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Original Article

Antibacterial activity, metabolites and elemental analysis of *Saussurea candicans* C. B. Clarke

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ABSTRACT

Phytochemical analysis of ethanol and water extracts of *Saussurea candicans* has indicated the presence of flavonoides, phenolics, tannins, etc., that supports its use in traditional medicine both for human and animals in different parts of the world. *S. candicans* is a plant of medicinal interest hence selected for analysis. Antibacterial activity of the two extracts was tested against *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Only the ethanol extract was found to be effective. Both aqueous and ethanol extracts were prepared and studied for different phytochemicals such as alkaloids, flavonoides, phenolics, and steroids and also for their antibacterial activity using agar well diffusion method. The Fourier transform infrared and wavelength dispersive X-ray fluorescence spectroscopy of whole plant powder were done to study the functional groups and elemental profile, respectively. The aqueous extract contains more (11) phytochemicals as compared to ethanol (9). No zone of inhibition was observed at different volumes of the extract, i.e., 20 µl, 50 µl and 100 µl but at 150 µl, and 200 µl concentration of 230.7 mg/ml plant extracts, the zone of inhibition was reported. The dose of 150 µl concentration active only against *E. faecalis*, whereas, 200 µl concentration showed activity against all bacterial strains. Only the ethanol extract had antibacterial activity against all the tested bacteria. The medicinal activity of *S. candicans* is assumed to be because of the presence of different phytochemicals and elements. Further study can be conducted to understand the role of each and every reported phytochemicals and elements.

KEY WORDS: Bacterial strains, fourier transform infrared, medicinal value, phytochemicals, *Saussurea candicans*, wavelength dispersive X-ray fluorescence

INTRODUCTION

Saussurea DC. is an important genus of the family *Asteraceae* comprising nearly 410 species out of which 61 of these are growing in India. *Saussurea candicans* C. B. Clarke (syn. *Saussurea heteromalla*; accepted name *Himalaiella heteromalla* [D. Don] Raab-Straube) is an annual, tall, herbaceous plant. It grows profusely in the Shivalik ranges, the foothills of Himalayas extending to higher altitudes of Himalayas from Jammu and Kashmir Eastwards to Arunachal Pradesh (Saklani and Rao, 2000).

The leaves are arranged in a dense basal rosette form having lyrate-pinnatifid basal leaves and upper leaves with white tomentose on abaxial side. Capitulam inflorescence has purple flowers with cottony involucral bracts in 3-5 series. The fruits are achenes; 4-5 angled with muricated surface and possess white pappus (Hajra, 1996). The whole plant or different parts have high ethnomedicinal importance. The seeds have carminative and styptic properties and cooling effect; leaves are useful in the treatment of horse bite (Jain, 1991; Ambasta, 1994; Rana and Samant, 2011; Bhatia *et al.*, 2014; Shedayi *et al.*, 2014). Plant extract is used to cure inflammation, rheumatoid arthritis, cough with cold, stomach-ache, dysmenorrhea, altitude sickness, cancer, bacterial diseases, wounds and fatigue (Ahmad *et al.*, 2009; Bisht and Purohit, 2010; Habiba *et al.*, 2016). In view of its importance in traditional and modern medicines, this study has been undertaken to analyze the antibacterial activity of this species. Efforts have also been made to screen various phytochemicals using different extracts and to study the elemental profile of this plant species. This will help in establishing the relationship between chemical properties of the plant and its curative potential. This investigation is likely be useful for different research groups as there are no such previous reports for this plant to the best of our knowledge and literature.

MATERIALS AND METHODS

Collection of Material and Preparation of Extracts

The plant material (whole plant) was gathered from the campus, Panjab University, Chandigarh. The washing of material was done with running tap and distilled water (DW). A herbarium sheet was prepared from the dried plant specimen. The plant specimens were compared with the herbarium of the Botany Department, Panjab University, Chandigarh, and deposited in the herbarium (PAN No. 20330). The powder was made from a dried material with the help of electric grinder and kept in a polythene bags. The extracts were prepared as follows:

Aqueous extract

Plant powder (20 g) was mixed into 100 ml DW taken in a conical flask and kept for 24 h on orbital shaker. The mixture was filtered through muslin cloth and then through Whatmann's No. 1 filter paper. The extract was added to vials and kept in the refrigerator at 4°C until further use (Gupta *et al.*, 1996). The extract was 200 mg/ml in concentration.

