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ASSESSMENT OF ANTIMICROBIAL POTENTIAL AND PHYTOCHEMICAL PROFILING OF ETHNOMEDICINAL PLANT *BERGENIA CILIATA* (HAW.) STERNB. IN WESTERN HIMALAYA

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ABSTRACT

Western Himalaya is endowed with rich treasure of medicinal plant wealth. *Bergenia ciliata* is one of the important ethnomedicinal plants collected from Western Himalaya and used for dissolving kidney stones by the local inhabitants. In present work ethanolic rhizome extract of plant was evaluated for its medicinal potential by assessing its antimicrobial and phytochemical properties. In antimicrobial studies the ethanolic rhizome extract of the plant was used against standard and clinical isolates of selected pathogenic bacterial strains. Maximum inhibition zone (12±1.0mm) was observed against clinical isolate of *S. typhimurium* followed by clinical isolate of *E. coli* with an inhibition zone of 11.33±0.57mm. The MIC of *B. ciliata* rhizome extract ranged from 100 to 200µg/100µl against different pathogenic strains. The rhizome extract showed presence of reducing sugars, alkaloids, saponins, tannins, flavonoids and cardiac glycosides. Further, GC-MS analysis of rhizome extract confirmed the presence of phytochemicals viz., beta sitasterol, ethyl iso-allocholate, hexadecanoic acid, cetene, monolinolein TMS, berginin, gallic acid, linolool, β-caryophyllene, calcitriol, carotene, oleic acid and astaxanthin with significant ethnomedicinal importance.

Keywords: Ethnomedicinal plant, Western Himalaya, Antimicrobial potential, MIC, GC-MS analysis

INTRODUCTION

Medicinal and aromatic plants are predominantly present in the forest areas and a very few of these are also cultivated in agricultural fields. Some of these plants are used by the local people for their food, medicine and for other domestic purposes (**Semwal *et al.*, 2010**). Ethnobotany has been variously defined and interpreted by different workers as this study follow methods based on knowledge and use of anthropological techniques (**Rawat and Kharwal, 2013**). **Guleria and Vasishth (2009)** documented the ethnobotanical use of plants by nomadic communities in Western Himalaya for varied medicinal purposes i.e. for curing stomach diseases, eye infection, fever, arthritis, blackening of hair, refreshing mind, loss of hairs, general weakness, loss of alertness, diabetes, loose motions and in impotency. Medicinal plants are used for preventive, promotive and curative purposes and serves as a main source of medicine in India **(Sonowal and Barua, 2012)**. Herbal medicines have also been recognized by WHO (World Health Organization) as an important component for primary health care and about 11% of the 252 drugs are derived from plants sources **(Shakya, 2016)**.
The Indian Himalayan Region (IHR) is a mega hotspot of biological diversity and comprises about 18% region of India with more than 2,800 km long and 220 to 300 km wide with altitudes ranging from 200-8000m **(Myers *et al.*, 2000; Chauhan *et al.*, 2014)**. Shimla region in Himachal Pradesh, India lies between longitude 77.00" and 78.19" east and latitude 30.45" and 31.44" north and due to variable altitudinal and climatic conditions it is a vast repository of medicinal plants **(Smith and Smith, 1899; Collet, 1902; Chauhan 1999; Verma *et al.*, 2012; Singh and Thakur, 2014; Rana and Masoodi, 2014; Thakur, 2015; Chauhan *et al.*, 2016; Verma *et al.*, 2016).** The western Himalayan region accounts for about 80% of herbal drugs in Ayurveda, 46% in Unani, and 33% of allopathic systems **(Baragi *et al.*, 2008)**. Since this region has diverse flora of medicinal plants, it has a great potential of raising the economy of its inhabitants **(Sharma *et al.*, 2014)**. *Bergenia ciliata*, an important plant of this region is used in the treatment of fever, diarrhoea and pulmonary infections **(Farooquee *et al.*, 2004)**. It is used as an antipyretic, antidiabetic (alpha-glucosidase inhibitor) and antibacterial agent **(Hsieh *et al.*, 2001)**. A growing interest in existence of wild medicinal and aromatic plants in a locality and its role in improving the economy and welfare of the society has led to an increased research in this area. The present study attempts to postulate the medicinal importance of *Bergenia ciliata* collected from western Himalaya in terms of its antimicrobial and phytochemical potential.

