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Study on comparative chemical composition and biological activities of *Lantana camera* L. oleoresins

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Abstract
Lantana camara L., commonly known as wild sage, is a widely distributed plant species with various medicinal properties. In this study, we aimed to evaluate the phytochemical constituents and explore the antibacterial, and herbicidal activities of the *Lantana camara* L. oleoresins obtained from two different districts of Uttarakhand. The comparative biological activities and phytochemical composition of *Lantana camera* Dehradun oleoresin (LCDO) and *Lantana camera* Udham Singh Nagar oleoresin (LCUO) revealed the presence of various phytoconstituents. In LCDO the major constituent identified as 2-acetylamino-3-(3,4,5-trimethoxyphenyl) with a percentage composition of 19.21%. In LCUO, major constituent identified as 3-Methylglutaric anhydride (25.02%). However, the oleoresin was evaluated for their antimicrobial activity against two-gram negative bacteria *Pseudomonas aeruginosa and Escherichia coli*, and herbicidal activity against *Raphanus raphanistrum sub sp. Sativus* seeds. The results revealed the presence of several bioactive compounds in both the oleoresins, which exhibited significant biological activities. This research provides valuable insights into the potential applications of *Lantana camara* as a source of natural products with diverse pharmacological properties.

Keywords: *Lantana camara*, antimicrobial, herbicidal, oleoresins, phytochemicals

Introduction
Lantana camara L. is a highly versatile and notorious plant species that belongs to the family Verbenaceae [1]. Originally native to tropical regions of the Americas, *L. camara* has spread widely across the globe due to its aggressive growth, adaptability, and ornamental value. The species is characterized by its opposite, serrated leaves, distinctive tubular flowers arranged in dense clusters, and small, berry-like fruits [2-4]. While appreciated for its vibrant flowers and attractiveness to pollinators, *L. camara* has become a major concern due to its invasive tendencies, ecological impact, and potential negative effects on native biodiversity [5]. The plant's name accurately reflects its tendency to grow vigorously and spread quickly in the wild. *Lantana camara* is known for its vibrant, multi-colored flower clusters, which are highly appealing to both gardeners and pollinators. The flowers, often seen in shades of red, orange, yellow, and pink, create a striking visual display and emit a pleasant fragrance [6]. It has a unique growth habit, forming dense shrubs or sprawling bushes that can reach a height of up to six feet (approx. 2m) [7]. The plants leaves are rough-textured, oppositely arranged, and emit a strong, pungent odor when crushed. This characteristic aroma acts as a natural deterrent against herbivores and pests [8]. *Lantana camara* L. is widely utilized as a medicinal herb and also as a source of firewood and mulch, particularly in India [9]. Despite its reputation as an ornamental plant, lantana possesses several medicinal properties due to its rich phytochemical composition. Phytochemical screening of *Lantana camera* L. leaves and flowers revealed the presence of various secondary metabolites like Alkaloids (lantanine, lantadene A, lantadene B, and lantadene C), flavonoids (quercetin, kaempferol, apigenin, and luteolin), phenolic compounds (gallic acid, caffeic acid, chlorogenic acid, and ellagic acid), terpenoids (oleanolic acid, ursolic acid, and betulinic acid), tannins, saponins, and glycosides were detected in both the oleoresins [10]. The presence of these compounds suggests their potential contribution to the medicinal properties of *Lantana camera* L. [11, 12]. Understanding the biology, ecology, and phytochemical analysis of *Lantana camara* is crucial for effective management and conservation strategies.

Materials and Method

Collection of Plant material

Lantana camara L., fresh leaves and flowers were collected from two different district (Udham Singh Nagar, Altitude- 230m, Latitude 29°9' 30.7224″ N and Longitude 79°8' 46.9932″ E) and Rajawala (Dehradun, Altitude- 648m, Latitude 30°21'2"N and Longitude 77° 59' 30" E) Uttarakhand, India, in the month of March, 2023.

