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In vitro Anthelmintic Activity of *Mentha longifolia* (L.) Leaves Against *Ascaridia galli*

[1]*Javid Ahmad,* [1]*Syed Tanveer and* [2]*Bilal A. Zargar*

[1]Department of Zoology, University of Kashmir, Srinagar-190 006, India
[2]Department of Pharmaceutical Sciences, University of Kashmir, Srinagar-190 006, India

Abstract: The *in vitro* anthelmintic activity of the aqueous and hydroalcoholic extracts of *Mentha longifolia* against *Ascardia galli* was evaluated in comparison to Fenbendazole. In folklore medicine, *M. longifolia* (Lamiaceae) is used in the treatment of various gastrointestinal ailments and other conditions. Worm Motility Inhibition (WMI) assay was used for *in vitro* assessment that revealed anthelmintic effects of crude hydroalcoholic extract (CHE) and crude aqueous extract (CAE) of *M. longifolia* on live *Ascardia galli* worms as evident from their paralysis and/or death at 6 h after exposure. Different concentrations (25 mg ml^{-1} and 50 mg ml^{-1}) of aqueous and hydroalcoholic extracts were used against *A. galli* which exhibited concentration and time dependent anthelmintic effects on *A. galli*. It is concluded that the leaves of *M. longifolia* possesses significant anthelmintic activity and could be a potential alternative for treating cases of *A. galli* infections in chickens.

Key words: *Mentha longifolia* · Anthelmintic · *Ascardia galli* · *In vitro* · Kashmir

INTRODUCTION

Domestic fowl (*Gallus gallusdomesticus*) is reared in most parts of the world either in the backyard or commercial production system. It acts as the source of meat, eggs, feathers and organic manure of high fertility. However, parasitism presents a main threat to the indigenous poultry production and cause heavy economic losses in the production of meat and eggs. Helminthosis is considered one of the most common diseases that affect free-range backyard chickens[1,2]. Like in other parts of the world, chickens are reared as the main backyard poultry bird in the households of Kashmir Valley and are known to be infected with different parasitic helminthes [3-6].*A. galli* shows the highest prevalence in different poultry production systems [7-9]. It is known to cause droopiness, hemorrhages and diarrhea in heavily infected chickens [10,11]. *A. galli* not only damages the integrity of intestinal mucosa but also affects utilization of nutrients, which results in reduced weight gain [12,13]. Heavy infection of *A. galli* may cause blockage of intestinal lumen and death of the host [14,15]. *A. galli* infections also result in economic losses due to treatment cost, decreased feedefficiency and impaired performance [10, 16]. *A. galli* acts as a vector for some infectious organisms like *Salmonella enterica* and *Escherichia coli* [17,18]. The development of drug resistance by helminthes against chemotherapeutical products and the associated risks of chemical residues in poultry products have drawn the attention to alternative approaches.

Different approaches have been utilized for the sustainable control of *A. galli* infections in poultry birds like utilization of genetic resistance [19-21], nutrition of host animal [13], biological control [22] and the use of plants with anti-parasitic properties as well as the use of traditional herbal remedies [23,24]. Because of easy availability, the use of *A. galli* worms as a suitable model for the screening of anthelmintic drugs had been advocated earlier [25-28]. To our knowledge, no scientific studies are available on the use of *M. longifolia* extracts against *A. galli*. Therefore, the present study was carried out to scientifically evaluate the *in vitro* and *in vivo*anthelmintic activityof different *M. longifolia* extracts against the common poultry nematode, *A. galli*.

Corresponding Author: Javid Ahmad, Department of Zoology,
University of Kashmir, Srinagar-190 006, India.

MATERIALS AND METHODS

Collection of Plant Material: *Mentha logifolia* (L.) L. is the scientifically accepted name for Horse Mint, locally known as *Ven*a or *Yen*a (family Lamiaceae). It grows along streamsides, waysides, bogs, irrigation channels and borders of paddy fields throughout the Kashmir Valley. Kashmir valley is a temperate North West Himalayan region of Jammu and Kashmir State in India and lies between 33°20' and 34°54'N latitudes and 73°55' and 75°35'E longitudes, covering an area of about 15,948 sq km [29].

