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IN VITRO ANTIHELMINTIC ACTIVITY OF SOME MEDICINAL PLANTS: A REVIEW

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ABSTRACT

Helminthiasis is infestation with one or more intestinal parasitic worms roundworms, whipworms , or hookworms in humans and animals. Presently, many synthetic drugs are available to treat Helminthiasis infection effectively, but suffer from number of side effects like abdominal pain, dizziness, headache, fever, nausea, vomiting, or temporary hair loss. Moreover, drug resistance is also another factor concerned with the use of these drugs. Thus, Herbal drugs need to be introduced as large number of medicinal plants are known for their antihelmintic activity with fewer or no side effects and are used by the ethnic groups across different parts of the world. But these medicinal plants need to be screened first for their *in vitro* and *in vivo* activity before putting them in use. Thus, many of these herbs have been scrutinized for their pharmacological and pre clinical studies. The present article describes review of promising *in vitro* efficacy of some medicinal plants having antihelmintic activity, which can be helpful in investigation and discovery of novel herbal drugs.

INTRODUCTION: Nowadays when the term “worms” is used clinically, it has a more restricted meaning and indicates various helminths. Worms can be classified into several groups like Annelida or Segmented worms, Platyhelminthes or Flatworms, Round worms or Nematodes, Hook worms, Tape worms, Whip worms and others. These worms vary greatly in size with different body structures.

They undergo a complicated life cycle. The larvae of some nematode species infect various human tissues. These are accidental infections, and do not represent the natural life cycle of the parasite. The larvae migrate through the liver, eyes, brain and so on, where they cause a granulomatous inflammatory reaction. These may spread through infected faeces, water, food, and via vectors. They after getting localized in any of the human tissue affect and damage it by causing infection.

Antihelmentics are the drugs that expel out parasitic worms either by killing them (Vermicide) or causing paralysis (Vermifuge).

Albendazole, Mebendazole, Pirenzepine citrate, Niclosamide, Ivermectin, Levamisole, Pyrantal palmoate are some of the drugs which are currently used as antihelmentics and are successful in treating the infection. But some of the side effects are common to these drugs like abdominal pain, diarrhoea, slight headache, fever, dizziness, exanthema, urticaria and angioedema, etc.

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Drug interaction and resistance are other concerned factors. Treatment with an antihelmentic drug kills worms whose phenotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs. Thus, this requires the use of herbal drugs which become a conventional therapy as these have a wide spectrum of action and fewer side effects without problem of drug resistance.

The *in vitro* antihelmintic activity of some of the medicinal plants was studied for their use in humans as well as in veterinary science. Commonly employed method of study was noting the time required for causing paralysis and death of the worms or determining the faecal egg count in a series of concentrations and comparing the observations with any of the standard drug in use. Generally, the worms used were *Pheretima posthmus*, *H. Diminuta*, *T. repens*, etc.

In all the herbs studied, mostly dose dependent nature was observed which gives a good sign of effectiveness of these herbs against the worms. Thus, this article becomes helpful in carrying out further research on the medicinal plants and isolates the active constituents responsible for the antihelmintic activity to establish their commercial use.

Antihelmintic Herbs:

1) ***Moringa oleifera***: (Moringaceae) It is commonly known as Drumstick. Ethanolic extracts of *Moringa oleifera* were taken for antihelmintic activity against Indian earthworm *Pheritima posthuma*. Various concentrations of extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10mg/ml) was used as a reference standard and distilled water as a control group. Dose dependent activity was observed in extracts of *M. oleifera*.

   The antihelmintic activity was performed according to the method of Ghosh *et al*., on adult Indian earthworm *Pheritima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Preliminary phytochemical screening has shown the presence of saponin, steroids, carbohydrates, alkaloids, tannins, proteins, flavonoids in ethanolic extracts of plants. From the results, it was observed that *M. oleifera* showed potent antihelmintic activity. It showed paralysis within 6-15 min while death is comparable with that of piperazine citrate as death of worms was observed at 64 min.

2) ***Melia azederach*** (Meliaceae): The extracts obtained from the drupes of *M. azedarach* were active against both the tapeworm and the earthworm tested. It is worth to mention that the drupe extracts were comparatively more active than piperazine phosphate against *Taenia solium*. The antiparasitic activity against this tapeworm was better than that of piperazine phosphate (80 min at 0.1 %, and 56 min at 0.2%) at drupe extract concentrations of 0.1 % and 0.2 % (mean death values of 52 and 32 min, respectively). These findings support the use of *Melia azedarach* drupes as antiparasitic in the traditional medicine.

