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Full Length Research Paper

Antidiarrheal activity of methanol extract and major essential oil contents of *Saussurea lappa* Clarke

J. S. Negi[1]*, V. K. Bisht[1], A. K. Bhandari[1], V. P. Bhatt[1], M. K. Sati[2], J. P. Mohanty[2] and R. C. Sundriyal[1,3]

[1]Herbal Research and Development Institute, Mandal, Gopeshwar - 246 401, Uttarakhand, India.
[2]Himalayan Pharmacy Institute, Majhitar, East Sikkim- 737 136, Sikkim, India.
[3]G. B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora-263643, Uttarakhand, India.

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Earlier folklore claims reveal that *Saussurea lappa* Clarke (family Asteraceae) is used in the treatment of abdominal pain, dysentery and chronic skin diseases. This species has a good economic potential as its essential oil is commercially valuable in the flavour and fragrance industry. The major bioactive components of *S. lappa* are costunolide and dehydrocostus lactones. However, other sesquiterpene lactones are also active constituents. The present study reports antidiarrheal activity and major constitutes of *S. lappa*. Five groups of Wistar rats (210 to 230 g), each group consisting of 5 animals were taken for the study. Group I was kept as control, providing only saline while group II, III and IV were considered as test group, and the plant extracts (100, 300 and 500 mg/kg body weight) were administrated orally. The fifth group received the standard drug loperamide (5 mg/kg body weight). Qualitative and quantitative analysis of extracted essential oil of *S. lappa* was performed on Perkin-Elmer Gas Chromatography equipped with Perkin-Elmer-Clarus-500 Mass Spectrometry (GC-MS). The individual constituents were identified by comparing their mass spectra to National Institute of Standards and Technology (NIST) and Wiley mass spectral libraries. We reported that application of three different doses of 100, 300 and 500 mg/kg inhibited diarrhea by 26.33, 32.28 and 66.77%, respectively. GC-MS analysis of extracted essential oil of *S. lappa* showed presence of sesquiterpenes, among these, β-castol and δ-elemene were found as major components. The methanol (MeOH) extract significantly exhibited antidiarrheal activity in dose dependent manner. The study supports the traditional claims of *S. lappa* as an antidiarrheal agent.

Key words: Qualitative and quantitative analysis, gas chromatography-mass spectrometry (GC-MS), castor oil, loperamide, wistar rats.

INTRODUCTION

Saussurea lappa Clarke [Synonym: *Saussurea costus* (Falc.) Lipschitz] (family Asteraceae) is a well known medicinal plant growing in the Himalayan region between 2500 to 3000 m above sea level. In view of increasing national and international market demand of *S. lappa*, it is also cultivated in a few states of India, including Uttarakhand and Himanchal Pradesh. Sesquiterpene lactones such as costunolide and dehydrocostus lactone, are major components of the roots, and have been reported to possess various biological activities such as antifungal (Barrero et al., 2000), anthelmintic (Seki et al., 1991), antidiabetic (Upadhyay et al., 1996), antitumor (Ko et al., 2005), antimicrobial (Khalid et al., 2011), immuno-stimulant (Kulkarni and Desai, 2001), antiulcer (Sutar et al., 2011), antiinflammatory (Yashvanth et al., 2010) and antihepatotoxic (Yaeesh et al., 2010). The medicinal importance of *S. lappa* is well reviewed (Pandey et al.,

*Corresponding author. E-mail: negijs@yahoo.com. Tel: +911372254210. Fax: +911372254273.

2007). Diarrhea disease is a leading cause of mortality and morbidity among children in many developing countries (Das et al., 1999). The majority of the people in developing countries rely on herbal medicine for the treatment of diarrhea. *S. lappa* is used traditionally for the treatment of abdominal pain, dysentery and chronic skin diseases (Kala and Manjrekar, 1999).

Essential oils are valuable natural products used as raw materials in perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition (Buchbauer, 2000). Earlier studies on essential oil from roots of *S. lappa* showed the presence of (−)-α-selinene, (+)-selina-4, 11-diene, (−)-α-*trans*-bergamotene, (−)-α-costol, (+)-γ-costol, (−)-elema-1,3,11(13)-trien-12-ol, (−)-α-costal, (+)-γ-costal, (−)-elema-1,3,11(13)-trien-12-al, (−)-(E)-*trans*-bergamota-2,12-dien-14-al, (−)-*ar*-curcumene, (−)-caryophyllene oxide (Maurer and Grieder, 1997) and 12-methoxydihydrodehydrocostuslactone (Dhillon et al., 1987). α-Pinene, p-cymene, δ-elemene, β-selinene, α-selinene and caryophyllene oxide were also reported from *Artemisia arborescens, Michelia macclurei* and Atractylodis rhizomes (Younes et al., 2012; Zhou et al., 2012; Wang et al., 2012).

