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IN VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF ESSENTIAL OILS FROM DIFFERENT PARTS OF *SKIMMIA LAUREOLA* (DC.) ZUCC. EX WALP., VER. NAIR

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Abstract

In this study 'anthelmintic activity of essential oils (EOs), of leaf, stem and root of *Skimmia laureola* (DC.) Zucc. Ex Walp., ver. Nair was evaluated. 'Adult motility assay' was employed using *Haemoncus contortus* adult worms. EO was applied at three levels viz. 10, 35 and 50µL/10 ml in phosphate buffer solution (PBS) plus 10µL of tween 20 (as carrier/emulsifier). Levamisole was used as a positive control at 0.55 mg/ml concentration. Each concentration of essential oils obtained from different plant parts exhibited varied anthelmintic activity. The best dose dependant effect on adult mortality was by EOs from *S. laureola* root and stem (R^2 values 0.801). Change in the color of the dead worms was an indicator parameter of the fact that the EOs might have damaged the skin of the worms or after transcutaneous penetration into the body has disrupted the circulatory system by causing constriction of blood vessels. It is concluded that EOs extracted from *Skimmia laureola* have anthelmintic properties.

Introduction

Helminthiasis, a cosmopolitan disease of grazing animals, is of especial occurrence in developing countries along with improper handling and control activities (Lateef *et al.,* 2003). Synthetic anthelmintics have been used extensively for controlling parasitic infections. These synthetic chemicals are costly on one hand and often are not available to the farmers in rural areas on the other hand. Therefore, livestock producers have continued to use local plants as dewormers. Many other problems are associated with the use of synthetic chemicals for control of worms such as development of resistance in helminthes to various anthelmintic compounds (Adrian *et al.,* 2004) and groups along with chemical residue and toxicity problems. Therefore, biologists have prompted the search for medicinally important plants for their 'anthelmintic activity' besides the too much use of synthetically prepared chemicals in the clinical practices underway recently the world-over (Lateef *et al*., 2003). Therefore, the use of essential oils is thought to be more important and environment friendly.

It is well reported that essential oils obtained from various plants like, *Croton zehntneri* and *Lippia sidoides* (Camurça *et al*., 2007) and that from *Eucalyptus staigeriana* (Lara *et al.,* 2010) inhibited larval development of *Haemonchus contortus.* Similarly, extracts from different parts of plants had 'anthelmintic effects on the eggs and mature *Haemonchus contortus* (Eguale *et al.,* 2007; Gbolade & Adeyemi 2008; Tariq *et al*., 2008; Fall *et al*., 2008; Sujon *et al*., 2008; Nery *et al.,* 2010; Ademola *et al.,* 2010).

The family Rutaceae is of great economic importance for its numerous edible fruits of Citrus and *Aegle* genera. Other species like those of *Murraya, Zanthoxylum,* and *Skimmia* have medicinal properties. The presence of essential oils in members of family Rutaceae with diverse activities has an increasing demand for natural sources of anthelmintics. Thus, this study was conducted to explore the anthelmintic activity of essential oil obtained from different parts of *Skimmia laureola* (DC.) Zucc. Ex Walp., ver. Nair.

Materials and Methods

Extraction of essential oils and analysis: Different parts i.e., root, stem and leaves of *Skimmia laureola* (DC.) Zucc. Ex. Walp., ver. Nair were chosen to analyze the 'anthelmintic activity' of essential oil. The above mentioned plant parts were chosen keeping in mind their ethno-botanical use in Pakistan and were obtained from their natural habitat, identified and authenticated by an expert, at the Department of Botany, G.C. University, Lahore. The respective plant parts were separated and subjected to hydro-distillation for about four hours. The EOs obtained thus were dried with anhydrous sodium sulphate and put in brown colored glass bottles, at temperature of about 4°C. Chemical composition was determined using a GC-MS.

