

Original Research Article

Phytochemical profiling of Costus (*Saussurea lappa* Clarke) root essential oil, and its antimicrobial and toxicological effects

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Abstract

Purpose: To carry out gas chromatography-mass spectrometry (GC-MS) analysis of the phytochemical content of the root essential oil of *Saussurea lappa* Clarke Asteraceae (Costus, SLEO), and to evaluate its physicochemical, antimicrobial and cytotoxic properties.

Methods: The oil was extracted from the plant's roots by steam distillation using a Clevenger system. Various physicochemical parameters for the oil including refractive index, color, acid value, saponification number, ester and peroxide values were measured. Flavonoid content was assessed using thin layer chromatography (TLC). Thermoscientific trace ultra gas chromatograph equipped with a Thermoscientific capillary TR-5MS column was utilized to determine the volatile components of SLEO. Antimicrobial activity of SLEO was performed against various Gram (+ve) and Gram (-ve) microorganisms, viz, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, while cytotoxic effect was monitored using *Artemia salina* (brine shrimp) lethality assay.

Results: Essential oil yield was good (3 %). Concentration-dependent antimicrobial effects were observed on all test microorganisms and no marked difference in lethality levels was observed among the tested SLEO concentrations on brine shrimp ($p < 0.05$). The main component of SLEO was costunolide or eudesma-5,11(13)-dien-8,12-olide (52.01 %).

Conclusion: The results indicate the promising therapeutic properties of *S. lappa*. However, further phytochemical and biological investigations are required to establish the mechanism of action and toxicological the extract.

Keywords: *Saussurea lappa*; Antimicrobial, Essential oil, Brine shrimp lethality

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INTRODUCTION

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locally known as Costus or kuth, is native to South Asia [2]. Thin layer chromatography was utilized for the determination of flavonoid content [11]. Essential oils from the roots of this plant are utilized intensively in conventional medicine [3].

As a folklore medicine, this plant has been used in a variety of conditions, including convulsion, duodenal ulcer, tumors, liver injuries, arthritis and microbial infections [4,5].

These traditional uses were mainly reported in India and Arabic region [5]. A review published by Bajrai in 2010 reported that this plant is used in Saudi Arabia for the treatment of female complaints, carminative and cough [6]. It has also been used for the treatment of tonsillitis in children [7]. *Saussurea lappa* contains various compounds including costunolide and isodihydrocostunolides which are known to be biologically active and promising resource for developing new medications [4].

Owing to its diverse and important biological activities, *S. lappa* has shown to have substantial possibility to emerge as a new drug. Antimicrobial agents obtained from plant sources are presently contributing to human health. Previously, antibacterial, antiviral, antifungal and antiprotozoal studies carried out on *S. lappa* collected from other regions, have revealed promising results [8]. This spectrometer coupled to thermo GC-MS ultra led to the present investigation of the essential oils obtained from the root of *S. lappa* obtained from southern Saudi Arabia against various bacterial and fungal strains. No previous reports have shown the biological activities of *S. lappa* root essential oil (SLEO) extracted from the plants used in Saudi Arabia.

EXPERIMENTAL

Extraction of essential oil

The plant roots were collected from an exclusive shop in Jazan, Saudi Arabia. Oil extraction was performed on 400 g coarsely powdered roots by steam distillation using Clevenger system, operated for 3 h. The aqueous residue obtained was extracted with dichloromethane (3 × 50 mL) and the combined organic phase was dried with sodium sulphate, filtered and the evaporated under reduced pressure until dryness. The Stock of SLEO obtained was kept in the refrigerator till further use.

