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Anthelminthic Activity of *Lavandula Angustifolia Hidcote* Extracts Using *Pheretima Posthuma*

Sarnim G.,[1] Jignesh S.,[1] Sanjay S.T.,[1] Roshan A.,[1] Vedamurthy A.B.,[1] Krishna V.,[2] Joy Hoskeri H.[1,2*]

*Corresponding author:

Joy Hoskeri H

[1] Dept. of Biotechnology, The Oxford College of Science, Bangalore, India
[2] Department of P.G. Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Shankarghatta – 577 451, Shimoga, Karnataka, India.

Abstract

In the present investigation, the in vitro anthelminthic activity of Lavandula angustifolia Hidcote extracts was carried out against Indian adult earthworm Pheretima posthuma. Piperazine citrate was used as the standard reference and normal saline as the control. Five different concentrations (2.5, 5.0, 7.5, 10.0 and 12.5 mg/ml) of chloroform and methanol extracts were used to determine their effect as time taken for paralysis (vermifuge) and time for death (vermicidal activity) of the worms. Both the extracts showed significant action in paralyzing and also killing the earthworms in a dose dependant manner. Among both the extracts, chloroform extract showed significant anthelminthic activity. Among the various concentrations of chloroform and methanol extracts, 12.5 mg/ml showed efficient anthelminthic activity. On the basis of these observations, we conclude that both extracts possess potential anthelminthic property and also suggest that further investigations on identification of active principles, standardization of dose and toxicity studies of this plant for drug development need to be carried out.

Keywords: *Lavandula angustifolia Hidcote*, Anthelminthic activity, *Pheretima posthuma*, Piperazine citrate, Methanol extract, Chloroform extract.

Introduction

Anthelminthics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. Intestinal infections with worms can be treated more easily than other infections. This is because the intestinal worms are killed by the drug and the drug need not be absorbed when administered through oral route. More than one third of the world's population is infected with worms (helminthes). There are many different types, but the most common are soil-transmitted helminthes (roundworm, whipworm and hookworm) and schistosomiasis which can negatively affect children's health, nutrition and education. Helminthes are classified as eukaryotic endoparasites because they live inside the body, unlike parasites like lice and fleas that live outside their host. Although helminthes infections can affect anyone, children in developing nations are at higher risks for helminthes infections. Many helminthes infections occur in poverty-stricken and developing countries with warm, moist environments with poor sanitary conditions. Helminthes can live in humans or animals and are usually transmitted through contaminated food, water, feces, unwashed hands or contact with any contaminated objects. Helminthes infections normally found in livestock can be transferred to human through a process called zoonosis and can then cause increased prevalence among humans. Although few helminthes infections lead to death, most of them do cause severe physical impairment. However, increasing problems of development of resistance in helminthes [1,2] against anthelminthics have led to the proposal of screening medicinal plants for their anthelminthic property. Plants are known to provide a rich source of potent botanical anthelminthics.[3,4] A number of medicinal plants have been used to treat parasitic infections in human and animals.[5,6,7,8]

Lavandula angustifolia hidcote is also known as Lavender angustifolia hidcote blue, Lavandula spica or Spike Lavender. Lavender angustifolia hidcote grows at lower altitudes, has coarser leaves and foliage and much higher oil content. Lavender angustifolia hidcote is a evergreen shrubby perennial which has been grown commercially for various uses including Lavender aromatherapy. Blue gray flowers are produced on this plant held up on long stems (hence the name spica). It is used a great deal commercially in Lavender perfumes, soaps and laundry products. The flowers are hermaphrodite and are pollinated by Bees, lepidoptera. It is noted for attracting wildlife. The flowering stems, and the essential oil obtained from them, is abortifacient, antibacterial, antiseptic, antispasmodic, carminative and emmenagogue. They can be used in all the ways that common lavender is used, externally to treat wounds, burns, insect stings etc and internally to treat digestive disorders. In the present investigation, we made an attempt to evaluate the anthelminthic

property of chloroform and methanol extract of Lavandula angustifolia hidcote.

