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Antioxidant, antimicrobial, and GC-MS profiling of *Saussurea obvallata* (Brahma Kamal) from Uttarakhand Himalaya

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Abstract

Background: *Saussurea obvallata* (DC.) Edgew. (*S. obvallata*) is an endangered medicinal herb of the high altitude Himalayan region with immense cultural significance. This study is one of the first report of pharmacological evaluation and GC-MS analysis of methanolic extracts of leaves and flowers of *S. obvallata*.

Methods: Total flavonoid content (TFC) and total phenolic content (TPC) were performed according to standard protocols. The antioxidant activities of both the extracts (methanol and aqueous) were examined using two complementary methods, namely diphenylpicrylhydrazyl (DPPH) and Hydrogen peroxide (H_2O_2) assay. While, antimicrobial potential examined by using classical assays on pathogenic microbial strains along with appropriate controls of antimicrobial agents. Gas Chromatographic-Mass Spectrometric analyses of methanolic extracts of *S. obvallata* were performed for the identification of bioactive components. Statistical analysis was performed by using SPSS version 16.0.

Results: The extracts of leaves and flowers of *S. obvallata* showed significant results for all pharmacological experiments. TPC was recorded from a range of 132.67 ± 0.17 to 358.67 ± 0.17 mg GAE gm^{-1} dw while TFC was recorded from a range of 65.73 ± 1.55 to 326.88 ± 2.11 mg QE gm^{-1} dw. Per cent DPPH free radical scavenging activity was recorded from a range of 29.25 ± 0.86 to 82.88 ± 0.48% whereas per cent H_2O_2 free radical scavenging activity were recorded from a range of 39.75 ± 0.36 to 41.05 ± 0.46%. Antimicrobial activity was analyzed against four bacterial strains and three fungal strains. *S. obvallata* extracts showed fine zone of inhibition against *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus,* but *Escherichia coli* showed resistant (in terms of lower zone) against the extract compared to three bacteria. In antifungal activity, extract showed maximum zone of inhibition against *Candida glabrata* followed by *Candida albicans* and *Candida tropicalis*. GC-MS analysis of methanolic extracts of leaves and flowers of *S. obvallata* showed the presence of 36 and 48 components, respectively, based on retention time (Rt), and area per cent.

Conclusion: These findings confirm the traditional claims and contribute in providing promising baseline information for the pharmacological use of *S. obvallata*. Additional highly developed research is essential for isolation and identification of specific active components which are responsible for pharmacological properties of the plant.

Keywords: Antioxidant, Antimicrobial, Microbial strains, Pharmacological evaluation, *Saussurea obvallata*, Traditional claims

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Background

Herbal drugs are used in the traditional Indian system of medicine and also in other systems of medicine in the world for times immemorial due to low cost, effectiveness and no or low side effects. These attributes of the so called "natural" medicines are attracting the attention of increasingly large number of people of the world. World Health Organization (WHO) survey reports that 80% of the population in developing countries are still dependent on the traditional and folk systems of medicine, 85% traditional medicines are prepared by the use of plant extracts and that nearly 70% of prescribed human medicines are derived from the plants [1–3]. International market of herbal drugs is around US $ 62 billion, which is poised to grow to US $ 5 trillion by 2050 in the Asian and International market [4]. Demand of herbal drugs in India alone is estimated at US $ 1 billion per year [5], and Uttarakhand is one of the richest source for a whole variety of medicinal plants.

Saussurea is a very important genus of the family Asteraceae due to a variety of reasons, and most species of this genus are well investigated at the international level [6–9]. However, little is known about *S. obvallata* (2n = 32). It is an endemic herb of the Himalayan region and distributed between 3000 and 4800 m amsl. Commonly it is known as Brahma Kamal and is the state flower of Uttarakhand (India). It is a well known plant across Uttarakhand due to traditional, medicinal, ornamental and religious purposes [10]. It is used for the treatment of various diseases or disorders like paralysis, cerebral ischemia, wounds, cardiac disorders and mental disorders; some people also use it as antiseptic, in healing cuts, etc. [10]. Preliminary investigations about the phytochemicals found in *S. obvallata* (qualitative) have been reported by Semwal et al. [11], and its mineral composition has been described by Mishra et al. [12]. The present study, deals with the estimation of its total phenolic and flavonoid contents, as well as antioxidant and antimicrobial activities associated with its extracts. Gas Chromatography-Mass Spectrometry (GC-MS) based analyses of crude methanolic extracts of *S. obvallata* leaves and flowers have been carried out for the identification of active components.

Methods

Collection of plant material

The plant material (leaves and flowers) was collected from Kedarnath valley (4335 m amsl, 30°40′73" N latitude and 79°06′20″ E longitude) in Uttarakhand, India, during the month of September, 2012. The identity was subsequently confirmed by Dr. Anup Chandra (Scientist–E) Systemic Botany, Forest Research Institute, Dehradun, Uttarakhand, India (Ref. n. GEU/DBT/AT-1PS/2013). The fresh material was kept in perforated poly bags and immediately brought to the laboratory.

Preparation of extracts

The extracts were prepared as per Sati and Joshi [13] with slight modification. The flower and leaf samples of *S. obvallata* were washed, dried, and powdered using an electric blender (Willey Grinder Mill, Micro Scientific, India). Two solvents, methanol or water, were used for the extraction of phyto-constituents from the dried powder.

Methanol

Methanolic extracts of leaves and flowers were prepared by the Soxhlet extraction procedure. In this method the powdered plant material (1.0 g) of leaves and flowers were extracted in 10 mL of 70.0% (*v*/v) methanol used and extraction was carried out for 5 h. Afterwards the extract was collected and filtered through two layers of muslin cloth and then the filtrate was transferred to a rotator vacuum evaporator (Strike-12, Steroglass, Italy) flask and dried under reduced pressure and temperature less than (50 °C). Then dried extract was stored at (– 20 ° C) until used. The percentage yield of the plant extract was calculated according to the formula.

$$[\text{Percent Yield of the extract } (\%) = C_X/C_Y \times 100] \quad (1)$$

Where, C_X = Plant material weight after extraction process, C_Y = Plant material weight taken for extraction.

