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Effect of altitudinal variations on biological activities of *Justicia adhatoda* L. growing wildly in Western Himalayas: An *in vitro* assessment

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ABSTRACT

An investigation was carried out to check the influence of altitude on antibacterial, antifungal, anti-inflammatory, and antioxidant activities displayed by ethanolic leaf extracts of *Justicia adhatoda* L. *Justicia adhatoda* is a medicinal plant, growing predominantly at an altitude of 1,300 m above sea level. The collections of leaves were made from plants growing across different altitudinal ranges. The antibacterial activity was tested against four different bacterial strains, namely *Escherichia coli* (MTCC 82), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 2453), and *Klebsiella pneumonia* (MTCC 39) by disk diffusion assay and subsequently antifungal activity was tested on *Rosellinia necatrix* (HG964402.1) and *Fusarium* spp. (SR266-9) by poisoned food technique. The leaf extracts exhibited a potent inhibitory effect on the tested bacterial and fungal strains. Consequently, the antioxidant activity was found to be significantly higher among the extracts from high altitudes, whereas the difference observed in antibacterial, antifungal, and anti-inflammatory activities is not statistically significant. The extracts were further characterized by GC–MS analysis and the presence of pharmacologically important compounds, viz. 1-hexyl-2-nitrocyclohexane, 2-naphthalenamine, 1-butanol, 3-methyl-, acetate, and 1-docosene, were reported.

INTRODUCTION

Justicia adhatoda L. is an intense restorative plant which is utilized in treating different sicknesses, exceptionally comparable to respiratory issues, and has different organic activities (Gangwar and Ghosh, 2014). The dynamic metabolites of a restorative plant assume the main part in deciding their remedial potential. The plants' active compounds vary with geographical locations and altitude (Chelghoum *et al*., 2021; Goyal *et al*., 2021). The environmental factors directly or indirectly affect the plants' active constituents and their biological activities (Jugran *et al*., 2016). Geographical regions are positioned far from one another possess diverse climatic conditions, thereby impacting the production of the plants' phytochemicals, and hence alter the efficacy of various biological activities, viz., antimicrobial activity, antioxidant activity, etc., (Mehdizadeh *et al*., 2018). Plant cells own the tendency to produce variable compounds, for instance, secondary metabolites, for their use in defense and also possess several biological activities (Bakhtiari *et al*., 2021; Compean and Ynalvez, 2014; Wallace, 2004). It is anticipated that the genuine and reliable medicines produced from plants in their native provenance contain adequate and best chemical compositions (Dong *et al*., 2011). Altitude can affect the potency of plant extracts, for instance, in a study, the antimicrobial efficiency of *Satureja thymbra* essential oils collected from different altitudes was tested against various pathogens, and plants from low altitude exhibited better antifungal activity, whereas those growing at a high altitude showed better antibacterial activity. Both low and

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high altitude essential oils displayed better anthelmintic activity in comparison to the standard drug piperazine citrate (Khalil *et al*., 2020). In a study, it was found that the antioxidant potential of highland populations of *Hibiscus rosa-sinensis* was greater in comparison to the lowland population (Wong *et al*., 2009). A study was conducted on the different grape stems in Portugal where the lower altitude regions were observed to have higher phenolic percentage and higher antioxidant activities (Gouvinhas *et al*., 2020). In another study, *Zataria multiflora* Boiss was investigated in Iran across its different natural habitats where it was demonstrated that the phytochemical content and biological activities were largely influenced by environmental conditions in different natural habitats (Niczad *et al*., 2019). Plant needles of *Taxus wallichiana* were gathered from various areas in Rudraprayag locale of Uttarakhand and the study exposed that the altitude considerably affected cancer prevention agent actions just as phenol, flavonoids, and other bioactive mixtures. Then again, the review divulged no relationship of tannins and taxols with altitude; however, they set up a connection with the counter microbial movement which thus suggested that the counter microbial action was primarily a result of those phytochemicals (Adhikari *et al*., 2020). Henceforth, considering the altitude at which a plant develops holds significance. Previously, studies have been carried out on the determination of biological activities and medicinal potential of *J. adhatoda* L., but this is the first report regarding the effect of different altitudes on the biological activities of *J. adhatoda* L.

