may be further explored for its phytochemical profile, isolation and characterizations and identification of the active constituent responsible for anthelmintic activity.

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