Oleoresin preparation

Leaves and flowers of *Lantana camara* L., were first thoroughly washed with distilled water and air dried in the laboratory at room temperature. Once dried, the plant material was crushed into a coarse powder. The pulverized leaves and flowers underwent extraction using acetone through the cold percolation method [13]. The resulting extract was then filtered and concentrated using a rotary evaporator. The concentrated sample was stored at a temperature of 40°C for future chemical analysis and determination of biological activities.

GC-MS analysis

The phytochemical analysis of the acetone oleoresin obtained from *Lantana camara* was conducted using Perkin Elmer GCMS-SQ8 equipment equipped with a GC capillary column (PE-5 column, 30 m × 0.25 mm, i.d. 0.25 µm). The injector temperature was set to 280 °C with a split ratio of 50:1, and the carrier gas used was helium with a flow rate of 1 mL/min. The identification of constituents in the oleoresin was performed by analyzing their mass spectral fragmentation patterns and comparing their retention index (RI) values with the NIST14.lib MS library, as well as referencing literature data [14].

Evaluation of Herbicidal activity

Using the technique developed by Sahu and Devkota in 2013 [15], the herbicidal activity of acetone oleoresin obtained from *Lantana camara* L. was assessed against radish (*Raphanus raphanistrum* sub sp. Sativus) seeds. Different concentrations of acetone oleoresin, ranging from 250 to 1000 L/mL in a 1% Tween-20 aqueous solution, were prepared in order to evaluate the inhibition of seed germination. The radish seeds were surface sterilized for 15 minutes with a 5% sodium hypochlorite solution before to the experiment. Each petri dish included seven sterilized radish seeds. To ensure proper moisture for germination, the bottom of each petri dish was lined with filter paper. After that, 4 mL of each acetone oleoresin concentration was poured to the plates, and the seeds were left to sprout for 24 hours at a temperature of 25±1 °C in an incubator. Once all of the seeds in the control group had germinated, the experiment was over. The herbicidal activity was assessed by comparing the germination rates of the treated seeds with the control group and a standard herbicide, pendimethalin.

Inhibition of seed germination

% Inhibition =100 × (1- Gt/Gc)

Where,
Gt – no. of seeds germination in treatment,
Gc – no. of seeds germination in control.

Inhibition of shoot length

% Inhibition =100 × (1- Ct/Cc)

Where,
Ct –shoot length in treatment,
Cc – shoot length in control

Inhibition of root length

% Inhibition =100 × (1- Rt/Rc)

Where,
Rt –root length in treatment,
Rc – root length in control

Evaluation of antibacterial activity

The antimicrobial activity of the oleoresin was assessed using the agar diffusion method [16]. Two pathogenic gram-negative bacterial strains, namely *Pseudomonas aeruginosa* and *Escherichia coli*, were employed for the screening. The standard drug Ciprofloxacin was obtained from the Fermentation Lab, Department of Pharmacy, Dev Bhoomi Institute of Pharmacy (DBIPR) in Dehradun. To cultivate the bacteria, nutrient agar was utilized as the growth medium. A loopful of the bacterial culture from a nutrient agar petri plate was transferred into 10 mL of nutrient agar and allowed to grow overnight in an incubator with shaking conditions. For the screening experiment, 20 mL of molten nutrient agar was poured into petri plates and left to solidify. Next, the respective nutrient agar plates were evenly spread with 100 µL of the bacterial strains using a sterilized spreader under aseptic conditions. Once the plates had solidified, wells with a diameter of 5 mm were created in the agar using a cork borer. Various concentrations (2 to 32 µg/mL) of the test samples were poured into each well, with 4 µL per well. Subsequently, the plates were incubated at a temperature of 32±2 °C for 24 hours. Following the incubation period, the diameter of the zone of inhibition, indicating the absence of bacterial growth, was measured in millimeters. Acetone served as a negative control for the oleoresin, while Ciprofloxacin acted as a positive control to compare the antimicrobial potential.