MATERIALS AND METHODS

Sample collection and preparation

Different provenances of Jammu which varied in altitude were selected and sample collection was carried out for two consecutive years, viz. 2019 and 2020. The samples were collected before the monsoon, i.e., during the month of June. In this study, purposive sampling was performed and three different altitudes, viz. 336, 688, and 1,330 m, were selected for sample collection according to the availability of plants in the specific areas. The collected plant specimens were verified by the Botanical Survey of India, Dehradun, and submitted in the herbarium of Shoolini University under voucher numbers SUBMS/BOT-4800 (Vijaypur, 336 m), SUBMS/BOT-4801 (Harotkot, 688 m), and SUBMS/BOT-4802 (Khetriar, 1,330 m). The leaf sample collection was carried out in triplicates from each selected altitude. The mature leaves were hand harvested from each plant. Fifth and sixth mature leaves from the top of the plant were taken. Leaves were collected from all the four directions of each plant. The collected leaves were cleaned and shade dried, and after that, the leaves were crushed in a mechanical grinder to obtain fine powder and stored at 4°C for further analysis.

Extraction of leaves

The ethanolic extract of leaf powder samples were prepared in order to perform further experiments. A 10 g leaf powder sample was dissolved in 100 ml of 80% ethanol and further kept in a rotary orbital shaker for 48 hours. The extracts were filtered through Whatman filter paper and dried at 40°C and stored under 4°C till further use.

Microorganisms

The *in vitro* antibacterial activity was assessed against four different strains, viz. *Escherichia coli* (MTCC 82), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 2453), and *Klebsiella pneumonia* (MTCC 39) by disk diffusion assay and subsequently antifungal activity was tested on *Rosellinia necatrix* (HG964402.1) and *Fusarium* spp. (SR266-9) by poisoned food technique.

Antimicrobial activity

Antibacterial activity was performed by disk diffusion method, following the protocol of Duraipandiyan *et al*. (2015). Petri plates were prepared by taking nutrient agar at a concentration of 20 ml. Nutrient agar for bacterial and fungal strains was prepared by autoclaving them at 121°C for 30 minutes. The material obtained was then poured into petri plates and allowed to solidify. A loop full of broth containing microbes was taken and uniformly spread on the nutrient agar plate. The loaded disk with different concentrations of the extract was placed on the medium. Distilled water was used for the negative control. Ampicillin served as a positive control. The plates were witnessed after incubation. Incubation was carried out for 24 hours at 37°C. The inhibition zones were produced because of the inhibitory activity of the extract and their diameters were measured and recorded.

Antifungal activity

The poisoned food technique was used for examination of antifungal efficacy by following the protocol given by Kumar and Garampalli (2015). Specific amounts of extracts were added in sterilized potato dextrose agar in petri plates. After that, actively growing mycelium disks of both the pathogens were taken and placed in the plates. Hygromycin was used in the place of positive control. Plates lacking plant extracts were reserved for negative control and all the plates were kept for incubation at a temperature of 27°C. After 7 days, the estimation of the growth of pathogen in respective plates was determined by measuring their diameter:

$$\text{Inhibition}\% = \frac{\text{Growth in control plate} - \text{Growth in poisoned plates}}{\text{Growth in control plate}} \times 100$$

Antioxidant activity

The antioxidant capacity was determined by following DPPH radical scavenging assay and the method of Braca *et al*. (2001) was followed. Methanol was used to prepare the stock solution by adding it to the extracts. DPPH solution was also added in a specific quantity to the extracts, followed by serial dilutions. After 10 minutes, the absorbance was recorded at 515 nm using a spectrophotometer. For reference, standard ascorbic acid was used and IC_{50} values were also calculated.

$$\text{Scavenging activity}\% = \frac{\text{Absorbance of the control} - \text{Absorbance of test sample}}{\text{Absorbance of the control}} \times 100$$

Anti-inflammatory activity

The protocol described by Sarveswaran *et al*. (2017) was followed. This assay was based on protein denaturation. Denaturation is a well-established cause of inflammation. Capability of the leaf extract to prevent denaturation was studied

in this assay. Bovine serum albumin was used instead of protein. Denaturation was persuaded by maintaining the reaction mixture in a water bath for 10 minutes at 70°C. The reaction mixture was made with different concentrations of the plant extract, i.e., 1,000 µl (100–500 µg/ml), 450 µl bovine serum albumin, and phosphate buffered saline, i.e., 1,400 µl. Then, the mixtures were incubated for 15 minutes at 37°C and after that they were maintained at 70°C for 5 minutes. Diclofenac sodium was used instead of the control and the absorbance was taken at 660 nm. The percentage inhibition of protein denaturation was measured with the help of following equation:

$$\text{Inhibition\%} = \frac{\text{Absorbance of the control} - \text{Absorbance of test sample}}{\text{Absorbance of the control}} \times 100$$

Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis of *J. adhatoda* L. extract was performed by using an instrument, viz. Thermo Scientific (Trace 1300 GC,TSQ Duo, Triplus RSH auto sampler) equipped with Thermo Scientific column DB-5MS with the following dimensions: length = 40 m, ID = 0.15 mm, film thickness = 0.15 µm. Carrier gas = helium, and flow rate = 0.7 ml/m. Oven temperature program was as follows: 80°C (1 minute hold), 10°C/minutes, 180°C (2 minutes hold), 10°C/min, 260°C (10 minutes hold). Split flow = 50 ml/minute and split ratio = 71.4. Injector temperature = 250°C and ion source temperature = 230°C. MS Foreline temperature = 250°C at 70 eV. Mass range = 45–450. The data were interpreted using Xcalibur software and NIST/EPA/NIH Mass spectral library, version 2.2, build 2014.

Statistical analysis

Data were analyzed by one-way ANOVA and Bonferroni's multiple comparison tests.

RESULTS AND DISCUSSION

Susceptibility of various microbes was tested against *J. adhatoda* L. leaf extracts from different elevations. The antibacterial efficacy of extracts was considerable against all the tested strains and the inhibition rates are described in Table 1.

All the extracts showed moderate to significant inhibitions of microbial growth in comparison to that of standard drug Ampicillin (Fig. 1).

Justicia adhatoda L. leaf extracts were able to inhibit mycelia growth better in *Rosellinia* in comparison to that of *Fusarium* (Fig. 2).

The comparison between antifungal activities from different altitudes was made and no significant result was observed. The difference between the resistances of tested fungi against *J. adhatoda* L. leaf extracts is described in Table 2.

The plants' antimicrobial activity is known to be mainly because of its phytochemicals, for example, taxol, tannins, and flavanols. Previous studies have suggested that antimicrobial activity depends on the phyto-constituents of plant extracts, for instance, the antimicrobial bustle of phenolic compounds is because they have a significant impact on the permeability of the membrane and consequently the ratio of penetration in bacterial cell which in turn promotes the damage and inactivation of the cellular profile (El-Jalel *et al*., 2018; Moreno *et al*., 2006; Russo *et al*., 2013). Besides this, other phenol imitative compounds, such as thymol, carvacrol, etc., are known to lay the basis of cellular profile disruption, viz. inhibition of ATPase activity and further components of numerous microorganisms such as *E. coli*, *P. aeruginosa*, *S. aureus*, and *Salmonella enteritidis* (Cetin-Karaca, 2011). However, the variation in potency of *J. adhatoda* L. leaf extracts from different elevations did not show any significant result (Table 1), which is in agreement with a study conducted on *Taxus wallichiana* in Uttarakhand, where the impact of altitude on the biological activities was checked, but the antimicrobial activity did not vary with elevation, perhaps displaying a positive correlation to secondary metabolites (Adhikari *et al*., 2020). *Justicia adhatoda* L. is mainly known for its bronchodilator and antihistaminic effects that are attributed to its active alkaloid content. Various bioactive molecules present in plants take part in antimicrobial action, for instance, alkaloids, flavonoids, terpenes, etc., contain an extremely variable chemical structures with high potential antibacterial activities (Bahman *et al*., 2019; Gorniak *et al*., 2018). Bioactive constituents of plants exhibit an extraordinary spectrum of pharmacological activities, such as antibacterial, antifungal, antiprotozol, astringent, and circulatory stimulant. In a study, the alkaloids were extracted from hot methanolic extracts of *J. adhatoda* L. and evaluated for antimicrobial activity against clinically important bacteria, viz. *E. coli*, *S. aureus*, *Proteus mirabilis*, *Salmonella typhi*, *P. aeruginosa*, *Candida albicans*, etc., where the isolated alkaloids exhibited potent activity against the tested microorganisms (Bailey-Shaw *et al*., 2018; Jasim *et al*., 2015; Joo *et al*., 2010; Karthikeyan *et al*., 2009; Keesara and Jat, 2017; Rahaman and Sharma, 2018; Sawant *et al*., 2013; Shahwar *et al*., 2012; Singh and Sharma, 2013). Plants grow defense strategies to guard themselves from various pathogens present in the environment. They accumulate defense compounds that are chiefly secondary metabolites comprising good antimicrobial activities. It is assumed that these bioactive plant-derived secondary metabolites play a chief role in synthesizing antimicrobial agents with some pharmacological effect which in turn helps if any bacteria or fungi attacks the plants (Reichling, 2009).