Materials and Methods

Collection and authentication of plant material

The Lavandula angustifolia hidcote plant material was collected from Ooty district in Tamil Nadu, India during the month of March. The whole plant material was washed in running tap water and then shade dried. After drying, the whole plant material was milled into coarse powder by a mechanical grinder and stored in a closed container for further use.

Drugs and chemicals

The standard drug piperazine citrate (SD Fine Chemicals Ltd., Mumbai). Methanol was purchased from NICE chemical Pvt. Limited, Kerala, India. And Chloroform from SDFCL (SDfine chemical limited), Mumbai-30, India.

Preparation of extract

The air dried coarse powder of the Lavandula angustifolia hidcote was subjected to cold extraction using organic solvents like chloroform, methanol and finally with water sucessively by sequential extraction. This powdered plant material was subjected to extraction using chloroform as the solvent system for about 48 h with shaking at regular intervals. Each time before extracting with next solvent, the marc was air dried and then repacked into the apparatus, similar process was followed using next higher polar solvent ethanol and then with water sequentially in the similar fashion. All the three extracts were allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The yield of crude ethanol, chloroform and water extract for 1 kg of powdered seed material was 37 g, 59 g and 32 g respectively.

Earthworms collection

Healthy adult Indian earthworm (Pheretima posthuma; Annelida; Megascolecidae) were collected from vermin composting division, The Indo-American Hybrid Seeds, Bangalore. Earthworms from moist soil were washed with normal saline and then used for the study. The earthworm of 3 -5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [9,10]

Extract preparation

The crude powdered material was used for the extract preparation. After extraction, the crude extracts were stored in dessicator until further use. Chloroform extracts were dissolved in 5% DMSO, methanol extracts in 2.5% DMSO. Standard drug piperazine citrate was dissolved in normal saline and was used for evaluation for anthelminthic activity.

Anthelminthic activity

Anthelminthic activity was carried as per the method reported by Ajaiyeoba et. al., [11] with minor modifications. Because of easy availability, earthworms have been widely used for the initial in vitro evaluation of anthelminthic compounds. [12,13,14,15] 20 ml of formulation containing five different concentrations, each of crude chloroform, methanol and aqueous extracts (50, 100, 150, 200 and 250 mg/ 20ml in normal saline). All the extracts and the standard drug solution were freshly prepared before starting the experiments. Earth worm were released in each plate and observed for paralysis and death. Mean time for paralysis (in min) was noted when no movement of any sort could be observed except when the worm was shaken vigorously; time for death of worms (in min) was recorded after ascertaining that worms lost their motility followed by fading away of their body color.[16] Piperazine citrate (50 mg/20ml) was used as reference standard.[17] This study was carried out in triplicates. The result of anthelminthic activity is depicted in Table 1.

Table 1. In vitro anthelminthic activity of methanol and chloroform extracts of Lavandula angustifolia hidcote against Pheretima posthuma.

Test samples	Concentration (mg/20ml)	Paralysis Time (min)	Death Time (min)
Control (Normal Saline)		189.33 ± 7.42	249.67 ± 19.24
Methanol extract of Lavandula angustifolia hidcote	50	92.0 ± 4.16**	107.67 ± 4.67**
	100	21.33 ± 2.03**	98.67 ± 3.18**
	150	19.0 ± 2.65**	90.33 ± 1.76**
	200	16.0 ± 1.53**	87.0 ± 1.53**
	250	14.33 ± 0.88**	62.33 ± 3.18**
Chloroform extract of Lavandula angustifolia hidcote	50	17.33 ± 2.03**	98.0 ± 2.08**
	100	16.33 ± 2.03**	95.33 ± 4.67**
	150	16.33 ± 0.88**	95.33 ± 4.98**
	200	15.67 ± 1.76**	91.33 ± 7.8**
	250	15.33 ± 1.2**	72.33 ± 0.88**
Piperazine citrate	50	15.0 ± 2.08**	32.0 ± 3.79**

Values are the mean ± S.E.M. of three earthworms. Symbols represent statistical significance. * P < 0.05, ** P < 0.01, ns: not significant as compared to compared to control group.