In recent times, the species has been introduced in farmers' field and successfully cultivated. Except antidiarrheal activity, *S. lappa* has been investigated for several types of biological activities. There is little published data on essential oil contents of *S. lappa*. Therefore, the present study is aimed at evaluating the antidiarrheal activity of *S. lappa* methanol extract and its major essential oil contents.

MATERIALS AND METHODS

Collection of plant material and extraction of essential oil

Roots of *S. lappa* (cultivated) were collected from the Herbal Garden of Herbal Research and Development Institute, Gopeshwar, India. The plant was authenticated by Dr. C. S. Rana, State Medicinal Plant Board, Dehradun, Uttarakhand, through existing literature (herbarium No. GUH 19278). The dried roots were pulverized and extracted with MeOH using Soxhlet apparatus. Thereafter, solvent was removed in a rotary evaporator. The methanol extract of *S. lappa* at 100, 300 and 500 mg/kg (body weight) and the standard drug loperamide 5 mg/kg (body weight) were prepared. For extraction of essential oil, the fresh roots of *S. lappa* were cut into small pieces and subjected to hydro-distillation in a Clevenger apparatus (5 h). The pale yellow colored essential oil was collected, dried over anhydrous sodium sulphate and stored in a sealed glass vial at low temperature (0 to 4°C) prior to analysis. All the chemicals and reagents were of Guaranteed Reagent (GR) quality of Merck.

Animals

Experiment was carried out on Wistar rats (210 to 230 g) housed in standard cages at room temperature (23 ± 1°C). Food and water *ad libitum* were provided to the animals and allowed seven days acclimatization period prior to commencement of the experiment. All the experiments were carried out according to the recommendation of International Association for the Study Pain (IASP) committee for research and ethical issues guidelines.

Acute toxicity test

The acute toxicity study was carried out on the basis of the method described by Lorke (1983). The MeOH extract of *S. lappa* was administered orally in doses of 2000, 3000 and 5000 mg/kg body weight to the animals, because lower doses were not found toxic. The animals were observed for signs and symptoms of toxicity including mortality for 48 h. The final lethal dosage (LD_{50}) was calculated as the square root of the product of the lowest lethal and highest non lethal dose (that is, geometric mean of consecutive doses for which 0 and 100% survival of rats were recorded). The extract had an LD_{50} of 2215.8 mg/kg body weight.

Castor oil induced diarrhea

Five groups of Wistar rats (210 to 230 g), each group consisting of 5 animals, were used for the study. Group I was kept as control, providing only saline while group II, III and IV were considered as test groups, and the plant extracts (100, 300 and 500 mg/kg body weight) were administered orally using gavage. These doses were prepared in saline as solutions and administered to respective rats groups. The fifth group received the standard drug loperamide (5 mg/kg body weight). After one hour, each experimental animal received castor oil (1 ml/kg body weight basis) orally by gavage. For an early protection of diarrhea, *S. lappa* was administered 1 h before castor oil administration. The animals were placed in individual transparent plastic container over clean non-wetting paper. Diarrhea was determined by the presence of fluid material in the stool, which stained the absorbent paper placed beneath at the cage. Stools were collected on non-wetting paper sheets of uniform weight, up to 24 h after administration of the castor oil. The urine was drained off every 15 min during the first 8 h by gravity, and the net stools weights were recorded. The time between castor oil administration and the occurrence of first diarrheal output was considered as diarrhea free period, while the period between the first and the last diarrheal output of the 8 h observation was considered as acute diarrheal phase. The stools occurring between 8 and 24 h after castor oil administration are considered as late diarrheal excretion (Pazhani et al., 2001). The total score of diarrheal stool for control group was considered as 100%, and results were expressed as percentage of inhibition of diarrhea.

Gas chromatography-mass spectrometry (GC-MS) analysis

Qualitative and quantitative analysis of extracted essential oil of *S. lappa* was performed on Perkin-Elmer-Clarus-500 GC equipped with Clarus-500 MS and capillary column (60 m × 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were 210 and 280°C, respectively, while helium was used as carrier gas. Oven temperature was held for 5 min at 50°C with 5 min solvent delay, then programmed at 3°C/min up to 220°C/min, and then maintained isothermally at 220°C for 20 min. GC-MS was operated in electron ionization (EI) mode at 70 eV.

Statistical analysis

Results were presented as mean ± standard deviation (SD), and the data were analyzed by one way Analysis of variance (ANOVA) at 99% confidence level ($p < 0.01$).