MATERIALS AND METHODS

Plant collection

Bergenia ciliata (Haw.) Sternb. plant was collected from the Theog region (2500m amsl) of district Shimla, Himachal Pradesh, India, in western Himalaya. The plant was identified at Himalayan Forest Research Institute (HFRI), Shimla and the verified sample (voucher no: SUBMS/BOT/565) was submitted in Herbarium at the School of Biological and Environmental Sciences, Shoolini University, Solan, India. The collected rhizomes were washed with water to remove soil and dust particles and then dried thoroughly in shaded place. The dried rhizomes were grinded to fine powder and stored in an airtight container at room temperature and used to prepare the extract for further study.

Preparation of bacterial strain

Standard culture of five bacterial strains viz. *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhimurium* (MTCC 98) and *Pseudomonas aeruginosa* (MTCC 741) were procured from IMTECH Chandigarh, India. Clinical isolates of all these bacterial strains were obtained from PGIMER, Chandigarh and Indira Gandhi Medical College (IGMC), Shimla, India. All isolates were maintained by sub-culturing once in a month on nutrient agar and stored at 4°C. All standard and clinical isolates of the above bacterial strains were used for determining the antimicrobial property using ethanolic extract of *B. ciliata*.

Preparation of plant extract for antimicrobial activity

The ethanolic rhizome extract of *B. ciliata* was prepared by using **Selvamohan *et al.* (2012)** method by dissolving 10g fine powder of rhizome of this plants in 50ml of ethanol. The contents were kept in rotary orbital shaker for 48h at 40°C. Finally the extract was filtered through Whatman filter paper No. 1, dried at 40°C and stored at 4°C for further studies.

Assay of antimicrobial activity using disc diffusion method

The antimicrobial activity of the rhizome extract was determined by using **Ahmady-Asbchin *et al.* (2013)** and **Tabaraki *et al.* (2013)** method. For this 20ml of sterilized Muller Hinton Agar (MHA) was poured into sterile petriplate. The turbidity of inoculum was compared with 0.5 McFarland standards, containing 1-2x10^{8}cfu/ml. After solidification of MHA plates, 100µl of bacterial inoculums adjusted to an optical density (OD) of 0.8 were swabbed on the respective plates. Stock solution of plant extract (20mg/ml) was prepared in ethanol and then different volumes of this extract i.e. 4µl, 6µl, 8µl and 10µl were transferred from stock on sterile Whatman No. 1 filter discs (at concentrations of 80µg, 120µg, 160µg and 200µg/disc). These discs were further placed on MHA plates and incubated for overnight at 37°C. After incubation the diameter of inhibition zone formed around each discs was measured by using HiMedia inhibition zone scale. Ampicillin at a concentration of 10µg/disc was used as a positive control and ethanol (10µl/disc) was used as negative control.

Minimum inhibitory concentration

The modified method of **Elshikh *et al.* (2016)** was adopted for determination of minimum inhibitory concentration **(**MIC) in ethanolic rhizome extract of *B. ciliata*. Rhizome extract of plant were dissolved at twice the concentration in 100µl of Mueller Hinton broth (MHB) in each well of microtitre plate in column 1, while columns 2-10 were dispensed with 50µl of MHB broth only. Column 11 contained 100µl of diluted standard inoculum and column 12 contained 100µl of the MHB (as a control to monitor sterility). Further, 50µl of MHB from column 1 was transferred with micropipette from column 1 till column 10, resulting in 50µl solution per well. A 50µl of bacterial suspension was then added to all the wells containing plant extract in MHB and also to the control wells. After incubation of microtitre plate for overnight at 37°C, resazurin (0.015 %) dye was added to all the wells (30µl per well), and incubated further for 2-4 h to observe the colour change. The microtiter well after which colour change was observed was scored as MIC of plant extract against respective culture under study.

Phytochemical screening

The phytochemical screening of ethanolic extract of *B. ciliata* rhizome was carried out to determine the presence of reducing sugars, alkaloids, saponins, tannins, flavonoids and cardiac glycosides as per the method given by **Solomon *et al.* (2013)**.

