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Comparative Anthelmintic activity of three Ayurvedic medicinal plants

Research Article

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Abstract

Helminthiasis, which is the infection by helminths or worms, is a major public health issue, especially in developing nations where it leads to malnutrition, anaemia, and death. The traditional treatment with synthetic anthelmintic drugs like albendazole and mebendazole is usually accompanied by adverse effects like gastrointestinal upsets and neurological manifestations. Additionally, the growing problem of drug resistance has created a need for safer and more effective natural alternatives. The present study aims to explore, evaluate and compare the anthelmintic potential of three traditionally used Ayurvedic medicinal plants *Annona squamosa, Murraya koenigii,* and *Cassia tora* as herbal alternatives to synthetic deworming agents. Method: Plant materials were obtained from parts of Maharashtra state and identified. Petroleum ether and hydroalcoholic extracts of each plant were obtained through Soxhlet and reflux processes. Phytochemical screening was carried out using standard procedures. Anthelmintic activity was analysed on *Pheretima posthuma* (Earthworm) at concentrations of 50, 100, and 150 mg/ mL, with Albendazole as reference standard. The activity was found to be dose dependent on concentration with maximum in *Murraya koenigii.* Results: All three plants exhibited dose-dependent anthelmintic activity, with hydroalcoholic extracts generally showing greater efficacy than petroleum ether extracts. Among them, the hydroalcoholic extract of *Murraya koenigii* exhibited the highest efficacy, compared to the standard drug. Conclusion: The findings support the traditional use of these plants in managing helminth infections. Given their promising efficacy and presumed safety, these herbal candidates may serve as potential alternatives or complementary therapies to conventional anthelmintic drugs. Further studies involving phytochemical analysis and in vivo evaluations are recommended to validate their therapeutic potential and mechanisms of action.

Keywords: Helminthiasis, Anthelmintic Activity, *Krimi*, *Annona squamosa, Murraya koenigii, Cassia tora*.

Introduction

Helminth infections is one of the most common parasitic infections that affect a large portion of the human population. In developing countries, the parasitic infection causes a larger threat to public health, which leads to anaemia, eosinophilia, malnutrition, and even death if untreated. Males are more prone to helminth infections than that of females. More than 24% of the total population depends on the native frameworks of medication such as Ayurveda, Unani & Sidha in India. (1)

Helminths the word is derived from the Greek meaning "worms".(2) Helminthiasis is an infectious disease caused by parasitic worms called helminths. These parasites are classified into tapeworms, roundworms and flukes. These worms live in the gastrointestinal tract or sometimes in other organs, inducing physiological damage. Present treatments for helminthiasis include drugs like albendazole, mebendazole, piperazine citrate, levamisole etc. However, these treatments exhibit side effects like dizziness, diarrhoea, undesirable neurological side effects etc. Therefore, herbal drugs are preferred over Allopathy. (3)

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Anthelminthic agents

In modern medicine, anthelmintic is a drug that kills or removes gastrointestinal worms. "Dewormer" or "wormer" are the more popular names. (4) Anthelmintics are deworming agents that either kill (vermicide) or expel (vermifuge) infesting helminths. (5) An anthelminthic drug can act by causing paralysis of the worm, or by damaging its cuticle, which lead to partial digestion or rejection by immune mechanisms (6).

In contrast, Ayurveda refers to worm infestations as *Krimiroga* and treats them with specific herbal drugs known as *Krimighna Dravyas*. These herbal formulations either destroy the *Krimi* (worms), similar to vermicidal action (*Krimivadha*), or facilitate their expulsion (*Kriminirharana*), much like modern vermifuges. Ayurvedic theory attributes the cause of *Krimiroga* to imbalances in *doshas*, especially *Kapha* and *Pitta*, and

impaired *Agni* (digestive fire), which create a favorable environment for parasites to thrive. (7)

Plants showing Anthelminthic properties

Several medicinal plants are said to be effective in the traditional medical system in killing worms. Some of them are as follows: (8, 9, 10, 11, 12, 13, 14, 15).