Determination of physicochemical properties

The refractive index and density of *S. lappa* essential oil (SLEO) were measured using standard methods [9]. A digital refractometer (Mettler Toledo, Columbus, USA) was used for the determination of refractive index of oil. Standard procedures were followed for the determination of color, acid value, saponification number, ester value and peroxide value of the oil

Antimicrobial activity

The paper disc diffusion method was used to screen the antibacterial activity of SLEO and performed by using Mueller Hinton agar (MHA) medium. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [12]. Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/mL (turbidity = McFarland standard 0.5). An aliquot of 100 μ L of bacterial suspension was swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 min. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of MHA and soaked with 20 μ L solutions of each SLEO concentration prepared in DMSO. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured. Tested microorganisms were four bacterial strains *Bacillus subtilis* (NCTC 8236, Gram +ve), *Staphylococcus aureus* (ATCC 25923, Gram +ve), *Escherichia coli* (ATCC 25922, Gram -ve), *Pseudomonas aeruginosa* (ATCC 27853, Gram -ve) as well as two fungal strains, *Aspergillus*

niger (ATCC9763) and *Candida albicans* (ATCC7596).

Brine shrimp lethality assay

Bio-safety and possible cytotoxicity of SLEO was monitored using the *Artemia salina* (brine shrimp) lethality assay [13]. Approximately, 50 mg of *A. salina* (leach) eggs were added to a hatching chamber containing artificial sea water (75 mL). The hatching chamber was kept under an inflorescent bulb for 48 h for the eggs to hatch into shrimp larvae. Accurately weighed quantity (20 mg) of SLEO was dissolved in 2 mL methanol, and 500, 50 and 5 µL of the resulting solution were transferred to vials corresponding to 1000, 100 and 10 µg/mL concentrations respectively. Ten larvae of *A. salina* leach (taken 48 to 72 h after the initiation of hatching) were added to each vial and the final volume of solution in each vial was adjusted to 5 mL with sea water immediately after adding the shrimps. One drop of dimethylsulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. Each dose was tested in triplicate.

Statistical analysis

Data were statistically analyzed using SPSS (IBM Inc, USA) version 20 software and are reported as mean ± standard error of mean (SEM). Differences between groups were accordingly assessed with the suitable inferential statistical test. *p*-value of less than 0.05 was considered as a border type-1 error.

RESULTS

Physicochemical properties

Various physicochemical properties of the essential oil were determined and the values obtained are summarized in Table 1. It was obtained as a reddish yellow liquid in good yield of 12 g (3 %). The thin layer chromatography

(TLC) analysis showed presence of flavonoids in high concentration.

Table 1: Physicochemical properties of *Saussurea lappa* root essential oil

Parameter	Value
Yield	3 %
Specific density	0.0479
Refractive index	15.5
Color	Reddish yellow
Acid value (mg KOH/g)	19.63:5
Saponification number	130.4007
Ester value	110.8172
Peroxide value	2.5
Flavonoid	+++

Antimicrobial activity

The antibacterial activity of SLEO was tested against strains of *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis* using paper disc diffusion method. The inhibition zone diameter (Mean ± SEM) was used to measure the efficacy of SLEO as antimicrobial agent. A concentration dependent antibacterial effect was observed on all tested bacterial strains. The fungal strain *C. Albicans* was also susceptible to SLEO and this effect was also dose-dependent as shown in Table 2.

Cytotoxicity

Brine shrimp (*Artemia salina*) lethality assay was performed to test the toxicity of SLEO and the results obtained are shown in Figure 1. *S. lappa* essential oil showed no major difference in the lethality levels at various concentrations tested. When tested at high concentration (1000 µg/mL), it showed *A. salina* larvae mortality rate of 13%.

Phytochemical composition of SLEO

Gas chromatography-mass spectrometry analysis of the SLEO was carried out using an improved method and different peaks corresponding to various constituents were obtained in the GC spectra as depicted in Figure 2.