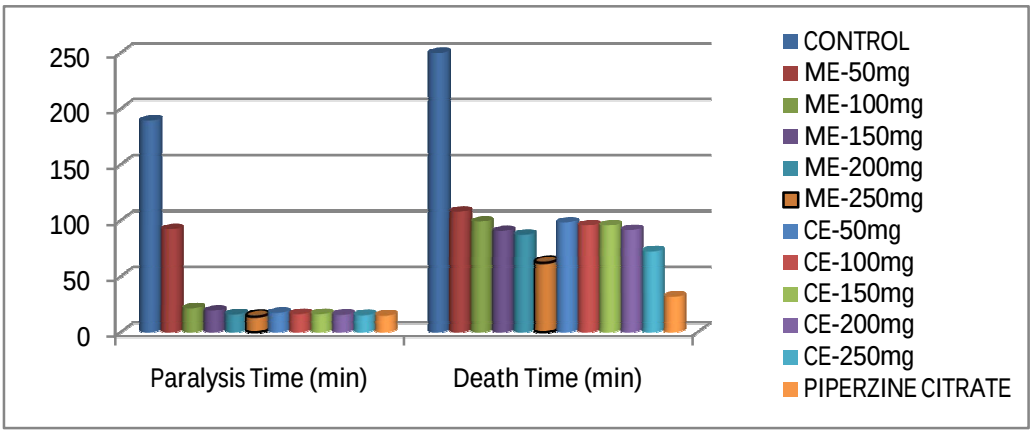

Figure 1. Bar chart illustrating the comparative in vitro anthelminthic effect of different concentrations of methanol and chloroform extracts of Lavandula angustifolia hidcote.

Statistical analysis

The data of anthelminthic evaluations were expressed as mean ± S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's t-test. The difference in values at P< 0.01 was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

Results and Discussion

The present investigation revealed that the chloroform and methanol extracts of the Lavandula angustifolia hidcote showed considerable anthelminthic activity against Pheretima posthuma as compared to the standard drug. Each crude extract at the concentration of 2.5, 5.0, 7.5, 10 and 12.5 mg/ml produced anthelminthic activity in a dose dependent manner giving shortest time of paralysis and death at 12.5 mg/ml concentration. Methanol extract at concentration of 12.5 mg/ml caused paralysis in 14.33 min and death in 62.33 min. While, chloroform extract showed paralysis in 15.33 min and death in 72.33 min. The reference drug

piperazine citrate at 2.5 mg/ml o paralysed the worm in 15.0 min and death in 32.0 min respectively.

Results of the present investigation were compared with other reports on anthelminthic effects of medicinal plant extracts. Nagaraja et. al., (2011) have reported that extract of Millingtonia hortensis stem bark possesses potent anthelminthic activity with paralysis time of 147.25 min and death time of 194.50 min at 20 mg/ml concentration of methanol extract. It was also reported that chloroform extract at 20 mg/ml showed paralysis time of 34.75 min and death time of 126.50 min.[18] Similarly, Rajesh et. al., (2011) also reported that methanol extract of aerial parts of Aerva lanata at 25 mg/ml showed paralysis time of 26.66 min and death time of 34.83 min.[19] In the present investigation, the plant used in the current study showed better activity at 12.5 mg/ml methanol extract with paralysis time of 14.33 min and death time of 62.33 min, while chloroform extract showed paralysis time of 15.33 min and death time of 72.33 min. Our findings on anthelminthic activity were comparable with other reports in this field viz., Swati et. al , (2011)[20] Rajeshwar et. al., (2011)[21] and Joy et. al., (2011)[22]

The predominant effect of piperazine citrate on the worm is to cause paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [17].This investigation revealed that methanolic extract of Lavandula angustifolia hidcote showed significant anthelminthic activity against Pheretima posthuma when compared to chloroform extract.