3) ***Butea monosperma*** (Fabaceae): The antihelmintic activity of *Butea Monosperma* was evaluated using the earthworm *Pheretima Posthmus*. 50mg/ml, 20mg/ml, 10mg/ml, 7.5mg/ml, 5.0mg/ml concentration of aqueous extract of *Butea monosperma* (AEBM), ethanolic extract of *Butea monosperma* (EEBM) and standard drug albendazole were used. At 50mg/ml concentration, the AEBM showed time of paralysis 10.08 min and 18.11 min, while at same concentration EEBM showed time of paralysis 7.19 min and time of death 21.4 min against the standard drug Albendazole in which the time of paralysis and time of death were 11.12 min and 17.21 min.

   The antihelmintic effect of *Butea monosperma* aqueous extract as well as ethanolic extract is related to the possible presence of alkaloid and tannins in the extract. The activity revealed the concentration dependence nature of different extract.

4) ***Semicarpus anacardium***: (Anacardiaceae) The antihelmintic activity of different extracts of nuts of *Semecarpus anacardium* were evaluated separately on adult Indian earthworm (*Pheretima posthuma*). It was found that petroleum ether, chloroform extract of *S. anacardium* (PESA and CESA,

respectively) showed better antihelmintic activities than ethanol (EESA) and aqueous (AESA) extract of it. The antihelmintic effects of PESA and CESA at 10 mg/ml and EESA at 20 mg/ml concentration were comparable to that of the effects produced by the reference standards, Albendazole (10 mg/ml) and Piperazine citrate (10 mg/ml).

Albendazole (10mg/ml) caused paralysis of worms in 36.60±0.52 min and death in 63.15±0.68 min, Piperazine citrate (10mg/ml) caused paralysis of worms in 21.58±0.36 min and death in 138.50±2.90 min, while PESA (20mg/ml) showed paralysis of worms in 11.70±0.35 min and death in 25.10±0.46 min, similarly CESA (20mg/ml) showed paralysis of worms in 17.25±0.25 min and death in 36.50±0.60 min.

So, the activity revealed concentration dependent nature of all the four different extracts. Potency of these extracts was found to be inversely proportional to the time taken for paralysis/death of the worms.

5) ***Trifolium repens*** (Fabaceae): *Trifolium repens* L. is an herbal plant that is used in the folk medicine of the Naga tribes of India as a deworming remedy. People customarily use the plant(s) plant-derived preparations and consider them to be efficacious against intestinal worm infections without any scientific base to explain the action of such plants. *T. repens* is one such common plant whose aerial shoots have a reputation of being efficacious against tapeworm infections in the Naga tribes of northeast India.

The anticestodal activity of T. repens was evaluated using experimental *Hymenolepis diminuta* Rudolphi infections in albino rats. Doses of *T. repens* aerial shoots extract 200 and 500 mg/kg reduced the mean fecal egg counts of *H. diminuta* by 47.72% and 54.59% and worm recovery rate by 60.00% and 40.00%, respectively.

Praziquantel, the standard cestocidal drug, reduced the mean fecal egg count by 65.90% and worm recovery rate by 26.67%. The study suggests that the aerial shoots of *T. repens* bear anticestodal properties and supports its use in the traditional medicine system.

6) ***Abutilon indicum*** (Malvaceae): Antihelmintic Activity of the ethyl acetate and aqueous extract obtained from *Abutilon indicum* Sweet leaves was determined on Earth worm *Eudrillus eugeniae* and Round worm *Ascaris lumbricoids*. Both extracts showed significant and dose dependent activity compared to standard drug Albendazole. *Abutilon indicum* Sweet leaves solutions exhibited anti-helmintic activity in dose-dependent manner giving shortest time of paralysis and death with 100mg/ml concentration for earth worms.

The ethyl acetate extract solution showed paralysis within 17.00 min and time of death 28.16 min and aqueous extract solution showed paralysis within 21.16min and time of death 36.17. The ethyl acetate extract solutions exhibiting antihelmintic activity (100mg/ml) was found to be most potent among the all solutions.

7) ***Evodia Lunu-ankenda*** (Rutaceae): The Anti-helmintic activity of ethyl acetate and aqueous extract obtained from *Evodia Lunuankenda* (Gaertn) Merr. Bark were determined on Earth worm *Eudrillus eugeniae* and Round worm *Ascaric lumbricoidus*. Both extracts showed significant and dose dependent activity compared to standard drug Albendazole. These solutions exhibited antihelmintic activity in dose-dependent manner giving shortest time of paralysis and death with 100mg/ml concentration.

The ethyl acetate extract solution caused paralysis within 7.67min and time of death 14.00 min and aqueous extract solution caused paralysis wit in 13.66 min and time of death 16.00 min. The ethyl acetate extract solutions exhibiting antihelmintic activity (100mg/ml) was found to be most potent among the all solutions.