Significant variations were observed among antioxidant activities of extracts from different altitudes (Fig. 3). *Justicia adhatoda* L. displayed the highest antioxidant activity among the extracts from a higher elevation, viz. 1330 m, in comparison to that of low and mid-altitudes, viz. 336 and 688 m, which emphasizes that environmental differences attributed to the observed variation in the antioxidant potential of *J. adhatoda* L. (Table 3).

Phenolic and flavonoid compounds are chiefly responsible for the antioxidant property. In a study conducted on *Hedychium spicatum* in Uttarakhand, all the antioxidant assays carried out on plant extracts showed significant correlations with the total phenolic compounds. The assays work on a similar mechanistic approach, i.e., transfer of electrons from the antioxidant to reduce an oxidant (Rawat *et al*., 2011). In adverse environmental conditions, the free radicals are generated that cause damage in their system and in order to deal with the changing environmental conditions, plants either stimulate protection mechanisms or initiate the repair mechanism (Frohnmeyer and Staiger, 2003). Oxidation is a crucial process and a prerequisite in order to run the biological processes efficiently (Jha *et al*., 2014). Plants possess a powerful antioxidant system (Toldi *et al*., 2019). The antioxidant activity of plants comes mostly from the

Table 1. Antibacterial activity of *J. adhatoda* L. ethanolic leaf extracts collected from different provenances of Jammu against selected standard and clinical bacterial isolates. Data are represented as mean ± standard deviation.

Altitude (m.a.s.l.)	Bacterial strains	Year	Anti-bacterial zone (mm)				
			Control	Concentration of leaf extract (mg/ml)			
				0.5	1	1.5	2
336	*E. coli*	First year	21.5 ± 0.264	12.1 ± 0.529	13.1 ± 0.550	13.2 ± 1.011	13.3 ± 0.404
		Second year	21.2 ± 0.655	11.4 ± 0.173	13.7 ± 0.173	13.5 ± 0.493	13.3 ± 0.838
688	*E. coli*	First year	21.9 ± 0.781	11.8 ± 0.513	12.3 ± 0.251	12.3 ± 0.173	13.4 ± 0.435
		Second year	22.5 ± 0.793	11.4 ± 0.513	13.7 ± 0.288	13.5 ± 0.7	13.3 ± 0.529
1,330	*E. coli*	First year	21.3 ± 0.1	11.2 ± 0.2	12.2 ± 0.624	12.1 ± 0.519	13.6 ± 0.152
		Second year	21.2 ± 0.115	12.1 ± 0.7	12.2 ± 0.472	12.2 ± 0.416	13.8 ± 0.404
336	*S. aureus*	First year	21.8 ± 0.55	11.2 ± 0.115	12.5 ± 0.305	12.8 ± 0.404	13.1 ± 0.435
		Second year	21.7 ± 0.568	11.6 ± 0.519	12.6 ± 0.115	13.3 ± 0.321	13.7 ± 0.152
688	*S. aureus*	First year	21.7 ± 0.351	11.9 ± 0.655	12 ± 1.096	13.6 ± 0.115	13.7 ± 0.264
		Second year	22.1 ± 0.814	12.1 ± 0.115	13.5 ± 0.305	13.6 ± 0.378	14.2 ± 0.208
1,330	*S. aureus*	First year	21.5 ± 0.264	12.2 ± 0.723	12.6 ± 0.152	13.1 ± 0.36	13.3 ± 0.251
		Second year	21.6 ± 0.2	11.3 ± 0.529	12.4 ± 0.36	12.5 ± 0.416	13.7 ± 0.152
336	*P. aeruginosa*	First year	21.4 ± 0.472	11.8 ± 0.173	12.7 ± 0.173	13 ± 0.152	13.7 ± 0.208
		Second year	21.2 ± 0.152	11.3 ± 0.4	12.3 ± 0.435	13.2 ± 0.493	13.6 ± 0.556
688	*P. aeruginosa*	First year	21.6 ± 0.264	11 ± 0.838	12.4 ± 0.321	13.2 ± 0.152	13.5 ± 0.351
		Second year	21.7 ± 0.493	11.7 ± 0.208	12 ± 1.04	13.5 ± 0.665	13.6 ± 0.152
1,330	*P. aeruginosa*	First year	21.4 ± 0.152	11.9 ± 0.493	12.5 ± 0.115	13.4 ± 0.173	13.7 ± 0.1
		Second year	21.7 ± 0.568	11.2 ± 0.4	12.7 ± 0.115	13.3 ± 0.556	13.3 ± 0.493
336	*K. pneumonia*	First year	22.3 ± 0.45	12.1 ± 0.602	12.3 ± 0.378	13.3 ± 0.7	13.8 ± 0.1
		Second year	22 ± 0.288	11.7 ± 0.6	12.6 ± 0.2	13.2 ± 0.416	13.7 ± 0.351
688	*K. pneumonia*	First year	21.9 ± 0.416	12.1 ± 0.55	12.4 ± 0.305	13.2 ± 0.152	13.4 ± 0.152
		Second year	21.8 ± 0.55	11.4 ± 0.251	12.4 ± 0.585	13 ± 0.321	13.7 ± 0.351
1,330	*K. pneumonia*	First year	22.3 ± 0.896	11.9 ± 0.692	12.2 ± 0.208	13.2 ± 0.36	13.4 ± 0.461
		Second year	21.6 ± 0.642	11.7 ± 0.556	12.2 ± 0.568	13.1 ± 0.493	13.8 ± 0.264