Determination of Minimum inhibitory concentration (MIC)

Using modified technique from the NCCLS (National Committee for Clinical Laboratory Standards) guidelines [17], the minimum inhibitory concentration (MIC) was determined using the agar dilution susceptibility test. To produce decreasing concentration ranges from 32 to 2 µg/mL, serial dilutions of the tested samples were made by dilution with a solvent (acetone and sterilized distilled water for oleoresin). On nutrient agar plates, the bacterial strains were dispersed in a 100 µL solution. The inoculated nutrient agar plates were made into wells on the plates and filled with 50 µg/mL of various quantities of the tested substances. The bacterial plates were then incubated at a temperature of 37±2 °C and a relative humidity of 62±5% for 24 hours. The MIC was determined as the lowest concentration of each tested sample that showed a clear zone of inhibition on the agar plate. Distilled water was used as the negative control, while Ciprofloxacin was used as the positive control.

Statistical analysis

The quantitative data collected from the experiments were subjected to calculate germination percentage and Seedling growth was statistically analyzed as mean ± SD by using one-way analysis of variance (ANOVA) at 5% level of

significance ($p<0.05$). Regression line method was used to calculate IC_{50}.

Result

Comparative GC/MS analysis of LCDO and LCUO

The phytoconstituents present in the LCDO and LCUO oleoresins were identified and are presented in (Table 1), based on their elution order on the PE-5 column in GC-MS. Twenty-five and thirty components were identified in LCDO and LCUO, respectively, contributing to 85.21% and 88.93% of the total oleoresins. In LCDO, the major constituent identified was 2-acetylamino-3-(3,4,5-trimethoxyphenyl), accounting for 19.21% of the composition. This was followed by 2-acetylamino-3-(3,4,5-trimethoxyphenyl) (12.03%), 1,3-butanediol diacetate (10.70%), ethanethioic acid, s-pentyl ester (8.62%), pentane (7.56%), and n-butyl n-hexyl disulfide (6.98%). Other minor constituents present in the acetone oleoresin included 2,4-di-tert-butyl-6-(5.2%), N-butyl n-hexyl disulfide (4.98%), 1-dodecanol (3.6%), disulfide, dipentyl (3.23%), 2,4-imidazolidinedione, 5-methyl- (2.10%), and furazan, 3-(dimethylaminom ethylene amino) (1.2%). In LCUO, 3-methylglutaric anhydride (25.02%) as the major component followed by benzaldehyde (12.94%), 2-acetylamino-3-(3,4,5-trimethoxyphenyl) (10.84%), 4-piperidone (8.8%), 2-hydroxyoctanoic Acid (7.63%), and isoxazolidine (6.2%). Other minor constituents present in the acetone oleoresin included methyl acetoxyacetate (5.87%), 2-hexen-1-ol, acetate (4.5%), 2(3h)-furanone, dihydro-4-methyl- (3.5%), Isobutane (3%), nitroxide, bis(1,1-dimethylethyl) (2.5%), and methanamine, n-butylidene (1.13%). Many of these identified constituents are known to possess several pharmacological activities. 7,10,13-hexadecatrienoic acid, methyl ester and 3-methylglutaric anhydride, a major phytoconstituent of *L. camara* acetone leaf extract, is known to possess strong antimicrobial activity/. Th dodecanol, is an important compound reported with antioxidant, cytotoxic, and antimicrobial properties (7). However, pharmacological activities of other compounds of *L. camara* oleoresins are yet to be determined. Therefore, we assume that the strong bioactivities exhibited by *L. camara* in this study are correlated to the occurrence of these bioactive compounds in the acetone solvent extract. However, further studies on the isolation, characterization, and biological evaluation of these identified compounds are necessary to confirm their potential benefits.

