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PHYTOCHEMICAL STUDIES AND QUANTIFICATION OF TOTAL PHENOLS, TANNINS AND FLAVONOIDS IN LESS KNOWN SEMI-ARID PLANTS OF BALLARI, KARNATAKA

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ABSTRACT: Four plant species *Senna italica*, *Prosopis juliflora*, *Cressa cretica* and *Schouwia purpurea* were selected to estimate phytoconstituents and quantify total phenols, tannins, and flavonoids. The total content of phenols, tannins, and flavonoids with the gallic acid curve by the standard method was estimated. The leaf extract of *Senna italica* contained maximum, and *Schouwia purpurea* contained a minimum amount of phenol. The maximum amount of tannins was found in *Schouwia purpurea*, and the minimum amount of flavonoids was recorded in *Senna italica*. It is further suggested that there are a need for systematic phytochemical, pharmaceutical and pharmacological investigations of these plants since there are less reports available. Moreover, these plants are accessible in abundant in Ballari region and can prove to be the best source for bioprospection studies since they contain high contents of phenols, tannins, and flavonoids.

INTRODUCTION: Human reliance to sustain their life and health on plants is increasing day by day with the increasing demand and supply of herbal products in the international market. Since the knowledge of valuable and unique phytochemicals and usage of plants as medicines, as food has increased swiftly with the spread of scientific information through various means during the last three decades, especially in the developed and developing countries. Herbal drugs have the most frequently proven to be safe with less side effects for consumption and have potential components to cure a wide range of ailments in comparison with synthetic medicines. Ballari is a semi-arid district in Karnataka where the majority of the vegetation are xerophytes, which have enormous quality of hidden treasure of medicinal properties like wound healing, antioxidant, anti-inflammatory, antimicrobial, anti-diabeticetc. Amongst many xerophytes of this region, four plants *Senna italica* Mill., *Cressa cretica* L., *Prosopis juliflora* (Sw.) DC and *Schouwia purpurea* (Forssk) Schwein were found growing abundantly in and around Vijayanagara Sri Krishnadevaraya University Ballari campus. Hence, these were selected based on a literature survey evidenced by several reports on ethnobotanical and medical uses. Senna italic Mill., (Caesalpinioideae) is used as a laxative and purgative also used against constipation, rheumatic, intestinal disorders, and to cure stomach ailments and treat typhoid fever, urinary infection [1].

Cressa cretica L. (Convolvulaceae) is a remarkable salt-tolerant plant common in coastal areas, and it is also found in and around Ballari district plenty. The plant is traditionally used as an expectorant and antibilious agent. Dry leaves of *Cressa cretica* crushed with sugar are used as an emetic in Sudan [2].

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Studies of Aqeel *et al.,* (1988, 1989) [3] showed that *Prosopis juliflora* (SW) DC. Belonging to the family, Mimosoideae is rich in alkaloids has proven anti-fungal and anti-bacterial activities. *Schouwia purpurea* (Forssk) Schwein, (Brassicaceae) is used as a food and source of fuel collected from the wild. It has entomological importance since it provides feed and shelter for the desert locust. Kavitha Sagar *et al.,* (2021) [4] have reported antimicrobial activity from *Senna italica, Schouwia purpurea, Cressa cretica,* and *Prosopis juliflora* leaf extract.

In most hydroxyl groups produced by plants mainly for protection against stress, phenolics play an important role in plants; phenolic compounds are one of the major chemical classes of plants' secondary metabolites. They play an important role in the defense of plants against pathogens, diseases, parasites & predators. They involve a number of physiological mechanisms, such as antioxidant activity. Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways & antioxidant effects. These molecules are found in a variety of fruits & vegetables. Flavonoids have antioxidant properties.

These molecules are found in a variety of fruits and vegetables. Tannins are water-soluble polyphenols that are present in many plant foods. Many tannins have also been shown to reduce the mutagenic activity of a number of mutagens. The antimicrobial activities of tannins are well documented. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf- life of certain foods such as catfish fillet. In the view of above, the present was conducted to determine total phenols, flavonoids, and tannins in the four plants mentioned above. As mentioned earlier, the present study was carried out considering the ethnobotanical reports and less known phytochemical reports of the plants.

METHODS AND MATERIALS:

Sample Collection: Four plants *Senna italic* Mill, *Cressa cretica* L., *Prosopis juliflora* (SW.) DC., *Schouwia purpurea* (Forssk.) Schwein was collected from surrounding areas of VSK University campus Ballari and was identified by using local and national floras followed by preparation of herbarium. Leaves of these plants were separated, washed carefully under tap water, rinsed with distilled water, air-dried for one hour, and shade dried at room temperature.