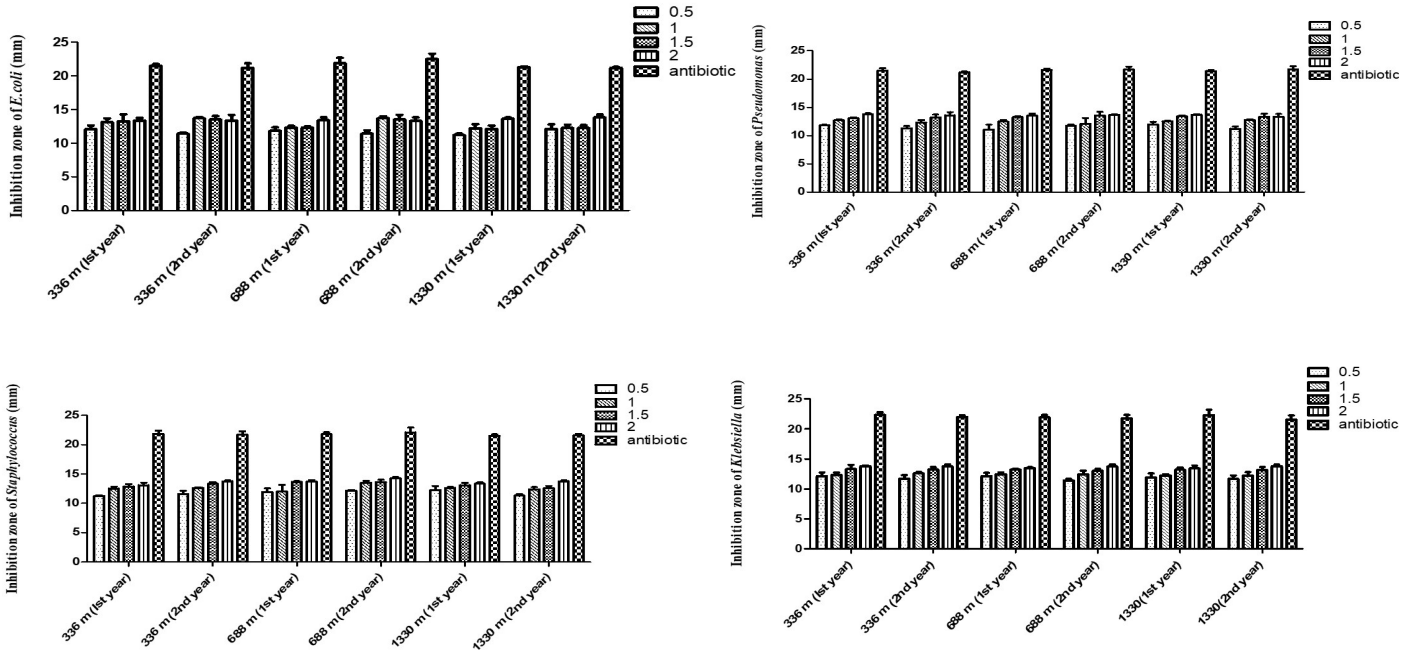

Figure 1. Effect of different concentrations of *J. adhatoda* L. ethanolic leaf extract from different altitudes on (a) *E. coli*, (b) *P. aeruginosa*, (c) *S. aureus*, and (d) *K. pneumonia*.