Ethanol extract

The ethanol extract of powdered plant material was prepared using the Soxhlet apparatus (Yadav and Agarwala, 2011). 10 g powder was extracted with 130 ml of ethanol at 60°C. The extract was evaporated to its 1/3rd volume at room temperature and stored in vials at 4°C.

Phytochemical Screening

Screening of phytochemicals both in aqueous and ethanol extracts of *S. candicans* was done following the procedure of the previous studies (Sidhu and Sharma, 2016; Thakur and Sidhu, 2013).

Antibacterial Assay

Bacterial strains

The plant extracts will be studied for their activity against following bacteria:

Gram-positive bacteria	*Enterococcus faecalis* (MTCC 439)
	Staphylococcus aureus (ATCC 25923)
Gram-negative bacteria	*Escherichia coli* (ATCC 25922)
	Pseudomonas aeruginosa (ATCC 27853)

Inoculum Preparation

From a streak plate, a single colony of bacterial strain was inoculated in 10 ml Mueller Hinton Broth (MHB) media (g/L: Beef infusion 300, casein acid hydrolysate 17.5, starch 1.5, and pH 7.4 ± 0.1) in the tubes. These tubes were incubated for 16-18 h at 37°C/200 rpm. This is a primary culture. Now, 100 µl of primary culture was inoculated into 10 ml MHB media. The tubes were incubated at 37°C/200 rpm for 16-18 h until absorbance reaches 0.3 at 600 nm.

Bacterial Susceptibility Test

The antibacterial activity of extracts was studied through agar well diffusion method. The zone of inhibition if any was measured (diameter in millimeter around the well). 100 µl of culture was spread on Mueller-Hinton agar plates and allowed to dry. A sterile cork borer was used to make wells in agar media. Sterile water and ethanol were used as a control. The sterile discs were filled aseptically with the different volumes of the extract, i.e., 20 µl, 50 µl, and 100 µl initially and plates were then kept at 37°C/overnight. Inhibition zone was checked after 24 h. If no zone of inhibition was observed, the experiment was repeated and this time each well was loaded with 150 µl and 200 µl concentration of 230.7 mg/ml plant extracts. Further, the time interval was also increased for 48 h. It has been noted that activity of the extracts remained similar to 24 h. However, contamination has been observed in some cases near 48 h.

Fourier transform infrared (FTIR) spectroscopy for functional groups has been done using Perkin Elmer Spectrum 400 FTIR/FTFIR spectrometer and wavelength dispersive X-ray fluorescence (WD-XRF) analysis for elemental detail was carried out at Sophisticated Analytical Instrumentation Facility, CIL and UCIM, Panjab University, Chandigarh.

RESULTS AND DISCUSSION

Phytochemical Study

Phytochemical screening of both (aqueous and ethanol extract) of whole plant powder of *S. candicans* has been done. Various phytochemicals reported during the analysis were presented in Table 1. The phytoconstituents such as gums and mucilages, phlobatannins, and saponins were not present in ethanol extract. The number of different phytochemicals obtained in ethanol extract was lesser as compared to aqueous extract but in higher concentration (Table 1). The ethanol is efficient in the cell wall and seed degradation thus supposes to release more chemical compound from the material under investigation (Lapornik *et al.*, 2005). Previously quantitative analysis of flavonoides carried out in the ether extract of *S. heteromalla* and recorded the amount of flavonoids as 3.9 ± 1.0%

in the extract (Yaseen *et al.*, 2014). However, the pharmacologically importance of arctigenin, arctiin and chlorojanerin of this species were examined (Saklani *et al.*, 2011). Arctigenin and arctiin were known to be anti-inflammatory whereas, chlorojanerin possess antiulcer and antiviral properties. Both the extracts were studied for their antibacterial activity against four bacteria during the present investigation (Table 1).

