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Phytochemical profiling and biological activity *of Leucas lanata* Benth. an important ethnomedicinal plant of Western Himalaya

Rachna Verma[a*], Ashwani Tapwal[b], Dinesh Kumar[a] and Sunil Puri[a]

[a]Shoolini University of Biotechnology and Management Sciences, Bajhol, PO Sultanpur, Distt. Solan 173 229 (HP), India.
[b]Himalayan Forest Research Institute, Shimla, H.P., India

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ABSTRACT

Western Himalaya is an abode of medicinal plant wealth witnessed by herbal practitioners since time immemorial. Ethnomedicinal plant *Leucas lanata* have therapeutic potential in curing skin problems. Ethanolic leaves extract of plant possess antimicrobial potential against both standard and clinical isolates of pathogenic bacteria. The preliminary phytochemical screening of ethanolic leaf extract for important phytochemicals confirmed presence of reducing sugars, alkaloids, cardiac glycosides, flavonoids and tannins in plant extract. GC-MS analysis of the plant showed presence of 6-octadecenoic acid (45.54%), cis-13-octadecenoic acid (42.76%) and l-(+)-ascorbic acid 2,6-dihexadecanoate (2.84%) as dominant phytochemicals. This is first report on GC-MS analysis of *Leucas lanata* plant. The phytochemical 6-octadecenoic acid is known for antimicrobial, anticancerous and antiaging property, cis-13-octadecenoic acid have therapeutic uses in medicine and surgery, while ascorbic acid 2,6-dihexadecanoate possess antioxidant, antinociceptive, antiinflammatory, antimutagenic and wound healing property. Plant also possess optimum antioxidant potential as observed by DPPH and FRAP assay.

Key words: *Leucas lanata, Ethnomedicinal plant, Antimicrobial potential, GC-MS, Antioxidant potential, MIC.*

Introduction

Plants have gained importance in the life of human beings because of their acquaintance in day to day life, for dependence on food, cloth and shelter, but besides these, they are also known for their ethnomedicinal significance (Verma *et al.*, 2019). The medicinal properties of plants may be due to presence of various secondary metabolites in them which prevents pathogen invasion (Sen and Batra, 2012). More than 8000 species of vascular plants are found in Indian Himalaya of which 1748 plants are known for their medicinal properties (Joshi *et al*., 2016). In the present scenario, if we look at the adverse effects of antibiotics the use of medicinal plants for curing diseases happens to be a better option over them as they are harmless and cheaper. *Leucas lanata* is one such important ethnomedicinal plant where leaves of the plant are used for curing skin problems. The aim of the present research work was to investigate selected plant's antimicrobial potential, phytochemical properties and antioxidant value to provide scientific support for its existing ethnomedicinal significance and its recommendation for future applications. *L. lanata* is first time reported for its GC-MS study in present research.

Materials and Methods

Plant collection

The *Leucas lanata* plants (Lamiaceae family) used in the present study were collected, from Jubbal region of district Shimla, Himachal Pradesh, India, in the western Himalaya. The plant was identified at Himalayan Forest Research Institute, Shimla (HFRI) and the verified sample (voucher specimen: SUBMS/BOT/546) was submitted in the Herbarium of School of Biological and Environmental Sciences, Shoolini University, Solan, India. The collected plants were washed with water to remove the soil and dust particles. The leaves were dried in shaded place, grinded to fine powder form and stored in an airtight container and used to prepare extract for further study.

Microbial culture

Standard culture of five bacterial strains viz. *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhimurium* (MTCC 98), *Staphylococcus aureus* (MTCC 737), *Pseudomonas aeruginosa* (MTCC 741) were procured from IMTECH Chandigarh, India. Clinical isolates of all these microbial culture were obtained from PGIMER, Chandigarh and IGMC, Shimla, India. All isolates were maintained by sub-culturing once in a month on nutrient agar and stored at a temperature of 4°C. All standard and clinical isolates of these bacterial strains were tested to determine the antimicrobial property of the ethanolic leaf extract of *L.lanata*.

