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Yearly variation in biochemical composition, morphological aspects, and antimicrobial activities of *Justicia adhatoda* L. Growing wildly in Western Himalayas

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ABSTRACT

This study was done to explore yearly variation in biochemical profile and important biological properties exhibited by medicinal plant, namely, *Justicia adhatoda* L. The plant is well-known for its beneficial properties. Its leaves, bark, fruits, and flowers are used in treating various ailments. It is known for its bronchodilatory, expectorant, and mucolytic properties. Significant variations were observed among carbohydrate content, crown spread, and number of the primary and secondary branches. 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 pneumoniae* (MTCC 39) by disk diffusion assay, and subsequently, anti-fungal activity was tested on *Rosellinia necatrix* (HG964402.1) and *Fusarium* spp. (SR2 66-9) by poisoned food technique. The leaf extracts exhibited potent inhibitory effect on the tested bacterial as well as fungal strains; however, no significant variation was observed among antimicrobial potential of the *J. adhatoda* L. leaf extracts.

1. INTRODUCTION

Plants have been considered an ultimate source of medicine. Plants offer numerous medicines to the mankind and the essentials of typical traditional schemes of medicines which have been beneficially explored for various years entirely rely on plants. Plant derived drugs have transfigured modern medicinal practice also [1]. Evaluation and analyzing of active plant compounds and plant based drugs could contribute a lot in the advent of pioneering epoch of healthcare scenario and medicaments. Isolation of plant compounds plays a significant role in discovery of different and new therapeutic reliefs. Moreover, demands for plant-derived products are increasing throughout the globe [2]. Besides that, the demands for plant-derived medicines have been increasing throughout the globe. *Justicia adhatoda* L. belongs to family acanthaceae and is widely acknowledged for its effectiveness in curing respiratory problems. It has been extensively utilized in treating issues such as asthma, chronic bronchitis, and other problems for eras in Ayurveda [3]. J. adhatoda L. embrace countless biologically active compounds and its phytochemical profile could be explored for the improvement of a number of plant based drugs [4]. The fresh leaves of *J. adhatoda* L. have been known to exhibit stimulant effect on the respiratory tract, it has been used by Yogis and Sadhus to get relief from various ailments [5]. The various biological activities exhibited by *J. adhatoda* L. could be ascertained as hepatoprotective, antiproliferative, cardioprotective, antimalarial, anti-inflammatory, and oxidative DNA damage protection and all these activities could be ascribed due to the occurrence of efficient bioactive compounds such as primary metabolites, vitamins, secondary metabolites, organic acids, and minerals [6]. Moreover, the environmental condition under which the plants develop holds a significant impact on its various attributes and that ultimately affects its medicinal efficacy [7,8]. Variation in factors such as fluctuation in day length and annual temperature range, solar radiations, and photoperiod affect plant's morphology and biological mechanisms [9]. Plants alter their performances to adapt to a certain environmental condition [10]. In this perspective, it stands paramount important to highlight variations in biochemicals which occur with the progression of time and different environmental factors.

2. MATERIALS AND METHODS

2.1. Morphological Analysis

The present study was done on *J. adhatoda* L. for two consecutive years, namely, 2019 and 2020 in Jammu region and in case of morphological analysis, measurements of 5 plants from each sampling

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site were taken and growth characteristics, namely, plant height, leaf length, and leaf width which were measured by the help of ruler and expressed in centimeters, whereas number of branches were counted. The leaf area was estimated by protocol of Green-armytage [11], In case of crown spread, method described by Blozan [12] was followed.

2.2. Biochemical Composition

Carbohydrates were determined by the method of Thangaraj [13]. Protein content was estimated by following the method of Thangaraj [13]. Amino acids were estimated by following protocol of Thangaraj [13]. Crude fiber was estimated by following the protocol of Chandaka *et al*. [14]. Crude fat was estimated by Thangaraj's [13] method. Potassium and sodium were determined in flame photometer by protocol given by Olaniyi *et al*. [15]. Alkaloids were determined by Harborne's [16] method. The flavonoids were determined by following Zao *et al*. [17]. Phenols were determined by following protocol of Sethi and Sharma [18]. Tannin was estimated by the protocol of Saxena *et al*. [19]. Saponin was determined by protocol of Mir *et al*. [20].