Conclusion

Among all the extract tested, methanol extract showed dose dependent anthelminthic action and was comparable with standard reference. Chloroform extract at 12.5 mg/ml concentration also showed similar activity comparable with piperazine citrate at dose of 2.5 mg/ml. On the basis of these investigations, we may partially conclude that Lavandula angustifolia hidcote could be a potent anthelminthic agent for next generation. Further studies are required on phytochemical profiling as well as isolation and identification of bioactive components responsible for anthelminthic activity.

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References

[1]. Geert S, Dorny P. Anthelminthic resistance in helminthes of animals of man in the tropics. Bullet des Sean, Academ Royal des Sci Dutre Mer. 1995;3:401–423.

[2]. Coles GC. Nematode control practices and anthelmintic resistance on British sheep farms. Vet Rec. 1997;141:91–93.

[3]. Satyavati GV, Raina MK, Sharma M. Medicinal Plants of India. Vol. I. New Delhi, India: Indian Council of Med. Res.; 1976;p. 201–206.

[4]. Lewis WH, Elvin LMPH. Medicinal Botany Plants Affecting Man's Health. New York: John Wiley & Sons; 1977.

[5]. Nadkarni AK. Indian Materia Medica, 3rd Ed. Bombay, India: Popular Prakashan; 1954.

[6]. Chopra RN, Nayyar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi, India: Council of Scientific and Industrial Research; 1956;p. 160-161.

[7]. Said M. Hamdard Pharmacopea of Eastern Medicine. Karachi, Pakistan: Hamdard National Foundation; 1969.

[8]. Akhtar MS, Zafar I, Khan MN, Muhammad L. Anthelminthic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent. Small Rum Res. 2000;38:99–107.

[9]. Thorn GW, Adams RD, Brundwal E, Isselbacher KJ, Petersdort RG. Harrison Principles of Internal Medicine, New York: McGraw Hill Co.; 1977;p. 1088- 1089.

[10]. Vigar Z. Atlas of Medical Parasitology. Singapore: PG Publishing House; 1984;p. 216- 217.

[11]. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. Invitro anthelminthic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. Pharm Biol. 2001;39:217- 220.

[12]. Sollmann T. Anthelminthics: their efficiency as tested on earth worms. J Pharmacol Exp Ther 1918;112:129-170.

[13]. Jain ML, Jain SR. Therapeutic utility of Ocimum basilicum var album. Plant Med. 1972;22:66-70.

[14]. Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB. Evaluation of Evolvulus alsinoids Linn for anthelminthic and antimicrobial activities. J Nat Rem. 2002;2:182-185.

[15]. Shivkar YM, Kumar VL. Anthelminthic activity of latex of Calotropis procera. Pharma Biol. 2003;41:263-65.

[16]. Mali RG, Shailaja M, Patil KS. Anthelminthic activity of root bark of Capparis spinosa. Indian J Nat Prod. 2005;21:50-51.

[17]. Martin RJ. γ-amino butyric acid and piperazine activated single channel current from Ascaris suum body muscle. Br J Pharmacol, 1985;84:445-461.

[18]. Nagaraja MS, Padmaa MP. In vitro anthelmintic activity of stem bark of Millingtonia hortensis linn. Inter J Pharm Bio Sci. 2011;2(2):15-19.

[19]. Rajesh R, Chitra K, Padmaa MP . In vitro anthelmintic activity of aerial parts of Aerva lanata Linn Juss. Inter Pharm Scia Drug Res. 2010;2(4):269-271.

[20]. Swati A, Simi J, Nikkita C, Saloni B, Ayesha T, Vedamurthy AB, Krishna V, Joy HH. Evaluation of in vitro anthelminthic activity of Leucas aspera

extracts. Pharmacog J. 2011;3(24):77-80.

[21]. Rajeshwar RM, Tirumal RK, Vedamurthy AB, Krishna V, Joy HH. A study on anthelminthic activity of Tinospora cordifolia extracts. Inter J Pharm Pharm Sci. 2011;3(5):78-80.

[22]. Joy HH, Krishna V. anthelminthic and bactericidal activity of extracts from Flaveria trinervia Spring C. Mohr. Euro J Med Plant. 2011;153-161.