Preparation of Plant Extract: The dried plant's parts were ground by using mortar and pestle to make a powder. The obtained powder samples were packed in sealed plastic covers or airtight bottles and stored at room temperature. The samples' extract was prepared by soaking 100gms of each powder sample, dissolved in 300 ml of ethanol in 500 ml of the conical flask, airtight them with aluminum foil, and kept in the dark place for 24 h. Later the extract was filtered and kept for evaporation of solvent for 2 days. Then the dried extract was carefully collected and preserved at 40°c in a tight screw cup tube.

Quantification of Total Phenols by Folin-Ciocalteu Method: Total Phenol was determined by the Folin-Ciocalteu reagent (FCR) method with modification. Each crude extract (1mg) was dissolved in methanol (1ml). A total of 10% FCR was prepared by adding Folin-Ciocalteu reagent (10ml) in water (90ml). Than 5% Na_2CO_3 (3gm) in water (50ml). Each crude sample (200µl) was taken in a test tube and added 10% folin-ciocalteu reagent (1.5ml). Then all test tubes were kept in the dark place for 5 minutes. 5% Na_2CO_3 (0.5ml) was added to the solution and mixed well by hand. Again all the test tubes were kept in the dark for two hour. The absorbance was measured for all solutions by using UV-spectrophotometer at a constant wavelength of 750 nm.

Gallic Acid Standard Curve: A standard curve was prepared using Gallic acid. For this 10mg, Gallic acid was dissolved in100% methanol. Serial dilution of Gallic acid methanol was prepared, viz. 50, 100, 150, 200, 250mg/ml. 1ml aliquot of each dilution was taken in a test tube and diluted with 9ml of distill water, and this 2.5ml FCR was added. This was followed by the addition of 2.5ml of 7.5% NaHCO in each test tube. The resulting mixture was left to stand for 30 minutes at room temperature. The absorbance of the standard was measured at 765 nm using a UV spectrophotometer against blank. Quantification was done on the basis of a standard curve of Gallic acid. Based on the measured concentration of phenols was read

(mg/ml) from the calibration line, then the content of phenols in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract). Gallic acid equivalent

$$(GAE)\ T = C \times V / M$$

Where GAE is the Gallic acid equivalence (mg/ml); V is the volume extract (ml), and M is the weight (g) of the pure plant extract. The % yield extract was calculated by using the following formula:

% Yield = Weight of extract obtained (g) / Weight of plant material (g) × 100

Quantification of Total Content of Flavonoids: The total flavonoid were determined according to the method described by Djeridane *et al.*, [5]. One ml of extract (0.5 mg/ml) was mixed with 1 ml $AlCl_3$ (2%). The mixture was stirred and kept at room temperature for 15 min. The absorbance was measured at 430nm using a UV-Visible spectrophotometer (LMSP-UV1000B). The total flavonoids were reported as mg routine equivalents (RE) per g weight (DW).

Quantification of Tannins: The tannins were determined by the Folin-Cocalteu method. For this 0.1 ml of the sample, the extract was added to a volumetric flask (10 ml) containing 7.5ml of distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid 20, 40, 60, 80, and 100mg/ml were prepared in the same manner as described earlier. Absorbance for test and standard solution were measured against the blank at 725 nm with a UV-Visible spectrophotometer (LMSP-UV1000B). The tannin content was expressed in terms of mg of GAE/g of extract. The total content of tannins is calculated by Schandrel S.H (1970) [6] method.

Tannic acid mg/100g = C ×Extract Volume× 100 / Aliquot volume × wt of sample ×100

RESULTS:

Preliminary Qualitative Analysis of Selected Plant Species: The presence or absence of phytochemical compounds are represented by positive (+) or negative (-) signs presented in **Table 1.**

TABLE-1: PRELIMINARY QUALITATIVE ANALYSIS OF SELECTED PLANT SPECIES

Sl. no.	Phytoconstituents / Plant species	*Senna italica* Mill. (leaves)	*Cressa cretica* L. (Whole plant)	*Prosopis juliflora* (SW.) DC (leaves)	*Schouwia purpurea* (Forssk) Schweinf. (leaves)
1	Glycoside	+	-	+	-
2	Phenols	+	+	+	+
3	Saponin	-	-	+	-
4	Sterols	+	+	+	+
5	Carbohydrates	+	+	+	+
6	Tannins	+	+	+	+
7	Coumarins	+	+	+	+
8	Proteins	-	-	-	-
9	Anthraquinones	+	-	+	-

Presence (+), absence (-)

Quantification of Total Phenols: The total phenolic contents in the examined plant extracts using FCR is expressed in terms of Gallic acid equivalent (the standard curve equation: Y = 0.0101x + 0.0352, R2 = 0.992). The values obtained for the concentration of total phenols are expressed as mg of GAE/g of extract.

