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On the chemical composition and antibacterial activity of *Saussurea lappa* (Asteraceae)

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Abstract

The black root of *Saussurea lappa* (Asteraceae), an aromatic perennial plant growing in the open slopes of India and Kashmir, are used in traditional medicine for treatment of several diseases and ailments. The present study was carried out to extract the root available in the local market with solvents increasing in polarity, to assess their antibacterial activity and to identify some biologically active constituents by chromatographic and spectroscopic methods. Phytochemical screening of the root extracts indicated the presence of alkaloids, coumarins, flavonoids, sterols, saponins and tannis. The petroleum ether, chloroform, methanol and water extracts were assessed by the cup plate diffusion method against two standard Gram positive and three standard Gram negative bacteria: *Bacillus subtilis*; *Staphylococcus aureus*; *Escherichia coli*; *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, respectively. The chloroform extract showed the highest activity. Five compounds have been identified in the active fraction based on ir-spectroscopic and HPLC-MS analysis data. The compounds were: Umbelliferonglucoside, Costunolide, Luteolin-7-O- β -D-glucoside, Rutin and Apigenin-7-O- β -D-glucoside.

Keywords: *Saussurea lappa*, Costus, Antibacterial activity, Chemical composition, ir and hplc analysis.

1. Introduction

The root of *Saussurea lappa* (Asteraceae) known as Costus is used in traditional medicine of many countries in the treatment of several diseases and ailments [1]. Pharmacologic investigations showed that the root possesses anti-cancer, antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, anti-parasitic and anti-ulcer activities [2]. Volatile oils, glycosides, alkaloids, sesquiterpene lactones, steroids and triterpenes have been reported in the plant [3, 4]. These modern investigations and findings provided many of the scientific bases for the traditional use of the plant. The nomenclature confusion between *Saussurea lappa* and *Costus spicatus* has been resolved through anatomical investigations where the former belongs to dicotyledoneae group and the latter to the monocotyledoneae group [5]. The present work attempts to report the activities in the current literature and to spot more light on the uses and chemical composition of samples available in Sudanese markets and to add new findings to the on-going research in this important plant.

2. Materials and Methods

Plant samples collection

The plant materials were collected from herbal shops in Khartoum City (Sudan) and Jeddah (Kingdom of Saudi Arabia) and authenticated by Dr. Nahed Mourad Waly, Associate professor as blackroot of *Saussurea lappa* (Asteraceae), at the taxonomy and Flora section, Botany Department, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia, during 2010.

Extraction of plant material

Powdered black root of *S. lappa* (100 g), was extracted successively with Pet. ether 40-60 °C, chloroform, methanol in a Soxhlet apparatus. The extracts were filtered and evaporated to dryness under reduced pressure using a rotary evaporator (Buchi, Switzerland). The residue after extraction was dried and extracted with distilled water at room temperature for 72 hrs, filtered and water was removed by freeze-drying. The four extracts were used for antibacterial assessment and phytochemical investigations.

Phytochemical screening of the prepared extracts

The prepared extracts were tested for the presence or absence of alkaloids, saponins, cardiac

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glycosides, flavonoids, sterols and triterpenes, sesquiterpene lactons, tannins and sugars, according to methods described by Harborne, (1984) [6] and Sofowora, (1993) [7].

Assessment of antibacterial activity

A total of five bacterial cultures (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were used for assessment against the pet. Ether, chloroform, methanol and water extracts of black root. The antibacterial activity was assessed according to the cup – plate – agar diffusion method described by Kavanagh, (1972) [8].

Chromatographic and Spectroscopic analysis

The chloroform active fraction was investigated by thin layer chromatography (TLC), Infra-red spectroscopy (IR-) and Liquid chromatography mass spectrometry analysis (LC/MS) [9]. TLC was carried out on precoated silica gel plates GF₂₅₄, 0.20 mm thickness and solvent systems (Toluene/ Ethyl acetate 8:2 and Hexane/ chloroform 5:5). Chromatograms were inspected under day light, UV-light at 254 and 366 nm and finally sprayed with vanillin/ conc sulphuric acid. Liquid chromatography mass spectrometry analysis was carried out using HPLC/ESI-MS (Shimadzu, Japan). LC separation was conducted using Shimadzu LCM- 2020 and C₁₈ reversed phase column (5 mm, 4.6 mm × 150 mm, GL, Sciences, Japan), at a flow rate 1 ml/ min. A gradient elution program was conducted for chromatographic separation with acetic acid/ acetonitrile mobile phases at column temperature 30 °C.