GC-MS analysis of the plant rhizome extract

GC-MS analysis of the ethanolic rhizome extract of *B. ciliata* was performed using Thermo Scientific Triple Quadrupole GC-MS (Trace 1300 GC, Tsq 8000 triple quadrupole MS) equipped with TG 5MS (30m X 0.25mm, 0.25µm) column. Helium was used as the carrier gas at a flow rate of 1ml/min using an injection volume of 1.0 µL. Injector temperature was kept at 250°C and ion source temperature was 230°C. The oven temperature was maintained at 50°C isothermal at 280°C. Mass spectra was taken at 70eV with scan interval of 0.5seconds and fragments from 45 to 450Da. Total GC running time used was 36 minutes. The phytochemicals of resolved components of test samples were identified by GC-MS NIST library to confirm the name, molecular weight and structure.

Statistical analysis

The values for each tests were calculated in triplicates as mean ± standard deviation. Results were analyzed by one way ANOVA followed by Tukey's multiple comparison test ($P < 0.05$) to find out the significant differences among the results. Graph Pad Prism software was applied for statistical analysis.

RESULTS AND DISCUSSION

B. ciliata an ethnomedicinal plant is used in the treatment of kidney stones by local people. The plant is found on the rocky surface or tree trunks at high altitude regions in Shimla district in western Himalaya. The scientific validation of medicinal potential of this plant was done by assessing its antimicrobial property and phytochemical analysis and the results of the study are discussed in following sections.

Antimicrobial potential of *B. ciliata*

Antimicrobial activity of *B. ciliata* revealed that at maximum concentration of rhizome extract (200µg/disc) highest inhibition zone (12±1.0mm) was observed against clinical isolate of *S. typhimurium* followed by 11.33±0.57mm inhibition zone against clinical isolate of *E coli.* No inhibition zone was observed in negative control. However, the inhibition zone in standard isolate of *K. pneumoniae* was 10.66±0.57mm, in standard isolate of *E coli* this inhibition zone was 10.33±0.57mm and in clinical isolate of *E. coli* it was 11.33±0.57mm. Results at all concentrations when compared with control were found significantly different while under different treatments (120µg/disc vs 160µg/ disc, 120µg/ disc vs 200µg/ disc) these were found non-significant. The results of antimicrobial activity and MIC activity of rhizome extract of *B. ciliata* are shown in Table 1 and Table 2 respectively. The results showed that MIC was recorded 200µg/100µl respectively against standard and clinical isolate of *P. aeruginosa* and 120µg/100µl against clinical isolate of *S. typhimurium*. However, the MIC in rest of test microbes was found to be 100µg/100µl. **Sinha *et al.* (2001)** while working on antibacterial activity of *B. ciliata* reported that maximum zone of inhibition (15mm) against *S. aureus* was observed by using concentration of 200µg/disc and the minimum activity was observed against *P. aeruginosa i.e.* 3mm with 200µg/disc. However, in present study an inhibition zone of 10mm was observed with *B. ciliata* rhizome extract with concentration of 200µg/disc which was also the MIC against same organism i.e. *P. aeruginosa*.

Table 1 Antimicrobial activity in rhizome extract of *B. ciliata* against different bacterial strains.

Bacterial strains	Antimicrobial inhibition zone (mm)				
	Control	80µg/disc	120µg/disc	160µg/disc	200µg/disc
***E.coli* (S)**	**20.33±0.57^a**	**ND**	**10.00±0.00^b**	**10.00±0.00^b**	**10.33±0.57^b**
***E.coli* (C)**	**21.00±0.00^a**	**ND**	**10.33±0.57^b**	**10.50±0.50^b**	**11.33±0.57^b**
***K.pneumoniae* (S)**	**19.00±0.00^a**	**ND**	**10.00±0.00^b**	**10.33±0.57^b**	**10.66±0.57^b**
***K.pneumoniae* (C)**	**19.33±0.57^a**	**ND**	**10.00±0.00^b**	**10.16±0.28^b**	**10.66±0.57^b**
***S.typhimurium* (S)**	**20.33±0.57^a**	**ND**	**10.00±0.00^b**	**10.33±0.57^b**	**11.00±1.00^b**
***S.typhimurium* (C)**	**19.66±0.57^a**	**ND**	**10.00±0.00^b**	**11.00±1.00^b**	**12.00±1.00^b**
***S.aureus* (S)**	**18.00±0.00^a**	**ND**	**10.66±0.57^b**	**10.50±0.86^b**	**11.00±1.00^b**
***S.aureus* (C)**	**18.33±0.57^a**	**ND**	**10.33±0.57^b**	**10.33±0.57^b**	**11.00±1.73^b**
***P.aeruginosa* (S)**	**20.00±0.00^a**	**ND**	**ND**	**ND**	**10.00±0.00^b**
***P.aeruginosa* (C)**	**19.33±0.57^a**	**ND**	**ND**	**ND**	**10.33±0.57^b**

