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CONTAINMENT OF HUMAN GASTROINTESTINAL PARASITES BY HERBAL DRUGS: AN *IN-VITRO* STUDY

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Keywords:

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ABSTRACT: Parasitic worms live in and feed in living hosts. They receive nourishment and protection while disrupting their hosts ability to absorb nutrients. The macroparasites called helminths, causes Helminthiasis. In India, the overall prevalence rates range from 12.5% to 66%. About 50% of the urban population and 68% of the rural population in India is affected. According to WHO, just few medications are consistently utilized for the treatment of these parasite diseases, these include albendazole, mebendazole, piperazine thiabendazole and levamisole. The resistance of helminths to drugs poses health complications to both man and animals which increased the demand for the use of herbal drugs. The present study was carried out to evaluate anthelmintic activity of leaves of *Trachyspermum ammi* and *Desmodium gangeticum*. Earthworms (*Eisenia fetida*) were chosen for studying anthelmintic activity as they have resemblance to intestinal round worms. The earthworms were exposed to series of concentrations 100mg/ml, 200mg/ml, 300mg/ml, 500mg/ml, 1000mg/ml of leaf extracts. Albendazole is used as a standard drug. Results revealed that a dose dependent decrease of paralysis time and death time were observed in both the extracts and albendazole treated groups. The Mean±SEM values of time of paralysis and time of death at 1000mg/ml of *Trachyspermum ammi* was found to be 6.6±0.881 and 8.3±1.20 (min) and that of 1000mg/ml of *Desmodium gangeticum* was found to be 7.3±1.201 and 10±1.527 (min). Hence, based on present results *Trachyspermum ammi* and *Desmodium gangeticum* have an anthelmintic activity and validates their traditional use for treating helminthiasis.

INTRODUCTION: Helminthiasis is an infectious disease caused by parasitic worms known as Helminths. These worms live in gastrointestinal tract or sometimes in other organs inducing physiological damage [1].

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Helminths infection is a huge challenge, both in developing and developed countries due to their continuous contamination of the environment with their eggs and larvae [2].

These helminths can cause intestinal blood loss, iron deficiency anaemia and protein malnutrition, especially in heavily infected individuals [3]. Approximately more than 10% of the population is infected by GI nematodes worldwide [4]. There are two major phyla of helminths known as nematodes and platyhelminths. Nematodes are also known as roundworms that include soil-transmitted helminths

and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis. Other phyla platyhelminths also called flatworms, which include flukes schistosomes and tapeworms such as the pork tapeworm that causes cysticercosis [5].

Almost all these parasites could be treated and the level of infection could be reduced below clinical significance with one or a combination of the following categories of anthelmintic drugs; benzimidazoles, macrocyclic lactones, levamisole, piperazine and amino-acetonitrile derivatives [6, 7]. The activity of any anthelmintic medication is to either deaden or kill the worm and oust from the body [8]. Currently, instances of nematode resistance to all available anthelmintic drugs have been documented. The knowledge of the genetics and mechanisms of helminths resistance to drugs is essential to prevent resistance; to newly develop anthelmintic drugs, to reduce the spread of resistant parasites and to better manage parasite control at all stages of their lifecycle [9].

Major problems associated with the treatment of helminthiasis are severe side effects and high cost of anthelmintic drugs [10]. The high medical, educational, and economic burden of helminth infections, together with their co-endemicity with malaria and AIDS, provides an important rationale for launching a global assault on parasitic worms [11]. For these problems, there is a great need for new chemical entities with the least side effects and low cost. Scientific data obtained from different countries revealed that herbs and their active principles could play an important role in the development of the new anthelmintic drug [12].