3. Results and Discussion

The nomenclature confusion between *Saussurea lappa* and *Costusspicatus* under the same Arabic name (Costus) has been clarified. It was found that through anatomical investigations, the former belongs to dicotyledoneae roup and the latter to monocotyledoneae group [5]. Successive extraction of black root gave the highest yield with methanol followed by water, petroleum ether and finally chloroform: 15.23; 9.86; 4.00 and 2.53%. Phytochemical screening of black root extracts revealed the presence of alkaloids, coumarins, flavonoids, sterols, saponins and tannins (Table 1).

Results of phytochemical screening and % yield were compatible with published literature [12, 13, 14, 15]. The extracts were further assessed for their antibacterial activity against five Gram positive and Gram negative bacteria (Table 2).

Table 1: Results of phytochemical screening of extracts of black root of *S. lappa*

Test \ Extract	Pet. ether	Chloroform	Methanol	Water
Alkaloids	+	+	+	+
Anthraquinones	-	-	-	-
Cardiac glycosides	+	-	+	-
Coumarins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	-	+	+	+
Sterols	+	+	+	-
Tannins	-	-	+	+
Triterpenes	-	+	+	+

(+) - Positive, (-) - Negative

Table 2: Results of antibacterial activity of extracts of black root of *S. lappa*

Extract \ MDIZ (mm)	Pet.ether	Chloroform	Methanol	Water
<i>Bacillus subtilis</i>	21	22	17	-
<i>Staphylococcus aureus</i>	18	18	15	11
<i>Escherichia coli</i>	11	11	-	-
<i>Pseudomonas aeruginosa</i>	-	11	11	-
<i>Klebsiella pneumonia</i>	-	11	12	15

Concentration: 5% of extract in DMSO

MDIZ: Mean Diameter of Inhibition Zone

MDIZ: >15 mm = sensitive; <15 mm = resistant; (-) = no activity

The five bacterial cultures showed variable response to the extracts of the black root. The Gram positive bacteria were more sensitive to the extracts than the Gram negative bacteria. The chloroform extract of the root was the most active, compared to other extracts. Based on these findings, the chloroform extract of the black root was selected for more investigations and analysis by different chromatographic procedures. The active chloroform extract was fractionated using column packed with silica gel and eluted with hexane, chloroform and methanol and fractions were monitored by silica gel precoated plates developed in toluene/ ethyl acetate (8:2) and hexane/ chloroform (5:5) solvent systems. The column fractions were assayed for antibacterial activity to select the most active fraction for further investigations (Table 3).

Table 3: Results of antibacterial activity of column fractions of black root chloroform active fraction.

Column Fraction	Mean Diameter of Inhibition Zone, MDIZ (mm)				
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia Coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>
1	-	18	19	14	-
2	-	18	19	14	-
3	-	14	15	-	-
4	-	14	21	-	-
5	20	21	23	-	-
6	23	21	23	11	13
7	15	15	-	-	-
8	15	14	-	-	-
9	10	15	-	-	-
10	-	-	-	-	-

MDIZ: mean Diameter of Inhibition zone

Concentration: 10 % in methanol

MDIZ:>15 mm = sensitive; <15 mm = resistant; (-) = no activity

Based on the table results, column fraction no. (6) Was the most active and was subjected to HPLC analysis to spot more light on its chemical composition. Five compounds have been identified based on their retention times and the functional groups assigned from the IR-spectrum of the fraction. The presence of -OH groups (alcoholic, phenolic or acidic) was confirmed by a broad and strong absorption at 3402 CM⁻¹. A strong, broad absorption was observed at 1800 CM⁻¹ and extending to 1580 CM⁻¹ and could be interpreted for the presence of molecules with a carbonyl group; carboxylic acids;

anhydrides; acyclic and cyclic esters (lactones). Aliphatic and aromatic double bands were assigned at 1504 and 1469 CM⁻¹ and aromatic stretching at 900-700 CM⁻¹. Absorption at 1427 and 1379 CM⁻¹ were assigned to -CH₂- and -CH₃ groups. Bands at 1300 – 1000 CM⁻¹ confirmed the C-O stretching, while absorption at 1315 may be due to SO₄⁻ functionalities (Table (3);Table (4); Fig (1) and Fig (2)) [14, 15]. The identified compounds were: Umbelliferonglucoside (1); costunolide (2); Luteolin-7-O-β-D-glucoside (3); Rutin (4) and Apigenin-7-O-β-D-glucoside (5)