Antibacterial Activity

The extracts showed inhibitory activity against bacterial isolates *Enterococcus faecalis* (MTCC 439), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923) (Figure 1a-d). In this study, *E. coli, P. aeruginosa,* and *S. aureus* strains have shown resistance against *S. candicans* ethanol extract at 34.605 mg/ml conc. A 4 mm zone of inhibition was observed against *E. faecalis* (Figure 1a.6A). However, a dose of 46.2 mg/ml ethanol extract has formed 11 mm, 9 mm, 8 mm, and 5 mm inhibition zones against *P. aeruginosa*, *S. aureus*, *E. coli,* and *E. faecalis,* respectively, (Figure 1c6,d6,b6 and a6B). This study on antibacterial activity of *S. candicans* is likely to be the first. The anti-inflammatory activity of *Saussurea heteromalla* was due to the presence of chlorojanerin (a guaianolide type of sesquiterpene lactone) (Saklani *et al.*, 2012).

The size of zone of inhibition is related to the dose and concentration of the ethanol extract (Figure 1). The inhibition zone ranges between 4 and 11 mm (Figure 2). All the tested bacterial strains were resistant to different doses of aqueous extract. The minimum inhibitory concentration was varied from 4.620 mg/ml to 46.200 mg/µl.

Table 1: Phytoconstituents screening in two extracts of *Saussurea. candicans*

Phytochemical(s)	Test(s)	*S. candicans*	
		Aqueous	Ethanol
Alkaloids	Mayer's reagent test	+	+
Carbohydrates	Molisch test	+++	+++
Flavonoids	NaOH test	+	++
	H_2SO_4 test	+	++
Glycosides	$FeCl_3$ test	+++	+++
Gums and mucilages	-	++	-
Phenolics	-	+++	+
Phlobotannins	-	+++	-
Reducing sugar	-	+++	++
Saponins	Froth test	++	-
Steroids	H_2SO_4 test	++	+++
Tannins	$FeCl_3$ test	++	+++
	KOH test	-	++
Terpenoids	$CHCl_3$ test	+++	+++

Symbols: - (absent); + (present); ++ (moderately present); +++ (present in abundance)

The extracts have shown lesser activity for Gram-negative bacteria. This difference is likely due to variations in the structure of cell wall of bacterial strains. However, during this study, maximum inhibitory activity of the *S. candicans* has been reported against *P. aeruginosa*, Gram-negative bacteria.

FTIR

The FTIR spectra have provided the detail of various functional groups depending on the peak values. Accordingly, these spectra are considered as a "fingerprinting tool." The FTIR spectrum of *S. candicans* has confirmed the presence of different phytochemicals (Figure 3). The functional groups reported for this species have been examined with the available literature (Pavia *et al.*, 2006).

The researchers had applied FTIR spectroscopy as a tool to study the functional groups present in the powder

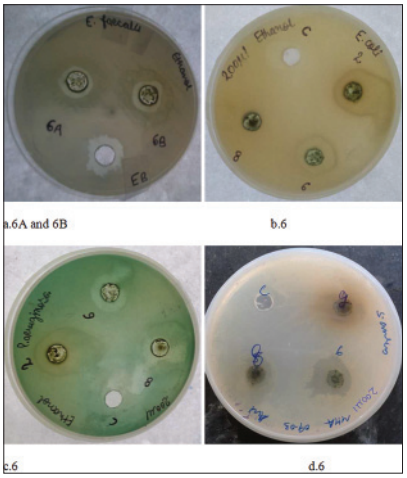

Figure 1: Antibacterial activity of *Saussurea candicans* whole plant extract. (a) *Enterococcus faecalis* (4 mm, 5 mm) (b) *Escherichia coli* (8 mm), (c) *Pseudomonas aeruginosa* (11 mm), and (d) *Staphylococcus aureus* (9 mm)