Preparation of plant extract for antimicrobial activity

The ethanolic extract of the plant was prepared by following Selvamohan *et al*. (2012) method by dissolving 10 g fine powder of leaves in 50 mL of ethanol. The contents were kept in rotary orbital shaker for 48h. Finally the leaf extract was filtered through Whatman filter paper No. 1, dried at 40 °C and stored under refrigeration at 4 °C till further studies.

Determination of antimicrobial activity using disc diffusion method

The antimicrobial activity of the plant extract was determined by following Bauer *et al*. (1966) method. For this 20 mL of sterilized Muller Hinton Agar (MHA) was poured into sterile petriplate. The turbidity of inoculum was compared with 0.5 McFarland standards, containing 1-2x10^8cfu/mL. After solidification of MHA plates, 100 µL of bacterial inoculums adjusted to an optical density (OD) of 0.8 were swabbed on the respective plates. Stock solution of plant extract (20 mg/mL) was prepared in ethanol and then different volumes of this extract i.e. 4 µL, 6 µL, 8 µL and 10 µL were transferred from stock on sterile Whatman No. 1 filter discs (at concentrations of 80µg, 120µg, 160µg and 200µg/disc) of 5mm in diameter. These discs were further placed on MHA plates and were 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 10 µg/disc was used as a positive control and ethanol (10 µL/disc) was used as negative control.

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

Screening for presence of phytochemicals

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

GC-MS analysis of the plant leaves extract

GC-MS analysis of the ethanol extract of *L.lanata* leaves 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, 0.25 µm) column. Carrier gas (Helium) was used at a flow rate of 1ml/min. using an injection volume of 1.0µL. 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 transfer line temperature. Mass spectra was taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time was 36 minutes. The phytochemicals were identified by NIST libraries available in the computer library attached to the GC-MS instrument and thus name, molecular weight and structure of phytochemicals present in test sample were ascertained.

Detection of antioxidant properties of selected plants

Antioxidant potential of selected plants was analyzed using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and Ferric Reducing Antioxidant Potential (FRAP) assay.

DPPH radical scavenging assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol (Sutharsingh *et al.,* 2011). DPPH solution (0.004% w/v) was prepared in 95% ethanol. A stock solution of ethanolic extracts and ascorbic acid were prepared in the concentration of 10 mg/100 mL (100 µg/mL). From stock solution 4 mL, 6 mL, 8 mL and 10 mL of this solution were taken in four test tubes respectively. With the same solvent made the final volume of each test tube up to 10 mL whose concentration was then 40 µg/mL, 60 µg/mL, 80 µg/mL and 100µg/mL respectively. 2 mL of freshly prepared DPPH solution (0.004 % w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 minutes and thereafter the optical density was recorded at 523 nm against the blank. For the control, 2 mL of DPPH solution in ethanol was mixed with 10 mL of ethanol and the optical density of the solution was recorded after 30 minutes. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation (Koleva *et al*., 2002) with little modification

$$\text{Percentage (\%) inhibition of DPPH activity} = \frac{A - B}{A} X\ 100$$

Where, A= Absorbance of DPPH radical + ethanol

B= Absorbance of DPPH radical + sample extract/standards

IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals

Ferric Reducing Antioxidant Potential Assay

Antioxidant potential of plant was also determined by FRAP assay as given by Banerjee *et al*. (2008). Plant extracts with different concentrations (40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL) were taken. One ml of this test sample was added to a test tube followed by addition of one mL of 0.2M sodium phosphate, and one ml of potassium ferrocyanide. This mixture was incubated at 50 °C for 20 minutes. After incubation one ml of trichloroacetic acid was added to the test tubes and centrifuged for 10 minutes at 5000 rpm. To one mL of supernatant, one ml of deionized water was added, followed by addition of 200 µL of ferric chloride solution and spectrophotometer readings were taken at 700 nm. The antioxidant compound present in the plant extracts forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride. For preparing blank distilled water was used instead of potassium ferrocyanide. The readings against each concentration were taken in triplicates at 700 nm.