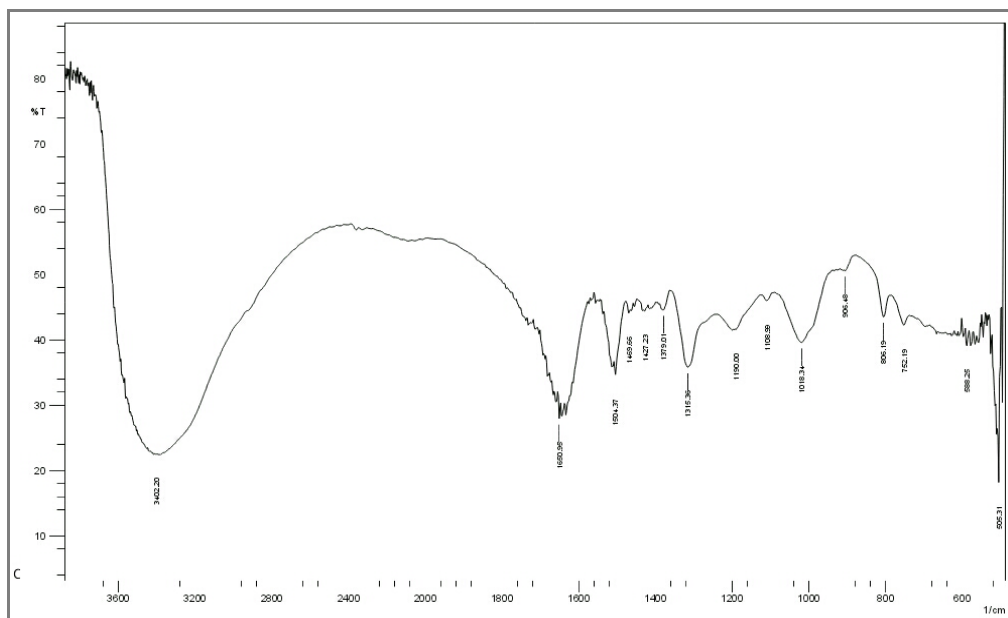


Fig 1: IR- spectrum of the active chloroform fraction of the Black root of *S. lappa*

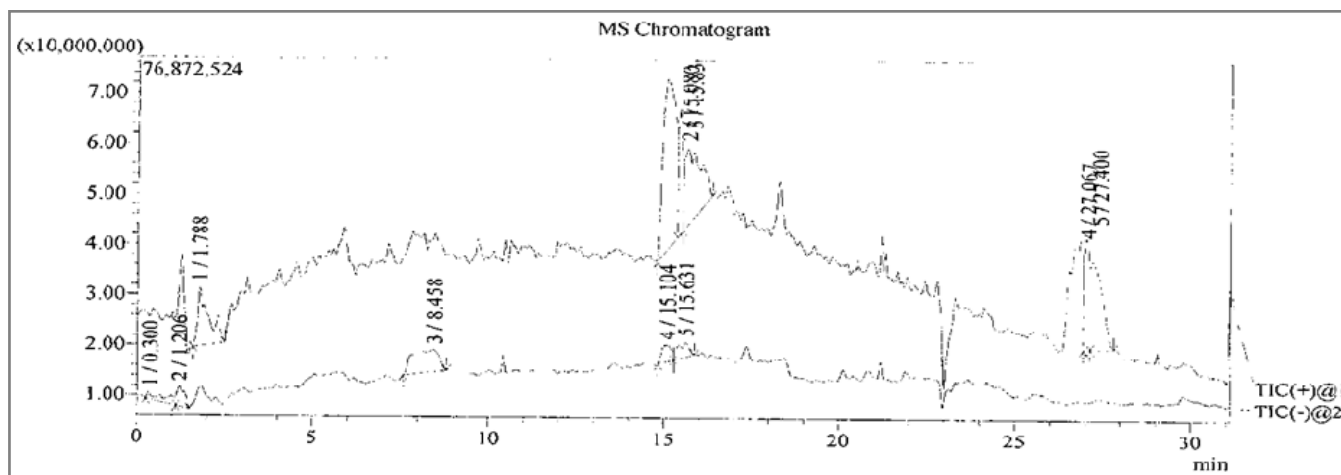
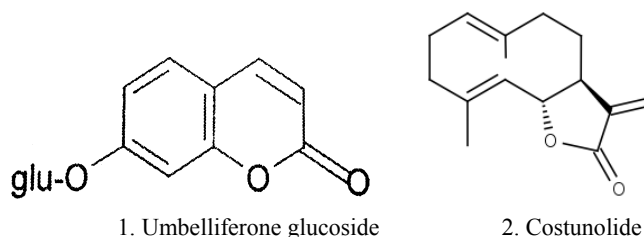
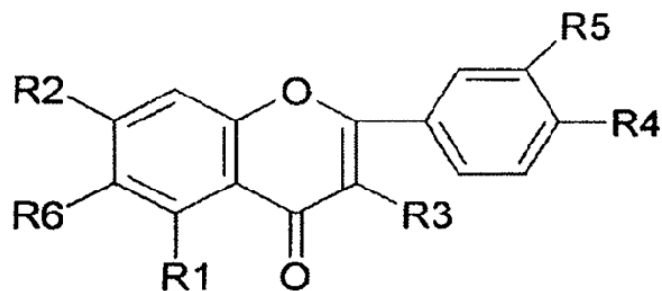