Table 1: Comparative GC/MS analysis of LCDO and LCUO

S. No	Compound identified	RI	% composition	
			LCDO	LCUO
1	N-butyl n-hexyl disulfide	533	4.98	_
2	Pentane	833	7.56	_
3	Ethanethioic acid, S-pentyl ester	313	8.62	_
4	1,3-butanediol, Diacetate	306	10.70	_
5	2-Acetylamino-3-(3,4,5-trimethoxyphenyl)	307	12.03	_
6	1-Dodecanol	1455	3.6	_
7	2-Acetylamino-3-(3,4,5-trimethoxyphenyl)	365	19.21	_
8	7,10,13-Hexadecatrienoic acid, methyl ester	2380	1.36	_
9	Disulfide, dipentyl	314	5.23	_
10	2,4-di-tert-butyl-6-	363	5.2	_
11	Furazan,3(Dimethylaminom ethylen amino)	307	1.2	_
12	2,4-Imidazolidinedione, 5-methyl-	591	2.10	_
13	Methenamine, N-butylidene-	849	_	1.13
14	Isobutane	650	_	3
15	(+-)-2-Hydroxyoctanoic acid, acetate	564	_	7.63
16	Methyl acetoxyacetate	328	_	5.87
17	2-Acetylamino-3-(3,4,5-Trimethoxyphenyl)	360	_	10.84
18	Benzaldehyde	363	_	12.94
19	3-Methylglutaric anhydride	591	_	25.02
20	Isoxazolidine	542	_	6.2
21	Nitroxide, bis (1,1-dimethylethyl)	525	_	2.5
22	2(3h)-furanone, dihydro-4-methyl-	509	_	3.5
23	4-Piperidone	495	_	8.8
24	n-Hexane	594	_	3.25
25	2-Hexen-1-ol, acetate	527	_	4.5

LCDO - *Lantana camera* L. Dehradun oleoresin; LCUO - *Lantana camera* L. Udham Singh Nagar oleoresin.; RI=Retention index

Herbicidal activity

Inhibition of seed germination

The mean percent seed germination inhibition of oleoresin from two altitudinal regions LCDO and LCUO of *L. camera* at different concentrations (250, 500, 750 and 1000 µL/mL) has been depicted in (Table 5). The herbicidal activity of LCDO and LCUO oleoresin of *L. camera* at the highest concentration (1000 µL/mL) was found in the order of LCDO (94.66%) > LCUO (92.33%). *L. camera* oleoresins have also been reported to have significant herbicidal activity against *Raphanus raphanistrum* at concentrations (250-1000 µL/mL). The IC50 was computed at the point where 100% germination was reached in the control. It is used to compare the relative herbicidal activities of the samples. The order in which the activity was observed in terms of IC_{50} was as follows: LCDO (88.97±4.28µL/mL) > LCUO (104.46±13.30 µL/mL) (Table 2 and Fig. 1). Regarding the herbicidal activity of the oleoresins from *Lantana camara*, there have been some studies conducted to investigate its potential as a natural herbicide. The plant contains various chemical compounds, including

pentacyclic triterpenes, flavonoids, and essential oils, which are believed to contribute to its herbicidal properties.

Table 2: IC_{50} values for seed germination

S. No.	Sample name	IC_{50} values (µL/mL) in triplicates			Mean IC_{50} values (µL/mL) ±SD
		I	II	III	
1	LCDO	93.75	87.71	85.47	88.97±4.28
2	LCUO	89.28	105.04	85.47	104.46±13.30

LCDO - *Lantana camera* L. Dehradun oleoresin; LCUO - *Lantana camera* L. Udham Singh Nagar oleoresin.

Inhibition of root length of LCDO

The percent inhibition of root length was calculated at the time when 100% germination was achieved at various concentrations range of 250, 500, 750, 1000 µL/mL. In the case of LCDO the percent inhibition of root length was recorded as 58%,66.33%, 80.66% and 92.33%, while in the case of LCUO, the percent inhibition was measured as 58.66%, 67.33%, 80.66% and 93% respectively from lowest to highest concentrations (Table 5). The order in which the activity was observed was as follows: LCUO (89.01±31.12 µL/mL) > LCDO (105.47±32.91µL/mL) (Table 3 and Fig.1).