Table 1. Antidiarrheal activity of methanol extract of S. *lappa* on castor oil induced diarrhea in rats.

Treatment	Dose (mg/kg)	Weight of stool (g) mean ± SD	% inhibition of diarrhea
Saline (control) + castor oil	5 ml	3.19±0.24	-
Lopeamide (standard) + castor oil	5	1.02±0.72*	68.02
MeOH extract + castor oil	100	2.35±0.21*	26.33
MeOH extract + castor oil	300	2.16±0.35*	32.28
MeOH extract + castor oil	500	1.06±0.14*	66.77

*****Statistically significant ($p < 0.01$) relative to control, SD = standard deviation.

RESULTS AND DISCUSSION

The data of antidiarrheal activity test of methanol extract of *S. lappa* roots on Wistar rats are presented in Table 1. It was observed that administration of a dose of 100, 300 and 500 mg/kg body weight showed 26.33, 32.28 and 66.77% inhibition of diarrhea, respectively. The standard drug (loperamide) at the dose of 5 mg/kg body weight showed significant reduction (68.02%) in diarrheal stool. Our results revealed that the dose of 500 mg/kg body weight showed almost similar effect to that of standard drug loperamide in reducing diarrheal stool, while the extract at doses of 100 and 300 mg/kg body weight also exhibited remarkable reduction in diarrhea.

Hemamalini et al. (2011) reported that methanol extracts of *Anogessius acuminate* at the dose of 300 mg/kg body weight exhibited 57.05% inhibition of diarrhea similar to standard drug (5 mg/kg diphenoxylate), while in present study, methanol extract of *S. lappa* roots exhibited 32.28% inhibition of diarrhea at this dose. The protective role of the extract at 500 mg/kg was comparable to that of the reference drug, loperamide (5 mg/kg). Comparison of our results with these findings clearly shows that the MeOH extract of *S. lappa* exhibited significant antidiarrheal activity.

Hydro-distillation of *S. lappa* (roots) yielded pale yellow colored oil (yield 0.23% w/v). The individual constituents were separated by gas chromatography and identified by comparing their MS to those of National Institute of Standards and Technology, U.S. Department of Commerce (NIST) and Wiley (John Wiley & Sons Ltd) mass spectral libraries. Upon GC-MS analysis, the hydro-distilled oil was found to contain 42 constituents eluted between 10 to 65 min. Among these constituents, 12 are found to be major constituents representing 58.18% of the oil, which are mainly comprised of sesquiterpenes.

In this study, the major component of the essential oil resulted to be β-costol (13.55%) and δ-elemene (12.69%). Other compounds characterizing from *S. lappa* essential oil were α-selinene (5.02%), β-selinene (4.47%), α-costol (4.02%), 4-terpinol (3.38%), elemol (3.21%), α-ionone (3.13%), β-elemene (3.00%), (-)-γ-elemene (2.08%), p-cymene (1.96%) and 2-β-pinene (1.57%). Maurer and Grieder (1997) had also reported (-)-α-selinene, (+)-selina-4, 11-diene, (-)-α-*trans*-bergamotene, (-)-α-costol, (+)-γ-costol, (-)-elema-1,3,11 (13)-trien-12-ol, (-)-α-costal, (+)-γ-costal, (-)-elema-1,3,11(13)-trien-12-al, (-)-(E)-*trans*-bergamota-2,12-dien-14-al, (-)-*ar*-curcumene and (-)-caryophyllene oxide as major components of *S. lappa* essential oil.

In another study, Liu et al. (2012) identified 39 components from the essential oil of *S. lappa* roots. The principal compounds in *S. lappa* essential oil were dehydrocostus lactone (46.75%), costunolide (9.26%), 8-cedren-13-ol (5.06%) and α-curcumene (4.33%). However, the percentages of all these compounds greatly varied in the other reported studies. Existing variations in essential oil composition of *S. lappa* may be attributed to factors related to ecotype, chemotype, phenophases and the variations in environment conditions such as temperature, relative humidity, irradiance and photoperiod. Moreover, the genetic background may also affect the chemistry of secondary metabolites of plants (Marotti et al., 1994).

Conclusion

The MeOH extract of *S. lappa* significantly protected the rats against diarrhea evoked by castor oil in dose dependent manner. β-Castol and δ-elemene were found as major components in the extracted essential oil. Based on variations in the composition of the essential oil, it can be concluded that the studied accession seems quite different from those investigated in the past. The results of the present study would be useful in promoting research, aiming at the development of a new agent for diarrhea control based on bioactive chemical compounds from *S. lappa*. Further research is needed to fractionate the MeOH extract and isolate components responsible for the antidiarrheal activity.

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