The plant material was collected from the stream sides and waysides of Bugam-Chadoora area of District Budgam,Kashmir. The mature plant material at the peak of flowering was collected in polythene bags and processed by standard technique adopted by Kashmir University Herbarium (KASH). The labeled fresh specimen was discussed with local people regarding its uses/abuses. The data were reconfirmed by discussing the plants usage with other populations. The plant was identified and authenticated by a plant taxonomist, Dr. Aijaz Ahmad at the Department of Botany, University of Kashmir, Srinagar, India. A voucher specimen (Voucher no.401) was deposited in KASH.

Preparation of Plant Extracts: The whole plants collected were processed for shade drying in a well-ventilated room (Drying Room at Animal House, Development of Zoology, University of Kashmir, Srinagar). The dried leaves were powdered mechanically in electric grinder. The powdered plant material was then stored in an airtight container at 4°C until extraction. Two types of extracts (aqueous andmethanolic) of the powdered plant leaves were prepared following standard procedures. The crude aqueous extracts (CAE) of leaves were prepared according to the techniques described by Iqbal *et al*. [30]. The powdered plant leaves (500 g) were extracted with distilled water (2000 ml) at 90-100°C in a Soxhlet extractor for 8 h. The extracts were filtered and solvent was allowed to evaporate under reduced pressure of 22-26 mmHg in a vacuum rotary evaporator (R-201, Shanghai Shenshen) at 55°C yielding few grams of dry aqueous extracts. Similarly, the powdered leaves (500 g) were extracted with methanol (2,800 ml, Qualigens) at 60°C in a Soxhletextractor for 8 h, yielding few grams of dry methanolic extracts. These dry extracts were stored at 4°C until their use in *in vitro* and *in vivo* experiments.

***In vitro* Experiment:** The *in vitro* trails for anthelmintic activity of different extracts of *M. logifolia* were conducted on adult live *A. galli* worms. Naturally infected chickens were purchased from local market in Srinagar, Jammu and Kashmir State, India. The adult *A. galli* worms were collected from the intestine of freshly slaughtered birds (*Gallus gallus domesticus*) and immediately transferred to phosphate buffered saline (PBS). The adult worms of both sexes were randomly distributed to 6 treatment groups. These six groups were concurrently exposed to one of the following treatments at controlled temperature (37 ± 1°C) up to the maximum period of 6 ½ hours:

- Group 1 worms were exposed to 50 mg/ml of CHE
- Group 2 worms were exposed to 25 mg/ml of CHE
- Group 3 worms were exposed to 50 mg/ml of CAE
- Group 4 worms were exposed to 50 mg/ml of CAE
- Group 5 worms were exposed to 0.50 mg/ml of Fenbendazole (Positive Control)
- Group 6 worms were exposed to 0.9 % of PBS (Negative Control)

Twenty *A. galli* worms were suspended in each of the above concentrations in separate Petri dishes, each containing Fifty milliliter of the formulation. The inhibition of motility of the worms kept in the above treatments (followed by the fading away of their body colour) was used as the criterion for anthelmintic activity [31]. Worm motility was observed at 0, 1, 2, 4 and 6 hour intervals. After 6h all worms were withdrawn, washed and re-suspended in Luke warm fresh PBS for 30 min to observe the revival of the worms. There were five replicates for each treatment, to avoid any observational bias and to minimize other sources of errors.

Percentage worm motility inhibition (%WMI) was determined according to Rabel *et al*. [32] using the following formula: [(number of mobile worms in negative control Petri dish number of mobile worms in treatment Petri dish) /number of mobile worms in negative control Petri dish] × 100.

The data were statistically analysed using statistical Package for the social Sciences (SPSS) version 11.5.