Table 3: IC_{50} values for root length

S. No.	Sample name	IC_{50} values (µL/mL) in triplicates			Mean IC_{50} values (µL/mL) ±SD
		I	II	III	
1	LCDO	78.82	95.33	142.27	105.47±32.91
2	LCUO	55.80	93.75	117.52	89.01±31.12

LCDO - *Lantana camera* L. Dehradun oleoresin; LCUO - *Lantana camera* L. Udham Singh Nagar oleoresin

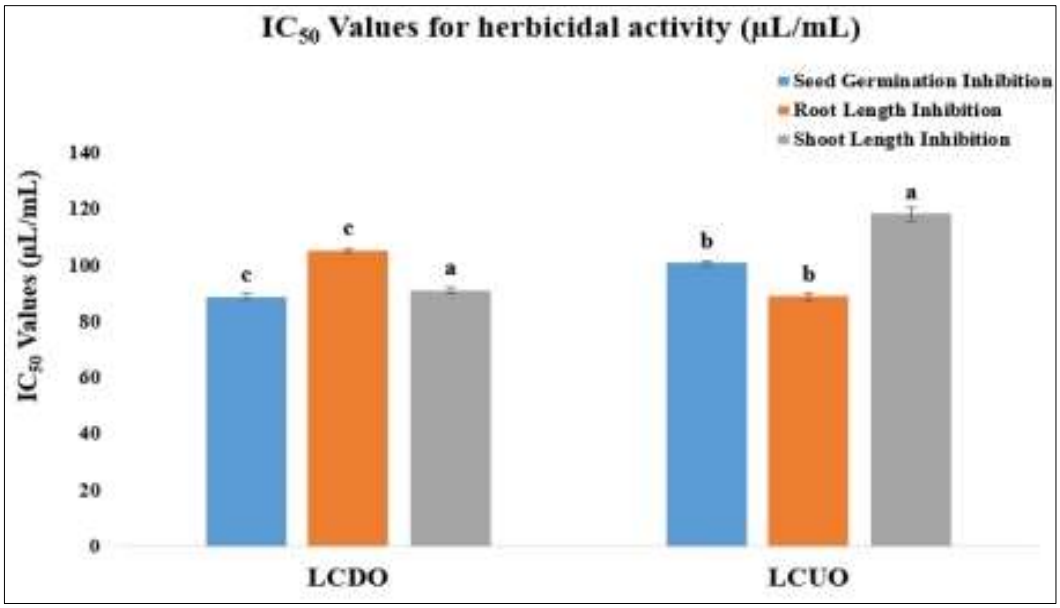
Inhibition of shoot length

The percent inhibition of shoot length was calculated at the time when 100% germination was achieved at various concentrations range of 250, 500, 750, 1000 µL/mL. In the case of, the percent inhibition of root length was recorded as 58.66%, 67.33%, 80.66% and 93% and in the case of LCDO the percent inhibition of root length was recorded as60%, 66.33%, 80.33% and 94.66% respectively (Table 5). The order in which the activity was observed was as follows: LCDO (91.22±30.11 µL/mL) > LCUO (118.52±37.28µL/mL) (Table 4 and Fig.1).

Table 4: IC_{50} values for shoot length

S. No.	Sample name	IC_{50} values (µL/mL) in triplicates			Mean IC_{50} values (µL/mL) ±SD
		I	II	III	
1	LCDO	63.02	122.95	87.71	91.22±30.11
2	LCUO	88.49	106.83	160.25	118.52±37.28

LCDO - *Lantana camera* L. Dehradun oleoresin, LCUO - *Lantana camera* L. Udham Singh Nagar oleoresin



LCDO - *Lantana camera* L. Dehradun oleoresin; LCUO - *Lantana camera* L. Udham Singh Nagar oleoresin.

Fig 1: IC_{50} values of seed germination, root length, shoot length of *Lantana camera* L. oleoresins

Table 5: Percent inhibition of seed germination, root length, shoot length of *Lantana camera* L.