Table 1: List of plant possessing anthelmintic properties

Andrographis paniculata	*Pumica grantum Linn.*
Azadirachta indica	*Psoralea corylifolia*
Acacia auriculaeformis	*Piper longum Linn*
Adhatoda vasica	*Phyllanthus embilica*
Allium sativum	*Piliostigma thonningii (Schum.)*
Anacardium occidentale	*Plumbago indica*
Annona squamosa	*Ricinus communis*
Avicennia marina	*Suregada multiflora*
Butea monosperma	*Saraca asoca*
Cannabis sativa Linn.	*Semecarpus anacardium Linn*
Capparis decidua	*Strobilanthes discolour*
Capparis spinosa	*Trichilia emetic*
Carica papaya Linn	*Trifolium repens Linn*
Cassia tora	*Trachyspermum ammi*
Commiphora mukul	*Tinospora cordifolia*
Curcubita maxima	*Tamilnadia uliginosa*
Cleome icosandra Linn.	*Tamarindus indica L.*
Calatropis procera	*Terminalia catappa*
Evolvulus alsinoides Linn	*Trianthema portulacastrum*
Ficus religiosa	*Turraea vogelii*
Ferula asafoetida	*Terminalia arjuna*
Ficus carica	*Thymus vulgarisL.*
Gyanadropsis gynandra Linn.	*Uncaria gambier*
Murraya koenigii	*Urtica dioica*
Mimusops elengi Linn	*Vernonia anthelmintica*
Moringa oleifera	*Vernonia amygdalina*
Melia azedarach Linn.	*Valeriana officinalis*
Moghania vestita	*Withania somnifera*
Neolamarckia cadamba Roxb.	*Xylopia aethiopica*
Nyctanthes arbortristis	*Zingiber zerumbet*
Nicotiana tabacum	*Ziziphus mauritiana*
Ocimum sanctum Linn	*Zingiber officinale*
Oroxylum indicum	*Zanthoxylum armatum*

The plants mentioned above are known for its anthelmintic properties. The three plants *Annona squamosa, Murraya koenigii* and *Cassia tora* were selected for the present study.

Annona squamosa

Annona squamosa Linn belonging to family Annonaceae commonly known as Sugar apple has its origin from West Indies and is cultivated throughout Asia. It is a small, semi deciduous, much branched shrub or small tree about 3- 8 m tall. Leaves are singly, pale green on both surfaces and mostly hairless while flowers are solitary in nature, greenish yellow in color. (16)

In Traditional System of Medicine, the leaf is used as an insecticide, in skin infections, mucosae, laxative, diarrhea, dysentery, pregnancy, antiaborifacients, for treating cancerous tumors (1)

Murraya koenigii

Murraya koenigii commonly known as Kadi patta or curry leaves or as Meethi neem belongs to family Rutaceae. (17) It is an aromatic more or less deciduous shrub or a small tree up to 6 m in height found throughout India up to an altitude of 1500 m and are cultivated for ite aromatic leaves.

In traditional system of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavouring agent in curries and chutneys. (18)

Cassia Tora L.

Cassia tora L., a seasonal weed, belongs to the Fabaceae family, traditionally reported to have medicinal properties, like laxative, antiperiodic, antihelmintic, ophthalmic, and effective for leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders, etc (7, 19).

Cassia tora is medicinal plant but it is known to us as weed because of lack of advance technology in our country to know the active chemical constituents of the easily available plant as their agricultural benefits to the medicinal point of view. It is an edible wild plant having remarkable nutritional as well as therapeutic property. This plant of seeds are roasted and dried then are used as substitute of coffee in many developing countries. (20) Thus in present study, the anthelmintic potential of three traditionally used Ayurvedic medicinal plants *Annona squamosa, Murraya koenigii,* and *Cassia tora* are explored as herbal alternatives to synthetic deworming agents. The anthelmintic efficacy of all the three plants was compared with standard.

Materials and methods

Collection and Authentication of Plant

The plant materials of *Annona squamosa and Murraya koenigii* were collected from the medicinal garden of Priyadarshini J. L college of Pharmacy, Nagpur and the seeds of *Cassia tora* were collected from cultivated plant of *Cassia tora* in farm of Yavatmal district. All the three plants were authenticated by Dr. N. M. Dongarwar, Head of Department of Botany, R. T. M. Nagpur University, Nagpur. The specimen herbarium sheets of *Annona squamosa, Murraya koenigii* and *Cassia tora* were submitted with the specimen number 67, 66 and 65 respectively.

Hydroalcoholic Extraction of Plant Material

The plant materials were extracted to obtain petroleum ether extract and hydroalcoholic extract. The petroleum ether extract was obtained by Soxhlet extraction method. The petroleum ether extract of each plant was evaporated to dryness and labelled as Petroleum extract of *Annona squamosa* (PEAS), Petroleum extract of *Murraya koenigii* (PEMK) and

Petroleum extract of *Cassia tora* (PECT). The marc obtained from Soxhlet extraction method was subjected to extraction by hydroalcoholic solvent. The dried marc of *Annona sqamosa, Murraya koenigii*, and seeds of *Cassia tora*, were refluxed with 750 ml of distilled water and 250 ml of Methanol for 30 minutes. It was filtered and evaporated to dryness to get Hydroalcoholic Extract of *Annona squamosa* (HAAS), Hydroalcoholic Extract of *Murraya koenigii (HAMK),* Hydroalcoholic Extract of *Cassia tora* (HACT). The percentage yield was calculated and the percentage yield of each is reported in table 2.