Table 2: Antimicrobial activities of *Costus roots essential oil* (*Saussurea lappa* Clarke)

Conc. of SLEO (µg/mL)	Zone of inhibition (mm, mean ± SEM)				
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>
DMSO	--	--	--	--	--
6.25	16 ± 0.89	14 ± 0.4	20 ± 2.8	20 ± 0.2	12 ± 1.7
12.5	17 ± 1.8	14 ± 0.8	25 ± 0.9	21 ± 0.6	13 ± 0.5
25	18 ± 2.5	15 ± 2.7	27 ± 1.4	21 ± 1.8	14 ± 2.6
50	19 ± 0.9	16 ± 0.9	28 ± 2.4	22 ± 2.1	14 ± 1.9
100	21 ± 1.2	17 ± 1.1	29 ± 5.2	25 ± 2.8	15 ± 1.1

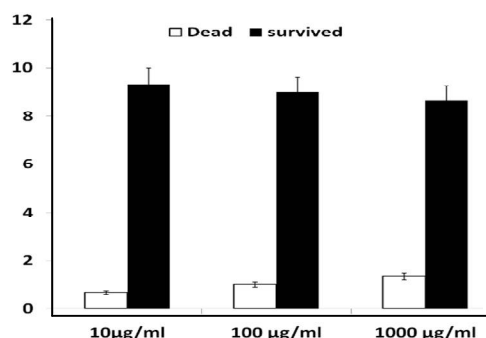


Figure 1: Brine shrimp lethality of *Costus roots* essential oil (*Saussurea lappa* Clarke). Brine shrimp were incubated at 10 shrimps/vial. Various concentrations of SLEO were tested and number of dead and survived larvae of *A. salina* was obtained

The major components of SLEO are listed in Table 3. Costunolides, which is chemically, Eudesma-5,11(13)-dien-8,12-olide (52.01 %; Figure 3), was the major compound detected in SLEO followed by elemene (7.18 %), phenanthrene (2.97 %), caryophyllene oxide (2.39%), 9,12-octadecadienoic acid (Z,Z)-(2.13 %), cyclohexane (2.12 %), germacra-1(10), 4,11(13)-trien-12-oic acid, 6 α -hydroxy-, γ -lactone, (E,E)- (1.96 %), androstan-17-one, 3-ethyl-3-hydroxy-, (5 α)- (0.82 %), bicyclo[10.1.0]tridec-1-ene (0.81 %), naphthalene (0.74 %), cedren-13-ol, 8- (0.62 %) and 4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene (0.47 %). Minor Compounds with less than 0.4 % were not reported.

DISCUSSION

Physicochemical properties, spectroscopic investigations, antimicrobial assay and lethality test were conducted to characterize and evaluate the biological activities of the essential oil of *Saussurea lappa* roots. Physicochemical properties of SLEO investigated in this study included specific density,

refractive index, acid value, saponification number, ester value and peroxide value. Previously, Liu *et al* has reported the extraction of essential oil of *S. lappa* with a yield of 0.89 % (v/w), and the density of the essential oil was calculated to be 0.99 g/mL [14]. Physicochemical parameters such as specific gravity and refractive index are significant in quantitative assessment of oxidative stability of fat and oils.

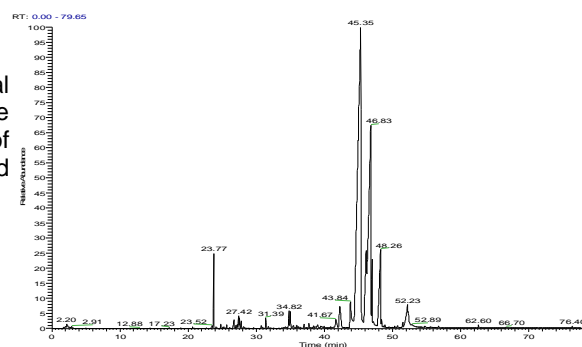


Figure 2: Total ion chromatogram of SLEO

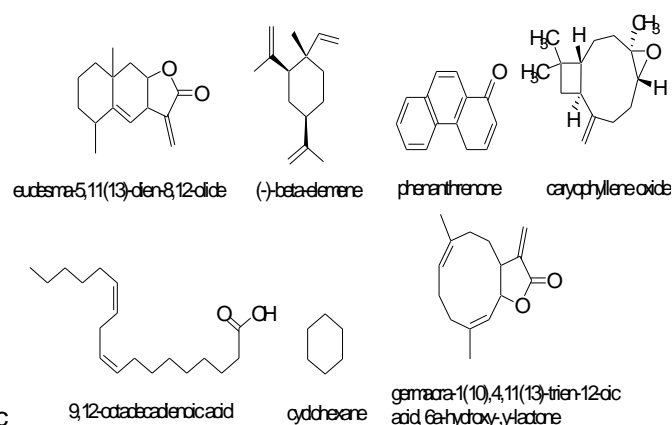


Figure 3: Major components detected in the essential oil of *Saussurea lappa*

Table 3: The major components of *Costus roots* essential oil (*Saussurea lappa* Clarke)