Statistical Analysis

The values in triplicates for each tests were calculated 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 antimicrobial inhibition zones of ethnomedicinal plant extract. Graph Pad prism software was used for statistical analysis.

Results and Discussion

Antimicrobial potential

The plant extract shows antimicrobial activity of *L. lanata* leaves extract in micrograms of concentration per disc(Fig. 1). The plant showed maximum zone of inhibition against standard isolate of *S. aureus*

(14.50±0.50mm), followed by clinical isolate of *S. typhimurium* (14.33±1.52 mm) at 200 µg/disc concentration with no inhibition zone in negative control. Results were statistically significant at all concentrations when compared with control, however non-significant results were observed only between concentration 120µg/disc and 160µg/disc. The MIC against pathogenic bacteria varied from 20 µg/100 µL, 80 µg/100 µL and 100 µg/100 µL.

Dixit *et al*. (2015) evaluated ethanolic extract of the *L. lanata* for antibacterial activity against *Salmonella typhi* and recorded maximum inhibition zone (20.56±0.37mm) followed by *Salmonella typhimurium* (19.76±0.37mm) at 200 µg/disc concentration. While, at lowest concentration (50 µg/disc) it showed no activity or partial antimicrobial activity. However, in the present study leaf extract of the plant had shown MIC at 20 µg/100 µL for clinical isolate of *S. typhimurium* and for standard isolate of *S. aureus.*

Antioxidant Potential

The plant extracts have substantial antioxidant activity against DPPH radical and optimum antioxidant activity in FRAP assay (Table 5). Dixit *et al*. (2015) reported *Leucas lanata* from Madhya Pradesh,

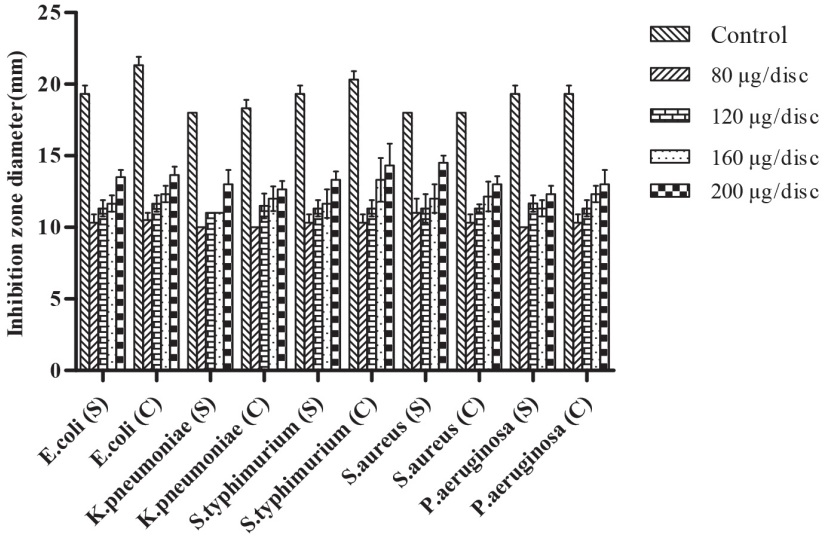

Fig. 1. Antimicrobial activity in ethanolic leaf extract of *L. lanata* against different bacteria. Where S= Standard isolate, C= Clinical isolate, Control (positive) = Ampicillin and bars on figure indicate standard deviation. Values are calculated as mean ± standard deviation (n=3).