RESULTS

In vitro assessment of anthelmintic activity of *M. longifolia* revealed that the effect CAE and CHE was

Table 1:*In vitro* anthelmintic efficacy of CAEs and CHEs of *M. longifolia* on *A.galli* of Chickens

		Mean±SEM of number of worms showing motility/survival at different hours (Percent Mortality)					
Treatment	Conc. (mg/ml)	0 h	1h	2 h	4h	6 h	Fresh PBS for 30 Min[a]
CHE	50	20±0.00	14.4±0.50 (28)	11.4±0.50 (43)	7.0±0.31 (65)	3.6±0.24 (82)	3.4±0.24 (83)
	25	20±0.00	17.0±0.31(15)	15.6±0.24 (22)	11.6±0.40 (42)	7.2±0.37 (64)	7.2±0.37 (64)
CAE	50	20±0.00	18.8±0.20 (6)	16.6±0.40 (17)	14.4±0.24 (28)	10.8±0.37 (46)	11.6±0.37 (41)
	25	20±0.00	19.4±0.24 (3)	17.6±0.40 (12)	15.2±0.20 (24)	14.8±0.20 (26)	16.4±0.24 (18)
Fenbendazole	0.50	20±0.00	8.2±0.37 (59)	3.6±0.40 (82)	0.8±0.20 (96)	0±0.00 (100)	0±0.00 (100)
PBS[b]	0.9%	20±0.00	20±0.00	20±0.00	20±0.00	19.8±0.20	19.8±0.20

CAE, Crude aqueous extract; CHE, crude hydroalcoholic extract; h, hour; SEM, standard error of mean.

a= Worms were exposed to phosphate-buffered saline for 30 min after exposure to the different treatments to confirm their mortality.

b =PBS (phosphate-buffered saline) indicates negative control.

concentration dependent. Both CHE @ 50 mg ml^{-1} and Fenbendazole showed a significant ($P < 0.001$) overall *in vitro* anthelmintic activity, with Fenbendazole exerting a greater effect ($P < 0.05$) than CHE, against adult *A. galli* worms in terms of worm paralysis when compared to the negative control (PBS). Highest mortality (83.00%) of worms was observed for CHE @ 50 mg ml^{-1} 6 hours (h) post exposure (PE) (Table 1). The CAE of *M. longifolia* @ 25 mg ml^{-1} resulted in paralysis of *A. galli*; however, their motility was revived after they were placed in PBS for 30 minutes. The CHE and CAE of *M. longifolia* exhibited time- dependent anthelmintic activity against *A. galli* of chickens, as revealed from the inhibition of motality and/or death of worms. The CHE of *M. longifolia* @ 50 mg ml^{-1} resulted in mean percentage worm motility inhibition (%WMI) of 83.00%, while CAE @ 50 mg ml^{-1} resulted in mean %WMI of 41.00%, as observed after the worms were put in PBS for 30 min after exposure to different treatments. Hydroalcoholic extracts of *M. longifolia* were thus more potent than the aqueous extracts. These findings suggested the presence of varying degrees of anthelmintic activity in CHE and CAE.

There was 100% mortality of worms in Fenbendazole (used as a reference drug) within 6 h PE. Almost all the worms (98%) that served for the negative control treatment (PBS) survived till 6 h PE.

DISCUSSIONS

In vitro trials confirmed the potential of *M. logifolia* extracts (CHE and CAE) to control the common poultry nematode *A. galli*. Fenbendazole and *M. logifolia* extracts (CHE and CAE) showed a significant *in vitro* anthelmintic activity against adult *A. galli* worms in terms of worm motility. Fenbendazole extremely paralyzed all worms after 6 h of exposure, while CHE and CAE @ 50 mg ml^{-1} resulted in 83 % and 41 % mortality respectively after the same interval. Fenbendazole caused a rapid and significant ($P < 0.001$) decrease in worm motility during the first hour of exposure and at all successive intervals. In contrast, CHE and CAE showed a time-dependant decrease in worm motility. However, Fenbendazole was more effective than CHE @ 50 mg ml^{-1}, the results showed a significant ($P < 0.05$) *in vitro* anthelmintic activity of CHE @ 50 mg ml^{-1} against the nematode after 6 h of *in vitro* exposure. *A. galli* worms showed a long time of active motility in PBS which provides a reliable potential for *in vitro* investigations.

