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Original Article

In vitro Anthelmintic Activity of Three Vegetative Herbs

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ABSTRACT

Background: Parasitic infections have become a serious threat to the lives of human beings and animals and helminthiasis is the major parasitic infection. A wide range of anthelmintic agents have been used to treat these parasitic infections. Synthetic anthelmintic has various limitations like resistance. So, the major practical way of developing efficacious and cheaper anthelmintics is to consider herbal plants. **Materials and Methods:** The three plants selected in the study are *Citrus medica, Coleus aromaticus* and *Murraya koenigii,* belonging to the family Rutaceae, Lamiaceae and Rutaceae respectively. Traditionally, all these plants are used as vermicides. The main goal of the study was to examine the anthelmintic activity of these three plants using earthworms (*Pheretima posthuma*). 50% Hydroethanolic extracts of the three plants at different concentrations (20, 50, 80 mg/ml) showed dose-dependent anthelmintic response. **Results:** At concentrations of 20, 50, 80 mg/ml, all three plant extracts caused paralysis at 16.4, 28.33, 28.17, 11.35, 23.17, 22.4, 9.1, 19.33, 19.21 min. and death at 60.1, 84.56, 40.06, 46.08, 67.06, 37.42, 25.54, 56.56, 29.35 min. respectively. Albendazole was used as a reference standard at three different concentrations 20, 50, 80 mg/ml and caused paralysis at 40.37, 36.12, 24.44 min and death at 50.1, 39, 30.49 min. **Conclusion:** After analysing results, it was concluded that the three plants possess effective anthelmintic activity when compared with standard drug Albendazole and *Citrus medica* was found to be much more effective than all.

Keywords: *Citrus medica*, *Coleus aromaticus*, *Murraya koenigii*, Anthelmintic activity, Albendazole.

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INTRODUCTION

Helminthiasis has been a major health issue in both humans and animals from ancient times. All kinds of helminth infections affect every second individual worldwide. Many efforts have been made to control parasitic infections with a level of utility and security over prototypes. Besides human complications, it also causes significant economic losses.[1] Major causes of these infections are poor sanitation, malnutrition, lack of knowledge and practice about hygiene and an overcrowded population.[2] The mode of transmission for helminthiasis is via humans and animals, acting as primary and secondary hosts. Insects act as the vector for the host, contaminating soil, water and food. It is a wide-spread, neglected tropical disease of developing countries and affects majorly poor people. Over 200 million people and 600 million school-year-aged children are diagnosed with parasites.[3] The helminthic parasites weaken the immune system and increase susceptibility to HIV/AIDS, pneumonia, tuberculosis and malaria.[4]

Citrus medica (galgal, vetas) belongs to the family Rutaceae. It resembles a lemon but its size is larger than lemon, originate in the ignoble sections of Himalaya and after Gadwall to Sikkim, Assam and Ghats in the western direction. Leaves are beneficial in fatigue, asthma, cancer, diarrhoea, hyperthermia, halitosis, tumours, haemorrhoids, intestinal complaints, jaundice, skin diseases, and dysmenorrhoea and worm infections. Pharmacologically, it is analgesic, hypoglycaemic, antiulcer, anthelmintic, anticancer, hepatoprotective, estrogenic, hypolipidemic activity. Erucylamide and isolimonen are the important constituents in leaf with linalool, γ- terpinene, citral derivatives, isopulegol, cymene, geranial and α-terineol.[5-15]

Coleus aromaticus (Patta ajvaayana, Mexican mint) belongs to the family Lamiaceae. *C. aromaticus* is a green, persistent bush; leaves are aromatic like oregano cultivated throughout India, majorly found in Ceylon and Moluccas. Leaves Decoction governing severe cough, asthma, spasm. It is beneficial in epilepsy, nervous tension, rheumatism and dyspepsia. It is also used as a remedy for kidney stones, vaginal discharge, and dysfunction of the liver. Pharmacologically, it is antiepileptic, antitumor and nephron-protective. Mains of essential oil are carvacrol, thymol with rosmarinic acid, eugenol, chavicol, eucalyptol, pinene, caryophyllene, 1, 8-cineole, phellandrene.[16-26]