Table 1. MIC in ethanolic extract of *L. lanata* leaves against selected bacterial strains

Bacterial strains	Concentrations of plant extract (µg/100 µL)									
	80	40	20	10	5	2.5	1.25	0.62	0.31	**MIC**
E. coli (S)	-	-	+	+	+	+	+	+	+	40
E. coli (C)	-	-	+	+	+	+	+	+	+	40
K. pneumoniae (S)	-	-	+	+	+	+	+	+	+	40
K. pneumoniae (C)	-	-	+	+	+	+	+	+	+	40
S. typhimurium (S)	-	+	+	+	+	+	+	+	+	80
S typhimurium (C)	-	-	-	+	+	+	+	+	+	20
S. aureus (S)	-	-	-	+	+	+	+	+	+	20
S. aureus (C)	-	-	+	+	+	+	+	+	+	40
P. aeruginosa (S)	-	-	+	+	+	+	+	+	+	40
P. aeruginosa (C)	-	+	+	+	+	+	+	+	+	80

Where S= Standard strain and C= Clinical isolate, MHB used as a control to monitor sterility, - = no bacterial growth, + = bacterial growth.

India, for its antioxidant potential by DPPH assay and IC_{50} value for 50% hydro alcohol extract was observed as 122.56 µg/mL however, in present study IC_{50} value for the same plant was 51.87 µg/mL. Lesser the IC_{50} value greater the antioxidant potential hence plant in the present research possess more antioxidant potential.

Phytochemical screening

Standard preliminary screening tests on leaves extract of *L.lanata* showed presence of flavonoids, tannins, cardiac glycosides, alkaloids and reducing sugars (Table 2). *L. aspera* showed the presence of phenols, alkaloids, flavonoids, carbohydrate, saponins, tannins and terpenoids (Kaur and Kumar, 2016). Earlier phytochemicals observed in the *Leucas* species included fatty acids, flavonoids, steroids, lignans, coumarins, terpenes and aliphatic long chain compounds (Chouhan and Singh, 2011).

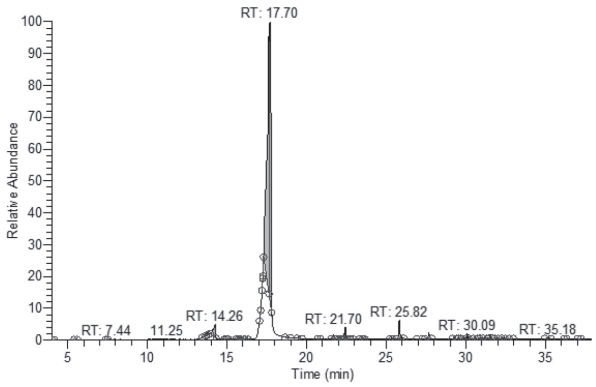

Fig. 2. GC-MS analysis of ethanolic leaves extract of *L. lanata*

Table 2. Phytochemical screening of *L. lanata* leaves extract

Phytochemicals	Tests	Inference
Flavonoids	Alkaline reagent test	+
Tannins	Braymer's test	+
Cardiac Glycosides	Keller Kelliani's test	+
Alkaloids	Wagner's reagent	+
Reducing sugars	Fehling's test	+
Saponins	Foam test	-

Where + = Present, - = absent

GC-MS study of ethanolic leaf extract of *L. lanata*

The prominent phytochemicals present in the *L. lanata* leaves were identified using GC-MS analysis (Fig. 2). Five important phytochemicals were identified at significant peaks in the GC-MS analysis (Table 3) and among these three phytochemicals were observed as medicinally important (Table 4 and Fig. 3). Besides the mentioned phytochemicals in Table 3 some other chemical compounds were also identified at non signficant peak areas in the GC-MS analysis. Moreover the chemical compounds which were not of plant origin includes Methylenebis (2,4,6-triisopropyl phenyl phosphine (0.89%), Milbemycin b,13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-,(6R,13R,25R)-(0.44%), Pregn-5-en-20-one, 3, 16, 17, 21-tetrakis[(trimethylsilyl)oxy]-,O-(phenylmethyl)oxime,(3á,16à)-(0.03%), Glycine, N-[(3à,5á,7à,12à)-24-oxo-3, 7,12-tris [(trimethylsilyl) oxy]cholan-24-yl]-, methyl ester (0.22%). There may be many reasons behind their presence in the plant extract as reported by Venditti (2018).