8) ***Cinnamomum comiphora*** (Lauraceae): The aqueous extract of *Cinnamomum camphor* Leaves was investigated for antihelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*).Various concentrations (10-70 mg/ml) of plant extract were tested in the bioassay. Piperazine citrate (10mg/ml) was used as reference standard drug whereas distilled water as control.

Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant antihelmintic activity in dose dependent manner at the concentration of 50 mg/ml. The result showed that aqueous extract possesses vermicidal activity and found to be effective as an antihelmintic.

9) ***Cissampelos parera***: (Menispermaceae) The activity was checked by *in-vitro* antihelmintic model by using earthworm. The alcoholic and aqueous extract at various concentrations (5, 10, 25, 50, 100 mg/ml) were used and studied for paralysis and death of earthworm *Pheretima posthuma*. The aqueous extract of *Cissampelos pareira* at 10mg/ml concentration showed paralysis at 29.33 min and death at 49.67 min, whereas 25 mg/ml showed paralysis at 14.33min and death at 27.50 min.

   At 50mg/ml concentration time taken for paralysis was 7.50 min and death at 14.67 min, while at the maximum concentration of drug i.e. 100 mg/ml required time for paralysis 3.67 min and for death of earthworms 5.67 min. The standard drug Piperazine citrate showed paralysis at 18.50 min and death at 60.29 min at 15 mg/ml concentration. The two concentrations (50, 100 mg/ml) of this plant show good antihelmintic activity as compared to standard drug. All the values are expressed as mean ± SEM (n=6). The extract *of Cissampelos pareira* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml in shorter time as compared to reference drug Piperazine citrate.

10) ***Embilia ribes*** (Myrsinaceae): The dried fruit of the plant is considered as antihelmintic, astringent, carminative, alternative and stimulant. It has been employed in India, since ancient times, as antihelmintic and is administered as powder, usually with milk, followed by a purgative. The oil sample of *Embelia ribes* was tested for its antihelmintic activity. It showed significant effect causing death of worms of the species *Pheretima posthmus* at the concentrations 10 mg/ml, 50 mg/ml and 100 mg/ml as compared to worms, which were treated with standard Peparazine citrate at the concentration 10 mg/ml.

11) ***Amorphophallus paeoniifolius*** (Araceae): The methanolic extract showed dose dependent antihelmintic activity as compared to a standard drug piperazine citrate. The mean paralyzing time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 45.66, 37.33 and 22.33 minutes respectively. In the meantime, Piperazine citrate at a dose of 10 mg/ml caused paralysis in the above helminth in 25 minutes. The mean death time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 81.32, 66.66 and 38.66 minutes respectively.

    In the meantime, piperazine citrate at a dose of 10 mg/ml causes paralysis in the above helminth in 64 minutes. The mean paralyzing time of Tubifex tubifex with the dose of 25, 50 and 100 mg/ml were found to be 94.66, 52.00 and 12.33 minutes respectively. In the meantime, Piperazine citrate at a dose of 10mg/ml caused paralysis in the above helminth in 22.66 minutes. The mean death time of *Tubifex tubifex* with the dose of 25, 50 and 100 mg/ml were found to be 113.99, 65.00 and 17.66 minutes respectively. In the meantime, Piperazine citrate at a dose of 10 mg/ml causes death in the above helminth in 45.33 minutes.

12) ***Helicteres isora*** (Sterculiaceae): The antihelmintic activity of aqueous extract of *Helicteres Isora* was determined on earthworm (*Pheretima Posthuma*). Time required for paralysis of earthworm by using *H. isora* aq. extract (500 mg/10ml) was found to be 17.38±0.3701 min, and for death it was 21.38 ±0.3302 min. While for 1000mg/10ml paralysis time was 13.04±0.42 min and for death, it was 17.01±0.37 min.

    By using Albendazole, Earthworms were paralyzed in 7.83±0.2193min and death occurs in 12.02±0.2560 min. Aq. Extracts (1000mg/10ml) took the least time to cause paralysis and death of earthworms.

    Results of preliminary phytochemical tests suggest that aqueous extract contains Carbohydrates (gums, sugars), Glycosides, Tannins, Oxalic Acid. Thus, the active constituents present in aqueous extracts responsible for antihelmintic activity.

13) ***Mimusops elengi*** (Sapotaceae): The methanolic bark extract of *Mimusops elengi* was screened for *in vitro* antihelmintic activity against earthworms (*Pheretima posthuma*) and cytotoxic activity by brine shrimp lethality bioassay. The extract showed antihelmintic activity at all the concentrations of 25mg/ml, 50mg/ml and 100mg/ml. But the potent antihelmintic activity was observed at the concentration of 100 mg/ml.