Murraya koenigii (Kari patta) belongs to the family Rutaceae. It is a defenceless shrub found in the Himalaya, Uttarakhand, Sikkim, Bengal, Garhwal, Western Ghats, Assam and Travancore-Cochin. Paste of leaves applied on bruises and eruptions cure itching and inflammation. This is also useful in poisonous animal bites, piles, leukoderma and other skin problems owing to its blood purifier activity. Pharmacologically, it is topoisomerase I and II inhibitor, antidiarrheal, mosquitocidal. Its main constituents present in roots are different types of alkaloids like mucoline, *o*-methyl murrayanine, 3-methyl carbazole, eustifolin-D, mahanine, mahanimbine, girinimbine, girinimbilol, mukoenin-A with essential oil like linalool, bornyl acetate, dehydro aromadendrene.[27-37]

MATERIALS AND METHODS

Plant Collection and Extraction

Leaves of *Citrus medica*, roots of *Murraya koenigii* and roots of *Coleus aromaticus* were collected from Malerkotla nursery, Punjab. Verification of all plant parts was done by NISCAIR, Delhi with authenticated numbers NISCAIR/RHMD/Consult/2019/3215-16-7, NISCAIR/RHMD/Consult/2019/3298-99-2, NISCAIR/RHMD/Consult/2019/3215-16-5 for *Citrus medica*, *Coleus aromaticus* and *Murraya koenigii* respectively. Minimum 500g quantity of each plant part was shade dried, powdered and kept in contact with solvent in a stopper container for 3 days (maceration technique). The extract was concentrated by rotary vacuum evaporator and evaporation was done

until it dried completely. The percentage yield of 50% hydroethanolic extract for each plant was calculated by the formula W1 (weight of residue after solvent evaporation) x100/ W_2 (weight of powder taken initially).

Extracts of three plants were i.e *Citrus medica* blackish green with percentage yield 4.80, *Coleus aromaticus* golden brown with percentage yield 25, *Murraya koenigii* dark greenish with percentage yield 16.

Animals

Healthy adult Indian earthworms, zoologically, *Pheretima posthuma* (3 to 5-centimeter-long and 0.1 to 0.2 cm in diameter) were collected locally in the rainy season from June to September and were identified via Zoologist. Worms washed with normal saline solution (0.9 % w/v NaCl) and then used for study.[38-39]

Drugs and Chemicals

The drug albendazole (Standard anthelmintic) was procured from Apple Biotech Ludhiana, Punjab on a gratis basis along with complete analytical data. All solvents (organic) and chemicals of analytical grade are obtained from SD Fine Chemical Limited, Mumbai.

Determination of Anthelmintic Activity

The dried plant extracts were investigated for *in vitro* anthelmintic activity on earthworms as per method of Sravani and Paarakh,[40] 2011. Extracts were suspended in normal saline with Tween 80 (0.1 % v/v). Albendazole (5 mg/ml) was used as a standard drug. Observations were made in minutes by calculating the time taken to cause paralysis and death of the individual earthworm up to four hours of the test dated. Paralysis was supposed to occur once. The worm didn't resuscitate even in 0.9 % w/v saline water. Death settles when a worm mislays its movement or integrity by vanishing away from its body colour. The consequences were recorded in terms of anthelmintic activity (minute) of each extractive. The anthelmintic activity of each plant 50 % hydroethanolic extract was evaluated on three changed attentions, 20, 50, 80 mg/ml as per the method of Sravani and Paarakh, 2011. All the dried plant extracts were suspended in normal saline Tween 80 (0.1% v/v) solution and properly labelled before starting the experiment. Total fourteen groups were made.

Group-1: (I control),

Group-2: (vehicle) I ml of 0.1 percent v/v Tween 80 in 0.9 percent w/v NaCl,

Group-3 Albendazole (20 mg/ml),

Group-4: *C. medica* (20 mg/ml),

Group-5: *C. aromaticus* (20 mg/ml),

Group-6: *M. koenigii* (20 mg/ml),

Group-7: Albendazole (50 mg/ml),

Group-8: *C. medica* (50 mg/ml),

Group-9: *C. aromaticus* (50 mg/ml),

Group-10: *M. koenigii* (50 mg/ml),

Group-11: Albendazole (80 mg/ml),

Group-12: *C. medica* (80 mg/ml),

Group-13: *C. aromaticus* (80 mg/ml),

Group-14: *M. koenigii* (80 mg/ml).