Compound	Area (%)	Retention time (min)
Eudesma-5,11(13)-dien-8,12-olide	52.01	45.30
Elemene	7.18	48.21
Phenanthrene	2.97	46.11
Caryophyllene oxide	2.39	42.27
9,12-Octadecadienoic acid (Z,Z)-	2.13	52.20
Cyclohexane	2.12	23.76
Germacra-1(10),4,11(13)-trien-12-oic acid,6 α -hydroxy-, γ -lactone, (E,E)-	1.96	43.84
Androstan-17-one, 3-ethyl-3-hydroxy-, (5 α)-	0.82	41.68
Bicyclo[10.1.0]tridec-1-ene	0.81	34.81
Naphthalene	0.74	27.42
Cedren-13-ol, 8-	0.62	35.00
4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	0.47	26.71

Polyphenolic content of extracts and oils of plant origin have shown tremendous biological and economic values in the past. Awareness regarding emerging natural nutritional antioxidants is rising due to their well-recognized impact on human wellbeing. The current study also investigated the flavonoid content of SLEO using TLC analysis which showed high content of flavonoids. Previous research showed that the *S. lappa* extract possesses alkaloids, carbohydrates, glycosides, phenolic compounds and saponins [15]. The brine shrimp lethality assay represents a quick, low-priced and straight-forward bioassay for the investigation of safety related to plant products. The findings of the toxicity assay of SLEO using brine shrimp revealed no significant difference in the lethality levels at the concentrations tested with 13 % mortality rate of the *A. salina* larvae at the highest concentration (1000 µg/mL) tested. The results obtained suggested the safety of SLEO use on human beings with no or little cytotoxic effects expected. However, further studies are required to establish the safety of SLEO on human. Disc diffusion assay is one of the most commonly used techniques for the assessment of antibacterial properties of medicinal plants. This method had already been used elsewhere to assess the antibacterial activity of *S. lappa* roots extract previously [18]. However, this recognized and reliable technique was re-applied to examine the antimicrobial activities of the obtained SLEO sample using two gram positive strains, *B. subtilis* and *S. aureus*; two gram negative strains, *E. coli* and *P. aeruginosa* and two fungal strains, *A. niger* and *C. albicans*. Concentration dependent antibacterial effects were observed on all the tested microbes. Acylated flavone glycosides were detected in the roots of the *S. lappa* and these glycosides are known to be the reason of antifungal activity of this plant [18]. Major components of SLEO represented 74.22 % of the total oil. The chemical composition of the essential oil of *S. lappa* essential oil was similar to that reported in other studies [17]. In this study, the essential oil from the roots was obtained in higher yield (3%) than previous studies. Two previous studies reported that the hydro-distillation of *S. lappa* (roots) yielded oil at 0.23% [18] and 0.89% [17] (v/w), respectively. Preceding 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 [18]. However, the chemical composition of the SLEO obtained in this study showed some dissimilarity from other reported studies, which may be due to time of harvesting, local, climatic and seasonal issues as well as the storage time of plant roots.

CONCLUSION

The extracted SLEO in this study showed different type and concentration of chemical constituents than the oil from other reported studies. It suggested that the oil composition depends upon a variety of environmental factors. The results of antimicrobial and toxicity studies supported the presence of active antimicrobial chemical constituents in SLEO with lesser toxicity. Additional studies are required to establish the mechanism of its antimicrobial effects and toxicity on other living systems. This may lead to the development of new plant based antimicrobial drug which could combat the problem of microbial resistance with lesser toxicity to the host.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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