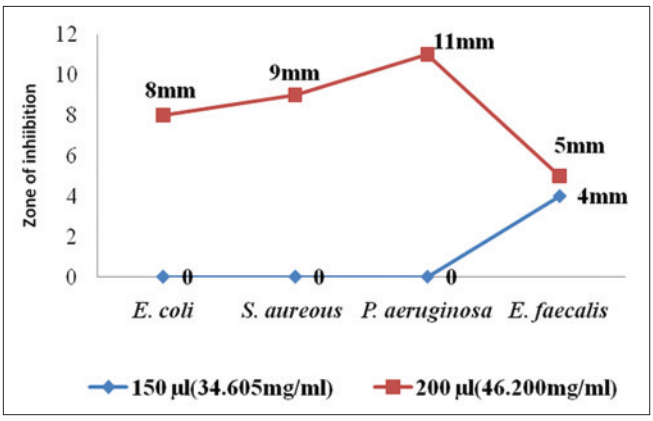

Figure 2: Antibacterial activity

for classification and differentiation between closely related plants and other organisms (Janakiraman *et al.*, 2011; Thenmozhi *et al.*, 2011). Similarly, this study on *S. candicans* has produced novel pharmacognostical and phytochemical markers (Table 2). However, further detailed studies would help to examine the chemistry of each and every chemical compounds.

WD-XRF Spectroscopy

In addition to this, elements such as Al, Ca, Cr, Cu, Fe, Hg, Mg, Ni, Si, Ti, and Zn were also known to inhibit the growth of *E. coli, S. aureus,* and some other bacteria (Niira *et al.*, 1990; Sawai, 2003; Tsuang *et al.*, 2008; Hetrick *et al.*, 2009; Baek and An, 2011; and Ravikumar *et al.*, 2012). Whereas, the inhibitory activity of elements such as S, Cl, Cu, and Zn against *E. faecalis* were also reported (Wright *et al.*, 1998; Aarestrup and Hasman, 2004). Alkaloids and flavonoids were found responsible for antibacterial activity (Phillipson and O'Neill, 1987; Tsuchiya *et al.*, 1996). These findings also supported by other researchers (Ogbulie *et al.*, 2007). According to them, antibacterial activity against *S. aureus* was due to alkaloids, flavonoids, and tannins. Screened alkaloids, flavonoids, saponins, tannins, and terpenoids of the leaf extracts of *Erythrophleum africanum* were active against *P. aeruginosa* (Mohammed *et al.*, 2014). All these phytochemicals are reported in the presently studied species and seems to be responsible for antibacterial activity. WD-XRF spectroscopy has provided data related to various macro and micro elements (Figure 4 and Table 3).

Zinc was also recorded in *H. heteromalla* (Sajad *et al.*, 2016). Earlier reports have suggested the role of different elements against bacteria. Therefore, these are believed to be antibacterial. Thus, it is assumed that antibacterial potential to this plant species may be imparted by the phytochemicals in association with elements.

Table 2: FTIR spectroscopy of *S. candicans*

Mode of vibration (functionality)	Range	IR frequencies
N-H stretch in amines	3500-3300/cm	3279/cm
N-H stretch in amides	3500-3180/cm	
Alkanes sp^3 C-H stretch	3000-2840/cm	2917/cm
C=O stretch in carboxylic acids, esters, aldehydes, ketones, Anhydrides and acid chlorides	1750-1700/cm	1732/cm
C=C stretch	1600/cm	1604/cm
C=O stretch in amides	1680-1630/cm	
N-H bend in 1° amines	1640-1560/cm	
$-CH_2$ bend in alkanes	1465/cm	1429/cm
C=C stretch	1475/cm	
O-H bend in carboxylic acids	1440-1400/cm	
C-F stretch	1400-1000/cm	1370/cm
$-CH_3$ bend in alkanes	1375/cm	
Nitro group has a strong absorption	1390-1300/cm	
C-N stretch in amines	1350-1000/cm	1315/cm
Asymmetric S=O stretch in sulfones, sulfonyl chlorides, sulfates, sulfonamides	1350-1140/cm	
C=O bending in ketones appears as a medium intensity peak	1300-1100/cm	1238/cm
Phenyl alkyl ethers give two strong bonds	1250-1040/cm	
C-O stretch in alcohols, ethers, esters, carboxylic acids, anhydrides	1300-1000/cm	
C-N stretch in amines	1350-1000/cm	
Aryl fluorides absorb	1250/cm	
C-N stretch in amines	1350-1000/cm	1027/cm
C-O stretch in alcohols, ethers, esters	1260-1000/cm	
S=O symmetric stretch, strong	1150/cm	
C-X for bromides/iodide	<667/cm	534 and 406/cm