Fig 2: HPLC chromatogram of column fraction (6) of the *S. lappa* black root chloroform extract

Table 4: HPLC analysis of fraction (6) of *S. lappa* black root chloroform extract

Peak No	Retention time RT	Area Under the peak (AUP %)	Name of compound
1	1.78	22	Umbelliferone Glucoside
2	15.08	25.16	Costunolide
3	15.83	34.42	Luteolin-7-O-β-D-glucoside
4	27.06	11.17	Rutin
5	27.40	27.33	Apigenin-7-O-β-D-glucoside





3. Luteolin-7-O- β -D-glucoside; $R_1 = R_4 = R_5 = OH$, $R_2 = O-\beta$ -glu, $R_3 = R_6 = H$
4. Rutin, $R_1 = R_2 = R_4 = R_5 = OH$; $R_3 = O-\beta$ -rha (2-1)-glu, $R_6 = H$
5. Apigenin-7-O- β -D-glucoside; $R_1 = R_4 = OH$; $R_2 = O-\beta$ -glu; $R_3 = R_5 = R_6 = H$

4. Acknowledgement

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5. References

1. Butola JS, Samant SS. *Saussurea* species in Indian Himalayan Region: diversity, distribution and indigenous uses. International journal of plant Biology 2010; 1:43-51.
2. Wang YF, Ni ZY, Dong M, Cong B, Shi QW, Gu YC. *et al.* Secondary metabolites of plants from the Genus *Saussurea*: Chemistry and Biological activity, Chemistry and Biodiversity 2010; 7(11):2633-2807.
3. Dawa Z, Bai Y, Zhou Y, Gesang SAP, Ding L. Chemical constituents of the whole plants of *Saussurea medusa*. J Nat Med 2009; 63:327-330.
4. Sun CM, Syu WJ, Don MJ, Lu JJ, Lee GH. Cytotoxic Sesquiterpene lactones from the root of *Saussurea lappa*. Nat Prod 2003; 66(9):1175-1180.
5. Waly NM. Verifying the Scientific name of Costus [*Saussurea lappa* ((Decne.) C.B. Clarke) – Asteraceae], Journal of king Abdulaziz University Sci 2009; 21(2):327-334.
6. Harborne JB. Phytochemical Methods, a Guide to Modern Techniques of plant analysis, Chapman A and Hall, London, 1984, 279.
7. Sofowora A. Medicinal plants and Traditional Medicines in Africa, Chichester, John Willey and Sons, New York, 1993, 256.
8. Kavanagh F. Analytical Microbiology, Academic press (Pub), New York and London, 1972, 211.
9. Phillipson JD. Phytochemistry and Medicinal Plants, Phytochemistry 2001; 56:237-243.
10. Thara KM, Zuhra KF. Comprehensive *In-vitro* Pharmacological activities of different extracts of *Saussurea lappa*. European Journal of Experimental Biology 2012; 2(2):417-420.
11. Bose BC, Saifi AQ, Vijayvargiya R, Sharma SK. Some aspects of phytochemical and pharmacological study of *Saussurea lappa*. Journal of Pharmaceutical Science, 1961; 50:679-681.
12. Lalla JK, Hamrapurkar PD, Mukherjee SA, Thorat UR. Sensitivity of HPTLC v/v HPLC for the analysis of alkaloid- Saussurine. In: 54th Indian Pharmaceutical Congress, Pune, India, 2002, 293.
13. Rao KS, Babu GV, Ramnareddy YV. Acylated flavone

glycosides from the roots of *Saussurea lappa* and their antifungal activity. Molecules 2007; 12(3):328-344.

14. Rao NR, Raju SS, Babu SK, Vadaparathi RP. HPLC determination of Costunolide as a marker of *Saussurea lappa* and its herbal formulations, international journal of Research in Pharmacy and chemistry 2013; 3(1):99-107.
15. Zhang X, Yu M, Chen J. Simultaneous Determination of Seven Compounds in Snow Lotus herb using High Performance Liquid Chromatography. Journal of chromatography 2003; 41(5-6):241-244.