Where S= Standard strain, C= Clinical isolate, Control=Ampicillin. Values are calculated as mean ± standard deviation (n=3) and significant results are represented by different letters in superscript.

Table 2 MIC in ethanolic rhizome extract of *B. ciliata* against selected bacterial strains

Bacterial strains	Concentrations of rhizome extract (µg/100µl)									
	200	100	50	25	12.5	6.25	3.12	1.56	0.78	MIC
E.coli (S)	-	-	+	+	+	+	+	+	+	100
E.coli (C)	-	-	+	+	+	+	+	+	+	100
K.pneumoniae (S)	-	-	+	+	+	+	+	+	+	100
K.pneumoniae (C)	-	-	+	+	+	+	+	+	+	100
S.typhimurium (S)	-	-	+	+	+	+	+	+	+	100
S typhimurium (C)	-	+	+	+	+	+	+	+	+	200
S.aureus (S)	-	-	+	+	+	+	+	+	+	100
S.aureus (C)	-	-	+	+	+	+	+	+	+	100
P.aeruginosa (S)	-	+	+	+	+	+	+	+	+	200
P.aeruginosa (C)	-	+	+	+	+	+	+	+	+	200

Where +indicates bacterial growth and - indicates no bacterial growth, S= Standard strain, C= Clinical isolate

Chauhan *et al.* (2012a) and **Pokhrel *et al.* (2014)** observed that the leaf extract of *B. ciliata* had much lower antibacterial activity than its rhizome extract owing to the presence of important phytocomponents in rhizome then in the leaves. This plant has also been assessed for its anti-cancerous **(Pokhrel *et al.*, 2014; Chauhan *et al.*, 2012a)**, antiulcer, antitussive **(Ruby *et al.*, 2012; Pokhrel *et al.*, 2014)**, antibacterial **(Hsieh *et al.*, 2001)**, antidiabetic **(Chauhan *et al.*, 2012b)**, antimalarial **(Rajput and Mandal, 2012)** and antiviral **(Rajbhandari *et al.*, 2003)** property in different regions.

Phytochemical analysis of *B. ciliata*

In phytochemical screening of *B. ciliata* all studied phytochemicals i.e. reducing sugars, alkaloids, saponins, tannins, flavonoids and cardiac glycosides were found present in the ethanolic rhizome extract (Table 3).

Table 3 Phytochemical study of selected ethnomedicinal plant collected from Shimla district in H.P., India

Saponins	Flavanoid	Reducing Sugars	Tannins	Alkaloids	Cardiac Glycosides
+	+	+	+	+	+

Where + indicates presence

Ahmad *et al.* (2018) also reported presence of terpenoids, tannins, flavonoids, saponins, steroids, alkaloids, tannins, flavonoids, coumarins and glycosides in the rhizome extract of *B. ciliata* plant in different regions of the world.

GC-MS analysis of *B. ciliata*

In rhizome extract of *B. ciliata* after GC-MS analysis, 26 phytochemicals were identified and most dominant were seen at RT 29.82, 33.38 and 25.17 occupying 12.94%, 12.92% and 9.53% area respectively (Fig. 1).