The present study showed that mortality of adult *A. galli* worms was a time and concentration-dependent variable when used as a parameter to evaluate the anthelmintic activity of *M. logifolia* extracts. Higher concentrations of CHE and CAE of *M. logifolia* with long exposure periods may result in higher anthelmintic activity against *A. galli.* Similar observations were confirmed in different medicinal plants [33-37].

Based on the findings of earlier studies, the active principles in medicinal plants responsible for the anthelmintic activity are non-polar compounds, therefore, water extracts of medicinal plants have shown lower anthelmintic activity than less polar solvent extracts (ethanol, methanol, hydro-alcohol, acetone) [35,38-41]. Eguale *et al*. (2007) [39] attributed the greater anthelmintic activity of ethanolic extract of *Coriandrum sativum* against *H. contortus* to its lipid soluble nature which enhances rapid transcuticular absorption of active ingredient into the body of the worms. In addition, different components of the crude extracts may work together in a synergistic fashion. However, the merits and demerits of the existing methodologies used to determine the acting of antiparasitic plants are to be given due consideration [42]. Furthermore, the active principles in plants are diverse and stable natural compounds with low molecular weight which can prevent the occurrence of anthelmintic resistance [35].

Anthelmintic property of *M. logifolia* represents a promising alternative to chemical treatments. This would be of great importance for poultry production in free-range or organic farming systems which will contribute in sustainable agriculture.

CONCLUSION

In conclusion, the results of the present study on *M. logifolia* against *A. galli* suggest a potential alternative to commercially available anthelmintics for the treatment of gastrointestinal nematodiasis in chickens. There is a need to undertake detailed Phytochemical, pharmacological and anthelmintic studies of *M. logifolia* to determine the active principles, their mode of action and proper doses. Research is recommended for evaluating anthelmintic activity of *M. logifolia* against other nematode infections *in vitro* and in vivo.