In *Senna italica* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total phenolic contents was 6.50, 8.88, 11.1, 16.11, and 19.52 mg GAE/g, respectively. In *Cressa cretica* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total phenolic contents was 0.16, 3.95, 4.19, 7.09, and 8.06 mg GAE/g, respectively. In *Prosopis juliflora* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total phenolic contents was 1.895, 2.523, 3.601, 4.028 and 4.075 mg GAE/g, respectively. In *Schouwia purpurea* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total phenolic contents was 0.06, 2.22, 2.4, 2.8 and 2.93 mg GAE/g, respectively.

Quantification of Total Flavanoids: Ethanol extracts of four plants *viz; Sennai talica, Prosopis juliflora, Cressa cretica* and *Schouwia purpurea* contains high flavonoid concentration. The concentration of flavonoids in plant extract of *Prosopis juliflora* at 2.5, 5, 7.5, 10, 12.5 mg/ml

concentration total Flavonoid contents was 0.19, 0.38, 3.23, 4.57 and 5.23 mg GAE/g, respectively. At 2.5, 5, 7.5, 10, 12.5 mg/ml concentration of *Senna italic*, total Flavonoid contents was 1.90, 3.17, 10.79, 26.66 and 31.74 mg GAE/g. In *Cressa cretica* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total Flavonoid contents was 0.645, 2.58, 2.25, 6.77 and 7.74 mg GAE/g and In *Schouwia purpurea* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total Flavonoid contents was 0.32, 5.2, 10.2, 10.9 and 13.3 mg GAE/g, respectively.

Quantification of Total Tannins: The total tannin content in the plant extract from *Senna italica* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total Tannin contents was 0.15, 0.571, 0.57, 0.66 and 0.79 mg TE/g respectively. In *Cressa cretica* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total Tannin contents was 0.064, 0.096, 0.129, 0.177 and 0.354 mg TE/g.

In *Prosopis juliflora* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total Tannin contents was 0.0189, 0.142, 0.284, 0.464 and 0.511 mg TE/g and in *Schouwia purpurea* at 2.5, 5, 7.5, 10, 12.5 mg/ml concentration total Tannin contents was 0.48, 0.53, 1.09, 1.21 and 1.22 mg TE/g, respectively.

