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Seasonal phytochemical evaluation of *Withania coagulans* - An important medicinal plant of the Indian Thar desert

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Abstract

Plant based medicines have gained much awareness in today's society due to their various well proven therapeutic effects with no or less side effects. This has provoked humans to go back to nature for safer herbal health solutions. The plant, Withania coagulans (Stocks) Dunal is such medicinal herb which is used to cure various ailments and under the category of folk medicines. A large numbers of phytochemicals have been isolated from this plant that are responsible for diverse pharmacological actions. The present investigation deals with the determination of seasonal (winter, summer and rainy) influence on phytochemical constituents (primary metabolites) parameters such as leaf pigments, osmotic potential (OP), proline, sugar, crude protein and phosphorus contents in W. coagulans. Results revealed that the higher amounts of Chl. a*, carotenoids and crude protein were present during the summer season, while phosphorus content was evaluated in rainy season. Remaining analysed parameters were maximum during the winter months. All parameters were significant at >0.01 level.*

INTRODUCTION

The traditional medicines are increasingly solicited by the traditional practitioners and herbalists for the treatment of infectious diseases. Medicinal plants are also playing a vital role for the development of new drugs [16]. Different herbal drugs technologies are used for converting botanical materials into medicines, where standardization and quality control in terms of their chemical profiles is of utmost important [20]. *Withania coagulans* (Stocks) Dunal (Family: Solanaceae) is used in Unani system as Tukm-e-Hayath, is a small genus of shrubs, popularly known as Indian cheese maker (Fig.1). The plant is commercially important for its ability to coagulate milk and in the treatment of ulcers, rheumatism, dropsy, consumption and sensile debility. The seeds are reported to be sedative, emetic, stomachic, blood purifier, febrifuge and diuretic in infants [10]. The potential of medicinal plants depends upon the presence of phytochemical synthesised during primary and secondary routes of metabolism. Among these, primary metabolites which are synthesised during photosynthesis are essential for plant life, growth and development [25]. Primary metabolites also serve as precursors for a large array of bioactive secondary metabolites. Among primary metabolites, chlorophyll is a green

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Fig. 1: *Withania coagulans* growing in a cultivated habitat

pigment found in leaves and green stems of plants that absorbs light energy to carry out photosynthesis. [5]. Carotenoids are accessory pigments, prevent photooxidative damage to chlorophyll. It usually absorbs red, orange or yellow wavelength. Osmotic potential is a good physiological parameter for measuring water status of plant cells and plays an important role in the synthesis of several primary and secondary metabolites under water stress conditions. Osmotic adjustments are important for maintaining turgor and limiting desiccation during water deficient situation in plants. It involves the uptake, internal production or transfer of osmotically active substances such as inorganic ions (K, Mg, Cl, Cu, NO_3), sugar and amino acids [17]. Proline is an amino acid with an exceptional conformational rigidity and hydrophilic characteristics and acts as one of the most common compatible osmolyte in plants under environmental stress [26]. Most of the dry weight of plants is carbohydrate of one kind or another. They are polar and the low molecular forms sugars [23]. Proteins are substrates of the first order in the metabolism of every organism. It has been reported that considerable hydrolysis of proteins occurs in stressed plants and is accompanied by an increase in amino acid content [3]. The main characteristic of proteins is their high nitrogen content, play essential roles in plant metabolism being the primary products of inorganic assimilation and precursors of protein and nucleic acid [6]. Phosphorus is typically one of the most limiting nutrients for plant growth due to its immobility and propensity to form insoluble compound with metal ions [24].

The present study was therefore aimed to obtain a better understanding of adaptive mechanism in *Withania coagulans* under harsh desert environment and to assess how far the seasonal variations influence the production of primary metabolic products such as leaf pigments, osmolytic accumulation, proline, total sugars, crude protein and phosphorus as a function of seasonal variations.

MATERIALS AND METHODS

The experiment was conducted during different seasons, *viz*. rainy (July-October), winter (November-February) and summer (March-June) for two consecutive years to evaluate effects of seasonal variability in production of different primary metabolites. The plant material was raised at New Campus of JNV University, Jodhpur. The samples were analysed for leaf pigments, proline, OP, total sugars, crude protein and phosphorus. Leaf pigments, proline and OP were analysed from fresh leaves, while other parameters from oven-dried ones. Leaf pigments were estimated according to the method suggested by Arnon [2]. Proline, OP and total sugars were estimated according to Bates *et al*. [4], Janardhan *et al*. [11] and Plummer [19] respectively. Crude protein was estimated by Microkjeldhal method as described by Peach and Tracey [18], while phosphorus as per Allen *et al*. [1]. The mean values of data obtained from three replicates for each parameter during both years were analysed statistically as per Gomez and Gomez [9].