***In vitro* anthelmintic activity (Adult motility assay):** *In vitro* trials of the essential oils were carried out on adult '*Haemonchus contortus'* of sheep as by Sharma *et al*., (1971) with slight modifications. The adult worms of either sex were obtained from the 'abomasums' of freshly sacrificed sheep in the local slaughterhouse. These worms were given washing and then put in petri-dish having phosphate buffer (PBS) in it. Ten worms, *Haemonchus contortus* were given the following treatments, in triplicates, in petri dishes separately at room temperature (25-30°C) and 10 µL of Tween 20 were added to each concentration of essential oils so as to dissolve essential oils in 10 ml PBS uniformly.

1. 10 ml PBS + 10 µL (oil) + 10 µL Tween-20
2. 10 ml PBS + 35 µL (oil) + 10 µL Tween-20
3. 10 ml PBS + 50 µL (oil) + 10 µL Tween-20
4. Levamisole at rate of 0.55 mg/ml of was taken as a positive control.
5. 10 ml PBS + 10 µL Tween-20 (control)

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On the basis of slight change in their body color and inhibition of movement, dead worms were recognized easily. The time taken during motility was noted. The dead worms were put in the lukewarm (37ºC) fresh PBS for 30 minutes to see revival of motility in worms if any. Following criteria was used to interpret the results of adult motility assay in the present work were (a) time taken for mortality of worms (*H. contortus*) and (b) response of worms to different concentrations of essential oils, i.e., dose dependant effect.

Statistical analysis: One-way ANOVA, LSD and Probit-regression tests were applied, using SPSS 13.0 (statistical software) on the data for statistical analysis to draw conclusions.

Results

Table 1. shows that on hydro distillation percent yield of EOs was maximum from *Skimmia laureola* stem i.e., 1% followed from leaves 0.7% and roots 0.28 %.

Table 1. Yield and physical characteristics of essential oils obtained from different parts of *Skimmia laureola.*

Skimmia laureola	Leaves	Roots	Stem
Weight of specimen (kg)	2.2	1.765	1.56
Weight of oil (g)	70.30	0.05	1.50
Percent yield	0.7	0.28	1
Color of oil	Clear yellowish	very light yellow	Clear light pale

LC_{50} and regression co-efficient: Essential oils from *S. laureola* root and stem had LC_{50} 12.26, for leaves it was 35.12. R^2 value for *S. laureola* root and stem was 0.801, while leaves showed 0.208.

Major compounds in essential oils: Table 2 indicates that β-linalool dominated in EOs from leaves and stem while 1,3-cycloheptadiene was of high percentage in EO from root followed by α- terpineol and Linalyl isobutarate respectively.

Table 2. Percentage composition of major compounds in essential oils obtained from different parts of *Skimmia laureola.*

Name of plant part/compound	Retention time	Area (%)
***Skimmia laureola* leaves**		
β-linalool	4.49	32.32
α- terpineol	6.79	16.68
Linalool acetate	8.18	23.53
***Skimmia laureola* stem**		
β-linalool	4.54	43.61
α terpineol	6.802	16.22
Solanone	8.193	12.68
***Skimmia laureola* root**		
1,3-cycloheptadiene	5.577	36.86
Linalyl isobutarate	4.560	19.78
Nerolacetate	12.518	19.06

Comparison of '*in vitro* anthelmintic activity: Comparison of '*in vitro* anthelmintic activity' of three (10, 35 & 50µL) concentrations of each essential oil with reference to 'anthelmintic activity' of 'Levamisole' (as 'positive control') and control (PBS + Tween 20) revealed a significant difference as indicated in the results of ANOVA and LSD in Figs 1-3 (Mean ± S.E), while comparison of *in vitro* anthelmintic activity of 10, 35 & 50 µL of all essential oils showed almost similar effect.

Does dependant toxicity with respect to time is shown in Fig. 4. The results show that the application of EOs limited the survival time of the worms, almost half to the control treatment.