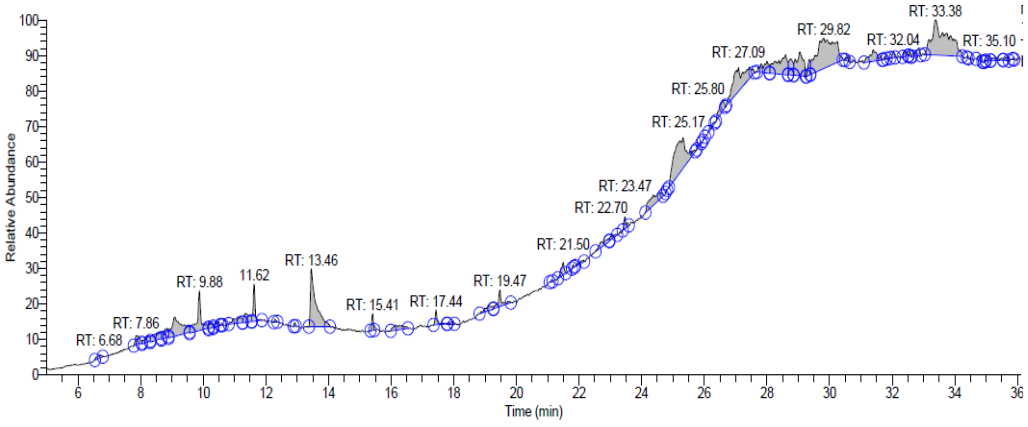

Figure 1 GC-MS analysis of rhizome extract of *B. ciliata*

The dominant phytochemicals includes 9,12,15 octadecatrienoic acid, 2,3bis[(trimethylsilyl) oxy]propyl ester, (Z,Z,Z)/ 1-linolenoylglycerol at RT 29.82 followed by sitasterol at 33.38 RT. Sitasterol possess antioxidant, anthelmintic, anti-mutagenic, antidiabetic, hypocholesterolemic, anti-inflammatory **(Soodabeh *et al.*, 2014)** and cholestrol inhibition property **(Ahmad *et al.*, 2017)** and 1-monolinoleoylglyceroltrimethylsilyl ether/ Monolinolein TMS (RT 25.17) possess antimicrobial, antioxidant, anti-inflammatory, anti-arthritic, anti-asthmatic and diuretic property **(Hugar and Londonkar, 2017)**. All other identified phytochemicals in the rhizome extract are mentioned in Table 4 and the medicinally important phytochemicals are mentioned in Table 5.

Table 4 Phytochemicals present in the rhizome extract of *B. ciliata* using GC-MS analysis.

S. No.	Chemical	Chemical Formula	Mol wt	Area%	Retention Time
1.	**9,12,15 Octadecatrienoic acid, 2,3bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)/ 1-Linolenoylglycerol**	**$C_{27}H_{52}O_4Si_2$**	**496**	**12.94**	**29.82**
2.	**Sitosterol**	**$C_{29}H_{50}O$**	**414**	**12.92**	**33.38**
3.	**1Monolinoleoylglyceroltrimethylsilyl ether/Monolinolein TMS**	**$C_{27}H_{54}O_4Si_2$**	**498**	**9.53**	**25.17**
4.	**Phthalic acid, butyl hex3ylester**	**$C_{18}H_{26}O_4$**	**306**	**7.53**	**13.46**
5.	**2-Decenal**	**$C_{10}H_{18}O$**	**154**	**7.08**	**27.09**
6.	**Astaxanthin**	**$C_{40}H_{52}O_4$**	**596**	**4.87**	**28.60**
7.	**4 Cetene/ 1-Hexadecene**	**$C_{16}H_{32}$**	**224**	**4.64**	**9.09**
8.	***Tetradecamethyl* hexasiloxane**	**$C_{14}H_{42}O_7Si_7$**	**593**	**3.83**	**9.88**
9.	**Psi psi Carotene**	**$C_{40}H_{56}$**	**536**	**1.86**	**28.00**
10.	**Octadecamethyl cyclononasiloxane**	**$C_{18}H_{54}O_9Si_9$**	**667**	**1.66**	**11.62**
11.	**9 Octadecenoic acid/oleic acid**	**$C_{18}H_{34}O_2$**	**282**	**1.43**	**8.62**
12.	**Linalool**	**$C_{10}H_{18}O$**	**154**	**1.37**	**26.59**
13.	**Beta-eudesmol**	**$C_{15}H_{26}O$**	**222**	**1.33**	**19.47**
14.	**Z10Tetradecen1ol acetate**	**$C_{16}H_{30}O_2$**	**254**	**1.27**	**11.35**
15.	**4Octadecenal**	**$C_{18}H_{34}O$**	**266**	**1.27**	**11.35**
16.	**Ethyl iso-allocholate**	**$C_{26}H_{44}O_5$**	**436**	**1.26**	**8.85**
17.	**9Octadecenoicacid,(2phenyl1,3dioxolan4yl) methyl ester, cis**	**$C_{28}H_{44}O_4$**	**442**	**1.13**	**11.11**
18.	**Hexanoic acid/Caproic acid**	**$C_6H_{12}O_2$**	**116**	**1.07**	**16.14**
19.	**Damascenone**	**$C_{13}H_{18}O$**	**190**	**0.94**	**21.50**
20.	**Hexadecanoic acid/Palmitic acid**	**$C_{16}H_{32}O_2$**	**256**	**0.79**	**32.85**
21.	***n*-Pentacosane**	**$C_{25}H_{52}$**	**352**	**0.78**	**17.44**
22.	**Berginin**	**$C_{14}H_{16}O_9$**	**328**	**0.59**	**15.41**
23.	**Azafrin**	**$C_{27}H_{38}O_4$**	**426**	**0.41**	**34.79**
24.	**Gallic acid**	**$C_7H_6O_5$**	**170**	**0.34**	**10.25**
25.	**Caryophyllene**	**$C_{15}H_{24}$**	**204**	**0.11**	**12.92**
26.	**Calcitriol**	**$C_{27}H_{44}O_3$**	**416**	**0.05**	**10.60**

