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Analysis of Allelopathic and Osmotic Constituents of *Prosopis juliflora* (Sw.) DC.

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ABSTRACT

Secondary metabolites like phenols, tannins and phytic acid reported from the plants have been associated with a variety of biological activities including allelopathy. Allelopathy is a natural phenomenon, which includes biochemical compounds produced by an organism influencing the growth, development and reproduction of other organisms. It is in this context that the present report evaluates the phytochemical constituents in different parts like leaf, stem, root, bark, pod and seeds of *Prosopis juliflora (Sw.)* DC., commonly known as vilayati babool in India. The results showed that total phenols were maximum in bark; tannins in roots; phytic acid and total flavonoids in leaf. These constituents present in *Prosopis juliflora* may have an allelopathic impact on the plants growing in its vicinity and their application in controlling weeds can also be assessed as allelopathic constituents of natural origin may be a better substitute for synthetic herbicides and pesticides. As *Prosopis juliflora* has been found growing even under adverse environmental conditions osmotic constituents in its different parts were also analysed. Pods contain greater amount of free sugars and free amino acid whereas leaf had greater amount of free proline. Osmolytes have crucial function in protecting sub-cellular structures through osmotic adjustment and scavenging reactive oxygen species which probably helps growth under water/salt stress conditions.

KEYWORDS: Allelopathy, Secondary metabolites and Osmotic constituents.

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INTRODUCTION

Invasions by exotic species may have negative effects on native flora and fauna causing alteration in biodiversity, natural system functioning and aesthetic values of various habitats globally [1, 2]. Various components of the soil like chemical, physical and microbial may affect allelochemical interactions in the environment. Soil plays an important role in facilitating exotic species [3]. Allelopathic studies on invasive alien species may focus on phytotoxins having direct bearing on invaders and native species. Allelopathic studies may help understanding the invasion of alien species and their direct and indirect influence over plant community organisation and functioning of ecosystems [4].

Allelopathy pertains to plant-plant, plant-microorganisms and microorganisms-plant interactions having either negative or positive effect [5].

Present study has mainly focused on the analysis of different secondary metabolites which may possess allelopathic potential in *P. juliflora* along with osmotic constituents which are of significance for its survival under stressed conditions.

MATERIAL AND METHODS

Plant parts (leaves, stem, root, bark and pods) of *Prosopis juliflora* (Sw.) DC." were collected from plants growing in Jiwaji University Campus, Gwalior (MP). Plant parts were separated and washed with tap water followed by distilled water and subsequently oven dried at 70^0C for 48 hours. Oven dried plant samples were ground with the help of a mechanical grinder and used for analysis following standard methods.

TOTAL PHENOLS

Total phenols were estimated following the method of Malick and Singh [6]. Powdered plant sample was homogenized in 80% ethanol (ten times volume) and centrifuged at 10,000 g for 20 minutes. Supernatant was evaporated to dryness and dissolved in 5.0 ml distilled water. Folin-Ciocalteau's reagent was then added and after three minutes Na_2CO_3 (2.0 ml) was added and kept in boiling water-bath for one minute. Absorbance was recorded at 650 nm and phenols were expressed in mg g^{-1} dry weight, equivalent to catechol.

TANNINS

Tannins were estimated according to Swain and Hills [7]. Powdered plant sample was taken in test tubes and 10mL distilled water was added to it and kept in a boiling water bath. After 30 minutes it was brought to room temperature and centrifuged at 2000g for 20 minutes. Supernatant was

collected and after addition of Folin-Denis reagent and Na_2CO_3 was incubated for 45 minutes at room temperature. Absorbance was recorded at 700 nm with spectrophotometer and tannin content of was expressed as mg g^{-1} dry weight tannic acid equivalents.

PHYTIC ACID

Dry plant sample was extracted using 0.4 m MHCl followed by centrifugation at 10,000g for 20 minutes. Homogenate was mixed with 1.0 mL of colorimetric reagent and left for one hour at room temperature. Thereafter, optical density was measured against blank at 650nm with spectrophotometer. Concentration of phytic acid was calculated using K_2HPO_4 as standard and expressed in mg $^{-1}$ dry weight Wilcox et al. [8].