RESULTS AND DISCUSSION

The data on leaf pigments (Chl. *a*, *b*, total chlorophyll and carotenoids), sugars, crude protein and phosphorus contents during different seasons are presented in Table 1. It is evident from this data that values of chlorophylls *a* and *b* during different seasons varied from 0.4625 to 0.5497 and 0.2056 to 0.4378 mg g^{-1} f. wt., respectively. Total chlorophylls were maximum during winter season followed by summer and minimum in rainy season.

Table 1: Various primary metabolic parameters in *W. coagulans* during different seasons

Parameters	Seasons			
	Winter	Summer	Rainy	CD
Chlorophyll *a* (mg g^{-1} f. wt.)	0.5389	0.5497	0.4625	16.874**
Chlorophyll *b* (mg g^{-1} f. wt.)	0.4378	0.2208	0.2056	30.969**
Total chlorophylls (mg g^{-1} f. wt.)	0.8529	0.7723	0.6365	14.650**
Carotenoids (mg g^{-1} f. wt.)	0.00022	0.00049	0.00041	4.307**
Proline (µg g^{-1} f. wt.)	4.816	3.506	1.811	9.514**
Osmotic potential (-MPa)	0.1696	0.2793	0.3182	41.388**
Soluble sugar (mg g^{-1} d. wt.)	14.822	7.801	9.892	28.374**
Insoluble sugar (mg g^{-1} d. wt.)	6.917	6.741	7.352	15.904**
Total sugars (mg g^{-1} d. wt.)	21.740	12.966	17.244	43.527**
Crude protein (mg g^{-1} d. wt.)	7.951	9.625	7.231	9.543**
Phosphorus (mg g^{-1} d. wt.)	0.269	0.330	0.369	5.832**

** = Significant at 1% probability levels.

Carotenoids were maximum during summer season. Chlorophyll is of prime importance and varies with species, plant age and growing season. Kedia *et al.* [13] and Sahoo and Kasera [22] observed maximum values of leaf pigments and carotenoids in *Phyllanthus fraternus* and *Boerhavia diffusa* during winter season, respectively. In the present study, the highest values of total chlorophylls were reported in winter might be due to the appearance of new leaves.

The OP values ranged from -0.1696 to -0.3182 MPa, being highest during winter. The values of proline during three seasons ranged from 1.8117 to 4.8162 µg g^{-1} f. wt., and maximum values were observed during winter and minimum in rainy season. Osmotic adjustment is recognized as an effective strategy to combat environmental stress in plants. Plants acclimatize to water deficit by accumulating compatible solutes or osmoprotectants such as proline to maintain their cellular homeostasis [21]. Kasera and Shukla [12] reported the highest OP in winter, while lowest during summer in *Leptadaenia reticulata*. The maximum and minimum values of OP in *Eclipta alba* during winter and rainy seasons, respectively were reported by Kedia *et al.* [14]. In the present investigation, the values of proline and OP were recorded to be maximum during winter season, and showed negative correlations.

The soluble and total sugars content varied from 7.801 to 14.822 and 12.966 to 21.7407 mg g^{-1} d. wt., respectively during different seasons, being maximum in winter and minimum in summer season (Table 1). Kasera and Shukla [12] and Gehlot *et al.* [8] reported maximum values of total sugars during winter season in *Leptadaenia reticulata* and *Withania coagulans*, respectively. In the present study, maximum total sugars values were reported in the winter and minimum in summer season. The decline in carbohydrate content during summer season may result from an imbalance between carbon production in photosynthesis and consumption in respiration as stated by Liu and Huang [15].

Crude protein lavel ranged from 7.2310 to 9.6259 mg g^{-1} d. wt., during different seasons, being highest in summer season. The maximum values of phosphorus contents were reported during rainy followed by summer and minimum in winter. Gehlot and Kasera [7] observed higher values of crude protein during summer season in *Phyllanthus amarus*. In the present study, maximum amount of crude protein were recorded in summer season. During summer season plant faces to heat stress, which increases concentration of crude protein. Gehlot and Kasera [7] reported maximum phosphorus content in *P. amarus* during rainy season, which confirms the present findings. The high phosphorus content during rainy season, can be explained by the simple fact that solubility of microbial phosphorus increases and results in increase uptake of phosphorus from soil medium during rainy season.

The ANOVA showed that temporal variations were significant at (P>0.01) for all phytochemical parameters.

Thus, it can be concluded from the present findings that winter is the most favourable season for maximum accumulation of leaf pigments, OP, proline, soluble sugar, total sugars, while summer for carotenoids and crude protein. However, the highest phosphorus was reported during rainy season.

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