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Screening of Phytoconstituents from *Curcuma longa*, *Piper nigrum* and *Murraya koenigii* Methanolic Extracts, and *In Vitro* Efficacy of Their Combinations on Nematode Parasite (*Ascaridia galli*) of *Gallus gallus domesticus*

Ahmad Rehan S.[1*] and Masuldar Asma[2]

[1]Department of Zoology, H. M. M. College for Women Kolkata, West Bengal, India
[2]Department of Zoology, D.B.F. Dayanand College of Arts and Science, Solapur, Maharashtra, India

**Corresponding Author*

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Abstract: Plants are a natural gift from God to humans. They play a crucial role in maintaining the health of humans as well as the environment. Humans have used medicinal plants to cure a variety of diseases since ancient times, including skin disorders, gastrointestinal issues, respiratory issues, bacterial infections, viral diseases, and parasitic diseases, as they contain a variety of active components (phytoconstituents). Nematidiasis is a parasitic ailment in livestock animals and birds caused by nematode parasites that reduces livestock output. The current work examines the phytoconstituents found in methanolic extracts of *Curcuma longa* (rhizome), *Piper nigrum* (seed), and *Murraya koenigii* (leaf), as well as their interactions. Their extracts were tested *in vitro* against the parasitic worm *Ascaridia galli* from the host *Gallus gallus domesticus*. They contain significant phytoconstituents such as alkaloids, tannins, flavonoids, phenols, glycosides, steroids, and others. When their extracts were combined, they have a high level of action against parasites, indicating a synergetic effect. The amount of activity displayed was dose-dependents. Researchers may find the findings useful in controlling nematode infection in farm animals and birds by combining these important natural plants as an alternative source of anthelmintics.

Keywords: Nematidiasis, Phytoconstituents, *Curcuma longa*, *Piper nigrum*, *Murrayakoenigii*, Ascaridiagalli, *Gallus gallus domesticus*

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Introduction

Medicinal plants are used as herbal remedies by human beings for treating various diseases. There are certain organisms that cause diseases to humans, livestock animals, and birds. These may be of different types such as-- protozoans, bacteria, viruses, and different endo as well as ectoparasites, etc. The endoparasites of livestock animals and birds include trematodes, cestodes, and nematodes. Nematode parasites cause Nematodiasis in animals and birds. Nowadays a lot of work has been done on the identification and prevalence of endoparasites from livestock

animals and birds. *Gallus gallus domesticus* has maximum chances of helminth infection due to its free-range feeding habitat. Among Helminth parasites, Nematode parasites are also abundantly found in *Gallus gallus domesticus* which causes production loss by affecting the health of the bird. Hence, the control of these nematode parasites has become an open challenge for researchers.

Helminthes infection in *Gallus gallus domesticus* (indigenous chicken) causes production loss (Siamba *et al.*, 2007). The gastro-intestinal nematode infection causes anemia, diarrhea, and severe weight loss which leads to loss of production and is responsible for the reduction in the livestock economy. No doubt there are different chemical anthelmintics which are being used for controlling helminth infections, but nematode parasites have become resistant to certain anthelmintics and the price sizes of chemical anthelmintics are an emerging problem for poor farmers hence, they rely on medicinal plants for treating their livestock animals and birds.

Nematode parasites have been developing-resistant capacity in them at different developmental stages even the same concen-tration drugs of plants with anthelmintic properties can be used as an alternate source to chemical anthelmintics for controlling nematodes infection (Mawahib, 2010). By considering these emerging issues about the use of chemical anthelmintics and their side effects, the present study was aimed to evaluate the use of valuable medicinal plants for controlling Nematodiasis.

Curcuma longa (Zingiberaceae), *Piper nigrum* (Piperaceae) *Murraya koenigii* (Rutaceae) are valuable medicinal plants used for various purposes. These are a rich source of various phytoconstituents and hence show different activities like-- antibacterial, antifungal, anti-protozoal, insecticidal, antidiabetic, astringent-inflammatory, anticancer, Molluscicides, anti-oxidant, pesticidal, hepato-protective, lipolytic, anthelmintic and many more.

Various anthelmintic drugs have been used by different researchers which are of natural plant origin while many of the reports from researchers concluded that extracts used in combination give high efficacy due to their synergetic activity. Different Practitioners says that using of combining herbs improves efficacy and decreases adverse effect which proves the principles of synergy and buffering system of combinations of plants (Pal and Shukla, 2003). Therefore, the present study focused on the nematicidal activity of cold methanolic extracts (CME) of *Curcuma longa* rhizome extract, *Piper nigrum* seed extract, and *Murraya koenigii* leaf extract in combination against nematode parasite (*Ascaridia galli*) of *Gallus gallus domesticus. Gallus gallus domesticus* is an important livestock poultry bird and nowadays it is getting affected by various diseases which may lead to loss of poultry production. Nematode parasites (*Ascaridia galli*) were found abundantly in the intestines as compared to cestodes hence they are selected for the present study. According to Mawahib (2010) especially environmental conditions such as temperature, humidity and rainfall are responsible factors for the living stages of nematodes and their egg production.