Phytochemical screening

The phytochemical screening of PEAS, PEMK, PECT, HEAS, HECT, and HAMK was conducted to identify presence of various phytoconstituents such as alkaloids, tannins, phenol, proteins, amino acids, flavonoids, glycosides and others. Phytochemical screening was carried according to the standard procedures.(21, 22) The results of phytochemical screening were shown in Table 3.

Evaluation of Antihelminthic activity

Anthelmintic activity was carried out on Indian adult earthworms (*Pheretima posthuma*) collected from and dump soil and washed with water to remove all foreign matter. Earthworms of 5-7 cm long and 0.1-0.2 cm wide were used throughout the experimental period due to their physical and physiological resemblance to human intestinal roundworm parasites. [4, 16] Earthworms were divided in eight groups containing 5 earthworms in each group. Earthworms were placed in I- VIII groups for mentioned treatment.

The petroleum ether extracts were dissolved in DMF, the hydroalcoholic extract were dissolved in distilled water and the Albendazole was dissolved in DMSO and then in saline water.

All the extracts, PEAS, PEMK, PECT, HAAS, HAMK AND HACT were dissolved in various concentrations such as 50, 100, and 150 mg/ml in saline water. The standard drug selected for this study was Albendazole.

- Group I - Earthworms were placed into 10 ml of the saline water in clean petri dish, It serves as control.
- Group II - Earthworms were placed in 10 ml of PEAS at concentrations of 50, 100, and 150 mg/ml separately.
- Group III - Earthworms were placed in 10 ml of PEMK at concentrations 50, 100, and 150 mg/ml.
- Group IV - Earthworms were exposed to 10 ml of PECT at concentrations 50, 100, and 150 mg/ml.
- Group V - Earthworms were placed in 10 ml of HAAS at concentrations 50, 100, and 150 mg/ml.
- Group VI - Earthworms were placed in 10 ml of HAMK at concentrations 50, 100, and 150 mg/ml.
- Group VII - Earthworms were placed in 10 ml of HACT at concentrations 50, 100, and 150 mg/ml.
- Group VIII - Earthworms were placed in 10 ml of standard drug Albendazole at concentrations 25 mg/ml.

The entire study was conducted in different petri dishes. Earthworms were observed for their movement. The time taken for paralysis and the time taken for death were monitored and documented in minutes. Paralysis was said to occur based on the behaviour of earthworms with no revival body state in the normal saline medium. Death was concluded after confirming that the earthworm neither moved when shaken vigorously nor when dipped in warm water (50 °C) with faded body colour. (23) The results of anthelmintic activity of each extract are reported in table 4.

Results and Discussion

The results of all the evaluations are depicted in below tables.

The percentage yield of all petroleum ether and hydroalcoholic extract of all the plant materials is reported in table 2.

Table 2: Percentage yield of Plant extract

SN.	Plant Extracts	% Yield
1	**PEAS**	8.05%w/w
2	**PEMK**	5.1%w/w
3	**PECT**	8.24%w/w
4	**HAAS**	24% w/w
5	**HAMK**	26% w/w
6	**HECT**	8.7% w/w

Table 2 shows that the percentage yield of HAMK is higher compared to the other extracts. This suggest that HAMK contains more quantity of polar components.

The preliminary phytochemical screening of petroleum ether and hydroalcoholic extracts of all the plant materials is reported in table 3.

Table 3: Preliminary phytochemical screening of extracts for various phytoconstituents

SN.	Phyto-constituents	Chemical test	Observation					
			PEAS	PEMK	PECT	HAAS	HAMK	HACT
1	Carbohydrate	Molisch's test	-	-	-	+	+	+
2	Protein	Biuret test	-	-	-	+	-	-
3	Flavonoid	Shinoda test	-	-	-	+	+	+
		Modified shinoda	-	-	-	+	+	+
		Sulphuric acid test	-	-	-	+	+	+
		Lead acetate test	-	-	-	+	+	+
		Alkali test	-	-	-	+	+	+
4	Amino acid	Ninhydrin test	-	-	-	+	+	+

5	Alkaloid	Dragendorff's	-	-	-	+	+	+
		Hager's reagent	-	-	-	+	+	+
		Mayer's reagent	-	-	-	+	+	+
		Wagner's reagent	-	-	-	+	+	+
6	Phenolic compounds	Ferric chloride test	-	-	-	+	+	+
		Lead acetate test	-	-	-	+	+	+
7	Steroid	Salkowski test	+	+	+	+	+	+
		Zimmermann test	+	+	+	+	+	+
		Pinus test	+	+	+	+	+	+
8	Triterpenoids	Noller's test	+	+	+	+	+	+

In table 3, + indicates Present is mentioned as, Absent

Table 3 illustrates the phytochemical screening results, which reveal that PEAS, PEMK, and PECT do not contain alkaloids, flavonoids, tannins, and phenolic compounds, while HAAS, HAMK, and HACT include carbohydrates, alkaloids, flavonoids, saponins, tannins and phenols.