FTIR: Fourier transform infrared, *S. candicans: Saussurea candicans*

CONCLUSION

The ethanol extract of *S. candicans* possess activity against different bacteria under consideration. This species has been designated as an antibacterial based on the activity shown by extracts and quantification of various useful elements. Thus, its medicinal potential especially antibacterial activity of whole plant ethanol extract is

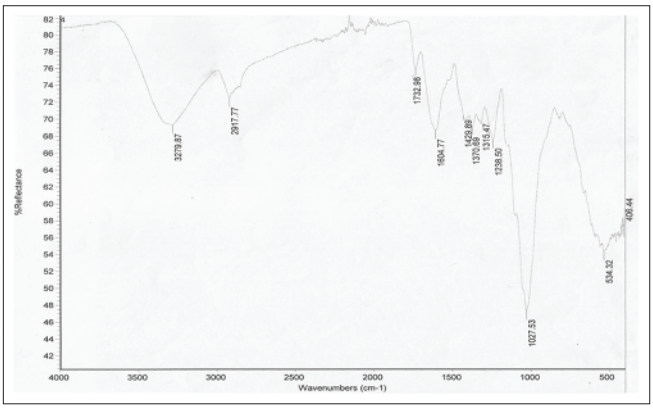

Figure 3: Fourier transform infrared spectrum of whole plant powder of *Saussurea candicans*

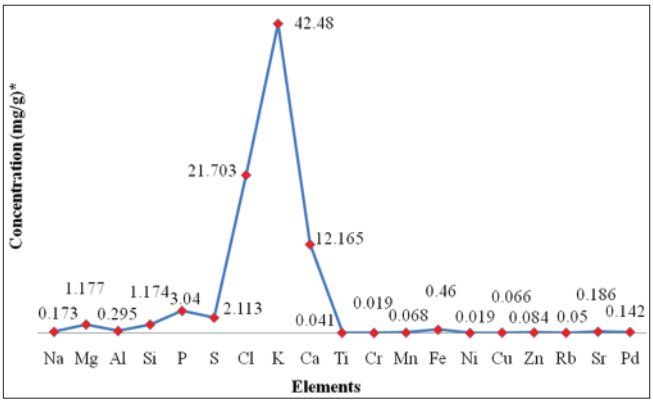

Figure 4: Wavelength dispersive X-ray fluorescence analysis of *Saussurea candicans*

Table 3: Elemental analysis of *S. candicans* (Concentration in mg/g)*

Elements	Concentration	Elements	Concentration
Na	0.173	Cr	0.019
Mg	1.177	Mn	0.068
Al	0.295	Fe	0.46
Si	1.174	Ni	0.019
P	3.040	Cu	0.066
S	2.113	Zn	0.084
Cl	21.703	Rb	0.05
K	42.480	Sr	0.186
Ca	12.165	Pd	0.142
Ti	0.041		

*The error in concentration values of the elements ranges from ~5% up to 10%. *S. candicans: Saussurea candicans*

likely to be because of the presence of phytochemicals and elements. Further investigation is required to understand the role of individual and specific phytoconstituents and elements. To the best of our knowledge and available literature, this study seems to be pioneer in terms of phytochemical, antibacterial, FTIR, and WD-XRF analysis of *S. candicans*.

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