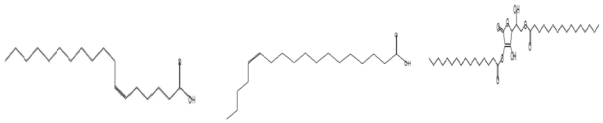

Fig. 3. Medicinally important phytochemicals identified in GC-MS analysis of *Leucaslanata* leaves: 1- 6-Octadecenoic acid; 2 cis-13-Octadecenoic acid; 3- l-(+)-Ascorbic acid 2,6-dihexadecanoate

L. lanata is first time reported for its GC-MS study in the present research, however it's another species *Leucasvirgata Balf.f.* was analysed for GC-MS assay by Mothana *et al.* (2013) and identified β-eudesmol (6.1%) and caryophyllene oxide (5.1%) as major

Table 3. Phytochemicals present in the extract of *L. lanata* leaves using GC-MS analysis.

S. No.	Chemical compound	Chemical Formula	Molwt	Area%	Retention Time
1.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	45.54	17.60
2.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	42.76	17.70
3.	l-(+)-Ascorbic acid 2,6-dihexa decanoate	$C_{38}H_{68}O_8$	652	2.84	14.26
4.	9-Octadecenoic acid(Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester/2-Monoolein	$C_{21}H_{40}O_4$	356	1.88	25.82
5.	2,3-Dihydroxypropyl elaidate/Monoelaidin	$C_{21}H_{40}O_4$	356	0.80	22.44

Table 5. DPPH scavenging activity and Ferric reducing antioxidant power (FRAP) of ethanolic leaves extract of *L.lanata.*

S. No.	Plant extract concentration	DPPH scavenging activity	Standard for DPPH assay (Ascorbic acid)	FRAP activity	Standard for FRAP assay (Ascorbic acid)
1.	40 µg/ml	47.11±0.64	51.60±0.0	0.61±0.051	1.82±0.02
2.	60 µg/ml	50.68±0.38	55.40±0.18	0.79±0.04	1.97±0.02
3.	80 µg/ml	61.18±0.84	62.61±0.22	0.89±0.00	2.09±0.03
4.	100 µg/ml	72.99±0.84	65.57±0.11	1.02±0.03	2.25±0.021

The values are expressed as mean ± standard deviation, n=3

Table 4. Medicinally important phytochemicals in the *L. lanata* leaves extract

S.No.	Chemical compound	Nature of compound	Uses	Reference
1.	6-Octadecenoic acid/ Petroselenic acid	Monounsatur-ated omega fatty acid	Anticancerous	Vijisaral and Arumugam, 2014.
			Anti-aging agent and as a skin-irritation reducing agent	Delbeke *et al*., 2016.
2.	cis-13-Octadecenoic acid	Elaidic acid	Therapeutic uses in medicine, surgery	Arora *et al*., 2017.
3.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Polyphenolic	Antinociceptive, antioxidant, antiinflammatory anti-mutagenic, wound healing property	Mathavi *et al*., 2015.

compounds in the oxygenated sesquiterpenes of plant which differs with the outcome of present findings in *L. lanata*.

Conclusion

The ethnomedicinal plant *L. lanata* is locally used for curing skin problems like wound healing, skin rashes, bruises and herpes disease treatment in Shimla district, Himachal Pradesh, India. The ethanolic leaf extract of plant showed significant antimicrobial potential against pathogenic microorganisms and also optimum antioxidant potential. GC-MS analysis revealed presence of important phytochemicals with anti-nociceptive, anti-cancerous, antioxidant, anti-inflammatory, anti-mutagenic, antiaging and wound healing property (Table 4), which supports the ethnomedicinal significance of plant leaves.

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