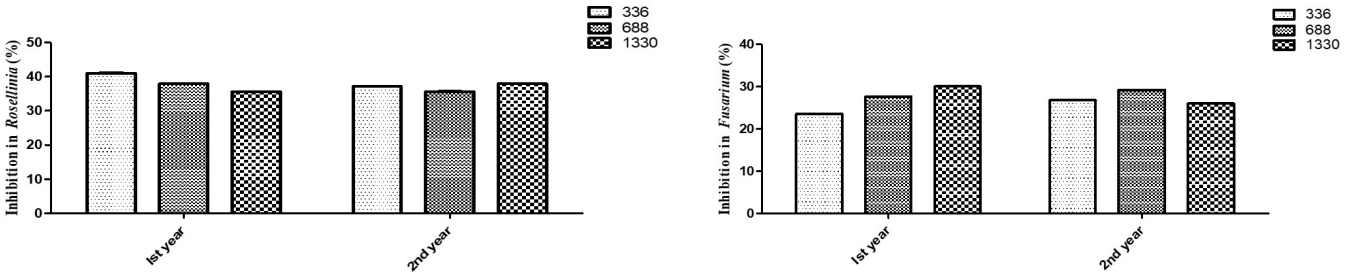

Figure 2. Effect of different concentrations of *J. adhatoda* L. ethanolic leaf extract from different altitudes on (a) *R. necatrix* and (b) *Fusarium* ssp.

Table 2. Antifungal activity of *J. adhatoda* L. ethanolic leaf extracts collected from different provenances of Jammu.

Altitude (m.a.s.l.)	Year	Antifungal activity			
		R. necatrix		*Fusarium* ssp.	
		(%) Mean inhibition	Standard deviation	(%) Mean inhibition	Standard deviation
336	First year	41.08	0.013	23.58	0.014
	Second year	37.20	0.023	26.82	0.024
688	First year	37.98	0.048	27.64	0.037
	Second year	35.65	0.053	29.26	0.024
1,330	First year	35.65	0.013	30.08	0.05
	Second year	37.98	0.026	26.01	0.014

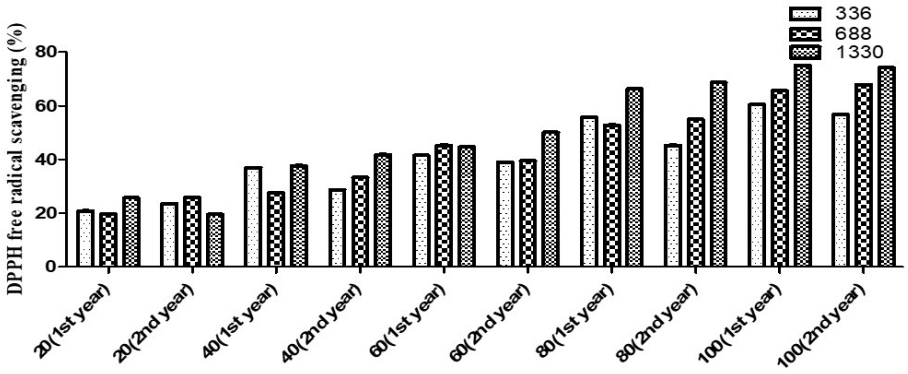

Figure 3. Antioxidant activity of *J. adhatoda* L. leaf extracts from different altitudes.

phytochemical compounds they contain (Wojdylo *et al*., 2007). Change in altitude can bring about variations in antioxidant activity (Jin *et al*., 2017). To deal with oxidative stress, plants are equipped with defense strategies which prevent the loss caused by reactive oxygen species (Khan *et al*., 2016). Natural antioxidants prevent or delay oxidative stress. Free radicals may mount up as a consequence of imbalance between oxidation and antioxidation processes and ultimately cause damage to the plants' biological scenario (Liu and Huang, 2014). Antioxidants quench free radicals as they act as electron donors and hence prevent various diseases induced because of free radicals (Nyanhongo *et al*., 2013). Not just altitudinal variations, seasonal variations also play a major role in bringing about change in the antioxidant levels of plants (Ahmed *et al*., 2017). The presence of polyphenolic compounds could be the possible reason behind the antioxidant potential of the plant (Rao *et al*., 2013). Besides, the secondary metabolites can also act as antimicrobial and anti-inflammatory agents (Moeini *et al*., 2020). In case of anti-inflammatory activity, the variation was not statistically significant among different altitudes (Fig. 4). The values are described in Table 4.

The GC–MS chromatogram of ethanolic leaf extracts of *J. adhatoda* L. revealed nine prominent peaks at retention time: 4.27, 5.27, 6.27, 9.16, 11.11, 17.08, 17.09, 18.71, and 20.29. 1-Butanol, 3-methyl-, acetate ($C_7H_{14}O_2$), benzaldehyde,

Table 3. Antioxidant activity of *J. adhatoda* L. ethanolic leaf extracts collected from different provenances of Jammu using DPPH radical scavenging assay. Data are represented as mean ± standard deviation.

