COMPARATIVE ANTHELMINTIC ACTIVITY OF LEAVES, STEMS AND ROOTS OF *LEUCAS LAVENDULIFOLIA* SPRENG. (LAMIACEAE)

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Summary

Stems, leaves and roots of *Leucas lavendulifolia* Spreng (Lamiacea) were separately dried, powdered and successively extracted with petroleum ether, chloroform, ethyl acetate and methanol in Soxhlet extractor. Anthelmintic activity of these extracts was evaluated on Indian adult earthworms, *Pherentima posthuma*. Results showed that the stems of *Leucas lavendulifolia* took less time to cause paralysis and death of the earthworms as compare to leaves and roots. Each extract was studied for their anthelmintic activity, which involved determination of the time of paralysis and time of death of the worms. Results showed that the petroleum ether extract and methanol extract of stems of *Leucas lavendulifolia* at 20 mg/ml was most potent as compared to other extracts and standard drug albendazole. The order of sensitivity of the extracts to the worms was found to be methanolic extract > pet ether extract > chloroform extract > ethyl acetate extract. It can be concluded that anthelmintic activity of the stems and leaves of *Leucas lavendulifolia* is due to the active principles present in the petroleum ether and methanol extracts. The purpose of this project was to evaluate *invitro* anthelmintic activity of various extract of *Leucas lavendulifolia*. Albendazole (20 mg/ml) and distilled water were included in the assay as standard drug and control, respectively.

Keywords: *Leucas lavendulifolia*, *Pheritima posthuma, In-vitro* anthelmintic activity, Lamiaceae.

Introduction

Leucas lavendulifolia is erect, slender and annual herb 30-60 cm high found as a weed in field. The leaves are linear-lanceolate, entire serrate and flowers are white with oblong and pale brown nutlets. [1, 2] Acacetin and chrysoeriol are isolated from aerial parts. A new flavonoid compound linifolioside was isolated and characterized asisopimara -8, 15 – diene - 7 – keto – 3 – o – α – L – rhamnopyranosyl (1→2) – β -D-glucopyranoside.[3] The aromatic plant used as flavouring leaves eaten as a potent herb, decoction of leaves used as a sedative, stomachic and vermifuge.[4] Poultice of fresh leaves applied to old sores and dermatitis. It is also used as stimulant and diaphoretic, used in rheumatism and snake bite. The root, stem and leaves are cynogenetic.[5] The *Leucas lavendulifolia* is also shows psychopharmacological activity.[6]

Material and method

Plant material and Preparation of extracts

The leaves, stems and roots of *Leucas lavendulifolia* were collected from Ahmednagar district, Maharashtra (India) in August 2007. The plant specimen was authenticated from Botanical Survey of India, Pune (Voucher specimen no. - LRM1). Dried and coarsely powdered stems, leaves and roots (500 g, each) of *Leucas lavendulifolia* were separately subjected to successive extraction using petroleum ether, chloroform, ethyl acetate and methanol in Soxhlet extractor. The extracts of various parts were concentrated by vacuum distillation and then dried in open air.

Animals:

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil of the Government Horticulture Department, Kopargaon and washed with normal saline to remove all the feacal matter, were used for the anthelmintic study. The earthworms 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 human being.

Chemicals:

Drugs: Albendazole (Pfizer Ltd., Bangalore), Chemicals: Pet ether (PCL, Pune), Chloroform (PCL, Pune), Ethyl acetate (PCL, Pune), Methanol A. R (PCL, Pune), DMF (PCL, Pune), Saline water (Nurilife, Ahmedabad).

Anthelmintic activity: [7, 8, 9]

In each case, six earthworms were released into 10 ml of desired formulations as follows; vehicles (5 % DMF in normal saline), Albendazole (20 mg/ml), or total pet ether, chloroform, ethyl acetate and methanol extracts of leaf, stem and roots of *Leucas lavendulifolia* (20 mg/ml, 40 mg/ml, 60 mg/ml) in normal saline containing 5 % DMF. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading of their body colors.

Statistical Significance

The results were analyzed for statistical significance using student't' test. $P<0.05$, $^{*}P<0.0001$ was considered significant.

Result and Discussion

It is evident from the result shown in Table 1, Table 2 and Table 3, all the extracts showed the dose dependant anthelmintic activity. The stems of *Leucas lavendulifolia* showed the significant anthelmintic activity as compared to the leaves and roots. The potency of extracts for the anthelmintic activity of stem, leaves and roots of *Leucas lavendulifolia* was found to be methanolic extract > pet ether extract > chloroform extract > ethyl acetate extract i.e. methanolic extract showed very less time to cause the paralysis of worms and death of the worms. All the extracts of stem, leaves and roots showed significant activity at a concentration of 20 mg/ml. Results were comparable with the standard drug, Albendazole, at the same concentration. The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feaces of human being and animals. The extracts not only demonstrated this property, they also caused death of the worms. The pH of the formulation (extract which is diluted to 10 ml with normal saline containing 5 % of DMF) was also found out during the activity which was found to be 5-6. The preliminary phytochemical investigation showed the presence of sterol, triterpines, flavonoids, tannins.[10] It can be concluded that active constituents responsible for anthelmintic activity are present in the methanol, petroleum ether and chloroform extracts of *Leucas lavendulifolia.*

This indicates that the anthelmintic principles are nonpolar and semipolar compounds. The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feaces of human being and animals. The extracts not only demonstrated this property, they also caused death of the worms. And further study was carried out for isolation of the chemical constituent responsible for anthelmintic activity. Further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of stem of *Leucas lavendulifolia* as an anthelmintic drug. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity.