Materials and Methods

Curcuma longa rhizome and *Piper nigrum* seeds were purchased from local shops in Solapur District while leaves of *Murraya koenigii* were collected from local areas of Solapur city. The leaves were air-dried in the shade at room temperature and made in powdered form. *Curcuma longa* rhizome (turmeric) and *Piper nigrum* (black pepper) were powdered and proceeded to the cold maceration method. By this method, Cold Methanolic Extract (CME) of all selected plants was prepared which was then used for phytochemical analysis and Dry Column Chromatographic technique.

Phytochemical analysis and Nematicidal activity of plant extracts:

Methanolic extracts of all selected plants were used for phytochemical analysis by using standard procedures (Table 1). The study was carried out in August 2015 and a total of 41 freshly slaughtered

Fig. 1: Nematode infection found in intestine of infected *Gallus gallus domesticus*.

Table1: Preliminary Phytochemical analysis of *Curcuma longa* rhizome, *Piper nigrum* seed, and *Murraya koenigii* leaf Methanolic extract

S. No.	Phytoconstituents in methanolic extract	*Curcuma longa*	*Piper nigrum*	*Murraya koenigii*
1	Alkaloids	++	+	++
2	Flavonoids	-	-	+
3	Saponins	+	-	+
4	Phenols	-	++	+
5	Cumarins	+	+	+
6	Tannins	+	+	++
7	Quinines	+	-	+
8	Glycosides	-	-	++
9	Terpenoids	-	+	-
10	Phlobatannins	-	-	+
11	Anthraquinones	-	+	+
12	Steroids	+	-	+

intestines of *Gallus gallus domesticus* were collected from different localities and studied for their gastrointestinal parasites. Out of them, 34 intestines were shown positive for the infection of parasites. Most of the nematodes were observed in the small and large intestine as well as in the rectum of *Gallus gallus domesticus.* The parasites collected from the intestine (Fig. 1) were washed with distilled water and the control media used for the Nematicidal activity is PBS (Phosphate broth solution). Different fractions from column chromatography of selected plants were collected and different concentrations of extracts were tested against the *Ascaridia galli* in combination (extracts from all plants i.e. *Curcuma longa* rhizome, *Piper nigrum* seeds and *Murraya koenigii* leaves were mixed in equal volume for combination study) as well as separately. Observations were recorded in terms of paralysis time and death time of parasites.

Results and Discussion

Methanolic extracts of all plant extracts separately (Tables 2, 3, 4) or in combination (Table 5) showed significant dose-dependent *in vitro* nematicidal activity against *Ascaridia galli*. Methanolic extract of combined extracts at the concentration of 0.1 mg/ml showed a potent activity which gives less Paralysis and Death time (193.5±0.61 and 218.8±0.31 min, respectively) than the effect of plant extracts which are used separately. *Curcuma longa* extracts showed significant dose-dependent anthelmintic activity against earthworm muscle cells as a model (Vidya *et al.*, 2012). Different extracts of leaves of *Murraya koenigii* showed significant anthelmintic

Table 2: Mean ± SEM values of *Curcuma longa* rhizome extracts on *Ascaridia galli* of *Gallus gallus domesticus*

Concentrations of extracts (mg/ml)	Paralysed Time in (min) Mean±SEM	Death Time in (min) Mean±SEM
0.1	202.5±0.49	245.3±0.24
0.2	191.0±0.66	216.6±0.19
0.3	183.5±0.38	194.0±0.67
0.4	172.3±0.42	188.2±0.70
0.5	150.5±1.07	163.4±1.01
1	124.6±1.22	149.5±1.32

Values represent Mean ± SEM values (N=6)

Table 3: Mean ± SEM values of *Piper nigrum* seed extracts on *Ascaridia galli* of *Gallus gallus domesticus*

Concentrations of extracts (mg/ml)	Paralysed Time in (min) Mean±SEM	Death Time in (min) Mean±SEM
0.1	194.5±0.36	220.0±0.63
0.2	180.4±0.52	192.6±0.88
0.3	167.8±0.31	180.0±0.54
0.4	151.6±0.29	168.5±0.93
0.5	133.1±1.02	157.4±1.25
1	95.2±1.23	119.5±1.33

Values represent Mean ± SEM values (N=6)

Table 4: Mean ± SEM values of *Murraya koenigii* leaf extracts on *Ascaridia galli* of *Gallus gallus domesticus*

Concentrations of extracts (mg/ml)	Paralysed Time in (min) Mean±SEM	Death Time in (min) Mean±SEM
0.1	223.1±0.51	248.0±0.36
0.2	197.4±0.29	229.0±0.51
0.3	188.0±0.71	195.2±0.87
0.4	176.3±0.54	180.5±1.30
0.5	165.1±1.12	174.4±1.50
1	140.6±1.05	163.0±1.38