    At this concentration, the time required for paralysis and death of earthworms was about 22 and 34 minutes respectively whereas time taken for paralysis and death by the standard drug Pyrantel pamoate at 10mg/ml was about 26 and 38 minutes respectively. The extract also exhibited good cytotoxic activity with LC50 value of 40µg/ml whereas LC50 of Vincristine sulphate was 0.078µg/ml. The experimental results suggest that *Mimusops elengi* Linn. has both *in vitro* antihelmintic and cytotoxic activities.

14) ***Zingiber Officinale***: (Zingiberaceae) This antihelmintic activity of *Zingiber officinale* rhizome, commonly known as ginger justifies its traditional use in veterinary medicine. Crude powder (CP) and crude aqueous extract (CAE) of dried ginger (1-3g/kg) were administered to sheep naturally infected with mixed species of gastrointestinal nematodes. Both CP and CAE exhibited a dose- and a time-dependent antihelmintic effect with respective maximum reduction of 25.6% and 66.6% in eggs per gram (EPG) of faeces on day 10 of post-treatment. Levamisole (7.5mg/kg), a standard antihelmintic agent, exhibited 99.2% reduction in EPG. This study showed that ginger possesses *in vivo* antihelmintic activity in sheep thus justifying the age-old traditional use of this plant in helminth infestation.

15) ***Balanites roxburghii***: (Simarubaceae) Evaluation of antihelmintic potential of crude methanolic and aqueous extract of dried leaves of *Balanites roxburghii* planch was done using *Pheretima posthuma* as test worms. Various concentrations (10, 50, 100mg/ml) of methanolic and aqueous extract were tested in bioassay, which involved determination of time of paralysis and time of death of worms.

    Piperazine citrate (50mg/ml) was used as reference standard and distilled water as control. Both the extracts showed antihelmintic activities in dose dependant manner giving shortest time of paralysis (P) and death with 100 mg/ml concentration, for both type of worms. The methanolic extract of *B. roxburghii* caused paralysis in 20.33±2.80min and death in 31.83±3.13min, while aqueous extract showed P in 23.0±2.00min and D in 35.16±3.02min against the earthworm *P. posthuma*.

    The reference standard drug Piperazine citrate showed P in 10.5±2.14min and D in 18.66±2.86min. The study confirmed that there is certain principle/s present in *Balanites roxburghii* leaves having antihelmintic activity and further studies are suggested to isolate the active principle/s responsible for the activity

16) ***Pongamia Pinnata***: (Fabaceae) The antihelmintic activity of *Pongamia Pinnata* wa evaluated against Indian adult earthworms, *Pherentima posthuma*. The total methanolic extracts of various parts of the plant *Pongamia pinnata* showed significant antihelmintic activity at 20 mg/ ml concentrations. Results were comparable with standard drugs Albendazole and Nitazoxamide, at same concentration.

    In order to find out active constituents from seeds, which are responsible for the activity, polar or nonpolar compounds, successive extracts of seeds of *Pongamia Pinnata* like petroleum ether, ethyl acetate and methanolic extracts were screened for antihelmintic activity. The results showed that ethyl acetate extract of seeds of *Pongamia Pinnata* took the least time to cause paralysis and death of the earthworms, followed by petroleum ether and methanolic extracts, respectively.

    Results of preliminary phytochemical tests suggest that petroleum ether extract of seeds contain sterols, triterpenes and fatty substances; ethyl acetate extract of seeds contain flavonoids and methanolic extract of seeds contain tannins and glycosides. It can be concluded that active constituents responsible for antihelmintic activity are present in the ethyl acetate and petroleum ether extracts of seeds.

17) ***Piper longum***: (Piperaceae) The essential oil from the fruits of Piper longum was screened for the antihelmintic activity against *Ascaris lumbricoids* as test worms. The experiments revealed that this oil has definite paralytic action on the nerve muscular junction of *Ascaris lumbricoids*.The activity of oil was found to be greater than Piperazine citrate used as standard in the study.

18) ***Carica papaya***: (Carecaceae) The Antihelmintic potential of latex of Carica papaya was established using *Pheretima posthuma* as test worms. Various concentrations (100%, 50%, and 20%) of *Carica papaya* latex were tested in the assay, which involved determination of time of paralysis (P) and time of death (D) of the worms. It showed shortest time of paralysis (P=24.5 min) and death (D= 56min) in 100% concentration, while the time of paralysis and death increased in 50% concentration (P=28 min & D= 64min) and in 20% concentration (P=34 min & D=74min) respectively as compare to Piperazine citrate (10mg/ml) used as standard reference (P= 24 min & D= 54) and distilled water as control.

This indicated that the latex of *Carica papaya* showed significant paralysis, and also caused death of worms especially at higher concentration as compared to standard reference Piperazine citrate and control.