Anthelmintic activity

The comparison between all the hydroalcoholic extracts of *Annona squamosa, Murraya koenigii,* and *Cassia tora* for anthelmintic activity was done. The plant extracts produced a significant anthelmintic activity in dose dependent manner as shown in below table 4.

Table 4: Anthelmintic activity with different extracts

SN.	Groups	Concentration	Time Taken in minutes	
			Paralysis (P)	Death (D)
1	Groups I- Control	-	-	-
2	Groups II- PEAS	50mg/ml	81.06 ± 0.30	113.69± 0.59
		100mg/ml	68.29 ± 0.18	98.85 ± 0.50
		150mg/ml	44.12 ± 0.06	70.99 ± 0.63
3	Groups III- PEMK	50mg/ml	72.35 ± 0.2	126.33 ± 0.20
		100mg/ml	55.01 ± 0.34	93.94 ± 0.40
		150mg/ml	43. 23 ± 0.21	82. 94 ± 0.52
4	Groups IV-PECT	50mg/ml	94.89 ± 0.40	132.9 ± 0.45
		100mg/ml	82.1 ± 0.39	111.77 ± 0.62
		150mg/ml	65.84 ± 0.36	87.01 ± 0.50
5	Groups V- HAAS	50mg/ml	52.42 ± 0.10	94.26 ± 0.19
		100mg/ml	35.26 ± 0.23	72.4 ± 0.1
		150mg/ml	30.21 ± 0.12	43.23 ± 0.21
6	Groups VI-HAMK	50mg/ml	44.77 ± 0.28	68.27 ± 0.17
		100mg/ml	37.19 ± 0.17	50.28 ± 0.17
		150mg/ml	24.19 ± 0.19	37.27 ± 0.18
7	Groups VII- HACT	50mg/ml	64. 86 ± 0.33	97.88 ± 0.33
		100 mg/ml	44.09± 0.38	84.93 ± 0.57
		150 mg/ml	29.21 ± 0.43	55.26 ± 0.17
8	GroupsVIII- Standard (Albendazole)	25 mg/ml	15.09 ± 0.40	25.24 ± 0.198

Results are expressed as Mean ± SD (n = 5)

Statistical analysis

F-value 9.420085 is greater than F- critical value 5.613591 and P (0.000956) < 0.05 indicates all of above three groups having different mean. Thus, the null hypothesis is that the mean anthelmintic effect is the same across all three groups i.e. standard (albendazole), petroleum ether extracts (PEAS, PECT, PEMK) and hydroalcoholic extracts (HAAS, HACT, HAMK). The alternative hypothesis is that at least one group has a different mean anthelmintic effect. Above data shows that hydroalcoholic extracts of all three plants found to have better anthelmintic activity when evaluated for paralysis time and death time upon increasing concentration of each extracts from 50 mg/ml to 150 mg/ml in comparison with petroleum ether extracts of all 3 plants. The standard albendazole dose of 25mg/ml shows mean paralysis time (min) and death time (min) 15.09 ± 0.4, 25.24 ± 0.19 respectively, whereas HAMK shows mean paralysis time (min) and death time (min) 24.19 ± 0.19 and 37.27 ± 0.18 respectively.

Conclusion

This research emphasizes the potential of three traditionally used medicinal plants—*Annona squamosa*, *Murraya koenigii*, and *Cassia tora* as natural and effective alternatives to synthetic deworming agents. The objective of this study was to compare hydroalcoholic extracts of these three medicinal plants for the anthelmintic activity. The results show that these plants possess dose-dependent anthelmintic activity, with hydroalcoholic extracts generally being more effective than petroleum ether extracts. The petroleum ether extract failed to show significant anthelmintic activity when compared to hydroalcoholic

extracts of all the three plants. The result suggested that as compared to HAAS and HACT, HAMK takes less time to paralyse the experimental animals. Among the plants studied, the hydroalcoholic extract of *Murraya koenigii* demonstrated the highest efficacy amongst other extracts. These findings support the traditional use of these plants for treating helminth infections and suggest they could serve as safer, more effective alternatives to synthetic anthelmintic drugs. However, further studies, including detailed quantitative analysis of phytoconstituents and in vivo testing, are needed to better understand their mechanisms of action and confirm their therapeutic potential. Given the rising concerns over drug resistance and side effects of synthetic treatments, these herbal remedies could offer a viable solution for managing helminthiasis, especially in developing regions.

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