Trachyspermum ammi (ajwain) is an erect, aromatic annual herbaceous plant belonging to the Apiaceae family, which is used worldwide as a spice and has medicinal properties [13]. It contains thymol and Carvacrol as the principal components, while γ-terpinene, camphene, ρ-cymene, δ-3-carene, β-pinene, myrcene, limonene, and sabinene as the minor constituents [14]. Ajwain exerts different pharmacological activities like antifungal, antioxidant, antimicrobial, anti-nociceptive, cytotoxic, hypolipidemic, antihyper-tensive, antispasmodic, broncho-dilating actions, antilithiasis, diuretic, abortifacient, antitussive, nematicidal and antifilarial [15].

FIG. 1: *TRACHYSPERMUM AMMI*

Desmodium gangeticum commonly called Shalparni is a subtropical perennial herb belonging to the family Leguminosae (Fabaceae) [16]. Desmodium contains tryptamine alkaloid and chemical investigation concludes the presence of isoflavones, glycosyl-flavonoids, coumarone-chromones, pterocarpons, triterpenoids, saponins, tetrahydroiso-quinolones, phenylethylamines, indole-3-alkylamines, lipids and glycolipids [17].

Pharmacological studies revealed the potentiality of *Desmodium gangeticum* extract as anti-amnesic, immunomodulator, anti-diabetic, antioxidant, cardio-protective, hepato-protective, anti-inflammatory drug [18].

FIG. 2: *DESMODIUM GANGETICUM*

MATERIALS AND METHOD:

Collection of Plant Material: The aqueous extract of leaf powder of *Trachyspermum ammi* and *Desmodium gangeticum* were collected from Shipra Ayurvedic store, Hyderabad, Telangana. The plant authentification was done by botonist, S.V University, Tirupathi. The voucher number is 1243. Indian earthworms *Eisenia fetida* were used to study anthelmintic activity.

The earthworms were collected from the Maa enterprises and washed with water.

Experimental Model: The assay was performed on *Eisenia fetida* due to its anatomical and physiological resemblance to the intestinal roundworm parasite of human beings [19]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds.

Phytochemical Screening: Crude leaf extract was subjected to phyto-chemical analysis for qualitative and quantitative determination of phytonutrients. *Desmodium gangeticum* and *Trachyspermum* ammi have some common chemicals such as alkaloids, phenols, flavanoids, terpenoids and tannins.

Phytochemical analysis of *Trachyspermum ammi* and *Desmodium gangeticum* were performed. The methods used are:

Test for Alkaloids: Solvent free extract, 50 mg was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid test reagents as follows:

Mayer's Test: Few drops of Mayer's reagent were added to 1 mL of extract. Yellowish or white precipitate formation indicates the presence of alkaloids [20].

Test for Carbohydrates Benedict's test: 0.5 ml of Benedict's reagent was added to 0.5 ml of the filtrate. The mixture was heated on boiling water bath for 2 min. A characteristic red colored precipitate indicates the presence of sugar.

Detection of Saponins by foam test: The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins [21].

Test for Phenolic Compounds:
Ferric Chloride Test: The extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds [22].

Test for Tannins: A few drops of lead acetate was added to 1 mL of extract. A large white-brown precipitate formation was considered a positive test for tannin [23].

Test for Terpenoids (Salkowski Test): 5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish-brown coloration of the interface showed the presence of terpenoids.

Preparation of Concentrations of Extract for the Activity: Series of concentrations of both the herbal aqueous extracts were prepared as 100mg/ml, 200mg/ml, 300mg/ml, 500mg/ml and 1000mg/ml.

Anthelmintic Activity: *Eisenia fetida* of size approximately 6-8cm were used for the study. Each worm was placed in petri dish and all the worms were treated with 100mg/ml, 200mg/ml, 300mg/ml, 500mg/ml and 1000mg/ml of extracts of *Trachyspermum ammi* and *Desmodium gangeticum*. Observations were made from the time taken to paralyse and death of individual worm. Death was concluded when worms lost their motility followed by fading away of their body color.