Values represent Mean ± SEM values (N=6)

Table 5: Mean ± SEM values of *Curcuma longa* rhizome, *Piper nigrum* seed, *Murraya koenigii* leaf extracts in combination on Gastrointestinal Nematodes of *Gallus gallus domesticus*

Concentrations of extracts (mg/ml)	Paralysed Time in (min) Mean±SEM	Death Time in (min) Mean±SEM
0.1	193.5 ± 0.61	218.8±0.31
0.2	176.1±0.38	207.0±0.26
0.3	162.5±0.27	177.5±0.33
0.4	150.2±0.34	166.2±0.70
0.5	132.4±1.15	153.10±1.01
1	93.17±0.03	116.5±1.32

Values represent Mean ± SEM values (N=6)

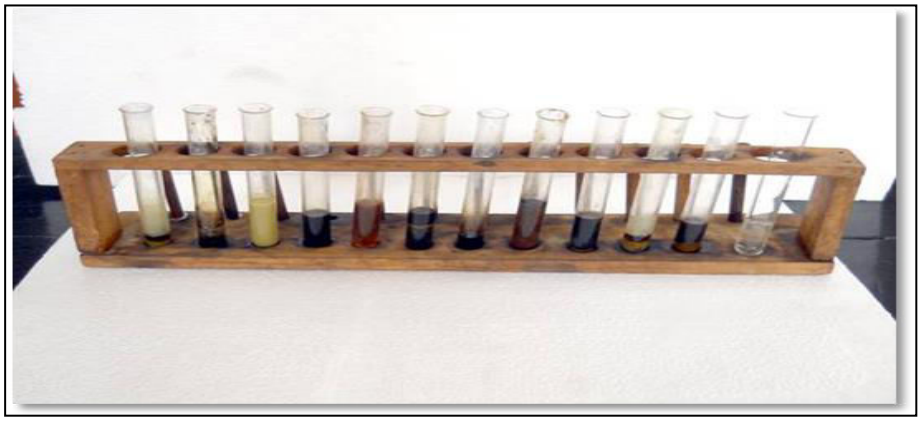

Fig. 2.1: Phytochemical analysis of *Piper nigrum* seed extract.

Fig. 2.2: Phytochemical analysis of *Curcuma longa* rhizome extract.

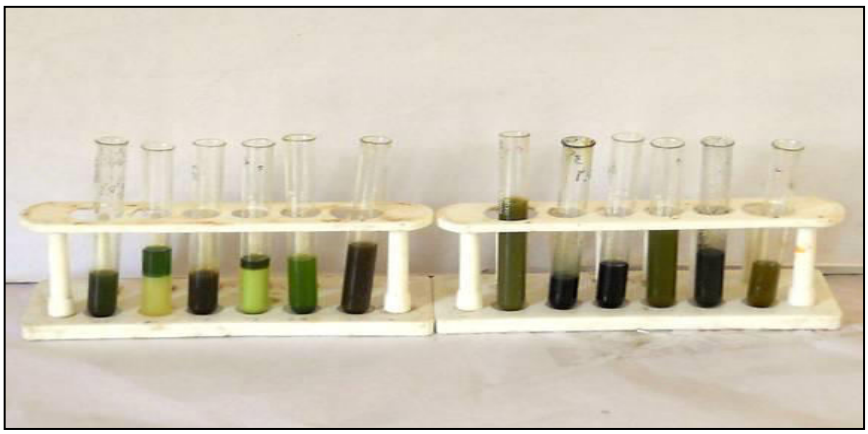

Fig. 2.3: Phytochemical analysis of *Murraya koenigii l*eaf extract.

activity against Indian earthworm (*Pheretima posthuma*) at 100 mg/ml of concentration Khuntia and Panda, 2011). Preliminary phytochemical analysis showed the presence of Alkaloids, Phenols, Cumarins, Tannins, Terpenoids, and Anthraquinones in *Piper nigrum* seed extract (Fig. 2.1). Phytochemical investigations of *Piper nigrum* seed extract are in resemblance to the report

given by Rai *et al.* (2012). *Curcuma longa* rhizome extracts showed the presence of phytoconstituents like Alkaloids, Cumarins, Saponins, Quinines, Tannins, and Steroids (Fig. 2.2). *Murraya koenigii* leaf extract showed the presence of Phytochemicals such as carbohydrates, alkaloids, sterols, tannins, volatile oils, saponins, an anthraquinone lycosides, and flavonoids (Fig. 2.3). The result showed a resemblance with the results of Handral *et al.* (2010).

Conclusion

There are certain problems regarding the use of chemical anthelmintics such as poor farmers are unable to purchase high price anthelmintics, while medicinal plants are nowadays used as an alternate source of anthelmintic, therefore present study was focused on the use of medicinal plants against nematode parasites, which will provide the basic platform for researchers in the field of parasitology and gives benefits of valuable medicinal plants (a noble gift) for us.

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