S. No.	Sample name	% Inhibition of seed germination				% Inhibition of root length				% Inhibition of shoot length			
		250 µL/mL	500 µL/mL	750 µL/mL	1000 µL/mL	250 µL/mL	500 µL/mL	750 µL/mL	1000 µL/mL	250 µL/mL	500 µL/mL	750 µL/mL	1000 µL/mL
1	LCDO	60±1.00	66.33±1.52	79.33±1.15	94.66±1.15	58.00±2.64	66.33.±1.52	80.66±1.15	92.33±0.57	60.00±2.00	66.33±1.73	80.33±1.52	94.66±2.88
2	LCUO	59.00±1.52	64.66±0.57	80.66±1.15	92.33±0.57	58.66±2.08	67.33.±2.51	80.66±1.15	93±1.00	59.00±1.00	63±2.08	79.00±2.64	92.33±0.57
3	Pendimethalin*	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

*Standard herbicide; LCDO - *Lantana camera* L. Dehradun oleoresin; LCUO - *Lantana camera* L. Udham Singh Nagar oleoresin.

Antibacterial activity

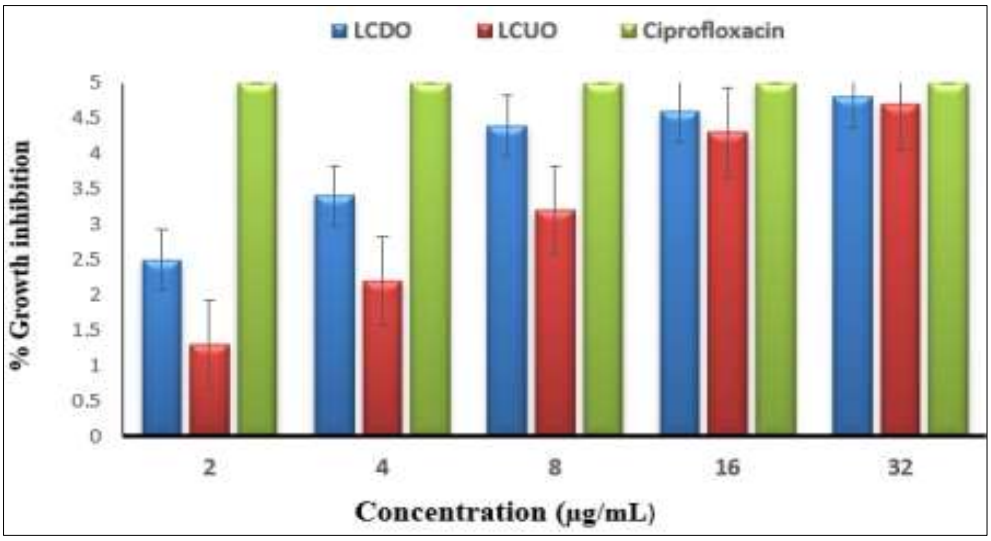
Lantana camara oleoresins and their chemical constituents have been reported to have effective action against various bacterial strains. In this study, we have explored the antibacterial activity of LCDO and LCUO using zones of inhibition assay against Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*. The agar diffusion method confirmed that both LCDO and LCUO

showed antibacterial activity against both the bacterial pathogens. However, LCDO showed a higher zone of inhibition against both Gram-negative bacteria, *Pseudomonas aeruginosa* causes infections in the blood, lungs (pneumonia), or other parts of the body after surgery. *Escherichia coli* is a common and clinically significant Gram-negative pathogenic bacterium that causes cause diarrhea/bloody diarrhea, vomiting and stomach pains and cramps. Results showed that, LCDO had potential antibacterial activity against both bacterial pathogens. The antibacterial activity of LCDO and LCUO at 32 µg/mL concentration against *Pseudomonas aeruginosa* were compared with Ciprofloxacin and found in order: Ciprofloxacin (ZOI= 5.0 mm) > LCDO (ZOI= 4.8 mm) > LCUO (ZOI= 4.7mm). Against *E. coli* at the same concentration LCDO showed good antibacterial activity (ZOI= 4.7 mm) whereas LCUO showed the moderate (ZOI= 4.2 mm) in comparison standard Ciprofloxacin (ZOI= 5.0mm). LCDO exhibited MIC of 8 µg/mL against *P. aeruginosa* while for *E. coli* the MIC was found to be 16 µg/mL. LCUO showed MIC of 16 µg/mL against for both *P. aeruginosa* and *E. coli*. The results of the antibacterial activity have been represented in Table 6 and Fig. 2, 3.