RESULTS AND DISCUSSION:

TABLE 1: PHYTOCHEMICAL SCREENING OF *DESMODIUM GANGETICUM* AND *TRACHYSPERMUM AMMI*

Phytochemicals	Inference of *Desmodium gangeticum*	Inference of *Trachyspermum ammi*
Alkaloids	+	+
Carbohydrates	+	+
Saponins	-	+
Phenols	+	+
Tannins	+	+
Terpenoids	+	+

Phytochemical screening of *desmodium gangeticum* shows the presence of alkaloids, carbohydrates, phenols, tannins, terpenoids and saponins are absent.

Phytochemical screening of *Trachyspermum ammi* shows the presence of alkaloids, carbohydrates, phenols, saponins, tannins, terpenoids.

Results of Paralysis of worms at various concentrations of *Trachyspermum ammi* leaf extract.

FIG. 3: PARALYSIS OF WORM AT 100mg/ml

FIG. 4: PARALYSIS OF WORM AT 200mg/ml

FIG. 5: PARALYSIS OF WORM AT 300mg/ml

FIG. 6: PARALYSIS OF WORM AT 500mg/ml

FIG. 7: PARALYSIS OF WORM AT 1000mg/ml

Results of Paralysis of worms at various concentrations of *Desmodium gangeticum* leaf extract.

FIG. 8: PARALYSIS OF WORM AT 100mg/ml

FIG. 9: PARALYSIS OF WORM AT 200mg/ml

FIG. 10: PARALYSIS OF WORM AT 300mg/ml

FIG. 11: PARALYSIS OF WORM AT 500mg/ml

FIG. 12: PARALYSIS OF WORM AT 1000mg/ml

FIG. 13: PARALYSIS OF WORM AT 500mg/ml STANDARD

The paralysis time and time of death are observed with both the drugs. The results are tabulated as mean ± SEM. The Mean±SEM values for time of paralysis at 100mg/ml, 200mg/ml, 300mg/ml, 500mg/ml, 1000mg/ml are 83.66±1.453, 41.3±1.201, 25±1.154, 11.3±0.88, 6.6±0.881 respectively. The anthelmintic activity of *Trachyspermum ammi* is higher at concentration 1000mg/ml as time of paralysis is less when compared to other concentrations.

The Mean±SEM values for time of death at 100mg/ml, 200mg/ml, 300mg/ml, 500mg/ml, 1000mg/ml are 92.6 ±1.201, 52.6 ±1.855, 33 ±1.154, 15.6± 0.881, 8.3±1.20 respectively is mentioned in **Table 2**. The anthelmintic activity of *Trachyspermum ammi* is higher at concentration 1000mg/ml as time of death is less when compared to other concentrations. The Mean ±SEM values are compared with standard. The Mean ±SEM values of time of death of standard is 22±1.52.

TABLE 2: EFFECT OF DIFFERENT CONCENTRATIONS OF *TRACHYSPERMUM AMMI* SHOWING PARALYSIS TIME AND TIME OF DEATH. VALUES ARE EXPRESSED AS MEAN±SEM

Concentration (mg/ml)	Time of paralysis Mean±SEM	Time of death Mean±SEM
100mg/ml	83.66±1.453	92.6±1.201
200mg/ml	41.3 ±1.201	52.6±1.855
300mg/ml	25 ±1.154	33±1.154
500mg/ml	11.3± 0.88	15.6±0.881
*1000mg/ml	6.6 ±0.881	8.3±1.20

Values are expressed as Mean±SEM, *P<0.001 when compared to standard.

The Mean ±SEM values for time of paralysis at 500mg/ml is 17 ±0.57 and Mean ±SEM value for time of death is 22 ±1.52 in **Table 3.**

TABLE 3: EFFECT OF CONCENTRATION OF ALBENDAZOLE SHOWING TIME OF PARALYSIS AND TIME OF DEATH

Concentration (mg/ml)	Time of paralysis Mean±SEM	Time of death Mean±SEM
500mg/ml	17±0.57	22±1.52

Values are expressed as Mean±SEM.