19) ***Capparis zylenica***: (Capparidaceae) The antihelmintic activity of *Capparis Zylenica* was evaluated using the earthworm *Pheretima posthmus*. The different successive extracts namely petroleum ether, ethanol and aqueous were used. The different concentrations (0.1%, 0.2% and 0.5%) of various extracts were tested in the bioassay which involved determination of the time of paralysis (P) and time of death (D) of the worms. Albendazole was included as standard reference and normal saline as control.

The results indicated that the crude ethanolic extract and aqueous extracts significantly demonstrated paralysis and also caused death of worms in dose dependent manner, as compared to standard reference Albendazole.

20) ***Punica granatum***: (Punicaceae) Ethyl acetate extracts of *Punica granatum* were investigated for antitrypanosomal activities in Balb albino mice infected with *Trypanosoma brucei* brucei. The plant extracts at 100mg/kg, 200kg/kg and 300mg/kg body weight were administered intraperitoneally twice daily for 7 days to determine their trypanocidal activity. Ethyl acetate extract had a lethal dose (LD50) of 900 mg/kg. Treatment commenced on day 8 post infection in all groups except the negative control group.

Parasitaemia and disappearance of clinical signs were used as indices for monitoring the efficacy of the extracts using the rapid matching method. Mean survival time showed that mice treated with diminazene aceturate survived for 15 days. The group which was administered 200mg/kg ethyl acetate leaf extract of *Punica granatum* had the highest mean survival time(9±2.94 days) than those that received 100mg/kg((8±0.82 days) and 300 mg/kg (6.75±0.5 days). Packed cell volume of the mice changed significantly ($p<0.05$) in the group which received 200mg/kg ethyl acetate extract of *Punica granatum*. This dose has promising trypanocidal activity *in vivo* against *Trypanosoma brucei* brucei.

21) ***Neolamarckia Cadamba*** (Rubiaceae): The antihelmintic activity of *Neolamarckia cadamba* was evaluated using earth worm *Pheretima Posthmus* against standard drug Piperazine citrate. Various extracts of the drug were prepared using different solvents. All the extracts showed dose dependent action evaluated as time of death and time of paralysis over the worms. Among the extracts, the chloroform extract and ethanolic extracts showed promising results. This study thus justifies traditional use of *Neolamarckia cadambia* as an antihelmintic medicine.

22) ***Thespespesia Lampas*** (Malvaceae): Satpuda hills region of Maharashtra is inhabited by several tribes and they are using several plants or plant-based preparations for the treatment of various ailments in their traditional system of medicine. During the course of studies on ethnomedicine of this region, the plant being used as antihelmintic is root of *Thespesia lampas* (Cav.).

This plant has a wide reputation among natives of being curative for intestinal-worm infections in the form of aqueous extract. Based on this, an attempt was made to evaluate the antihelmintic potential of this plant. The aqueous extract of *Thespesia lampas* (Cav.) roots (Ranibhendi) was investigated for antihelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (10-50 mg/ml) of plant extract were tested in the bioassay.

Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant antihelmintic activity at highest concentration of 50 mg/ml. The result shows that aqueous extract possesses vermicidal activity and found to be effective as an antihelmintic.

23) ***Tecoma Stans*** (Bigponiaceae): The evaluation of antihelmintic activity of aqueous, alcoholic, hydro-alcoholic and methanolic extract (sohxlet) of leaves of *Tecoma stans* was carried out on adult Indian earthworm (*Pheretima posthuma*). All leaf extracts showed antihelmintic activity at 100, 200 and 500 µg/ml concentration. The activities were compared with the standard drug Albendazole. Aqueous, alcoholic, hydro-alcoholic methanolic extract (Soxhlet) of leaves of *Tecoma stans* showed better antihelmintic activity than the standard drugs.

When the dose of the extract increased, a gradual increase in antihelmintic activity was observed. Alcoholic extract showed better antihelmintic activity in comparison to the hydro-alcoholic, aqueous and methanolic extracts (sohxlet) of *Tecoma stans*. The data were verified as statistically significant by using one way ANOVA at 1 % level of significance ($p<0.001$)

24) ***Trachyspermum ammi*** (Apiaceae) The *in vitro* activity of a methanolic extract of fruits of *Trachyspermum ammi* against adult bovine filarial *Setaria digitata* worms has been investigated. A bioassay-guided fractionation was carried out by subjecting the crude extract to flash chromatography. HPLC analysis was done for the crude extract and active fraction. The crude extract and the active fraction showed significant activity against the adult *S. digitata* by both a worm motility and MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide reduction assays. The isolated active principle was chemically characterized by IR, 1H-NMR and MS analysis and identified as a phenolic monoterpene.

It was screened for *in vivo* antifilarial activity against the human filarial worm *B. malayi* in *Mastomys coucha*, showing macrofilaricidal activity and female worm sterility *in vivo* against *B. malayi*. The findings thus provide a new lead for development of a macrofilaricidal drug from natural products. The *in vitro* macrofilaricidal activity was assessed by worm motility and MTT reduction assay. Motility assay showed complete inhibition of motility at higher test concentrations tested.