FLAVONOIDS

Flavonoid contents were measured according to Zhishen et al. [9]. Dry plant sample was homogenized in 80% ethanol and homogenate was centrifuged at 10,000g for 20 min at room temperature. Supernatant was mixed with $NaNO_2$ (5%) and left for 5 minutes. Thereafter, 10% $AlCl_3$ was added and incubated for 6 min followed by addition of NaOH (1M). After 15 minutes optical density was measured at 510 nm. Flavonoid contents were measured by comparing with quercetin standards.

FREE AMINO ACIDS

Free amino acids were estimated following the method outlined in Sadasivam and Manickam[10]. Powdered sample was homogenized in ten times volume of ethanol. Homogenate was centrifuged at 2000g for 20 minutes and supernatant was collected. Supernatant was reacted with acid ninhydrinand kept in boiling water bath. After 20 minutes diluent was added to it and absorbance was recorded at 570 nm using spectrophotometer. Concentration of total free amino acid is expressed in mg g^{-1} dry weight as glycine equivalent.

TOTAL FREE SUGARS

Total free sugars were determined using anthrone method following Fong et al. [11]; Jain andGuruprasad[12]. Plant sample was extracted using 80% aqueous ethyl alcohol followed by centrifugation at 5000g for ten minutes. The supernatant was evaporated to 0.2 mL in a water bath and the volume was made up to 1mL with distilled water followed by the addition of 1mL HCl (1N) and hydrolysis was allowed in boiling water bath. Amount of free sugars in were determined after reacting it with anthrone reagent. Optical density was recorded at 620 nm. Computation of total free sugars was done using glucose standards.

STARCH

Starch was determined by anthrone method as outlined in Sadasivam and Manickam[10]. Residue left after free sugars estimation was dried in a boiling water bath followed by addition of 2ml distilled water and subsequently keeping on a water bath for 15 minutes. After cooling 2mL perchloric acid (9.2N) was added and stirred for 15 minutes, followed by centrifugation at 5000 g for ten minutes. Supernatant was collected and residue was again treated with 2mL perchloric acid (4.2N) and stirred for 15 minutes and then centrifuged. Finally supernatants were combined and made 10mL using distilled water. Aliquot (0.1mL) was mixed with 4mL of anthrone reagent. The tubes were then kept in a boiling water bath for 10 minutes and subsequently cooled on ice and brought to room temperature and optical density was recorded at 630 nm.

FREE PROLINE

Free proline was estimated following Bates et al. [13]. Dry plant sample was extracted in 3%(w/v) sulphosalicylic acid followed by centrifugation at 3000 g for 10 min. 2mL supernatant was mixed with acid ninhydrin and glacial acetic acid followed by incubation for 1h at 100℃. The reaction was terminated on ice. Free proline was separated using 4mL toluene in a separating funnel and optical density was recorded at 520 nm. Concentration of proline is expressed in µ moles g^{-1} dry weight as L-proline equivalent.

STATISTICAL ANALYSIS

Data presented are mean of four replicates with standard error (Mean ± SE) calculated.

RESULTS AND DISCUSSION

Total phenolic compounds were higher in bark and roots ie 29.50±0.60 mg gm^{-1} dr. wt. and 26.63±1.62 mg gm^{-1} dr. wt. respectively and minimum in seeds (3.19±0.06 mg gm^{-1} dr. wt.) of *P. juliflora* (Fig 1A). Tannins were found to be maximum in roots and bark ie105.80±5.41 mg gm^{-1} dr. wt. and 97.15±2.24 mg gm^{-1} dr. wt. respectively and minimum in stem (33.60±1.29 mg gm^{-1} dr. wt.;Fig1B).

Phenolic compounds are the secondary metabolites frequently reported acting as antioxidants due to their capability to donate electrons and effectiveness in stabilizing radicals and preventing oxidation at cellular and physiological level [14].

Song et al. [15] found higher total phenolic contents in chinese medicinal plant (*Dioscorea bulfifera, Eriobotrya japonica, Tussilago farfara and Ephedra sinica*) which has been associated with the higher antioxidant and free radical scavenging potential of these plants.

Leaf of *P. juliflora* possessed higher contents of phytic acid (6.80±0.22 mg gm^{-1} dr. wt.) and flavonoids (29.88±1.26 mg gm^{-1} dr. wt.) whereas, bark showed minimum content of phytic acid (0.41±0.04 mg gm^{-1} dr. wt.) and seeds showed minimum content of flavonoids ie 1.76±0.13 mg gm^{-1} dr. wt. (Fig 1C and D).