Every time, a 20 ml volume of an extract solution was poured into petridish containing six healthy earthworms and repeated over three times (n=3) for each concentration. Three different concentrations 20, 50, 80 mg/ml, were prepared by dissolving 1.2 g, 3 g, 4.8 g in 60 ml of 0.9 percent w/v NaCl normal saline respectively. Each solution also contains 1 ml of Tween 80 (0.1% v/v). Remarks ended up to 4 hr because of the time taken for paralysis and death of worms in minutes.

RESULTS

The results of the anthelmintic activity of the above-mentioned plants at three different concentrations, i.e. 20, 50, 80 mg/ml respectively are summarized in Table 1. Remarks ended up to 4 hr for the time taken to paralysis and death of worms in minutes.

DISCUSSION

The problem of anthelmintic resistance, toxicity, and the increasing concern over the presence of drug deposits in animal products has led to a rebirth of curiosity in the use of plant based drugs.[41] The use of natural dietary and herbal constituents has control of it which may decrease this dependence on drug treatment, and reduce the development of resistance.[42] Plant materials estimated in the present-day study have been identified from several sources to serve as anthelmintic agents by traditional healers of Ethiopia. *In vitro* techniques are chosen over *in vivo* methods due to their low cost, simplicity, and rapid turnover.[41]

The present study involves a statistically significant relation between various graded concentrations of the extracts, the exposure test-time interval, and adult parasite mortality.[43] The earthworms selected for the anthelmintic activity were the utmost sensitive to the 50% hydroethanolic extract of *Citrus medica* as can be seen in Figure 1. The graph defines the dose-dependent paralysis ranging from loss of movement to loss of response to external stimuli, which eventually proceeds to death. Our results define the importance of assessing anthelmintic compounds against not only free-living parasites but also parasitic stages. At 20, 50 and 80 mg/ml concentrations, *Citrus medica* extract caused paralysis at 16.40, 11.35, 9.1 min and death at 60.1, 46.08, 25.54 respectively. The hydroethanolic extracts of *C. aromaticus* and *M. koenigii* also exhibited dose dependent anthelmintic response that caused paralysis at 28.33, 28.17 min (20 mg/ml); 23.17, 22.4 min (50 mg/ml); 19.33, 19.21 min (80 mg/ml) and death at 84.56, 40.06 min (20 mg/ml); 67.06, 37.42 min (50 mg/ml); 56.56, 29.35 min (80 mg/ml) post treatment (Figure 1). All the hydroethanolic extracts of three plants were highly effective in causing the death of the worms as well as causing paralysis.

The anthelmintic activity of all the three plants may be due to the presence of polyphenolic compounds. The activity of *Citrus medica* to cause paralysis of worms in a short time suggests that it could be effective against parasitic infections of humans. These classes of plant secondary metabolites are considered to be the main sources of chemical constituents responsible for a large range of therapeutic activities of several medicinal plants. Some studies are available for anthelmintic activity of polyphenols, tannins, alkaloids, and flavonoids.[44] The presence of these phytochemicals may be responsible for the observed anthelmintic activity of plant extracts in the present study.

Further studies are required to identify the actual constituents present in the extract which are responsible for activity against earthworms. It would be worthwhile to isolate pure compounds present in this plant responsible for activity as prototypes to synthesize their congeners, also to establish the effectiveness and pharmacological rationale for the use of *Citrus medica* as an anthelmintic drug.

CONCLUSION

It is concluded on the origin of the fallouts found by current study that floras can be considered as the substantial cause of accepted anthelmintic compounds in contradiction of digestive infections. The traditional use of these plants as folk remedies and putative anthelmintics by the people of India is also supported by the present study. All the plants showed concentration-dependent response against earthworm motility. Out of all the plants, *Citrus medica* was found to be highly active and potent to cause paralysis. The drug albendazole was used as a standard drug

Table 1: Summarized paralysis and death time (minute) at three concentrations 20, 50, 80 mg/ml in comparison to standard drug Albendazole.