Screening was carried out at varying concentrations ranging from 0.001-1.0mg/mL at two different incubation periods viz., 24 and 48 hrs to find out the dose response relationship as well as the effect of duration of exposure to the test material on the viability of the filarial worms.

25) ***Cucurbita maxima***: (Cucurbitaceae) Seeds of *Cucurbita maxima* have been reported to possess antihelmintic activity. The antihelmintic activity of seeds of this herb was evaluated against earthworm *Pheretima posthmus* using Albendazole as standard drug in normal saline (0.9% NaCl). Concentrations of 25, 50 and 100 mg/ml were tested over the earthworms in saline, while ethanolic extracts of seeds were used. From the study, the ethanolic extracts showed dose dependent nature of the drug compared to Albendazole. Thus, extensive research is needed to isolate individual component responsible for antihelmintic activity of the seeds.

26) ***Zingiber zerumbet***: (Zingiberaceae) This herb is commonly known as Nakachur. Rhizomes of *Zingiber zerumbet* have been reported to possess antihelmintic activity. The antihelmintic activity of rhizomes of this herb was evaluated against

earthworm *Pheretima posthmus* using Albendazole as standard drug in normal saline (0.9% NaCl).

Concentrations of 25, 50 and 100 mg/ml were tested over the earthworms in saline, while ethanolic extracts of seeds were used. From the study, the ethanolic extracts showed dose dependent nature of the drug and the antihelmintic activity was found to be more compared to Albendazole. Thus, extensive research is needed to isolate individual component responsible for antihelmintic activity of the rhizomes.

27) ***Vernonia anthelmintica*** (Compositae): *In vitro* studies revealed higher antihelmintic effects ($p>0.05$) of crude methanol extract (CME) as compared with crude aqueous extract (CAE) of *V. anthelmintica* seeds on live *Haemonchus contortus* as evident from their mortality. For *in vivo* studies, seeds of *V. anthelmintica* were administered as crude powder (CP), CAE, and CME to sheep naturally infected with mixed species of gastrointestinal nematodes.

In vivo, maximum reduction (73.9%) in fecal egg counts per gram (EPG) was recorded in sheep treated with *V. anthelmintica* CAE at 3 g kg^{-1} body weight on day 5 post-treatment (PT) followed by CP at 3g kg^{-1} (55.6%) on day 3 PT. It was found whereas *V. anthelmintica* seeds possess anti-helmintic activity against nematodes, it was not comparable to levamisole (97.8% to 100% reduction in EPG). It may be suggested that further research on a large scale be carried out with a large number of animals on higher doses for the identification of active principles and for standardization of the doses and toxicity studies for drug development.

28) ***Chenopodium album*** (Amaranthaceae): The study was carried out to determine the antihelmintic activity of *Chenopodium album* whole plant in order to justify its traditional use in veterinary medicine. *In vitro* antihelmintic activity of crude aqueous methanolic extract (AME) of the plants was determined using mature *Haemonchus contortus* and their eggs in adult motility assay and egg hatch test, respectively. *In vivo* antihelmintic activity was evaluated in sheep naturally infected with mixed species of gastrointestinal nematodes by administering crude powder (CP) and AME in increasing doses (1.0-3.0 g/kg). The plant exhibited dose- and time-dependent antihelmintic effects by causing mortality of worms and inhibition of egg hatching. *Chenopodium album* (LC50=0.449 mg/mL) was used in egg hatch test.

In vivo, maximum reduction in eggs per gram (EPG) of faeces was recorded as 82.2% for *Chenopodium album* AME at 3.0 g/kg on day 13 and 5 post-treatment. Levamisole (7.5 mg/kg), a standard antihelmintic agent, showed 95.1-95.6% reduction in EPG. Thus, *Chenopodium album* possesses antihelmintic activity *in vitro* and *in vivo,* justifying its use in the traditional medicine system of Pakistan.

29) ***Caeselpinia crista***: (Fabaceae) The study was carried out to determine the antihelmintic activity of *Caesalpinia crista* seed kernel in order to justify its traditional use in veterinary medicine. *In vitro* antihelmintic activity of crude aqueous methanolic extract (AME) of the plant was determined using mature *Haemonchus contortus* and their eggs in adult motility assay and egg hatch test, respectively. *In vivo* antihelmintic activity was evaluated in sheep naturally infected with mixed species of gastrointestinal nematodes by administering crude powder (CP) and AME in increasing doses (1.0-3.0 g/kg).

