Annals of Agri-Bio Research **27** (2) : 183-186, 2022 **Impact Score : 0.28 (Scopus)**

Evaluation and Yearly Variation in Phytochemicals of *Justicia adhatoda* L. Growing Wildly in Western Himalayas

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(Received : January 08, 2022; Accepted : January 29, 2022)

ABSTRACT

This study was done to investigate the yearly variation for phytochemical compounds in medicinal plant, *Justicia adhatoda* L. Significant variations were witnessed among the flavonoids, whereas insignificant variations were observed in alkaloids, saponins and tannins. Higher amount of flavonoid was observed in the second year i. e. 2.7±0.67 mg/g and lesser amount of flavonoid content was observed during first year viz., 1.7±1.88 mg/g. The study revealed that the plant's phytochemical composition responded to daily as well as seasonal environmental fluxes that happened throughout the year. The plant chemically adapted itself according to the specific environmental conditions.

Key words : *Justicia adhatoda* L., yearly, variation, medicinal, plants

INTRODUCTION

The environmental conditions, under which the plants grow, affect various plant attributes (Hatfield and Prueger, 2015; Schumann *et al.*, 2017; Wang *et al.*, 2020). Variation in the environmental parameters affects plant's growth and biological mechanisms. The factors like day length and temperature, solar radiations and photoperiod play a significant role in fluctuation of phytochemicals with time. Plant's biological mechanisms also get certainly affected by varying biotic and abiotic factors (Rapinski *et al.*, 2014). Along these gradients, plants adjust their performances to adapt to a certain climatic condition (Read *et al.*, 2014). For instance, in a study remarkable variations in phytochemical parameters were observed in a medicinal plant viz., *Glycyrrhiza glabra* and the factors like yearly average temperature, yearly average precipitation were held responsible for recorded variations (Esmaeili *et al.*, 2019). Plants phytochemical play a crucial part in maintaining and synchronizing molecular activities that in turn rely on daily and seasonal change. Phytochemical exploration of plants play a chief role and matter of great interest for pharmaceutical industries in order to produce medicines to cure various ailments (Kumar *et al.*, 2017). Not just phytochemicals, morphological and physiological variations in plants could be associated with change in habitat conditions (Classen *et al.*, 2016; Roux *et al.*, 2017; Schneider *et al.*, 2017; Henn *et al.*, 2018). In this perspective, it stood utmost important to highlight variations in phytochemicals that occur and as a consequence of which plants adjust their performances as a response to changing environmental conditions.

MATERIALS AND METHODS

The present study was done on *Justicia adhatoda* L. which is an evergreen sub-herbaceous bush mostly distributed up to altitude of 1300 m. The present study was done for two consecutive years i. e. 2019 and 2020 in Jammu region. The mature leaves were sampled from three different sites of Jammu region and samples were collected in triplicates for both the years and then shade-dried. The leaf samples were stored and analyzed in the laboratory for different parameters using standard techniques.

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Alkaloids were determined by following the method of Harborne (1973). 0.5 g of the powdered leaf sample and 50 ml of 10% acetic acid were taken. Ethanol was added to it in a beaker and covered with foil. The beaker was kept for 4 h at room temperature. The extract was filtered and transferred to a china dish and kept on a water bath to reduce its volume. To this extract concentrated ammonium hydroxide was added. Further, washings with 1% ammonium hydroxide were given and the extract was dried at 80°C in oven. The content was alkaloids.

Alkaloid (%) = (Weight of alkaloid content extracted/weight of powdered plant taken) × 100

Total flavonoid content was determined by using the protocol of (Zao *et al.*, 2004). One ml leaf extract prepared in methanol was added to a flask followed by further addition of 4 ml of distilled water. One ml of 5% sodium nitrite was added and it was left for 5 min. One ml of 10% aluminium chloride was added followed by further addition of 8 ml of sodium hydroxide. Absorbance was taken using spectrophotometer at 510 nm against blank containing all reagents except plant extract. The calibration curve was prepared by taking rutin as standard.
Phenolic content was determined by following method of Sethi and Sharma (2011). The calibration curve was prepared by taking gallic acid as standard. A stock solution of gallic acid was made by adding 80% ethanol (10 mg/10 ml), out of which 0.2, 0.4, 0.6, 0.8 and 1 ml concentrations were taken in other test tubes and the volume was made up to 1 ml by using 80% of ethanol. After that, 1 ml of Folin-Ciocalteu reagent was added to it which was again followed by addition of 20% sodium carbonate solution (2 ml). The test tubes were made to boil for 1 min. Each test tube was then diluted to 25 ml with distilled water. O. D. was taken in spectrophotometer at 750 nm. In the same way, plant extract was processed and O. D. was taken.
Tannin content was estimated by following the protocol of Saxena *et al.* (2013). 100 mg of the powdered leaf material was weighed and 1 ml of distilled water was added to it. The material was heated and centrifuged at 2000 rpm for 20 min and the supernatant was taken. 60 µl of Folin-Denis reagent and 100 µl of sodium carbonate solution was added and diluted to 1.5 ml. For the preparation of standard curve, standard tannic acid solution was made. The absorbance was taken at 700 nm after 30 min against reagent blank.
Saponin content was determined following Mir *et al.* (2013). Five gram of leaf sample was taken into a flask and 20% of 50 ml ethanol was added to it. The flask was then heated for about 4 h at a temperature of 55°C and constant stirring was done. The filtered extract was reduced to 40 ml of its volume keeping it at a temperature of 90°C. After that, 5 ml diethyl ether was added to it in a separating funnel and shaken well. The aqueous layer was recovered and ether layer was removed. 15 ml of n-butanol was added to it. The solution was then washed with 2.5 ml of 5% aqueous NaCl. After that, the solution was evaporated in water bath and the residue was collected and weighed.