2.3. Antimicrobial Activity

Antibacterial activity was performed by disk diffusion method, following the protocol of Duraipandiyan *et al*. [21]. For anti-fungal activity, poisoned food technique given by Kumar and Garampalli [22] was followed.

3. RESULTS AND DISCUSSION

In the present study, significant variation was observed in the carbohydrate content. The varied from 30 ± 4.88 mg/g (1st year) to 25.8 ± 2.9 mg/g (2nd year) [Figure 1]. Carbohydrates act as a source of several metabolic reactions [23]. Previous studies have reported that plants adopt to different environmental conditions by altering several metabolic pathways, for example, carbohydrate synthesis. A significant increase or decrease in carbohydrate content can play an important role in plants, for example, increased carbohydrate content possess a significant impact on osmoregulation, accumulation of sucrose during the time of stress [24]. The protein content varied from 15.9 ± 1.87 mg/g (1st year) to 19.1 ± 2.07 mg/g (2nd year). Proteins play a key role in body development, hormone formation, maintaining fluid balance, enzyme formation, development of immunity, etc. The crude fiber content of *J. adhatoda* L. leaves varied from 5.6 ± 0.75% (1st year) to 4.9 ± 0.58% (2nd year). Crude fiber plays an essential role in absorbing trace elements in gut [25]. The crude fat varied from 5.6 ± 0.75% (1st year) to 4.9 ± 0.5% (2nd year) [Figure 2]. Fats have also been known to contribute to some medicinal applications [26]. Amino acids varied from 14.3 ± 0.69 mg/g (1st year) to 13.8 ± 0.7 mg/g (2nd year) [Figure 1]. Amino acids play a major role in plant's metabolism. They act as nitrogen donors in various metabolic reactions [27]. Potassium content was found to be 2.3 ± 0.42 ppm (1st year) and 2.7 ± 0.1 ppm (2nd year). Sodium content varied from 0.26 ± 0.2 ppm (1st year) to 0.14 ± 0.0 ppm (2nd year) [Figure 3]. Potassium and sodium both take part in sustaining ionic balance in cells and also help in production of gastric juice in stomach [28]. Phenols varied from 1.2 ± 0.18 mg/g (1st year) to 1.2 ± 0.03 (2nd year) [Figure 1]. Plants possess ultraviolet (UV) protective systems to cope up with the increasing harmful UV radiations. Phenolic compounds play a key role in defense as well as in other mechanisms of the plant and during stress conditions [29]. Flavonoids varied from 1.7 ± 1.88 mg/g to 2.7 ± 0.67 [Figure 1]. Flavonoids are also considered a major UV protectant compound due to their ability to absorb UV radiations and scavenge UV-induced free radicals [30]. The tannin content varied from 3.68 ± 0.05 mg/g (1st year) to 3.9 ± 0.19 mg/g (2nd year), alkaloids varied from 43.8 ± 2.9% (1st year) to 45.5 ± 0.6% (2nd year), and saponin content varied from 31.9 ± 5.1% (1st year) to 29.1 ± 7.57% (2nd year). However, the variation was not statistically significant [Figure 2]. In morphological analysis, *J. adhatoda* L. height, the plants varied from 143.6 ± 16.5 cm (1st year) to 156.8 ± 9.88% (2nd year) and crown spread from 198.6 ± 3.2 cm (1st year) to 180.2 ± 27.26 cm (2nd year). Plant's ability to adapt to the suitable environmental conditions can be related to the morphological or physiological properties displayed by its organs [31-33]. The factors such as competition for nutrition and light lay a strong impact on the growth of plants. In a study, growth of small mountain beech trees was affected due to the competition for light and nutrients [34]. Short stature of plants is also known to possess some benefits for instance, provides protection against stresses like winds, reduced nutrient availability [35]. The number of primary branches in our study were significantly low during 1st year, that is, 95.6 ± 19.26 and high during 2nd year at, that is, 133 ± 23.01 and maximum number of secondary branches were found during 2nd year, that is, 150.2 ± 38.44 and minimum during 1st year at, that is, 127 ± 30.14. No significant change was observed in number