Table 1. Anthelmintic activity of various extracts of stems of *Leucas lavendulifolia* Spreng.

Treatment	Dose mg/ml	Time of paralysis (min) ± SEM	Time of Death (min) ± SEM
PES	20	0.201 ± 0.00881	3.06 ± 0.01856
	40	0.212 ± 0.00152	2.51 ± 0.01155
	60	0.210 ± 0.00120	2.40 ± 0.00881
CHS	20	0.286 ± 0.00176	4.706± 0.3290
	40	0.270 ± 0.002186	4.51 ± 0.01155
	60	0.252 ± 0.00088	4.43 ±0.01764
EAS	20	0.536 ± 0.00176	7.03 ± 0.01528
	40	0.519 ± 0.00176	6.49 ± 0.01453
	60	0.485 ± 0.00264	6.386 ±0.0145
MES	20	0.116 ± 0.001764	2.17 ± 0.01155
	40	0.108 ± 0.00088	1.453 ± 0.0882
	60	0.103 ± 0.00081202	1.216 ± 0.0176
ALBENDAZOLE	20	0.084 ± 0.00057	2.133 ± 0.08819
	40	0.072 ± 0.00088	1.253 ± 0.0088
	60	0.0663 ±0.0008819	1.02 ± 0.01202
CONTROL	5% DMF in normal saline		

PES- pet ether extract of stem, CHS- chloroform extract of stem, EAS- ethyl acetate extract of stem, MES- methanol extract of stem. SEM- standard error of mean.

Table 2. Anthelmintic activity of various extracts of leaves of *Leucas lavendulifolia* Spreng.

Treatment	Dose mg/ml	Time of paralysis (min) ± SEM	Time of Death (min) ± SEM
PEL	20	0.260 ± 0.00296	3.47 ± 0.01155
	40	0.251 ± 0.00145	2.44 ± 0.01856
	60	0.247 ± 0.00088	2.23 ± 0.00115
CHL	20	0.349 ± 0.00577	6.12 ± 0.01202
	40	0.339 ± 0.000881	5.406 ± 0.00881
	60	0.315 ± 0.000881	5.31± 0.00577
EAL	20	0.602 ± 0.001202	8.11 ± 0.01764
	40	0.591 ± 0.001202	7.41 ± 0.008819
	60	0.5796 ± 0.00145	7.21 ± 0.00577
MEL	20	0.157 ± 0.008817	3.06 ± 0.00881
	40	0.139 ±0.00881	2.53 ± 0.01155
	60	0.112 ± 0.00208	2.31 ± 0.00577
ALBENDAZOLE	20	0.083 ± 0.00115	2.11 ± 0.0088
	40	0.072 ± 0.00088	1.253 ± 0.0081
	60	0.065 ± 0.0006	1.033 ± 0.0088
CONTROL	5% DMF in normal saline		

PES- pet ether extract of leaves, CHS- chloroform extract of leaves, EAS- ethyl acetate extract of leaves, MES- methanol extract of leaves. SEM- standard error of mean.

Table 3. Anthelmintic activity of various extracts of roots of *Leucas lavendulifolia* Spreng.

Treatment	Dose mg/ml	Time of paralysis (min) ± SEM	Time of Death (min) ± SEM
PER	20	0.328 ± 0.00384	4.48 ± 0.01528
	40	0.322 ± 0.00152	3.886 ± 0.01453
	60	0.297 ± 0.00145	3.24 ± 0.01732
CHR	20	0.417 ± 0.00101	6.19 ± 0.02906
	40	0.389 ± 0.000881	5.67 ± 0.0152
	60	0.3546 ± 0.00266	5.203 ± 0.0463
EAR	20	0.698 ± 0.000881	9.04 ±0.01453
	40	0.54 ± 0.00120	8.51 ± 0.03055
	60	0.469 ±0.00655	7.29 ± 0.03464
MER	20	0.201 ±0.00088	4.09 ± 0.0290
	40	0.191 ± 0.001155	3.5 ±0.01155
	60	0.1753 ± 0.00145	3.053 ±0.02186
ALBENDAZOLE	20	0.083 ± 0.001155	2.113 ± 0.00881
	40	0.0721 ± 0.0011	1.26 ± 0.01155
	60	0.065 ± 0.000881	1.04 ±0.008819
CONTROL	5% DMF in normal saline		

PES- pet ether extract of roots, CHS- chloroform extract of roots, EAS- ethyl acetate extract of roots, MES- methanol extract of roots. SEM- standard error of mean.

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