Flavonoid concentration of 20 different extracts of the plant *Teucrium montanum* L. var. montanum, *F. supinum* (L.) Reichenb. varied between 3.96 to 88.31 mg RU/gm [16]. Quality and quantity of phenolic contents of plants vary in different regions of the world because of many environmental factors, climatic conditions, soil composition, age and vegetation cycle stage [17].

Total phenolic contents of extracts of roots, stems and leaves of *Thymus numidicus* Poir. showed the highest amount of polyphenols, flavonoids in methanolic extract and root extract exhibited allelopathic properties restricting the shoot and root growth of *Medicago sativa* and *Triticum aestivum* seedlings [18].

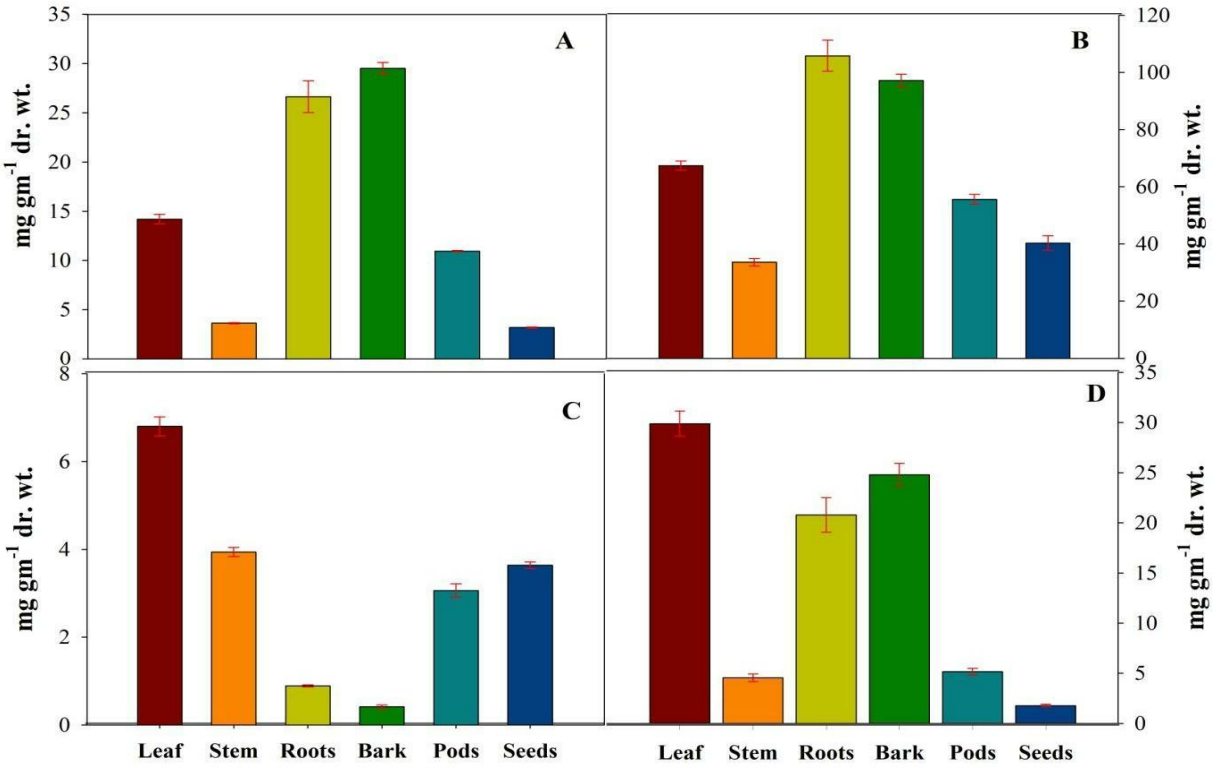

Fig 1: Total phenols (A), tannins (B), phytic acid (C) and flavonoids (D) in different parts of *Prosopis juliflora* (Sw.) DC.

Accumulation of osmotic constituents is one of the important strategies for withstanding harsh conditions of environment. Free amino acids and free sugars were higher in pods and free proline in leaf of *P. juliflora*. Starch content was higher in pods of *P. juliflora* (Fig 2 D).