Discussion

Essential oils obtained from *S. laureola* root and stem had higher *In vitro effects* against adult worms followed by EOs obtained from *S. laureola* leaves. Major phytochemicals in the EOs were monoterpenes and monoterpene alcohols. Monoterpenes have acarcidal activity (Cetin *et al.,* 2010). *β*- linalool from *S. laureola* leaves and stem is monoterpene alcohol and possesses insecticidal property (Chang *et al.,* 2009).

The anthelmintic efficacy of EOs may be due to single or a combined effect of the compounds or chemical groups. The speculated mechanism of action might be disruption of membrane by the lipophilic compounds (Suresh *et al.*, 1997; Amaral *et al.*, 1998). Variations in the 'anthelmintic activity' of the EOs tested in this experiment may be as a result of varied targets on the parasites for action of the active chemicals present in EOs, with differences in quality and/or quantity. The dermal application of the EOs indicates that EOs might interfere with the osmotic balance of the worm results into death of worms. Higher the dose, the more pronounced was the rate of mortality.

Different compounds/active principles of EOs may have different targets to exert anthelmintic effect on adults. The reported mechanisms are uncoupling of oxidative phosphorylation (Weinbach & Garbus, 1969). There are some similar targets among bacteria, fungi, protozoa, and helminths, which can also be used by the compounds with 'anthelmintic activity'. These may be inhibition of enzymes, making complexes with proteins, polysaccharides, forming channels of ions, etc. These specific actions might disturb the usual processes of biochemical and physiological nature, thus depriving of nutrition, changes in structure, disturbing neuromuscular aspects, and other effects on 'helminths'. These are the most recognized targets for 'anthelmintics' in common use, (Kohler 2001; Mottier *et al.*, 2006).

Nevertheless, EOs considered in this study have demonstrated *in vitro* anthelmintic activity. But due to a lot of variation in conditions faced *in vivo*, like biotransformations during metabolic pathways, interactions with 'feed materials' and uptake by the body, the results obtained by the *in vitro* method could not be considered applicable to *in vivo* activity. Thus, these results should be confirmed by *in vivo* experimentation, so as to standardize doses and develop a drug.

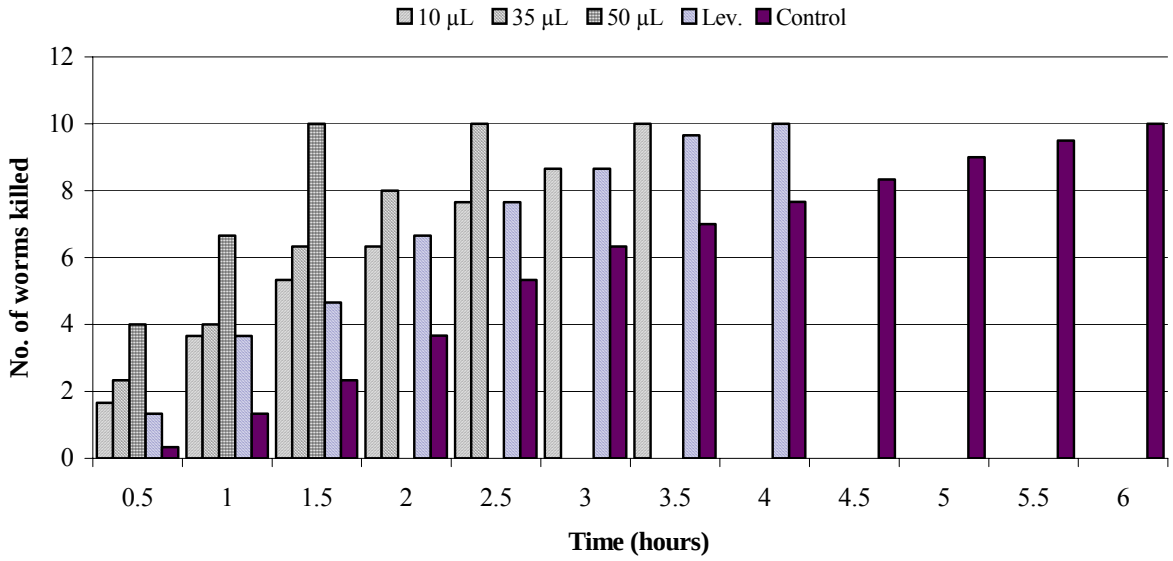

Fig. 1. Time dependant *In vitro* anthelmintic effect of essential oils from stem.