Water

Aqueous extracts of leaves and flowers were prepared using maceration technique. In this method the powdered plant material (1.0 g) of leaves and flowers were extracted in 10 mL of 100% (*v*/v) distilled water and allowed it to stand for 48 h (Shaker, 25 ± 1 °C). Afterwards the extract was filtered through two layers of muslin cloth and transferred to a rotator vacuum evaporator (Strike-12, Steroglass, Italy) flask and dried under reduced pressure and below 50 °C. The dried extract was stored at – 20 °C until used. The per cent yield of the extract was calculated using the formula giving in methanolic extraction.

Determination of total phenolics

The total phenolic content (TPC) in methanolic and aqueous extracts was determined by the Folin-Ciocalteu's reagent colorimetric method described by Anand et al. [14]. Both the methanolic and aqueous extracts of *S. obvallata* (20 μL) were taken from the stock solutions (1 mg mL^{-1}) of methanol and aqueous solutions, diluted with distilled water (80 μL); this was then mixed with Folin-Ciocalteu

reagent (500 μL) and allowed to react for 5 min in the dark (25 ± 1 °C). The mixture was neutralized by adding 400 μL of 7.5% sodium carbonate and kept in the dark (25 ± 1 °C, 30 min). The absorbance of this blue colored mixture was measured at 765 nm using a spectrophotometer (Thermo Scientific UV- 10). Quantification was done on the basis of a standard curve prepared using gallic acid (Sigma Aldrich, G7384-100G) at the concentration of 0.2, 0.4, 0.6, 0.8, and 1.0 mg mL^{-1} in 70.0% methanol. Based on the absorbance values, the TPC was estimated (mg mL^{-1}) from the calibration curve and the results expressed in terms of mg gallic acid equivalent dry weight (GAE g^{-1} dw). The whole process of TPC estimation was done and experiment was performed in triplicate.

Determination of total flavonoids

The total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method, as described by Anand et al. [14]. Briefly, the methanolic or aqueous extracts of *S. obvallata* (100 μL) from the respective stocks (1 mg mL^{-1}) of methanol or aqueous solutions were first diluted with distilled water (400 μL) and then 30 μL of 5.0% aq. $NaNO_2$ (sodium nitrite) solution was added to them. After 5 min, 30 μl of 10.0% aq. $AlCl_3$ (aluminium chloride) was added, and the mixture was allowed to stand for 5 min (25 ± 1 °C). Subsequently 20 μL of 4.0% aq. NaOH (sodium hydroxide) solution was added and the volume was made up to 1.0 mL with distilled water (420 μL). The absorbance of this mixture was immediately measured using a UV spectrophotometer (Thermo Scientific UV- 10) at 510 nm. Quantification was carried out on the basis of a standard curve prepared with quercetin (Sigma Aldrich, 337,951–25G) at the concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg mL^{-1} in 70.0% methanol. The results were expressed in terms of mg quercetin equivalent dry weight (QE g^{-1} dw). The whole process of TFC estimation was carried out in triplicate.

Determination of antioxidant activity by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) method

The free radical scavenging activity of samples was assessed according to the method of Azzahra et al. [15]. Briefly, first the stock solution of DPPH (Merck-Millipore, 300,267-50MG) was prepared by dissolving 24 mg DPPH in 100 mL methanol and the solution was stored at − 20 °C until used. Subsequently, the working solution was prepared by mixing 10 mL of stock with 60 mL of methanol, which gave an absorbance of 1.10 ± 0.02 units at 515 nm. Both the extracts (10 μL) were mixed with DPPH (990 μL) and the reaction was allowed to proceed for 2 h (25 ± 1 °C) in the dark, and then the absorbance was recorded at 515 nm. Ascorbic acid (Sigma Aldrich, A92902-25G) was used as a standard for making the calibration curve using 0.2, 0.4, 0.6, 0.8, and 1.0 mg mL^{-1} concentrations of the standard. The estimations were carried out in triplicate, and the per cent scavenging activity was calculated using the formula:

$$[\text{Scavenging rate } (\%) = [(\text{Ac}-\text{Ae})/\text{Ac}] \times 100] \quad (2)$$

Where, Ac and Ae represent the absorbance values of the control and extract, respectively.

Determination of antioxidant activity by hydrogen peroxide (H_2O_2) method

Determination of antioxidant activity by this method was carried out according to Panovska et al. [16]. Several oxidases generate H_2O_2, which is scavenged, either directly or indirectly via its reduction product, hydroxyl radical. In this method, when a scavenger is incubated with hydrogen peroxide, the decomposition or loss of H_2O_2 is measured using UV Spectrophotometer. Extracts (1 mL) were mixed with 20 mM H_2O_2 solution (2 mL) in phosphate buffer saline (PBS solution pH 7.5). The absorbance was measured after 10 min at 230 nm against phosphate buffer. H_2O_2 solution (2 mL) and the solvent (1 mL) were used as control and PBS as blank. Ascorbic acid (Sigma Aldrich, A92902-25G) was used as standard and the calibration curve was prepared using 0.2, 0.4, 0.6, 0.8, and 1.0 mg mL^{-1} concentrations of the standard. The per cent scavenging rate was calculated using the formula given for DPPH method.