Table 6: Percent growth inhibition by LCDO and LCUO against *Pseudomonas aeruginosa* and *Escherichia coli*

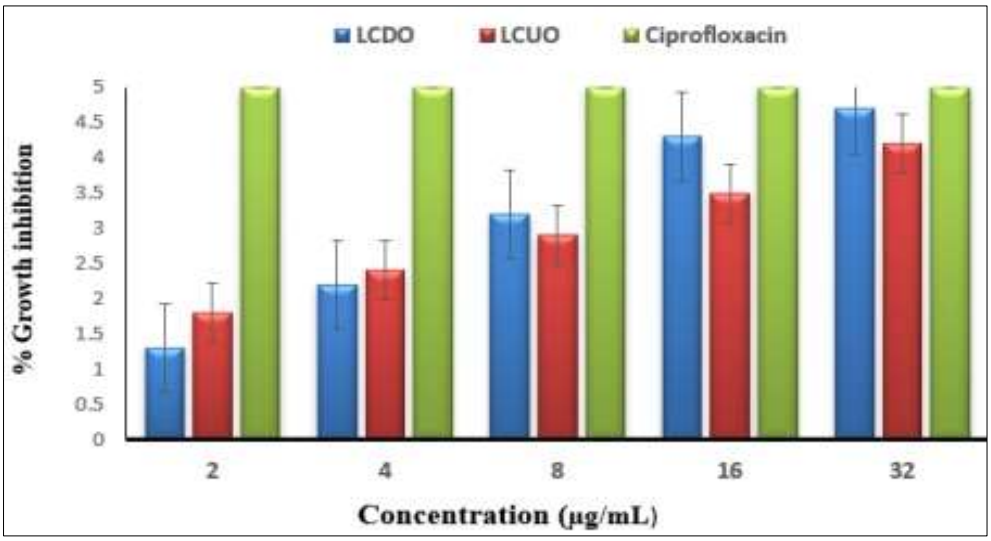
Sample	Concentration (µg/mL)	Percent growth inhibition	
		Pseudomonas aeruginosa	***Escherichia coli***
LCDO	2	2.5±0.02	1.3±0.02
	4	3.4±0.01	2.2±0.01
	8	4.4±0.03	3.2±0.03
	16	4.6±0.03	4.3±0.03
	32	4.8±0.02	4.7±0.03
LCUO	2	1.2±0.04	1.8±0.02
	4	2.6±0.01	2.4±0.02
	8	3.1±0.01	2.9±1.03
	16	3.9±0.02	3.5±0.05
	32	4.7±0.01	4.2±0.03
Ciprofloxacin	6	5.0±0.00	5.0±0.00

LCDO= *Lantana camera* Dehradun oleoresin; LCUO= *Lantana camera* Udham singh Nagar oleoresin; ZOI= Zone of inhibition



LCDO= *Lantana camera* Dehradun oleoresin; LCUO= *Lantana camera* Udham singh Nagar oleoresin

Fig 2: Percent growth inhibition by LCDO and LCUO against *Pseudomonas aeruginosa*



LCDO= *Lantana camera* Dehradun oleoresin; LCUO= *Lantana camera* Udham singh Nagar oleoresin

Fig 3: Percent growth inhibition by LCDO and LCUO against *Escherichia coli*

Conclusion

The phytochemical analysis of *Lantana camera* L. oleoresins revealed the presence of various bioactive compounds. The strong herbicidal properties and potential antibacterial activity of both the samples could lead in the formation of natural herbicide and antimicrobial pesticides. The oleoresins exhibited promising antimicrobial and herbicidal activity, indicating their potential application in pharmaceuticals, agriculture and can be useful in search of a more selective, biodegradable, naturally produced and environment friendly biopesticides. Further studies are warranted to isolate and identify specific bioactive compounds responsible for these activities and elucidate their mechanism of action. Additionally, studies on the toxicological profile and safety of these extracts are necessary to ensure their safe utilization in various applications.

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