Altitude (m.a.s.l.)	Year	Antioxidant activity (%)					IC_{50}
		Concentration of leaf extract (µg/ml)					
		20	40	60	80	100	
336	First year	20.81 ± 0.090	37.05 ± 0.014	41.49 ± 0.013	55.75 ± 0.068	60.53 ± 0.047	8.79
	Second year	23.56 ± 0.062	28.68 ± 0.083	39 ± 0.021	45.26 ± 0.014	56.85 ± 0.068	10.37
688	First year	19.62 ± 0.105	27.62 ± 0.075	45.17 ± 0.104	52.79 ± 0.056	65.6 ± 0.07	7.93
	Second year	25.8 ± 0.11	33.29 ± 0.105	39.5 ± 0.072	54.99 ± 0.067	67.89 ± 0.021	8.26
1,330	First year	25.8 ± 0.11	37.73 ± 0.068	44.71 ± 0.038	66.28 ± 0.039	75.04 ± 0.008	6.42
	Second year	19.58 ± 0.046	41.83 ± 0.047	50.12 ± 0.019	68.73 ± 0.05	74.23 ± 0.089	6.6

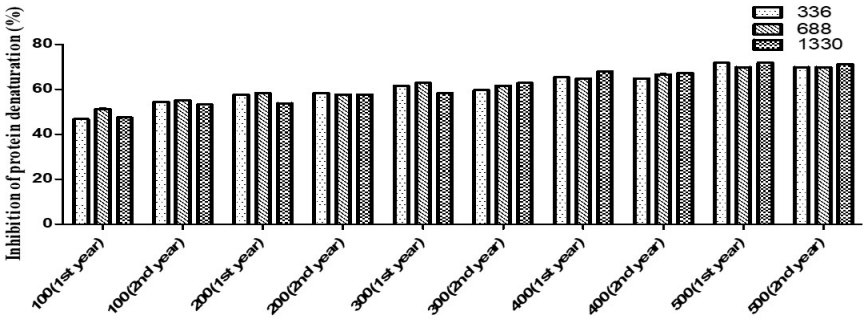

Figure 4. Anti-inflammatory activity of *J. adhatoda* L. leaf extracts from different altitudes.

Table 4. Anti-inflammatory activity of *J. adhatoda* L. ethanolic leaf extracts collected from different provenances of Jammu using inhibition of albumin denaturation technique. Data are represented as mean ± standard deviation.

Altitude (m.a.s.l.)	Year	Anti-inflammatory activity (%)				
		Concentration of leaf extract (µg/ml)				
		100	200	300	400	500
336	First year	46.79 ± 0.03	57.69 ± 0.01	61.53 ± 0.02	65.38 ± 0.01	71.79 ± 0.015
	Second year	54.48 ± 0.005	58.33 ± 0.005	59.61 ± 0.01	64.74 ± 0.025	69.87 ± 0.02
688	First year	51.28 ± 0.05	58.33 ± 0.02	62.82 ± 0.015	64.74 ± 0.005	69.87 ± 0.015
	Second year	55.12 ± 0.025	57.69 ± 0.017	61.53 ± 0.01	66.66 ± 0.02	69.87 ± 0.025
1,330	First year	47.43 ± 0.005	53.84 ± 0.02	58.33 ± 0.035	67.94 ± 0.01	71.79 ± 0.05
	Second year	53.2 ± 0.02	57.69 ± 0.026	62.82 ± 0.025	67.3 ± 0.01	71.15 ± 0.01

4-(1-phenyl-2-propenyloxy)-($C_{16}H_{14}O_2$), ethane, 1,1-dimethoxy-($C_4H_{10}O_2$), CYCLOPENTANE (C_5H_{10}), 1-hexadecanol ($C_{16}H_{34}O$), 1-docosene ($C_{22}H_{44}$) and 1-propanol, 3-(diethylamino)-2,2-dimethyl-, and p-amino benzoate (ester) ($C_{16}H_{26}N_2O_2$) are the compounds which were present in each altitudinal site. However, 1-hexyl-2-nitrocyclohexane ($C_{12}H_{23}NO_2$) was present in low and mid-altitude only and 2-naphthalenamine, 5,6,7,8-tetrahydro ($C_{13}H_{14}O_2$) was present only in mid-altitude. 2-Deoxy-d-ribose was the compound which was found to be present in high altitude only (Fig. 5).