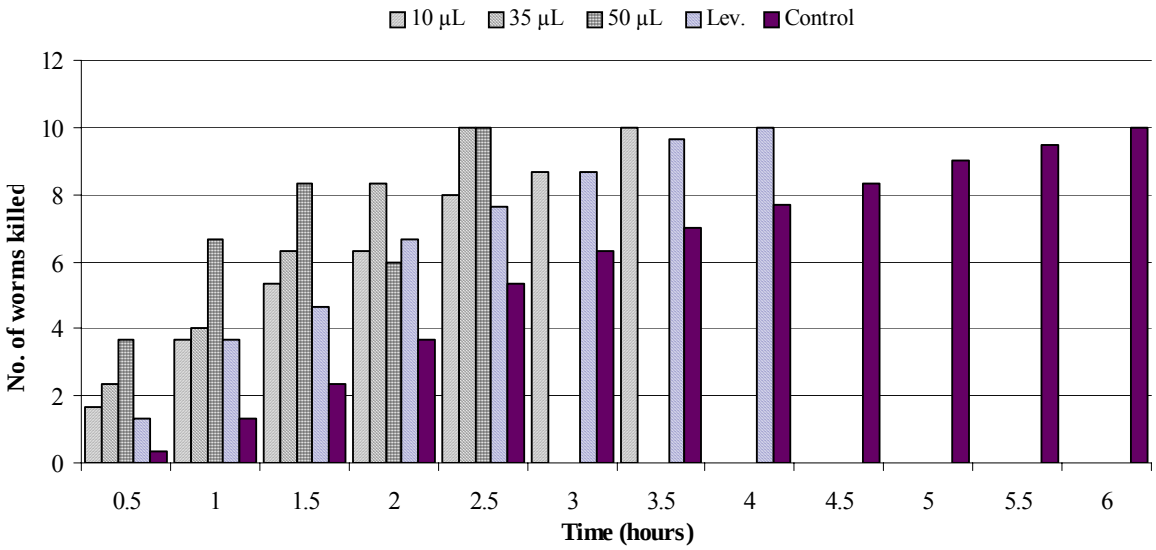

Fig. 2. Time dependant *In vitro* anthelmintic effect of essential oils from leaves.