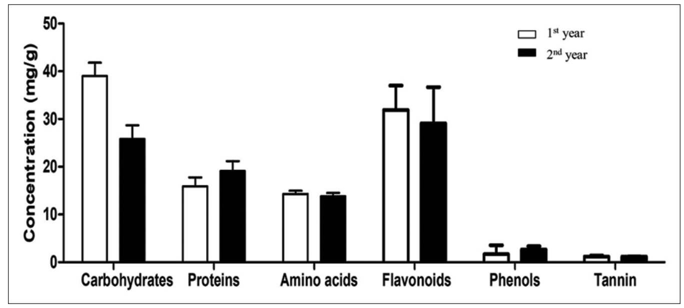

Figure 1: Concentration of carbohydrates, proteins, amino acids, flavonoids, phenols, and tannin in *Justicia adhatoda* L.

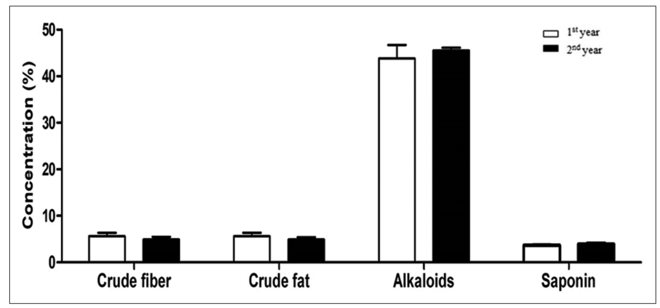

Figure 2: Concentration of crude fiber, crude fat, alkaloids, and saponin in *Justicia adhatoda* L.

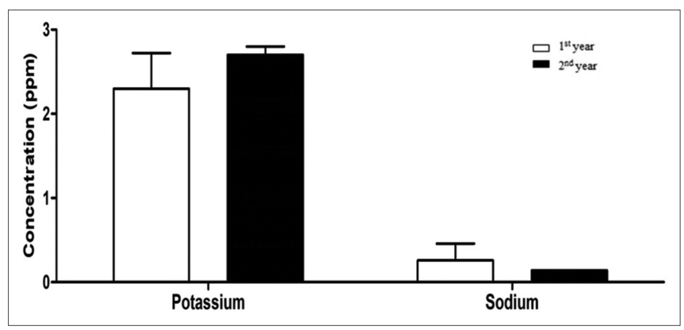

Figure 3: Potassium and Sodium of *Justicia adhatoda* L.

of tertiary branches, namely, 47.8 ± 10.96 and 52 ± 18.22 [Figure 4]. With decreasing height, mechanical damage in branches and trunks of the trees were also observed in some studies [36]. The environment in which the leaves are produced has a significant influence on leaf morphology [37,38]. Other abiotic and biotic factors, namely, exposure to strong wind regimes, soil mineralization and nitrification, transpiration rates, rate of photosynthesis, and differences in light intensity could be considered as contributing factors to the variations [39,40]. In the present study, variations in leaf morphology were also insignificant. Leaf length for the 1st year was 12.96 ± 0.76 cm and 14.3 ± 1.4 cm for 2nd year. Leaf width during 1st year was 5.54 ± 0.5 cm and 5.14 ± 0.23 cm for 2nd year [Figure 5]. The leaf area during the 1st year was 47 ± 1.58 cm^2 and 45.6 ± 1.14 cm^2 for 2nd year.