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REFERENCES

1. Soulsby, E.J.L., 1982. Helminths, Arthropods and Protozoa of Domesticated animals.7th ed. Bailliere Tindall, London, UK.
2. Permin, A., H. Magwisha, A.A. Kassuku, P. Nansen, M. Bisgaard, F. Frand-sen and L. Gibbons, 1997. A cross-sectional study of helminths in rural scavenging poultry in Tanzania in relation to season and climate. J. Helminthol., 71: 233-240.
3. Fotedar, D.N. and N.G. Khateeb, 1986. Occurrence and seasonal variation of helminth parasites of domestic fowl in Kashmir. Indian J. Helminthol., 38: 49-54.
4. Pandit, B.A., A.S. Mir, M.A.A. Banday and R.A. Shahardar 1991. Prevalence of helminth parasites in Indigenous fowls of Kashmir Valley. Poult. Adv., XXIV(X): 37-39.
5. Ganaie, N.A., A.A. Khan, M.S. Mir and G.A. Bhat, 2004. Effect of Levamisol on endoparasitic infestation of fowls in Kashmir valley. The Blue Cross Book. 22: 24-25.
6. Dar, J.A. and S. Tanveer, 2013. Prevalence of cestode parasites in free-range backyard chickens (*Gallus gallus domestics*) of Kashmir, India. Agric Biol. J. N. Am., 4(1): 67-70.
7. Permin, A., M. Bisgaard, F. Frandsen, M. Pearman, P. Nansen and J. Kold, 1999. The prevalence of gastrointestinal helminths in different poultry production systems. Br. Poult. Sci., 40: 439-443.
8. Abdelqader, A., M. Gauly, C.B. Wollny and M.N. Abo-Shehdada, 2008. Prevalence and burden of gastro-intestinal helminths among local chickens in northern Jordan. Prev. Vet. Med., 85: 17-22.
9. Kaufmann, F., G. Das, B. Sohnrey and M. Gauly, 2011. Helminth infections in laying hens kept in organic free range systems in Germany. Livest. Sci., 141: 182-187.
10. Ruff, M.D., 1999. Important parasites in poultry production systems. Vet. Parasitol., 84: 337-347.
11. Permin, A. and J.W. Hansen, 1998. The Epidemiology, Diagnosis and Control of Poultry Parasites. FAO, Rome, Italy.
12. Kilpinen, O., A. Roepstorff, A. Permin, G. Nørgaard-Nielsen, L.G. Lawson and H.B. Simonsen, 2005. Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behavior and health of laying hens (*Gallus gallus domesticus*). Br. Poult. Sci., 46: 26-34.
13. Das, G., F. Kaufmann, H. Abel and M. Gauly, 2010. Effect of extra dietary lysine in *Ascaridia galli* infected grower layers. Vet. Parasitol., 170: 238-243.
14. Ikeme, M.M., 1971. Observations on the pathogenicity and pathology of *Ascaridia galli*. Parasitology. 63: 169-179.
15. Ramadan, H.H. and A.N.Y. Znada, 1991. Some pathological and biochemical studies on experimental ascaridiasis in chickens. Nahrung. 35: 71-84.
16. Martín-Pacho, J.R., M.N. Montoya, T. Aranguena, C. Toro, R. Morchon, C. Marcos-Atxutegi and F. Simon, 2005. A coprological and serological survey for the prevalence of Ascaridia spp. in laying hens. J. Vet. Med. B Infect. Dis. Vet. Public Health. 52: 238-242.
17. Chadfield, M., A. Permin, P. Nansen and M. Bisgaard, 2001. Investigation of the parasitic nematode A. galli (Schrank 1788) as a potential vector for Salmonella enteric d issemination in poultry. Parasitol. Res., 87: 317-325.
18. Permin, A., J.P. Christensen and M. Bisgaard, 2006. Consequences of concurrent *A. galli* and *Escherichia coli* infections in chickens. Acta Vet. Scand., 47: 43-54.