Determination of antibacterial activity

The antimicrobial activity of *S. obvallata* extracts was carried out using standard protocols. Bacterial and fungal strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Selection of bacterial and fungal strains was made on the basis of their pathogenic nature, wider availability, antibiotic resistance and popularity. The bacterial strains included three Gram-negative strains namely, *Pseudomonas aeruginosa* (MTCC 4306), *Escherichia coli* (MTCC 1698), and *Klebsiella pneumoniae* (MTCC 9544) and one Gram-positive strain namely, *Staphylococcus aureus* (MTCC 6908). The antibacterial activity of the extracts was estimated using the well diffusion method [17, 18]. Briefly, the extracts (methanolic or aqueous) were tested on Luria Bertani agar (LB agar; Himedia, Delhi, India) plates to detect antibacterial activity, if any, against *P. aeruginosa, E. coli, K. pneumoniae* and *S. aureus*. Bacterial strains were cultured in LB broth at 37 °C for 18 h. The cultures were diluted to 1×10^6 cells mL^{-1} in LB broth and 100 μL of each was spreaded onto a separate LB agar plate. Wells of 6 mm diameter were bored using sterile cork borer. The extract (20 μL) from individual stocks (5 mg mL^{-1} concentration), and

ampicillin (20 μL) from stock (1 mg mL^{-1} concentration) were poured in the wells and the plates were further incubated at 37 °C for 24 h. Ampicillin was used as a positive control, whereas DMSO (5%) was used as a negative control. These tests were repeated three times to estimate consistency. Inhibition was measured in terms of zone of inhibition (ZOI) in mm formed on the plates.

Determination of antifungal activity

The well diffusion method was also used to screen the antifungal activity of *S. obvallata* extracts against three fungal strains of *Candida* namely, *C. albicans* (MTCC 3017), *C. glabrata* (MTCC 3019) and *C. tropicalis* (MTCC 3416) according to the same protocol [17, 18] giving in the previous section (antibacterial activity). These strains were also procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Potato dextrose agar (PDA; Himedia, Delhi, India) was used as the culture medium. Fluconazole (1 mg mL^{-1}) was used as a control drug (positive) and 5% DMSO was used as a negative control. The extract (20 μL) from individual stocks (5 mg mL^{-1}) was placed in each well, and the inoculated plates were incubated at 28 °C for 15 h. These tests were done in triplicate and the average of three separate measurements was taken for the colony diameter (mm) of the fungus.

Gas chromatography-mass spectrometry (GC–MS) analyses

GC-MS analyses and component identification of methanolic extracts of flowers and leaves of *Saussurea obvallata* were performed at the University Science Instrumentation Centre, Jawaharlal Nehru University, Delhi, India. The analyses were performed according to Ezhilan and Neelamegam [19], and Das et al. [20]. The GC-MS system (Shimadzu QP2010PLUS, Kyoto, Japan) was used for the analyses, which was equipped with an auto injector (AOC-20i), head space sampler (AOC-20s), and a fused silica capillary column Rtx-5 (30 m in length × 0.25 mm internal diameter × 0.25 μm film thickness). The oven temperature was set at 100 °C for 2 min, then increased to 250 °C at a rate of 5 °C per min, and finally to 280 °C at a rate of 10 °C per min. One μL of each sample (1 mg mL^{-1} stock) was injected to the column in split mode (split ratio 10) with helium (99.99% purity) as a carrier gas with a flow rate of 1.21 mL per min. The presence of unique peak fragmentation patterns for various metabolites/ phytocomponents was detected by an MS detector in full scan mode. Identification of components was based on their retention time (RT) and mass measured under identical GC-MS conditions. Quantitative determinations in the different samples were carried out using the peak areas. Identification of phytocomponents of *Saussurea obvallata* extracts was confirmed by comparing the spectral data of peaks with the standard mass spectra from the library data base [National Institute of Standards and Technology library (NIST08) and WILEY8]. Total running time of GC-MS was 50.74 min and the relative percentage of individual constituents was expressed on the basis of peak area normalization.

Statistical analysis

The results of phytochemical analyses and antimicrobial activity data were subjected to analysis of variance using SPSS version 16.0. The significance level was determined at $p < 0.05$ and the means were separated using Duncan's multiple range test (DMRT), to determine if the values were significantly different. Data have been presented as mean values ± standard error (SE).

Results and discussions

Total phenolic and flavonoid content

Significant variation ($p < 0.05$) was recorded in the total content of phenolics (TPC) and flavonoids (TFC) in *S. obvallata,* and the values for TPC and TFC were expressed in terms of mg gallic acid equivalent g^{-1} dw (the standard curve equation: y = 1.992x 0.226, R^2 = 0.977) and quercetin equivalent g^{-1} dw (the standard curve equation: y = 0.284x + 0.116, R^2 = 0.975), respectively. The maximum and minimum TPC values were found in methanolic leaf extract and aqueous flower extract of *S. obvallata* (358.66 ± 0.17 and 132.67 ± 0.17 mg GAE g^{-1} dw), respectively. Intermediate values were recorded for aqueous leaf extract (139.50 ± 0.28) and methanolic flower extract (185.00 ± 0.00). The maximum and minimum TFC values were recorded in aqueous flower extract and methanolic flower extract of *S. obvallata* (326.88 ± 2.12 and 65.73 ± 1.55 mg QE g^{-1} dw), respectively. Intermediate values were recorded for aqueous leaf extract (229.46 ± 1.54) and methanolic leaves extract (188.38 ± 1.01). The results of TPC and TFC estimations have been represented in Table 1. These types of poly phenolic compounds are known to show different types of biological effects, e.g., antioxidant and antimicrobial activities, etc. [21].