Total saponin (%) = (Weight of the residue obtained/Weight of sample taken) × 100

All the data were subjected to one way ANOVA analysis and Bonferroni multiple comparison test.

RESULTS AND DISCUSSION

The results displayed a significant change in *Justicia adhatoda* L. flavonoid content during both the years. Flavonoid content increased significantly during second year. The quantity of flavonoid during first and second year was observed to be 1.7 and 2.7 mg/g, respectively (Fig. 1). Previous studies have described the role of flavonoid as a chief UV protectant compound because of its ability to scavenge UV-induced free radicals in plant tissues. The increased flavonoid content occurred as a consequence of increasing UV radiations due to the varying environmental conditions (Murai *et al.*, 2015). Not only increased level of UV radiations but low temperatures in association with other abiotic and biotic factors like soil mineralization and nitrification, transpiration rates, rate of photosynthesis could be contributing to the observed variations (Barry *et al.*, 2016; Wang *et al.*, 2016; Korner and Hiltbrunner, 2017). The alkaloid content was observed to be 43.9 mg/g during first year and 45.5 mg/g during second year. The tannin

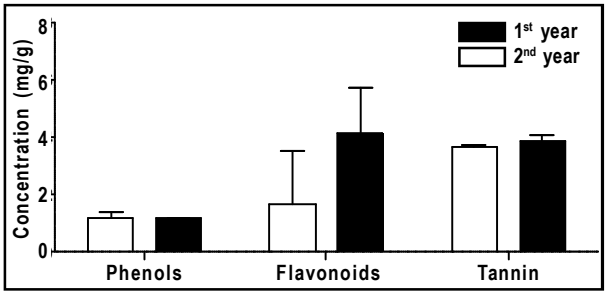

Fig. 1. Phenol, flavonoid and tannins in *J. adhatoda*.

content displayed a value of 3.68 mg/g in first year and 3.9 mg/g during second year. Phenols were found in similar quantity during both the years i. e. 1.2 mg/g. The saponin content was found to be higher viz., 31.9 mg/g during first year and lesser viz., 29.1 mg/g during second year but the variations were not statistically significant for all these parameters (Fig. 2). Daily and seasonal environmental instabilities in temperature, light, precipitation and humidity played a chief role in regulating plant's biological mechanisms (Liebelt *et al.*, 2019). In a study, significant variations in accumulation of phytochemicals were recorded in *Fragaria ananassa* when these plants were harvested at different times of the year. The study disclosed that when the plants were harvested previously, the phytoconstituents were present in low amounts (Ariza *et al.*, 2015). In another study, when *Rubus chamaemorus* genotypes were scrutinized throughout the year and the changing environmental situation according to different seasons led to significant change in quantity of phytochemicals. The study led to a conclusion that the amount of phytochemicals and nutritional quality of plants chiefly depended upon the time of day as well as the time of season in which they were harvested (Hykkerud *et al.*, 2018). Perhaps daily changes

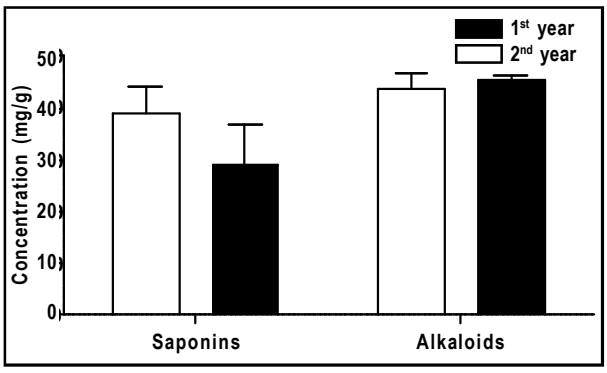

Fig. 2. Saponins and alkaloids in *J. adhatoda*.

in environmental gradients contribute to these variations.

CONCLUSION

The presence of various medicinally important phytoconstiuents from *Justicia adhatoda* L. justified the usage of this plant as a medicine. Phytochemical analysis in relation to their yearly variations in *J. adhatoda* described that the environmental conditions played a significant impact on chemical profile of the medicinal plant. It was evident from the study that suitable environmental conditions proved importance for commercial cultivation of *J. adhatoda* and its derived products.

ACKNOWLEDGEMENT

The authors acknowledge the facilities provided by the School of Biological and Environmental Sciences, Shoolini University, Solan, Himachal Pradesh.

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