Sl.no.	Group	Paralysis / Death	n1	n2	n3	Mean	Sd	Sem	M ± Sem (n= 3)
1	I	-	-	-	-	-	-	-	-
2	II								
3	III	Paralysis	36.58	45.16	39.37	40.37	4.38	1.46	40.37 ±1.46
		Death	46.12	54.59	49.59	50.10	4.26	1.42	50.10 ±1.42
4	IV	Paralysis	18.54	14.45	16.21	16.40	2.05	0.68	16.40 ±0.68
		Death	64.43	56.39	59.48	60.10	4.06	1.35	60.10 ±1.35
5	V	Paralysis	25.19	31.33	28.47	28.33	3.07	1.02	28.33 ±1.02
		Death	79.54	90.58	83.56	84.56	5.59	1.86	84.56 ±1.86
6	VI	Paralysis	27.53	30.57	26.41	28.17	2.15	0.72	28.17 ±0.72
		Death	39.51	44.44	36.23	40.06	4.13	1.38	40.06 ±1.38
7	VII	Paralysis	35.11	36.09	37.16	36.12	1.03	0.34	36.12 ±0.34
		Death	37.51	39.45	40.05	39.00	1.33	0.44	39± 0.44
8	VIII	Paralysis	10.35	11.55	12.15	11.35	0.92	0.31	11.35 ±0.31
		Death	45.14	46.07	47.02	46.08	0.94	0.31	46.08 ±0.31
9	IX	Paralysis	25.38	20.58	23.56	23.17	2.42	0.81	23.17 ±0.81
		Death	68.02	66.12	67.05	67.06	0.95	0.32	67.06 ±0.32
10	X	Paralysis	22.33	25.41	19.46	22.40	2.98	0.99	22.4 ±0.99
		Death	38	39.11	35.14	37.42	2.05	0.68	37.42 ±0.68
11	XI	Paralysis	22.56	24.45	26.3	24.44	1.87	0.62	24.44 ± 0.62
		Death	28.00	31.37	32.11	30.49	2.19	0.73	30.49 ±0.73
12	XII	Paralysis	9.02	9.14	9.14	9.10	0.07	0.02	9.1 ±0.02
		Death	24.16	25.12	27.33	25.54	1.63	0.54	25.54 ±0.54
13	XIII	Paralysis	18.36	20.21	19.42	19.33	0.93	0.31	19.33 ±0.31
		Death	58.26	56.16	55.25	56.56	1.54	0.51	56.56 ±0.51
14	XIV	Paralysis	18.05	20.06	19.53	19.21	1.04	0.35	19.21 ±0.35
		Death	28.55	30.42	29.09	29.35	0.96	0.32	29.35 ±0.32

n = 3 (number of times repetition done on each group, sd (standard deviation), M ± Sem (mean ± standard error mean

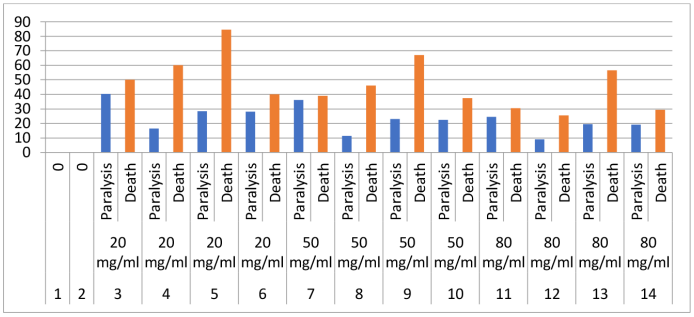

Figure 1: Summarized paralysis and death time (in minute) at concentrations 20,50,80 mg/ml in Comparison to Albendazole (standard drug).

for comparison. But there's a need to explore further this plant extract and precise mode of action in detail for delineation of their therapeutic efficacy, so that new drug molecules come into the market painstakingly as a principal of forthcoming that might avert helminthiasis and its associated health matters. It would be worthwhile to take pure compounds present in this plant as prototypes to synthesize their congeners that are edible for use in human and veterinary both. This will be a potent, effective and economical anthelmintic agent in future provided further studies are done focusing on the precise mechanism of action and *in vivo* study with other animal models.

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