The mean ± SEM values for time of paralysis at 100mg/ml, 200mg/ml, 300mg/ml, 500mg/ml, 1000mg/ml are 76.6±0.88, 56.3 ±2.185, 33±0.577, 16.3±1.201, 7.3±1.201 respectively. The anthelmintic activity of *Desmodium gangeticum* is higher at concentration 1000mg/ml as time of paralysis is less when compared to other concentrations.

The mean±SEM values for time of death at100mg/ml, 200mg/ml, 300mg/ml, 500mg/ml, 1000mg/ml are 86.3±1.76, 77.3±1.45, 43.6±2.027, 18.3±1.453, 10±1.527 respectively is mentioned in **Table 4**. The anthelmintic activity of *Desmodium gangeticum* is higher at concentration 1000mg/ml as time of death is less when compared to other concentrations.

TABLE 4: EFFECT OF DIFFERENT CONCENTRATIONS OF *DESMODIUM GANGETICUM* SHOWING PARALYSIS TIME AND TIME OF DEATH. VALUES ARE EXPRESSED AS MEAN±SEM

Concentration (mg/ml)	Time of paralysis Mean±SEM	Time of death Mean±SEM
100mg/ml	76.6 ±0.88	86.3±1.763
200mg/ml	56.3 ±2.185	77.3±1.453
300mg/ml	33±0.577	43.6±2.027
500mg/ml	16.3±1.201	18.3±1.453
*1000mg/ml	7.3±1.201	10±1.527

Values are expressed as Mean±SEM, *P<0.001 when compared to standard.

The Mean±SEM values of time of paralysis and time of death of *Trachyspermum ammi* at 1000mg/ml was analysed by T- test compared with standard at 500mg/ml and it was found to be significant. The Mean±SEM values of time of paralysis and time of death of *Desmodium gangeticum* at 1000mg/ml was analysed by T- test compared with standard at 500mg/ml and it was found to be significant.

CONCLUSION: On the basis of present results and available reports, *Trachyspermum ammi* and *Desmodium gangeticum* have an anthelmintic activity as it displayed activity against the worms. Ancient classical literature and ethanomedical surveys described the use of plants in the traditional system of medicines for the treatment of helminthic infections. This traditional medical wisdom is excellent proof of clinical efficacy and safety of medicinal plants. Due to the advancement in the research field there were many research studies conducted to reveal the power of the plant and its compound in the treatment of helminthic infection.

The chemically provided drugs are costlier and provide higher side effects when compared to the natural drugs that are obtained from the plant source were cheaper and provide lesser effect on the host organism. It was concluded based on findings of present study that extract of *Trachyspermum ammi* and *Desmodium gangeticum* passes varying degree of anthelmintic activity against *Eisenia fetida*. The increased dose of extract increases the activity of extract and is dose dependent. This study strongly supports the traditional use of herbs as anthelmintic.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Crompton DW and Nesheim MC: Nutritional impact of intestinal helminthiasis during the human life cycle. Annual Review of Nutrition 2002; 22(1): 35-59.
2. Naluke AS, Mbaria JM and Kimenju JW: *In-vitro* anthelmintic potential and phytochemical composition of ethanolic and water crude extracts of *Euphorbia heterophylla* Linn. J Med Plants Res Academic Journals 2013; 7(43): 3202-3210.
3. Coffeng LE, Stolk WA and De Vlas SJ: Predicting the risk and speed of drug resistance emerging in soil-transmitted helminths during preventive chemotherapy. Nat Commun 2024; 15: 1099.
4. Adak M and Kumar P: Herbal anthelmintic agents: a narrative review. J Tradit Chin Med 2022; 42(4): 641-651.
5. Al Amin ASM and Wadhwa R: Helminthiasis. Stat Pearls Treasure Island 2023.
6. James CE, Hudson L and Davey MW: Drug resistance mechanisms in helminths: is it survival of the fittest. Trends Parasitol 2009; 25(7): 328–335.
7. Aremu AO, Finnie JF and Van Staden J: Potential of South African medicinal plants used as anthelmintics - their efficacy, safety concerns and reappraisal of current screening methods. South Afr J Bot 2012; 134–150.
8. Marco A, Peter GS, Andrew H, Hababu MC, Kassim SA and Lorenzo S: A randomized controlled trial comparing mebendazole and albendazole against *Trichuris trichiura*, and *Ascaris lumbricoides* and hookworm infections. Trans R Soc Trop Med Hyg 1994; 8(5): 585-589.
9. Prangthip P, Tummatorn J and Adisakwattana P: Anthelmintic efficacy evaluation and mechanism of *N*-methylbenzo[d]oxazol-2-amine. Sci Rep 2023; 13: 22840.