DPPH and H_2O_2 free radical scavenging activity

Antioxidant activity was measured by two in-vitro assays and significant variation ($p < 0.05$) was recorded in antioxidant activity of *S. obvallata* extracts using DPPH assay, while significant variation ($p < 0.05$) was not recorded in H_2O_2 assay. The maximum and minimum per cent DPPH free radical scavenging activity were recorded in the methanolic and aqueous extracts of flowers (82.88 ± 0.48% and 29.25 ± 0.86%), respectively. Intermediate values of DPPH free radical scavenging activity were recorded for aqueous extract of leaves (49.66

Table 1 Quantitative estimation of total phenolic, total flavonoid and free radical scavenging activity by DPPH and H_2O_2 assays in aqueous and methanolic extracts of leaves and flowers of *Saussurea obvallata*

Plant extract	Yield of the extracts (%)	TPC (mg GAE g^{-1} dw ± SE)	TFC (mg QE g^{-1} dw ± SE)	DPPH (% S. activity ± SE)	H_2O_2 (% S. activity ± SE)
L1	13.70	139.50 ± 0.29^{c}	229.46 ± 1.55^{b}	49.67 ± 0.38^{c}	39.75 ± 0.36
L2	19.80	358.67 ± 0.17^{a}	188.38 ± 1.02^{c}	73.48 ± 0.44^{b}	41.05 ± 0.46
F1	13.14	132.67 ± 0.17^{d}	326.88 ± 2.11^{a}	29.25 ± 0.86^{d}	40.12 ± 0.40
F2	11.67	185.00 ± 0.00^{b}	65.73 ± 1.55^{d}	82.88 ± 0.48^{a}	40.76 ± 0.27

Values are expressed in mean ± standard error; Mean values followed by the same letter (s) in a column are not significantly different ($p < 0.05$) based on DMRT; *TPC* total phenolic content, *TFC* total flavonoid content, *DPPH* 1, 1-Diphenyl-2-picrylhydrazyl, *H_2O_2* hydrogen peroxide, *L1& L2* aqueous and methanolic extract of leaves, *F1 & F2* aqueous and methanolic extract of flowers, respectively

± 0.38%) and methanolic extract of leaves (73.48 ± 0.44%). On the other hand, the maximum and minimum per cent H_2O_2 free radical scavenging activity were recorded in methanolic leaf extract and aqueous leaf extract (41.05 ± 0.76% and 39.75 ± 0.36%), respectively. Intermediate values of H_2O_2 free radical scavenging activity were recorded for aqueous extract of flowers and methanolic extract of flowers (40.11 ± 1.10% and 40.76 ± 0.26%).

Antibacterial activity

Antibacterial activity of the extracts of leaves and flowers of *Saussurea obvallata* was measured in terms of zone of inhibition (ZOI) against *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), *Staphylococcus aureus* (SA), and *Klebsiella pneumoniae* (KP). The maximum zone of inhibition (ZOI) for the aqueous extract of leaves was 20.43 ± 0.30 mm for SA and minimum ZOI was 11.57 ± 0.23 mm for EC. Intermediate ZOI (16.60 ± 0.21, and 14.90 ± 0.21 mm) values were recorded for PA and KP, respectively. The maximum ZOI for methanolic extract of leaves was 19.90 ± 0.56 mm for KP and minimum ZOI was 10.83 ± 0.43 mm for EC, while intermediate values for ZOI (13.90 ± 0.21 mm and 13.60 ± 0.21 mm) were recorded for PA and SA, respectively. The maximum ZOI for the aqueous extract of the flowers was 20.50 ± 0.26 mm for PA and minimum value for ZOI was 13.90 ± 0.21 mm for EC, while intermediate values for ZOI of 16.53 ± 0.24 mm and 18.53 ± 0.24 mm were recorded for SA and KP, respectively. The maximum ZOI for methanolic extract of flowers of *S. obvallata* was found to be 16.33 ± 0.17 mm for PA and minimum ZOI value of 8.86 ± 0.18 mm was observed for EC, while 12.83 ± 0.16 mm and 11.53 ± 0.24 mm values for ZOI were recorded for SA and KP, respectively. Ampicillin (positive control) showed maximum ZOI against PA (34.33 ± 0.33 mm) followed by SA (31.33 ± 0.33 mm), EC (25.66 ± 0.33 mm), and KP (24.6 ± 0.30 mm). Five per cent DMSO (ne*gative control) did not show any ZOI for individual bacterial strains.* In conclusion, it was observed that the extract of *S. obvallata* caused lower values for ZOI against EC in comparison to other bacteria (SA, PA & KP). The results of antibacterial activity of extracts of *S. obvallata* against four bacterial strains have been represented in Table 2.

Antifungal activity

The antifungal activity of extracts of leaves and flower of *S. obvallata* were assessed against three fungal species, *Candida albicans* (CA), *Candida glabrata* (CG) and *Candida tropicalis* (CT) in terms of zone of inhibition (ZOI). The zone of inhibition observed for the aqueous extract of leaves was 15.43 ± 0.30 mm for CA, 8.30 ± 0.15 mm for CT, and 13.90 ± 0.17 mm for CG. The ZOI for methanolic extract of leaves was 15.90 ± 0.56 mm for CA, 13.17 ± 0.09 mm for CT, and 14.57 ± 0.23 mm for CG. The ZOI for the aqueous extract of flowers was 14.30 ± 0.12 mm for CG, 12.33

Table 2 The antibacterial and antifungal activity of the extracts of leaves and flowers of *Saussurea obvallata* determined using the well diffusion method