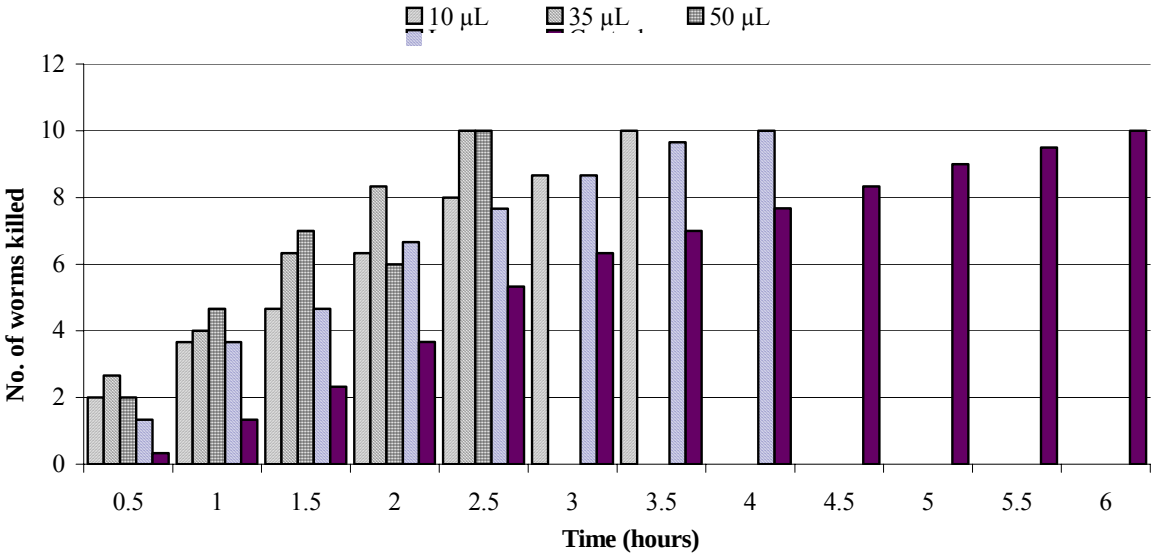

Fig. 3. Time dependant *In vitro* anthelmintic *effect* of essential oils from root.

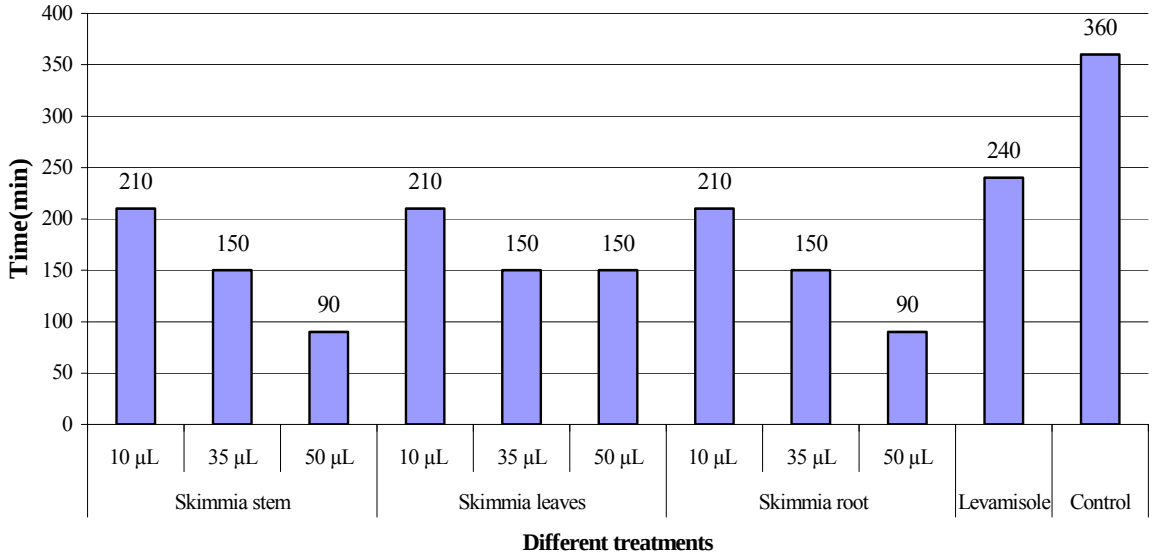

Fig. 4. A comparative profile of time and dose dependent anthelmintic activity of essential oils obtained from different parts of *Skimmia laureola*.

References

Ademola, I.O. and J. N. Eloff. 2010. *In vitro* anthelmintic activity of *Combretum molle* (R. Br. ex G. Don) (Combretaceae) against *Haemonchus contortus* ova and larvae. *Vet. Parasitol.,* 169(1-2): 198-203.

Adrian, J.W., I. Fairweather, R. Prichard, G.V. Samson, I. Himmelstjerna and N.C. Sangster. 2004. Drug resistance in veterinary helminthes. *Trends Parasitol.,* 20 (10): 469-476.

Amaral, J.A., A. Ekins, S.R. Richards and R. Knowles. 1998. Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. *Appl. Environ. Microbiol*., 64: 520-525.