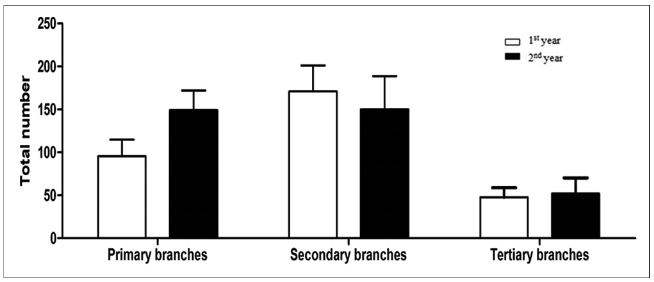

Figure 4: Different branches of *Justicia adhatoda* L.

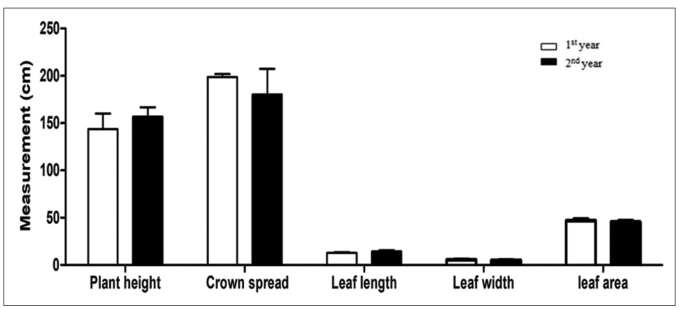

Figure 5: Plant height, crown spread, leaf length, leaf width, and leaf area of *Justicia adhatoda* L.

The antibacterial efficacy of *J. adhatoda* L. leaf extracts was considerable against all the tested strains. All the extracts showed inhibition of microbial growth in comparison to that of standard drug Ampicillin [Figure 6 and Table 1]. *J. adhatoda* L. leaf extracts were able to inhibit mycelia growth better in *Rosellinia* in comparison to that of *Fusarium* [Figure 7 and Table 2]. However, the comparison between antimicrobial activities from leaf extracts of both the years was made and no significant result was observed. Studies have suggested that plant's antimicrobial activity is mainly due to its phytochemicals, for instance, the antimicrobial effect of phenolic compounds is because they possess a significant impact on the permeability of membrane and ratio of penetration in bacterial cell to promote the damage and inactivation of cellular profile of microbes [41,42]. Yearly variations hold a significant impact on plant biochemical composition, for example, in a study, significant variations in quantity of phytochemicals were recorded in *Fragaria ananassa* when those plants were harvested at different times of the year. The study revealed that when the plants were harvested earlier in the year, the phytoconstituents were available in low amounts [43]. In another study, changing environmental situation throughout the year and impact of different seasons lead to significant change in quantity of phytochemicals of *Rubus chamaemorus*. The study lead to a deduction that the extent of phytochemicals and nutritional quality of plants primarily depended upon the time of day as well as the time of season, in which they were harvested [44]. Various bioactive molecules present in plants play their part in antimicrobial action, for example, alkaloids, flavonoids, and terpenes which contain tremendously variable chemical structures with high potential antibacterial activities [45,46]. Plants develop defense strategies to protect themselves from various microbes occurring in the environment. There is an accumulation of defense compounds chiefly, secondary metabolites involving potent antimicrobial activities. It is expected that these bioactive plant derived secondary metabolites

Table 1: Antibacterial activity of *Justicia adhatoda* L. ethanolic leaf extracts against selected standard and clinical bacterial isolates. Data are represented as mean±standard deviation.