Plant Extracts	Assay for antibacterial activity				Assay for antifungal activity		
	P. aeruginosa	*E. coli*	*S. aureus*	*K. pneumoniae*	*C. glabrata*	*C. albicans*	*C. tropicalis*
L1	16.60 ± 0.21^{c}	11.57 ± 0.23^{c}	20.43 ± 0.30^{b}	14.90 ± 0.21^{d}	13.90 ± 0.17^{b}	15.43 ± 0.30^{bc}	8.30 ± 0.15^{d}
L2	13.90 ± 0.21^{e}	10.83 ± 0.43^{c}	13.60 ± 0.21^{d}	19.90 ± 0.56^{b}	14.57 ± 0.23^{b}	15.90 ± 0.56^{b}	13.17 ± 0.09^{b}
F1	20.50 ± 0.26^{b}	13.90 ± 0.21^{b}	16.53 ± 0.24^{c}	18.53 ± 0.24^{c}	14.30 ± 0.12^{b}	14.27 ± 0.50^{c}	12.33 ± 0.18^{b}
F2	16.33 ± 0.17^{d}	8.87 ± 0.19^{d}	12.83 ± 0.17^{e}	11.53 ± 0.24^{e}	14.30 ± 0.47^{b}	15.27 ± 0.47^{bc}	10.90 ± 0.46^{c}
Drug	34.33 ± 0.33^{a}	25.67 ± 0.33^{a}	31.33 ± 0.33^{a}	24.60 ± 0.31^{a}	22.40 ± 0.31^{a}	25.93 ± 0.54^{a}	25.97 ± 0.49^{a}
DMSO	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00

Mean values ± SE followed by the same letter (s) in a column are not significantly different ($p < 0.05$) based on DMRT; *P. aeruginosa: Pseudomonas aeruginosa; E. coli: Escherichia coli; S. aureus: Staphylococcus aureus; K. pneumoniae: Klebsiella pneumoniae; C. albicans: Candida albicans; C. glabrata: Candida glabrata; C. tropicalis: Candida tropicalis*. L1& L2: aqueous and methanolic extract of leaves, respectively. F1 & F2: aqueous and methanolic extract of flowers, respectively. Ampicillin and fluconazole were used as positive controls for antibacterial and antifungal assays, respectively. DMSO used as a negative control

± 0.18 mm for CT, and 14.27 ± 0.50 mm for CA. The ZOI for methanolic extract of flowers was 15.27 ± 0.47 mm for CA, 10.90 ± 0.46 mm for CT, and 14.30 ± 0.47 mm for CG. Fluconazole (positive drug) showed maximum ZOI against CT (25.97 ± 0.49 mm), followed by CA (25.93 ± 0.54 mm), and CG (22.40 ± 0.31 mm). DMSO does not show ZOI against any of the strains (CT, CA, CG). The results of antifungal activity of extracts of *S. obvallata* against three fungal strains have been represented in Table 2.

GC-MS analyses of methanolic extracts of *Saussurea obvallata*

The results of GC-MS analyses of methanolic leaf and flower extracts of *Saussurea obvallata* showed the presence of 36 and 48 components, respectively based on separation of individual peaks through GC as per their retention time (Rt) and area per cent under individual peaks (Tables 3 & 4). The mass spectra of these compounds were matched with the spectra of known compounds listed in WILEY8.LIB and NIST08.LIB spectral databases/ libraries. Some of these components could not be identified by comparison using any of these libraries; such unidentified GC peaks numbered five (5; Rt: 9.113, 10.662, 10.887, 19.407, and 22.337 min) in the flower extract and one (1; Rt: 15.552 min) in the leaf extract. Most of the components presented in the extracts of leaves and flowers have been already reported in respect of different biological activities namely, Curumene (for anticancer), Methyl acetate (for hepatoprotective), Nerolidol acid (for antioxidant, anti-inflammatory and pesticide) and Piperine (malaria and respiratory disease), Stigmasterol (antioxidant, antimicrobial, anticancer) etc.

The major components in the methanolic extract of leaves of *S. obvallata* were Linoleic acid (22.50%); followed by Dehydrocostus lactone (21.98%); Palmitic acid (11.84%); Eltanolone (11.43%); and Doconexent (9%). However, major components in methanolic flower extract of *S. obvallata* was not be identified by the known libraries (Rt: 19.40; 38.85%,); followed by Methyle palmitate (12.18%); Linalyl acetate (4.94%); Palmitic acid (4.65%); Methyl stearate (3067%). After GC-MS analyses of both the extracts of *S. obvallata*, few components in leaves and flowers extract have already been reported for various biological activities, such as anti-oxidant, antimicrobial, hemolytic, cancer preventive, chemo preventive, anti-tumor, immuno-stimulant, etc. (Table 5). Furthermore, other uses of these components are in perfume industry, and in the preparation of pesticides, anti-androgenic formulations, flavour additives, and for sunscreens. Six (6) components, namely Palmitic acid, Methyl linoeate, Stearic acid, 1-Docosanol, Methyl oleate, Gama-Stearolactone, are common in both the extracts. While some components being common in both the extracts have not been reported to exhibit any biological activity.

To the best of our knowledge this would appear to be the first report regarding detailed phytochemical

