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

INTRODUCTION

Essential oils from herbal sources possess versatile properties and have been revealed presence of several important constituents. Aromatic and medicinal properties and have been utilized as cure for various diseases [1]. Phytochemical investigations of these substances

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locally known as Costus or kuth, is native to South[10]. Thin layer chromatography was utilized for Asia [2]. Essential oils from the roots of this plant the determination of flavonoid content [11], are utilized intensively in conventional medicine [3]. As a folklore medicine, this plant has been used in a Gas chromatography–mass spectrometry variety of conditions, including convulsion, duodenal(GC-MS) ulcer, tumors, liver injuries, arthritis and microbial infections [4,5]. These traditional uses were mainlyGC-MS analysis of SLEO was performed using reported in India and Arab region [5]. A review Thermoscientific trace ultra Gas Chromatograph published by Bajrai in 2010 reported that this plant(Thermo Fisher Scientific, MA) equipped with a used in Saudi Arabia for the treatment of female Thermoscientific capillary TR-5MS column (30 m complaints, carminative and cough [6]. It has also × 0.25 mm ID × 0.25 μm). For GC-MS detection, been used for the treatment of tonsillitis in children electron ionization system with ionization For S. lappa collected from other the total area of all components. Mass regions, have revealed promising results [8]. This spectrometer coupled to thermo GC-MS ultra led to the present investigation of the essential oil system was set to scan time 0.6 sec and mass obtained from the root of S. lappa obtained from range 50 – 800 amu. X-Calibur software was southern Saudi Arabia against various bacterial and used to interpret mass spectra and fungal strains. No previous reports have shown thechromatograms and the fragmentation patterns biological activities of S. lappa root essential oil of mass spectra were compared with those (SLEO) extracted from the plants used in Saudi stored in the spectrometer database using the NIST, MAINLIB and REPLIC built-in libraries.

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 physiochemical 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 10⁵ cfu/mL (turbidity = McFarland standard 0.5). An aliquot of 100 μL 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 μL 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.

| 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.
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 %), phenanthrenone (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, 6α-hydroxy-γ-lactone, (E,E) - (1.96 %), androstan-17-one, 3-ethyl-3hydroxy-(5α) - (0.82 %), bicyclo[10.1.0]tridec-1-ene (0.81 %), naphthalene (0.74 %), cedren-13ol, 8- (0.62 %) and 4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydrophenanthrene (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 % (w/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 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.

**Figure 2:** Total ion chromatogram of SLEO

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

**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 |
| Phenanthrenone | 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,6α-hydroxy-γ-lactone, (E,E) | 1.96 | 43.84 |
| Androstan-17-one, 3-ethyl-3-hydroxy-(5α) | 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-octahydrophenanthrene | 0.47 | 26.71 |

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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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