19. Gauly, M., C. Bauer, R. Preisinger and G. Erhardt, 2002. Genetic differences of *Ascaridia galli* egg output in laying hens following a single dose infection. Vet. Parasitol., 103: 99-107.
20. Abdelqader, A., M. Gauly and C.B.A. Wollny, 2007. Response of two breeds of chickens to *Ascaridia galli* infections from two geographic sources. Vet. Parasitol., 145: 176-180.
21. Kaufmann, F., G. Das, R. Preisinger, M. Schmulz, S. König and M. Gauly, 2011. Genetic resistance to natural helminth infections in two chicken layer lines. Vet. Parasitol., 176: 250-257.
22. Braga, F.R., J.V. Araújo, J.M. Araujo, L.N. Frassy, A.O. Tavela, F.E.F. Soares, R.O. Carvalho, L.M. Queiroz and J.H. Queiroz, 2011. *Pochonia chlamydosporia* fungal activity in a solid medium and its crude extract against eggs of *Ascaridia galli*. J. Helminthol., 85: 1-5.
23. Siamba, D.N., L.O. Okitoi, M.K. Watai, A.M. Wachira, F.B. Lukibisi and E.A. Mukisira, 2007. Efficacy of Tephrosia vogelli and *Vernonia amygdalina* as anthelminthics against *Ascaridia galli* in indigenous chicken. Livestock Research for Rural Development. 19(12), Article #176. Retrieved February 20, 2013, from http://www.lrrd.org/lrrd19/12/siam19176.htm.
24. Kholhring, L., R. Bishnupada and D.B. Kumar, 2009. Anthelmintic activity of *Acacia oxyphylla* stem bark against *Ascaridia galli*. Pharm. Biol., 47: 578-583.
25. Kaushik, R.K., J.C. Katiyar and A.B. Sen, 1974. Studies on the mode of action of anthelmentics with *Ascardia galli* as a test parasite. Indian Journal of Medical Research , 62: 1367-75.
26. Lal, J., S. Chandra, V.R. Prakash and M. Sabir, 1976. *In vitro* anthelmintic action of some indigenous medicinal plants on *Ascaridia galli* worms. Indian J. Physiol. Pharmacol., 20: 64-8.
27. Tandon, V., P. Pal, B. Roy, H.S.P. Rao and K.S. Reddy, 1997. *In vitro* anthelmintic activity of root tuber extract of Flemingia vestita, an indigenous plant in Shillong, India. Parasitol. Res., 83: 492- 498.
28. Kaushik, R.K., J.C. Katiyar and A.B. Sen, 1981. A new *In vitro* screening technique for anthelmintic activity using *Ascaridia galli* as a test parasite. Indian J. Anim. Sci., 51: 869-872.
29. Kaul, M.K., 1997. Medicinal plants of Kashmir and Ladakh-Temperate and Cold Arid Himalaya. Indus Publishing Company, New Delhi. pp: 131.
30. Iqbal, Z ., M. Lateef, M.S. Akhter, M.N. Gayur and A.H. Gilani, 2006. In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. Journal of Ethnopharmacology, 106: 285-287.
31. Yazwinski, T.A., H.D. Chapman, R.B. Davis, T. Letonja, L. Pote, L. Maes, J. Vercruysse and D.E. Jacobs, 2003. World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkeys. Vet. Parasitol., 116: 159-173.
32. Rabel, B., R. McGregor and P.G.C. Douch, 1994. Improved bioassay for estimation of inhibitory effect of ovine gastrointestinal mucus and anthelmintics on nematode larval migration. International Journal of Parasitology. 24: 671-676.
33. Asuzu, I.U. and O.U. Onu, 1994. Anthelmintic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascaridia galli* infected chickens. Fitoterapia, LXIV 65: 291-297.
34. Javed, I., M.S. Akhtar, Z.U. Rahman, T. Khaliq and M. Ahmad, 1994. Comparative anthelmintic efficacy and safety of Caesalpinia crista seed and piperazine adipate in chickens with artificially induced *Ascaridia galli* infection. Acta Vet. Hung., 42: 103-109.
35. Tariq, K.A., M.Z. Chishti, F. Ahmad and A.S. Shawl, 2009. Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. Vet. Parasitol., 160: 83-88.
36. Squires, J.M., J. Foster, D. Lindsay, D. Caudell and A. Zajac, 2010. Efficacy of an orange oil emulsion as an anthelmintic against *Haemonchus contortus* in gerbils (Meriones unguiculatus) and in sheep. Vet. Parasitol., 172: 95-99.
37. Abdelqader, A., B. Qarallah, D. Al-Ramamneh and G. Das, 2012. Anthelmintic effects of citrus peels ethanolic extracts against *Ascardia galli*. Veterinary Parasitology. 188: 78-84.
38. Iqbal, Z., M. Lateef, M. Ashraf and A. Jabbar, 2004. Anthelmintic activity of *Artemisia brevifolia* in sheep. J. Ethnopharmacol., 93: 265-268.
39. Eguale, T., G. Tilahun, A. Debella, A. Feleke and E. Makonnen, 2007. *In vitro* and *in vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus*. J. Ethnopharmacol., 110: 428-433.

40. Eguale, T., G. Tilahun, A. Debella, A. Feleke and E. Makonnen, 2007. *Haemonchus contortus*: *In vitro* and *in vivo* anthelmintic activity of aqueous and hydro-alcoholic extracts of *Hedera helix*. Exp. Parasitol., 116: 340-345.
41. Ademola, I.O. and J.N. Eloff, 2011. Anthelminthic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchus contortus* eggs and larvae. Trop. Anim. Health Prod., 43: 521-527.
42. Athanasiadou, S., J. Githiori and I. Kyriazakis, 2007. Medicinal plants for helminth parasite control: facts and fiction. Animal, 1(9): 1392-1400.