10. Aronson JK: Side effects of drugs annual: a world-wide yearly survey of new data and trends in adverse drug reactions. Elsevier First Edition 2003.
11. Hotez PJ: Control of neglected tropical diseases. N Engl J Med 2007; 357: 1018–1027.
12. Buhner SH: Herbal antibiotics: natural alternatives for treating drug-resistant bacteria. Storey Publishing Second Edition 2012.
13. Asangi H, Ravi Y, Ashoka N, Kavan Kumar V, Harisha CB and Arvind KV: Recent Advances in Ajwain (*Trachyspermum ammi* L.) Cultivation: A Review. International Journal of Environment and Climate Change 2023; 13(10): 2929-2938.
14. Sharma H, Yang H, Sharma N: An SSA *Trachyspermum ammi* Bioactives Promote Neuroprotection by Inhibiting Acetylcholinesterase, Aβ-Oligomerization/Fibrilization and Mitigating Oxidative Stress *In-vitro*. Antioxidants 2024; 13(1): 9.
15. Mirniyam G, Rahimmalek M, Arzani A, Yavari P, Sabzalian MR, Ehtemam MH and Szumny A: Phytochemical, morphological and physiological variation in different Ajowan (*Trachyspermum ammi* L.) Populations as Affected by Salt Stress, Genotype × Year Interaction and Pollination System. Int J Mol Sci 2023; 24(13): 10438.
16. Mohan PK, Adarsh Krishna TP, Senthil Kumar T and Ranjitha Kumari BD: Pharmaco-chemical profiling of *Desmodium gangeticum* (L.) DC. with special reference to soil chemistry. Futur J Pharm Sci 2021; 7(1): 210.
17. Joshi BR, Hakim MM and Patel IC: The biological active compounds and biological activities of Desmodium species from Indian region- a review. Beni-Suef Univ J Basic Appl Sci 2023; 12: 1.
18. Yahya SA, Vijay RC, Shruti S, Sudarshan S, Rahul M, Havagiray C, Naresh BC, Popat M, Ahmed MA and Masood MK: A multi-modal approach to investigate *Desmodium gangeticum's* influence on stress-induced male infertility: *in-vivo*, *in-vitro*, and *in-silico* assessments. Biomedicine & Pharmacotherapy 2024; 173.
19. Kainsa S, Kumar P and Dahiya RS: Investigation of *in-vitro* anthelmintic activity of *Cassia auriculata* leaves. J of Natural Product and Plant Resources 2012; 2(4) 460-4.
20. Kancherla N, Dhakshinamoothi A, Chitra K and Komaram RB: Preliminary Analysis of Phytoconstituents and Evaluation of anthelmintic Property of *C. auriculata (In-vitro)*. Maedica (Bucur) 2019; 14(4): 350-356.
21. Summi R, Ananda K, Hari PD and Ajaya B: Characterization of saponins from the leaves and stem bark of *Jatropha curcas* L. for surface-active properties. Heliyon 2023; 9(5).
22. Godlewska K, Pacyga P, Najda A and Michalak I: Investigation of Chemical Constituents and Antioxidant Activity of Biologically Active Plant-Derived Natural Products. Molecules 2023; 28(14): 5572.
23. Bakir CN, Yalçin E and Çavuşoglu K: Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. Sci Rep 2022; 12: 21421.

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