The plant exhibited dose- and time-dependent antihelmintic effects by causing mortality of worms and inhibition of egg hatching. *Caesalpinia crista* (LC50=0.134 mg/mL) was found to be potent in egg hatch test. *In vivo*, maximum reduction in eggs per gram (EPG) of faeces was recorded as 93.9 with *Caesalpinia crista* at 3.0 g/kg on day 13 and 5 post-treatment, respectively. Levamisole (7.5 mg/kg), a standard antihelmintic agent, showed 95.1-95.6% reduction in EPG. Thus, *Caesalpinia crista* possesses antihelmintic activity *in vitro* and *in vivo*, justifying its use in the traditional medicine system of Pakistan.

30) ***Swertia Chirata*** (Gentianaceae): *In vitro* studies revealed that at 25 mg/ml (P>/=0.05) the crude

aqueous (CAE) and methanolic extracts (CME) of *S. chirata* whole plant showed an antihelmintic effect on live *Haemonchus contortus*.

Moreover, in the *in vivo* study, the whole plant of *S. chirata* administered as crude powder (CP), CAE and CME at the dose of 3 g/kg to sheep naturally infected with mixed species of gastrointestinal nematodes, showed a significant reduction in egg per gram of faeces.

31) ***Cocus nucifera*** (Arecaceae): The ethyl acetate extract obtained from the liquid of green coconut husk fiber (LGCHF) was used for *in vitro* and *in vivo* tests. The *in vitro* assay was based on egg hatching (EHT) and larval development tests (LDT) with *Haemonchus contortus*. The concentrations tested in the EHT were 0.31, 0.62, 1.25, 2.5 and 5 mg ml (-1), while in the LDT they were 5, 10, 20, 40 and 80 mg/ml). The *in vivo* assay was a controlled test. In this experiment, 18 sheep infected with gastrointestinal nematodes were divided into three groups (n=6), with the following doses administered: G1-400 mg/kg LGCHF ethyl acetate extract, G2-0.2 mg/kg moxidectin (Cydectin) and G3-3% DMSO.

The worm burden was analyzed. The results of the *in vitro* and *in vivo* tests were submitted to ANOVA and analyzed by the Tukey and Kruskal-Wallis tests, respectively. The extract efficacy in the EHT and LDT, at the highest concentrations tested, was 100% on egg hatching and 99.77% on larval development. The parameters evaluated in the controlled test were not statistically different, showing that despite the significant results of the *in vitro* tests, the LGCHF ethyl acetate extract showed no activity against sheep gastrointestinal nematodes.

32) ***Salvadora persica*** (Salvadoraceae): The anti-helmintic activity of aqueous and alcoholic root extract of *S. persica* against Indian earthworms *Pheretima posthuma* was evaluated. Various concentrations (10, 20, 40 and 80 mg/ml) of aqueous and alcoholic root extract of *S. persica* were tested, which involved determination of time of paralysis and time of death of the worms.

It was compared with Piperazine citrate (10, 20, 40 and 80 mg/ml) as standard reference and normal saline as control. The aqueous and alcoholic root extracts *S. persica* have antihelmintic activity on dose dependent manner when comparable with standard drugs, which is effective against parasitic infections of humans. At 80mg/ml concentration, the alcoholic root extract showed time of death at 23.5±0.79 mins against the standard drug, Piperazine citrate showing time of death at 23.5±0.79 mins.

33) ***Trianthema portulacastrum*** (Aizoceae)**:** Evaluation of antihelmintic effects of *Trianthema* (*T*.) *portulacastrum* L. (Aizoaceae) whole plant against gastrointestinal worms of sheep was done that may justify their traditional use in veterinary clinical medicine. *In vitro* antihelmintic activity of the crude aqueous methanolic extract (CAME) of the plant was determined using mature female *Haemonchus* (*H*.) *contortus* and their eggs in adult motility assay (AMA) and egg hatch test (EHT), respectively.

In vivo antihelmintic activity of crude powder (CP) and CAME in increasing doses (1.0-8.0 g kg^{-1}) was determined in sheep naturally infected with mixed species of nematodes using fecal egg count reduction test (FECRT) and larval counts. The study design also included untreated as well as treated controls. Fecal egg count reduction and larval counts from coprocultures were performed pre- and post-treatments to assess the antihelmintic activity of the plant.

CAME of *T. portulacastrum* showed a strong *in vitro* antihelmintic activity and pronounced inhibitory effects on *H. contortus* egg hatching as observed through AMA and EHT, respectively. The plant exhibited dose and time dependent antihelmintic effects on live worms as well as egg hatching.

In vivo, maximum reduction in eggs per gram (EPG) of faeces was recorded as 85.6% with CAME of T. portulacastrum at 8.0 g kg^{-1}) on 15th day post-treatment as compared to that of Levamisole (7.5 mg kg^{-1}) that caused 97.0% reduction in EPG. All the species of gastrointestinal nematodes (GINs), i.e. *Haemonchus contortus, Trichostronglyus spp.,*

Oesophagostomum columbianum and *Trichuris ovis* which were prevalent, found susceptible (P<0.01) to the different doses of CP and CAME of both plants.