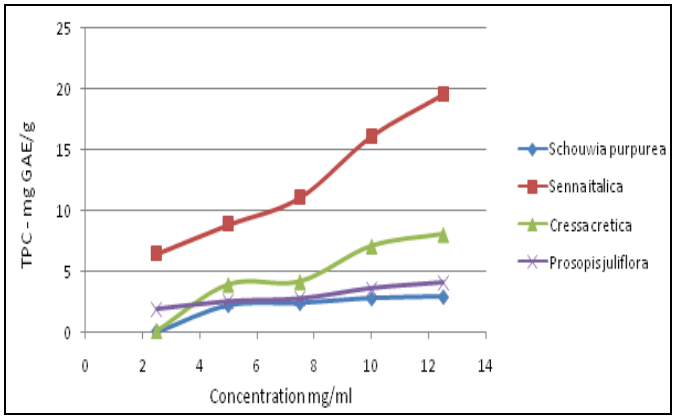

FIG. 1: QUANTIFICATION OF TOTAL PHENOLS

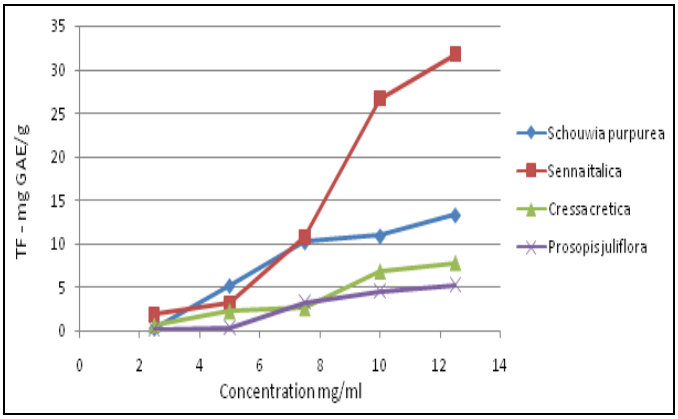

FIG. 2: QUANTIFICATION OF TOTAL FLAVONOIDS

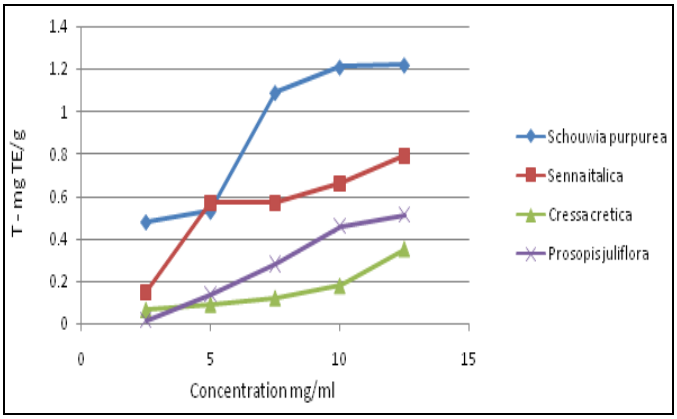

FIG. 3: QUANTIFICATION OF TOTAL TANNINS

DISCUSSION: Plants contain biological compounds responsible for several pharmacological activities that point to giving much attention to identifying crude plant materials. In this study, the phytochemical analysis of *Senna italica, Cressa cretica, Prosopis juliflora, Schouwia purprea* indicates the presence and absence of all the phytochemical constituents, as shown in **Table 1.** The result reveals that the proteins are absent in all four plant extracts, anthraquinones and glycosides absent in *Cressa cretica* and *Schouwia purpurea*. The saponins are absent in *Senna italica* and *Schouwia purpurea*. The presence or absence of the phytoconstituents may be due to the geographical distribution. Based on the preliminary phytochemical screening, one can understand whether to proceed with quantification studies or not. Based on the present results, it was felt necessary to go for quantification of phenols, Flavonoids, and Tannins, which were present in all four plants. The highest concentration of phenol is obtained from Senna Italica plant

extract, and in the other three plant extract of *Cressa cretica, Prosopis juliflora*, and *Schouwia purpurea*, considerably smaller concentrations of phenols were found. The total phenolic contents in plant extracts depend on the type of extracts, *i.e*., the polarity of solvent used in extraction. The high solubility of phenols in polar solvents provides a high concentration of these compounds in the extracts obtained using polar solvents for the extractions. The choice of solvent to extract phenolic compounds depends on the target compound solubility, the interaction between the solvent and plant material and factors such as constant and dielectric loss. Ethanol solvent has a good ability to solve phenolic compounds. Priyanka *et al.* (2015) [7] reported high phenolic content, 99.09 ± 0.10 µg/mg of petroleum ether extract of *Cressa cretica*. Also, the TPC in ethyl acetate and methanolic fractions of *Cressa cretica* 7.081 ± 1.033 and 12.833 ± 0.24 mg gallic acid equivalents (GAE)/g dry extract were obtained [8]. Similar results were reported in the plant extract of species *Marrubium peregrinum* [9]. The highest flavonoid concentration was observed in *Senna italica* leaves, and the lowest concentration was measured in *Prosopis juliflora* leaf extracts. The concentration of flavonoids in plant extracts depends on the solvent used for extraction.

The highest tannin content was obtained from *Schouwia purpurea* leaves extract, and in the other three plant extracts, considerably smaller amounts of tannins were recorded. The extraction value of Tannins greatly depends on the solvent polarity. In the present study highest tannin content was achieved in ethanolic extracts. The high solubility of tannin in polar solvent provides a high concentration of these compounds in the extract obtained using polar solvent for the extraction.

In the present study, the content of flavonoids is highest in *Senna italica*, when compared to Phenols and Tannins. Flavonoids content is more in *Senna italica* may be due to the ability of the phytoconstituent elements of the plant extract to dissolve into the ethanolic solvent. Phenols and tannins considerably were present in less quantity which may be due to the seasonal variation affecting the chemical composition of the plants; thus, the dissolution of the above phytoconstituents is less in ethanol solvent, and it may dissolve better in other solvents, and our work is in with agreement of the report given by Kawo (2007) [10] and Yusha'u *et al.*, (2008) [11]. Through the present investigation, it is further suggested to undertake detailed bioprospection studies in the above plant species, which may prove promising herbal drugs.

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