S. No.	Bacterial Strains	Year	Antibacterial zone (mm)				
			Control (Ampicillin)	Concentration of leaf extract (mg/ml)			
				0.5	1	1.5	2
1	*Escherichia coli*	1st year	21.5±0.264	12.1±0.529	13.1±0.550	13.2±1.011	13.3±0.404
		2nd year	21.2±0.655	11.4±0.173	13.7±0.173	13.5±0.493	13.3±0.838
2	*Pseudomonas aeruginosa*	1st year	21.4±0.472	11.8±0.173	12.7±0.173	13±0.152	13.7±0.208
		2nd year	21.2±0.152	11.3±0.4	12.3±0.435	13.2±0.493	13.6±0.556
3	*Staphylococcus aureus*	1st year	21.8±0.55	11.2±0.115	12.5±0.305	12.8±0.404	13.1±0.435
		2nd year	21.7±0.568	11.6±0.519	12.6±0.115	13.3±0.321	13.7±0.152
4	*Klebsiella pneumoniae*	1st year	22.3±0.45	12.1±0.602	12.3±0.378	13.3±0.7	13.8±0.1
		2nd year	22±0.288	11.7±0.6	12.6±0.2	13.2±0.416	13.7±0.351

Table 2: Anti-fungal activity of *Justicia adhatoda* L. ethanolic leaf extracts against selected fungal pathogens.

Year	Anti-fungal activity			
	Rosellinia necatrix		*Fusarium* ssp	
	(%) Mean Inhibition	Standard deviation	(%) Mean Inhibition	Standard deviation
1st year	41.08	0.013	23.58	0.014
2nd year	37.20	0.023	26.82	0.024

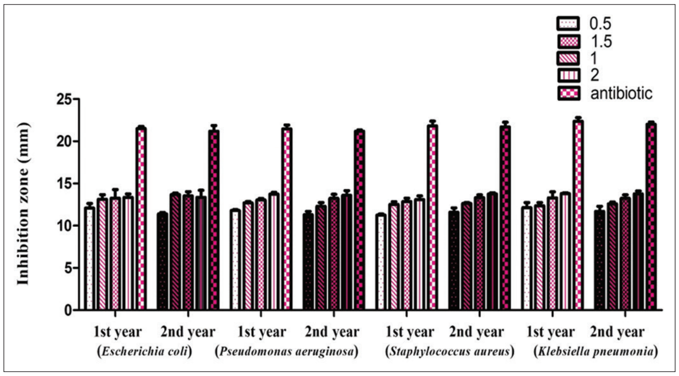

Figure 6: Effect of different concentrations of *Justicia adhatoda* L. ethanolic leaf extract on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Klebsiella pneumonia.*

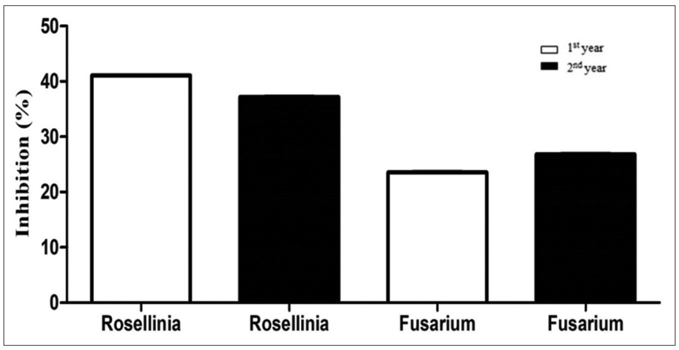

Figure 7: Anti-fungal activity of *Justicia adhatoda* L.

play a key role in synthesizing antimicrobial agents with certain pharmacological effect which, in turn, helps if any bacteria or fungi attacks the plants [47].

4. CONCLUSION

Plant's ability to adapt to suitable environmental conditions relies on the morphological and physiological properties exhibited by its organs. Daily and seasonal environmental fluxes in temperature, light, precipitation, and humidity play a chief role in regulating plant's biological mechanisms. The biochemicals of *J. adhatoda* L. build up its strong nutritional as well as pharmacological profile and have been known to contribute in various important metabolic reactions. The present study justifies its use as an important commercial plant with potent medicinal properties. *J. adhatoda* L. should be explored for further studies.

5. AUTHORS' 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 agreed 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 requirements/guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

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

9. DATA AVAILABILITY

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

10. PUBLISHER'S NOTE

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