Among these compounds, 1-butanol, 3-methyl-, acetate, and 1-docosene have been reported as important phytochemical compounds (Zambri *et al*., 2019); 1-hexadecanol is an antioxidant (Pejin *et al*., 2014). 1-Hexyl-2-nitrocyclohexane is an important anti-inflammatory compound. It is neuroactive and possesses analgesic property (Thirumalaisamy *et al*., 2018). 1-Hexyl-2-nitrocyclohexane has been reported to possess antibacterial and antifungal properties also (Dulara *et al*., 2019). 2-Naphthalenamine compounds have also been reported to possess anti-inflammatory, analgesic, and antimicrobial properties (Shareef *et al*., 2016).

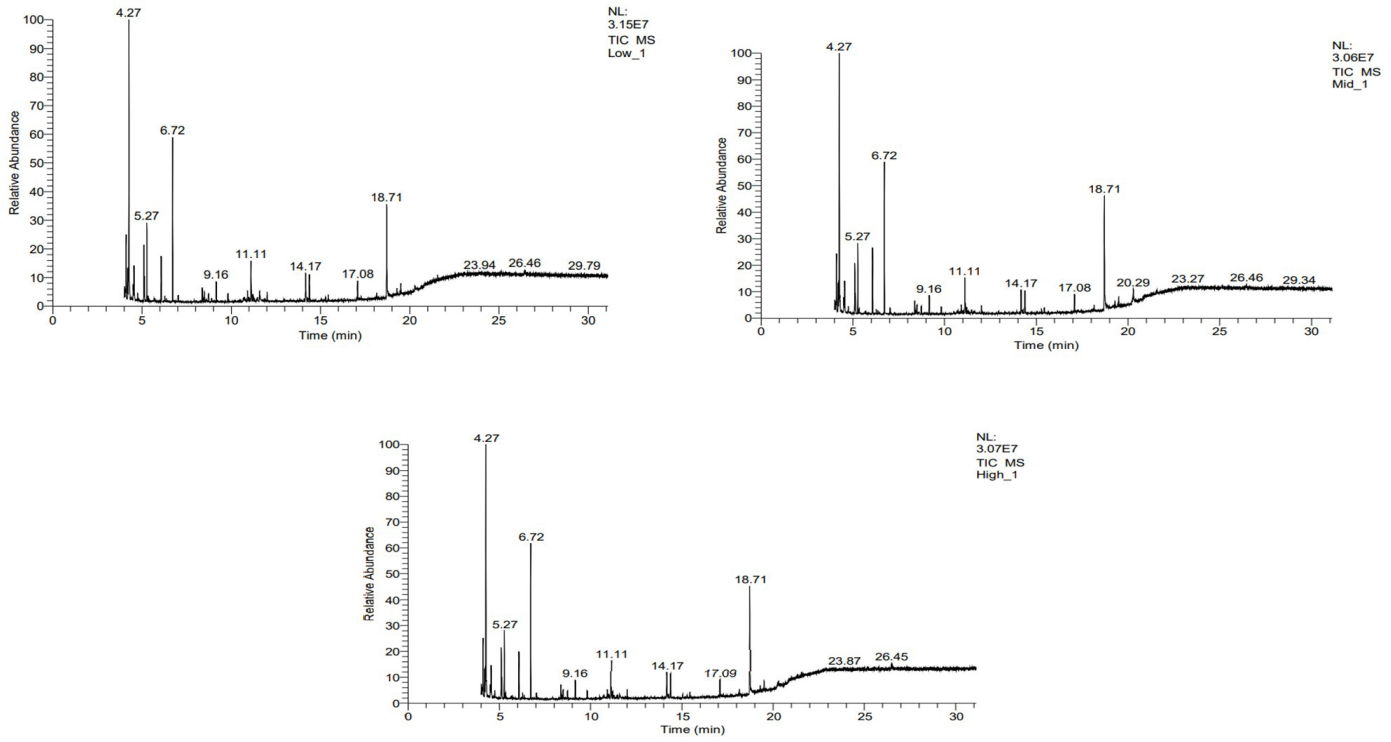

Figure 5. Gas chromatography–mass spectrometry of the leaf extracts of *J. adhatoda* L. from (a) 336 m, (b) 688 m, and (c) 1,330 m altitude.

CONCLUSION

In the present study, the potent biological activities of *J. adhatoda* L. leaf extracts justify the usage of this plant as a medicine. However, significant elevation variations were observed among the antioxidant property only. Various biotic and abiotic stresses present in the environment act as the driving forces behind the variations observed in chemical compositions and eventually the existence of medicinal plants. The assessment of the chemical composition of plants growing at different elevations can contribute to selecting the best genotype and better altitude for the commercial cultivation of plants. Apparently, the quality and therapeutic efficacy of medicinal plants depend on their chemical composition, which in turn depends on the environmental factors.

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CONFLICT OF INTEREST

All authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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