Table 3 Chemical composition of methanolic leaf extract of *Saussurea obvallata*

SN	Name of components	Mol. Formula	Area per cent (%)
1	Octyl cyanide	$C_9H_{17}N$	0.17
2	Benzeneacetaldehyde	C_8H_8O	0.10
3	Pyranone	$C_6H_8O_4$	0.72
4	Pentadecane	$C_{15}H_{32}$	0.21
5	(RS)-nicotine	$C_{10}H_{14}N_2$	0.07
6	Tetradecane	$C_{14}H_{30}$	0.27
7	Amantadine	$C_{10}H_{17}N$	0.18
8	Hexadecane	$C_{16}H_{34}$	0.16
9	α-Bisabol oxide B	$C_{15}H_{26}O_2$	0.15
10	Blumenol C	$C_{13}H_{22}O_2$	0.08
11	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.10
12	N-Acetylimidazole	$C_5H_6N_2O$	0.15
13	Androsterone, trifluoroacetate	$C_{21}H_{29}F_3O_3$	0.08
14	Phytol	$C_{20}H_{40}O$	0.25
15	Diisobutyl phthalate	$C_{16}H_{22}O_4$	0.06
16	Eltanolone	$C_{21}H_{34}O_2$	11.43
17	Proximadiol	$C_{15}H_{28}O_2$	0.24
18	Doconexent	$C_{22}H_{32}O_2$	9.00
19	Palmitic acid	$C_{16}H_{32}O_2$	11.84
20	Dehydrocostus lactone	$C_{15}H_{18}O_2$	21.98
21	Strophanthidin	$C_{23}H_{32}O_6$	0.25
22	n-Nonadecanol-1	$C_{19}H_{40}O$	0.11
23	Methyl linoleate	$C_{19}H_{34}O_2$	0.26
24	Linoleic acid	$C_{18}H_{32}O_2$	22.50
25	Stearic acid	$C_{18}H_{36}O_2$	2.38
26	1-Docosanol	$C_{22}H_{46}O$	1.75
27	Methyl oleate	$C_{19}H_{36}O_2$	0.09
28	Bupleuronol	$C_{17}H_{20}O_2$	1.23
29	Andrographolide	$C_{20}H_{30}O_5$	0.31
30	Nitrocyclododecane	$C_{12}H_{23}NO_2$	0.16
31	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	0.10
32	Stachydrine	$C_7H_{13}NO_2$	0.37
33	Gama-Stearolactone	$C_{18}H_{34}O_2$	0.91
34	Talaroconvolutin B	$C_{32}H_{43}NO_4$	0.11
35	22-Tricosenoic acid	$C_{23}H_{44}O_2$	0.68
36	Litsomentol	$C_{30}H_{52}O_2$	2.25
Total identified compounds			90.70%
Unknown compounds			00.36%

Table 4 Chemical composition of methanolic extract of flowers of *Saussurea obvallata*

SN	Name of components	Mol. Formula	Area per cent
1	α –Terpineol	$C_{10}H_{18}O$	3.3
2	Methyl octanoate	$C_9H_{18}O_2$	0.05
3	Cinnamaldehyde	C_9H_8O	0.44
4	Linalyl acetate	$C_{12}H_{20}O_2$	4.94
5	Eugenol	$C_{10}H_{12}O_2$	0.66
6	Methyl eugenol ether	$C_{11}H_{14}O_2$	0.21
7	Curumene	$C_{15}H_{24}$	2.79
8	Nerolidol	$C_{15}H_{26}O$	0.28
9	n-Heptadecanol-1	$C_{17}H_{36}O$	0.08
10	Caryophyllene oxide	$C_{15}H_{24}O$	0.56
11	Geranyl linalool isomer b	$C_{20}H_{34}O$	0.16
12	n-Pentadecanol	$C_{15}H_{32}O$	0.53
13	Heptadecane	$C_{17}H_{36}$	0.59
14	Pentadecyclic acid	$C_{15}H_{30}O_2$	0.42
15	Methyl palmitoleate	$C_{17}H_{32}O_2$	0.34
16	Methyl palmitate	$C_{17}H_{34}O_2$	12.18
17	Bicyclohexyl	$C_{12}H_{22}$	0.12
18	Palmitic acid	$C_{16}H_{32}O_2$	4.65
19	Stearic acid	$C_{18}H_{36}O_2$	0.43
20	Lignoceric acid	$C_{24}H_{48}O_2$	0.50
21	Methyl stearate	$C_{19}H_{38}O_2$	3.67
22	Camphor	$C_{10}H_{16}O$	0.96
23	Dibutyl sebacate	$C_{18}H_{34}O_4$	1.04
24	Methyl linoleate	$C_{19}H_{34}O_2$	0.31
25	Prodlure	$C_{16}H_{28}O_2$	0.14
26	Menthyl acetate	$C_{12}H_{22}O_2$	0.12
27	Rishitin	$C_{14}H_{22}O_2$	0.07
28	1-Docosanol	$C_{22}H_{46}O$	0.21
29	Methyl linolenate	$C_{19}H_{32}O_2$	0.14
30	Methyl oleate	$C_{19}H_{36}O_2$	1.02
31	Henicosanoic acid	$C_{21}H_{42}O_2$	2.24
32	Bornyl cinnamate	$C_{19}H_{24}O_2$	0.19
33	Gama-Stearolactone	$C_{18}H_{34}O_2$	0.12
34	Docosanoic acid	$C_{22}H_{44}O_2$	0.18
35	Tridecanedial	$C_{13}H_{24}O_2$	0.13
36	Gondoic acid	$C_{20}H_{38}O_2$	2.92
37	Tricosanoic acid	$C_{23}H_{46}O_2$	1.46
38	Deflazacortalcohol	$C_{23}H_{29}NO_5$	0.10
39	Methyl nervonate	$C_{25}H_{48}O_2$	0.15
40	Pentacosylic acid	$C_{25}H_{50}O_2$	0.93
41	Squalene	$C_{30}H_{50}$	0.52
42	17-Pentatriacontene	$C_{35}H_{70}$	0.06
43	Eicosane	$C_{20}H_{42}$	0.11
44	Piperine	$C_{17}H_{19}NO_3$	0.18
45	Nonacosane	$C_{29}H_{60}$	0.10
46	Stigmasterol	$C_{29}H_{48}O$	0.13
47	β- Sitosterol	$C_{29}H_{50}O$	0.17
48	Tetratetracontane	$C_{44}H_{90}$	0.10
Total identified compounds			50.70%
Unknown compounds			39.53%

analysis, medicinal and anti-microbial properties, and discussion on the bioactive components of *S. obvallata.* During the present study, basic information has been generated about total phenolic & flavonoid contents, antioxidant activity (using DPPH & H_2O_2 assays), and antimicrobial activity. GC-MS analyses were carried out to elucidate the chemical composition of *S. obvallata* leaf and flower extracts (methanolic). The composition of plant extracts depend on their origin, extraction techniques used, the time and temperature of extraction, nature of solvent used, its concentration and polarity, quantity and the secondary metabolite composition of a given extract [63]. Variations in the extraction methods like the duration of the extraction period, the solvent used, pH, temperature, particle size, and the solvent-to-sample ratio [64] can lead to different results. Phytochemical investigations of *S. obvallata* extracts showed that, as expected, it contains different types of constituents like alkaloids, flavonoids, terpenoids, glycosides, saponins, etc. using different solvents, e.g., methanol, ethanol, chloroform, and distilled water as solvents [11]. Similar pattern and constituents were reported by Alaagib & Ayoub [65] for *Saussurea lappa*.