Accumulation of these osmotic constituents is directly proportional to the external stresses protecting and maintaining the structure and function of cells [19]. Sugars function as osmoprotectants and as substrate for growth regulators for gene expression [20]. Free amino acids in seeds of *Abutilon theophrasti* inhibited germination of crop seeds suggesting that natural free amino acids possess allelopathic potential [21]. Plants of rice subjected to water deficit accumulated greater amounts of proline in leaves [22].

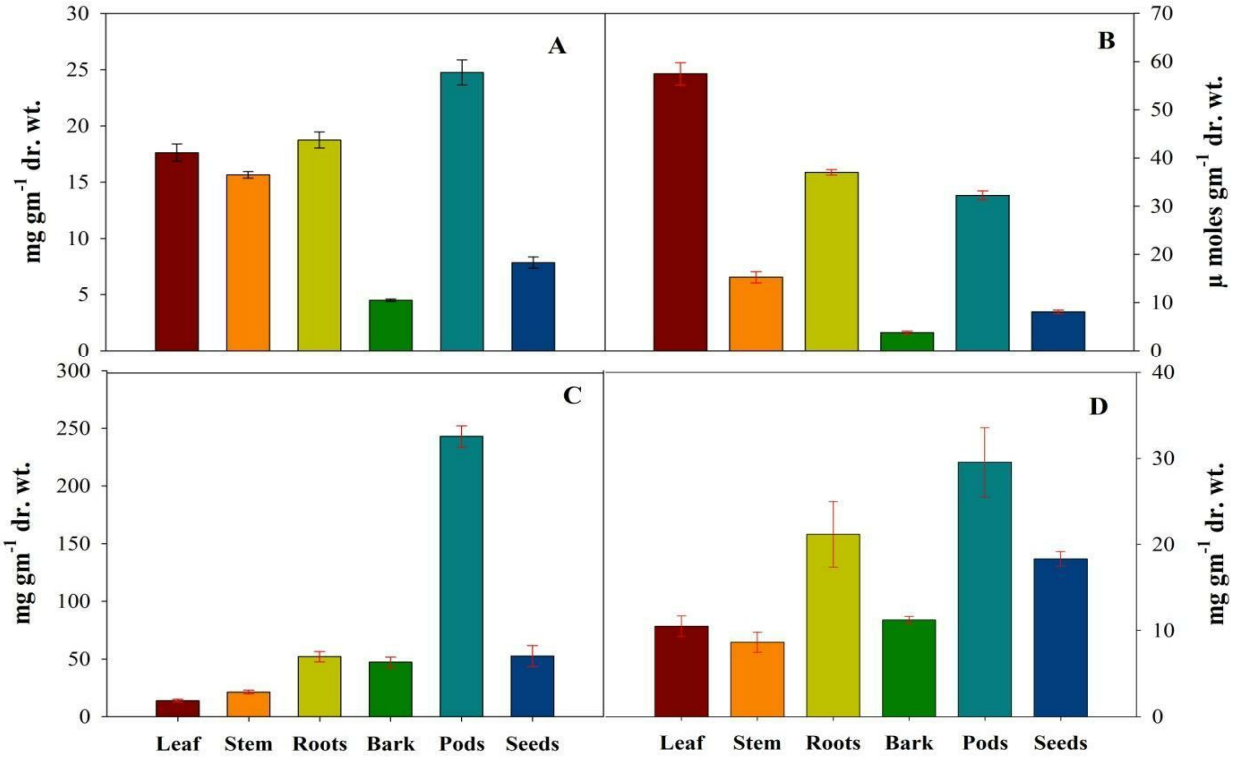

Fig 2: Free amino acids (A), proline (B), free sugars (C) and starch (D) in different parts of *Prosopis juliflora* (Sw.) DC.

CONCLUSION

The present study indicates the presence of phytochemical constituents of *Prosopis juliflora (Sw.)* DC. and its ability to accumulate osmotic constituents. The presence of secondary metabolites in the different parts may have allelopathic interactions over plants/crops growing in its vicinity and a possibility may be explored for their utility as natural herbicides and osmotic constituents present are probably contributing towards the growth and development of *Prosopis juliflora* even under stressed conditions.

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