Camurça, V.A.L.F., C.M.L. Bevilaqua, S.M. Morais, M.V. Maciel, C.T.C. Costa, I.T.F. Macedo, L.M.B. Oliveira, R.R. Braga, R.A. Silva and L.S. Vieira. 2007. Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils. *Vet. Parasitol.,* 148(3-4): 288-294.

Cetin, H., J.E. Cilek, E. Oz, L. Aydin, O. Deveci and A. Yanikoglu. 2010. Acaricidal activity of *Satureja thymbra* L. essential oil and its major components, carvacrol and γ-terpinene against adult *Hyalomma marginatum* (Acari: Ixodidae).*Vet. Parasitol.,* 170(3-4): 287-290.

Chang, C.L., I.K. Cho and Q.X. Li. 2009. Insecticidal activity of basil oil, trans-anethole, estragole, and linalool to adult fruit flies of *Ceratitis capitata, Bactrocera dorsalis*, and *Bactrocera cucurbitae*. *J. Econ. Entomol.,* 102(1): 203-9.

Eguale, T., G.A. Tilahun, A. Debella, Feleke and E. Makonnen. 2007. . *In vitro* and *In vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus. J. Ethnopharmacol*., 110(3): 428-33.

Fall, D., B. Sambou, M. Seck, A. Wele, I. Ndoye, C. Gleye and A. Laurens. 2008. Enhancing the anthelminthic activity roots of *Annona sengalensis*. *Dakar Med.,* 53(1): 61-7.

Gbolade, A.A and A.A. Adeyemi. 2008. Anthelmintic activities of three medicinal plants from Nigeria. *Fitoterapia,* 79: 223-5.

Lara, T.F., C.M.L. Bevilaqua, L.M.B. de Oliveira, A.L.F. Camurça-Vasconcelos, L. da S. Vieira, F.R. Oliveira, E.M. Queiroz-Junior, A. da R. Tomé and N.R.F. Nascimento. 2010. Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes.*Vet Parasitol.,* 173(1-2): 93-8.

Kohler, P. 2001. The biochemical basis of anthelmintic action and resistance. *Int. J. Parasitol.*, 31: 336-345.

Lateef, M., I. Zafer, M.N. Khan, M.S. Akhtar and A. Jabbar. 2003. Anthelmintic activity of *Adhatoda vesica* roots. *Int. J. Agri. Biol.,* 5(l): 86-90.

Mottier, L., L Alvarez, L. Ceballos and C. Lanusse. 2006. Drug transport mechanisms in helminth parasites: Passive diffusion of benzimidazole anthelmintics. *Exp. Parasitol.*, 113: 49-57.

Nery, P.S., F.A. Nogueira, E.R. Martins and E.R. Duarte. 2010. Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep. *Vet. Parasitol.*, 171(3-4): 361-364.

Sharma, L.D., H.S. Bhagra and P.S. Srivastava. 1971. *In vitro* anthelmintic screening of indigenous plants against *Haemonchus contortus* of sheep and goat. *Indian J. Anim. Res*., 5(1): 33-38.

Sujon, M.A., M. Mostofa, M.S. Jahan, A.R. Das and S. Rob. 2008. Studies on medicinal plants against gastro intestinal nematodes of goats. *Bang. J. Vet. Med.,* 6(2): 179-183.

Suresh, B, S. Sriram, S.A. Dhanaraj, K. Elango and K. Chinnaswamy. 1997. Anticandidal activity of *Santolina chamaecyparissus* volatile oil. *J. Ethnopharmacol.,* 55: 151-159.

Tariq, K.A., M.Z. Chishti, F. Ahmad and A.S. Shawl. 2008. Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. *Vet. Parasitol.,* 60(1-2): 83-88.

Weinbach E.C. and J. Garbus. 1969. Mechanism of action of reagents that uncouple oxidative phosphorylation. *Nature,* 221 : 1016-1018.

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