Phenolic and flavonoid compounds are produced by plants and are known to have strong anti-oxidant activity. They exert a positive effect on both plants and human health, and some derivatives of flavonoids possess antibacterial activity against a range of microbes. In the present study the maximum and minimum values for TPC were recorded for methanolic leaf extract and aqueous floral extract (358.67 ± 0.17 and 132.67 ± 0.17 mg GAE g^{-1} dw, respectively) of *S. obvallata,* while maximum TFC (326.88 ± 2.11 mg QE g^{-1} dw) was found in the aqueous floral extract, and the minimum TFC (65.73 ± 1.55 mg QE g^{-1} dw) was found in the methanolic extract of flowers. On the other hand, *Saussurea involucrata* was reported to possess significant anti-oxidant activity (high phenol and flavonoid content) and is known to be used by a large population in China for medicinal purpose [66]. Phenolic and flavonoid contents in *Saussurea obvallata*

Table 5 Bioactive components present in the methanolic extracts of leaves and flowers of *Saussurea obvallata*

SN	Components	Biological activity	References
1	Benzeneacetaldehyde	Anti-microbial, anti-inflammatory.	[22]
2	Pyranone	Anti-microbial, anti-inflammatory, anti-oxidant, anti-proliferative.	[23]
3	Tetradecanoic acid	Anti-fungal, anti-oxidant, cancer-preventive, cosmetic.	[24]
4	Phytol	Anti-microbial, anti-inflammatory, anti-nociceptive.	[25]
5	Palmitic acid	Anti-androgenic, hemolytic, cancer preventive, sunscreen, perfumery.	[26]
6	Methyl linoleate	Hepatoprotective, anti-microbial.	[27]
7	Linoleic acid	Anti-inflammatory, anti-microbial.	[28, 29]
8	Gama-Stearolactone	Anti-tumor, anti-inflammatory, anti-convulsant activity.	[27, 30–32]
9	α -Terpineol	Contractile activity; anti-bacterial; anti-microbial; Insecticidal.	[32–36]
10	Cinnamaldehyde	Anti-cancer, anti-microbial, anti-oxidant	[37]
11	Curumene	Anti-tumor, anti-bacterial, anti-inflammatory, sedative, fungicide.	[38–43]
12	Nerolidol	Anti-tumor, analgesic, anti-inflammatory, sedative, fungicide.	[44, 45]
13	n-Heptadecanol-1	Anti-microbial, anti-inflammatory.	[46, 47]
14	Caryophyllene oxide	Anti-microbial, anti-inflammatory.	[48–52]
15	Pentacosylic acid	Anti-bacterial.	[27, 52, 53]
16	1-Docosanol	Anti-microbial, anti-herpetic agent, wounds, anti-herpes.	[54–56]
17	Methyl acetate	Anti-oxidant, anti-microbial, anti-inflammatory and phytotoxic.	[57]
18	Piperine	Anti-oxidant, anti-microbial, anti-tumor, pesticide.	[58, 59]
19	β-Sitosterol	Anti-microbial, thyroid inhibitory, hypoglycemic effects.	[60, 61]
20	Stigmasterol	Anti-oxidant, thyroid inhibitory, hypoglycemic effects.	[60, 61]
21	Squalene	Anti-oxidant, anti-microbial, cancer preventive, pesticide.	[62]

are in general agreement with the previous findings on other species of *Saussurea*.

Free radicals can cause damage to essential proteins, DNA, lipids and also be attributed to various human diseases such as cancer, cardiovascular diseases, neurodegenerative disorders, etc. due to oxidative stress. The most important role of antioxidants is to suppress free radical mediated oxidation by inhibiting the production of free radicals through scavenging activity. Ascorbic acid was used as a standard antioxidant compound for DPPH assay, and H_2O_2 scavenging assay. Maximum per cent DPPH scavenging activity (82.88 ± 0.48%) was recorded in the methanolic extract of flowers, and the minimum (29.25 ± 0.86%) in the aqueous extract of flowers in *S. obvallata.* The maximum per cent H_2O_2 scavenging activity (41.05 ± 0.46%) was recorded in the methanolic leaf extract, and the minimum (39.75 ± 0.36%) in the aqueous leaf extract at 1 mg mL^{-1} concentration of extracts. The same pattern in respect of antioxidant activity by DPPH assay was observed by Yao et al. [67] from two polysaccharides (SCIP1–2 and CSIP2–3) isolated from *Saussurea involucrata,* and they reported 60.3% and 88.7% DPPH scavenging activity at a dose of 8 mg mL^{-1}, respectively for the two polysaccharides. *S. obvallata* therefore contains more antioxidant potential compared to *S. involucrata.* In this test, it was observed that methanol as a solvent resulted in higher values for TPC, DPPH and H_2O_2 assays, while the aqueous extract resulted in higher values for TFC.