Table 5 Medicinally important phytochemicals identified in the rhizome extract of *B. ciliata*

S.No.	Chemical	Nature of chemical compound	Uses	Reference
1.	**Beta sitasterol**	**Sterol compound**	**Antioxidant, anthelmintic and anti-mutagenic, antidiabetic, hypocholesterolemic and anti-inflammatory**	Soodabeh *et al.*, 2014
			Cholestrol inhibition	Ahmad *et al*., 2017
2.	**1Monolinoleoylglyceroltrimethylsilylether/ Monolinolein TMS**	**Steroid**	**Anti-microbial, anti-oxidant, anti-inflammatory, anti-arthritic, anti-asthmatic, diuretic**	**Hugar** and **Londonkar**, 2017
3.	**Astaxanthin**	**Tetraterpenoid**	**A potential therapeutic agent in cardiovascular disease**	Fassett and Coombes, 2011.
4.	**Cetene**	**Alkene**	**Antioxidant activity**	Abdel-Wahab *et al*., 2017
5.	**Carotene**	**Unsaturated hydrocarbon**	**Antioxidant, cancer preventive and treatment for cardiovascular diseases.**	Boominathan and Bakiyalakshmi, 2016
6.	**Oleic acid**	**Fatty acid**	**Cancer preventive, anemiagenic, insectifuge, antiandrogenic, dermatitigenic.**	Vijisaral and Arumugam, 2014
7.	**Linalool**	**Terpene alcohol**	**Food additives and shows bioactivity**	Ahmad *et al*., 2018
8.	**Ethyl iso-allocholate**	**Steroid**	**Anti-inflammatory, anticancer antimicrobial, antiasthmatic, diuretic**	Zekeya *et al*., 2014
9.	**Hexadecanoic acid**	**Fatty acid**	**Antioxidant, pesticide, hypocholesterolemic, nematicide, antiandrogenic, lubricant, flavor, hemolytic, 5-alpha reductase inhibitor**	Shah *et al.*, 2015
10.	**Berginin**	**Phenol**	**Antioxidant activity**	Ahmad *et al*., 2018
11.	**Gallic acid**	**Phenol**	**Antifungal, antiviral, cytotoxicity, antioxidant**	Ahmad *et al*., 2018
12.	**β-Caryophyllene**	**Sesquiterpene**	**Antimicrobial activity**	Ahmad *et al*., 2018
13.	**Calcitriol**	**Vitamin D3**	**Used in an ointment for the treatment of psoriasis (autoimmune disease)**	Smith *et al*., 1988

CONCLUSION

B. ciliata plant generally found at high altitudes in the western Himalaya is well known for its medicinal potential in dissolving kidney stones. The medicinal property of pant is supported scientifically with antimicrobial potential reported in the present study. The dominant phytochemicals in the rhizome extract as observed by GC-MS analysis includes 9,12,15 octadecatrienoic acid, 2,3bis[(trimethylsilyl)oxy]propylester,(Z,Z,Z)/1-linolenoylglycerol, sitasterol and Monolinolein TMS which further scientifically supports the medicinal use of this important plant of western Himalaya.

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