The data showed that both *T. portulacastrum* possesses strong antihelmintic activity *in vitro* and *in vivo*, thus, justifying its use in the traditional medicine system of Pakistan.

34) ***Musa paradisica***: (Musaceae) Evaluation of *Musa* (*M*.) *paradisiacal* leaves against gastrointestinal worms of sheep was done that may justify their traditional use in veterinary clinical medicine. *In vitro* antihelmintic activity of the crude aqueous methanolic extract (CAME) of the plant was determined using mature female *Haemonchus* (*H.*) *contortus* and their eggs in adult motility assay (AMA) and egg hatch test (EHT), respectively.

In vivo antihelmintic activity of crude powder (CP) and CAME in increasing doses (1.0-8.0 g kg^{-1}) was determined in sheep naturally infected with mixed species of nematodes using fecal egg count reduction test (FECRT) and larval counts. The study design also included untreated as well as treated controls. Fecal egg count reduction and larval counts from coprocultures were performed pre- and post-treatments to assess the antihelmintic activity of the plant.

CAME of *M. paradisiaca* showed a strong *in vitro* antihelmintic activity and pronounced inhibitory effects on *H. contortus* egg hatching as observed through AMA and EHT. The plant exhibited dose and time dependent antihelmintic effects on live worms as well as egg hatching. *M. paradisiaca* (LC_{50}= 2.13 µg mL^{-1}) was found to be very potent in EHT. However, *in vivo*, maximum reduction in eggs per gram (EPG) of faeces was recorded as 80.7% with CAME of *M. paradisiaca* at 8.0gkg^{-1} on 15th day post-treatment as compared to that of Levamisole (7.5 mg kg^{-1}) that caused 97.0% reduction in EPG.

All the species of gastrointestinal nematodes (GINs), i.e. *Haemonchus contortus, Trichostronglyus spp., Oesophagostomum columbianum* and *Trichuris ovis* which were prevalent, found susceptible (P<0.01) to the different doses of CP and CAME of both plants. The data showed that *M. paradisiaca* possesses strong antihelmintic activity *in vitro* and *in vivo*, thus, justifying their use in the traditional medicine system of Pakistan.

35) ***Crossandra infundibuliformis*** (Acanthaceae): The larvicidal activity of crude extracts obtained from petroleum ether, ethyl acetate and methanol of *C. infundibuliformis* were investigated against *Anopheles Stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Six fourth-instar mosquito larvae were used for the study. The activity of petroleum ether extracts against *Anopheles stephensi* showed good mortality level of 83 ± 0.000 when 20 ppm of the extract were used, it increased to 100±0.000 with 100 ppm of extract. With 40 and 60 ppm, petroleum ether extract showed the toxic level 88±7.857 mortality.

But, the petroleum ether extract showed 72±7.857 to 77±7.857 mortality against the other two species *Aedes aegypti* and *Culex quinquefasciatus* larvae. However, the percentage mortality increases with increase in the concentration of three extracts and in all three larvae. In case of ethyl acetate extract, the mortality rate was found to be 77±7.857 and it increases, as the dosage of the extract is increased with respect to the larvae *Anopheles stephensi*.

Similar observation is noted with respect to *Aedes aegypti* and *Culex quinquefasciatus*. But the amount of extract dosage required for good larvicidal activity is found to be 40 ppm only. The effect of methanol extract on *Anopheles stephensi* is found to be very good, showing the mortality rate of 100 % with 60 ppm concentration onwards where as the other two larvae, *Aedes aegypti* and *Culex quinquefasciatus* showed 100 % mortality on 100 ppm dosage. Hence, the methanol extract has good mortality against *Anopheles stephensi* than the other two species Aedes aegypti and *Culex quinquefasciatus* larvae.

The experimental results demonstrated that, petroleum ether extract (A) have significantly reduced the mortality of the larvae when compared with other two extracts. The results showed that the extracts of *C. infundibuliformis*

may be considered as a potent source for new drug. The bioactivity-guided fractionation, isolation and identification will bring out potential drug to mankind, especially with anti-infective properties.

CONCLUSION: Thus, the folk claims about the medicinal herbs holds true through the *in vitro* investigation of these herbs for their antihelmintic activity. But along with the use of drugs to treat the Helminth infection, sanitation and cleanliness should always be observed in order to prevent such infections.

To conclude, there is need in future to screen the herbs phytochemically and pharmacologically to determine the mechanism of action of the active molecules. In addition, efforts should be made to standardize the medicinal plants for their antihelmintic activity and formulate herbal drugs as complement or alternative source to the current drugs in use.

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