Nowadays, increased risk of infection and resistance against antibiotics has become a persistent and wide spread problem and antibiotics are often used indiscriminately against diseases causes by various microbes. Thus, it is an important task for investigators to be constantly in the lookout for an alternate chemical agent against theses human pathogens. In this study, four bacterial strains and three fungal strains were used for the analyses of antimicrobial activity of *S. obvallata* extracts. For aqueous leaf extract, the maximum ZOI (20.43 ± 0.30 mm) was found in respect of *S. aureus* while the minimum ZOI (11.57 ± 0.23 mm) was associated with *E. coli*. The maximum and minimum ZOIs in respect of methanolic leaf extract were 19.90 ± 0.56 mm for *K. pneumoniae* and 10.83 ± 0.43 mm for *E. coli,* respectively. The aqueous extract of flowers showed maximum ZOI (20.50 ± 0.26 mm) for *P. aeruginosa* and minimum ZOI (13.90 ± 0.21 mm) for *E. coli*. The methanolic extract of flowers caused maximum ZOI (16.33 ± 0.17 mm) for *P. aeruginosa* and minimum ZOI (08.87 ± 0.19 mm) for *E. coli*. In general, it was observed using this test

that *E. coli* exhibited resistance against both the extracts (lower ZOI).

In the case of antifungal activity of *S. obvallata* extracts, maximum ZOI (15.43 ± 0.30 mm) was recorded in respect of *C. albicans* and the minimum ZOI (08.30 ± 0.15) was recorded for *C. tropicalis.* However, maximum ZOI (15.90 ± 0.56 mm) in respect of *C. albicans* and the minimum ZOI (13.17 ± 0.09 mm) in case of *C. tropicalis* were recorded in methanolic extract of leaves. Aqueous floral extract exhibited maximum ZOI (14.30 ± 0.12 mm) for *C. glabrata* and minimum ZOI (12.33 ± 0.18 mm) for *C. tropicalis,* while the maximum ZOI (15.27 ± 0.47 mm) and minimum ZOI (10.90 ± 0.46 mm) were found in respect of *C. tropicalis*. In this experiment, it has been shown that *C. tropicalis* exhibited resistance against *S. obvallata* extracts (lower ZOI). Khalid et al. [68] have reported antimicrobial activity of *S. lappa* root extract against three Gram positive and two Gram negative bacteria. Methanol, hot water and cold water were used for the extraction, and the range of zone of inhibition was observed between 10 and 18 mm for methanolic extract, 8–11 mm for hot water extract and 9–13 mm for cold water extract.

Antimicrobial activity of methanolic and chloroform extracts of *S. lappa* against *E. coli, P. aeruginosa, K. pneumoniae, P. vulgaris, S. aureus, C. albicans, A. niger* were reported by Thara & Zuhra [69]. Both the extracts of *S. lappa* exhibited significant activity against all bacterial strains, but some fungal strains exhibited lower zone of inhibition. Antimicrobial activity of *Saussurea lappa* (roots) was reported by Negi et al. [70] the samples were collected from four different regions of Uttarakhand and methanol was used for the extraction. Negi et al. [70] tested six bacterial strains (*S. typhimurium*, *E. coli*, *C. freundii*, *P. vulgaris*, *E. faecalis* and *S. aureus).* The ZOIs were 6–8 mm for *E. coli*, 5–10 mm for *C. freundii*, 5–8 mm for *E. faecalis* and 6–10 mm for *S. aureus.* The extract of *S. lappa* exhibited excellent activity against these four bacterial strains compared to a known standard used. Two bacterial strains, *Proteus vulgaris and S. typhimurium* showed resistance against the root extract up to 100 mg mL^{-1} concentrations. Alaagib et al. [65] also reported antibacterial activity of *S. lappa* (black root) extract against five bacterial strains (*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*). Petroleum ether, chloroform, methanol, and water were used for extraction, and all five strains exhibited variable activity, Gram positive bacteria were more sensitive to the extract in comparison to Gram negative bacteria. The ZOIs caused by the root extract of *S. lappa* ranged from 11 to 21 mm (petroleum ether), 11–22 mm (chloroform), 11–17 mm (methanol), and 11–15 mm (water) extract. In respect of *E. coli* the zone of inhibition formed was quite low as also in the present study.

GC-MS analyses helped in the elucidation of chemical composition of both the extracts (leaves and flowers) of *S. obvallata.* Through these analyses thirty six (36) components were found to be present in the leaf extract, and forty eight (48) components in the flower extract. One (1) component in the leaf extract and five (5) components in the flower extract could not identified by known libraries. Total 21 components were enumerated as bioactive components in the two extracts based on previous studies, while 6 components were common in respect of both the extracts [71]. Many of the reported bioactive components were had been isolated from various sources, and already known for anti-oxidant and anti-microbial activity, etc. [72]. Thus, it can be concluded that the anti-oxidant and anti-microbial activities along with other medicinal properties associated with this plant may also be caused by theses bioactive components.

Conclusions

This study provides a fairly comprehensive report on TPC, TFC, anti-oxidant and anti-microbial activities, and GC-MS analyses of leaf and flower extracts of *Saussurea obvallata*. The results of present investigation are in general agreement with earlier studies on anti-oxidant, anti-microbial, and GC-MS analyses of other species of *Saussurea* genus. These results may provide a strong basis for traditionally known medicinal properties of *S. obvallata,* many of which are also mentioned in India holy books. Screening and identification of components and systematic evaluation of their medicinal properties may provide clues for a potential drug, and its other bioactivities may provide ground for these to be recommended for further investigations.

Abbreviations

DMSO: Dimethyl sulfoxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; GC-MS: Gas Chromatograpy – Mass Spectrometry; H_2O_2: Hydrogen peroxide; TFC: Total flavonoids content; TPC: Total phenolic content

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All data generated or analysed during this study are included in this article.

Authors' contributions

PS collected the samples, performed all the experiments, and drafting the manuscript with SP. Both authors read the manuscript and approved the final version.

Ethics approval and consent to participate

Not applicable.

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