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ORIGINAL ARTICLE **OPEN ACCESS**

Determination of primary and secondary metabolites in ethnobotanicals plants collected from Alwar district of Rajasthan

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ABSTRACT
Ethnobotany is the study of plants which are being used by tribal people to treat various diseases. In the present investigation, on the basis of tribal knowledge, we collected 6 ethnobotanical plants of various families (Cleome viscosa, Elytraria acaulis, Tephrosia villosa, Aerva javanica, Leucas aspera and Tridax procumbens). Plant parts used as traditional therapeutic purpose were selected for the study. Primary metabolites (TSS, proteins, free amino acids, and lipids) and secondary metabolites (total phenolic compounds, flavonoids, alkaloids, tannins, terpenoids, saponins) were determined quantitatively by using standard methods. Results revealed that these plant parts were rich in these metabolites. So, it can be concluded that the medicinal properties of these plant parts are due to presence of important phytochemicals.
Keywords: *Ethnobotany, tribal knowledge, therapeutic purpose, phytochemicals etc.*

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INTRODUCTION

The study of biological traits in relation to racial, anthropological, or cultural groupings is known as ethnobiology. Ethnology is the study of how people interact directly with the plants and animals in their environment. Ethnobotany is the study of plants that indigenous people have historically used medicinally. The term "aboriginal botany" was coined in 1875 to describe the study of all forms of the plant kingdom that the indigenous Australians used for a variety of purposes, including food, medicine, fabrics, clothing, ornaments, and so forth. Parallel to the formation and advancement of the science as a whole, the meaning of the term "ethnobotany" has experienced a process of change and development. Because of this, the study of the link between members of prehistoric cultures and their plant environment is known as ethnobotany [1]. India is one of the nations with the most in-depth knowledge of ethnobotany due to the presence of numerous ethnic groups with a lengthy history and a diverse array of plant life. roughly 550 separate tribal tribes are home to roughly 50 million people. The vast majority of these individuals reside in forested, hilly, and other naturally isolated regions. The complementary and alternative medicine practise known as Ayurveda, also referred to as Ayurvedic medicine, has its roots in India [2]. Plants that have been used medicinally for centuries produce a wide range of substances with established therapeutic uses [3]. These substances are called as secondary plant metabolites. Primary metabolites serve as the building blocks for the biosynthesis of secondary metabolites, which encompass a vast variety of active chemicals. Their presence across the plant kingdom is far less widespread than that of other organisms. When grown in different environments, the same plant species can produce varied amounts and qualities of these characteristics in varying degrees. They often accrue in lower quantities and have a tendency to be generated by particular cell types at various phases of development [4]. There are many plants secondary metabolites that are constitutive, meaning that they are present in healthy plants in their biologically active forms. Other plant secondary metabolites, on the other hand, occur as inactive precursors and are activated in response to tissue damage or the presence of pathogens [5]. In the present investigation, we investigated for determination of various primary and secondary metabolites in different ethnobotanical plants found in Alwar district of Rajasthan, India. In the district, about 12.44 percent of the population is comprised of people who belong to tribes such as the "Bhil, Bhil-Meena, Damor, Dhanka, Garasia, Kathodi, Kokna, Kolidhor, Naikara, Patelia, Meena, and Seharia and who live in places that lack" even the most fundamental infrastructure facilities. The cultural history of Rajasthan is further enriched by

the presence of several nomadic tribes, including the Banjara, Gadolia-Lohar, Kalbelia, Sikligar, Kanjar, Sansi, and Bagri.

MATERIAL AND METHODS

Study area – The plant sample were collected from three sacred groves Bherunath ki bani, Adawal ki devbanin, and Garwa ji ki devbani which are located in Alwar district on Rajasthan.

Collection and processing of plants

Healthy parts of various plants (selected on the basis of traditional knowledge) were collected from various localities of the districts. Those were- *Cleome viscosa, Elytraria acaulis, Tephrosia villosa, Aerva javanica, Leucas aspera and Tridax procumbens* The leaves of the selected plants were cleaned, air-dried, grinded and stored in airtight boxes.

Determination of primary metabolites

Total soluble sugars: "The total soluble carbohydrate content was determined according to the method described by Hedge and Hofreiter [6]. 1 ml of sample was mixed with 4 ml of anthrone reagent. Incubated in boiling water bath for 8 minutes after which the absorbance was read at 630 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample".

Proteins: "Protein content was determined according to the method of Lowry *et al*. [7]. 1 ml of sample was mixed with 0.5 ml of 0.1 N NaOH and 5 ml of alkaline copper reagent, incubated the mixture in room temperature for 30 minutes. Added 0.5 ml of Folin–Ciocalteau reagent and incubated again for 10 minutes at room temperature. Absorbance was read at 660 nm against a negative control (blank). The analysis was performed in triplicates and the results were expressed mg/g sample".

Determination of total free amino acids: "Total free amino acids (ninhydrin method) was determined according to the procedure given by Moore and Stein [8]. 1 ml of the sample was mixed with 1 ml of Ninhydrin in a test tube. Tubes were kept in boiling water bath for 20 minutes and then added 5 ml of diluent (equal volume of water and n-propanol) incubated at room temperature for 15 minutes and absorbance were recorded at 570 nm against a negative control (blank). The analysis was performed in triplicates, and the results were expressed as mg/g sample".

Lipids: "1gm of each dried sample was homogenised with 10 ml distilled water by using a mortar and pestle [9]. The paste obtained after homogenisation was mixed thoroughly with 30 ml of chloroform and methanol made in ration of 2: 1 by v/v and transferred in a conical flask. Each mixture was left overnight at room temperature and then 20 ml of chloroform with equal volume of d. H_2O was added and centrifuged. After centrifugation three layers were obtained. Of these 3 layers, a transparent lower layer of chloroform containing all the lipids was poured out in pre-weighted beakers. Rest of the two layers were discarded. After total evaporation, the weight of beakers was measured again. The difference weight was considered as total lipids/g of the dried plant sample. The procedure was carried out in a set of three replicas and the mean value was noted for each sample".

DETERMINATION OF SECONDARY METABOLITES

Total phenolic compounds: "The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract" [10].

Flavonoids: "Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract" [11, 12].

Alkaloids: "The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and

5 ml of phosphate buffer were added. The mixture was shaken with 1-4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract" [13, 14].

Tannins: "The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent, 1 ml of 35 % Na2CO3 solution and dilute to 10 ml with 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 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract" [15, 16].

Saponins: "Saponin content was determined by the procedure of Obadoni and Ochuko [17]. The samples were ground and 20 g of each were put into a conical flask and 100 Cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage".

Percentage of Saponin = (Weight of residue /Weight of sample taken) × 100

Terpenoids: "Terpenoids content was determined by the procedure of Ferguson, [18]. About 10 gm of leaves and bark powdered was taken and soaked in alcohol for 24 hours. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids" [19].

RESULTS AND DISCUSSION

Secondary metabolites are produced from primary metabolites in various metabolic pathways. These are stored in different plant parts as waste products. These secondary metabolites have various therapeutic uses for human beings. Traditional knowledge of using plants as therapeutic purpose is the basis of research on medicinal plants. On the basis on that traditional knowledge used by tribal people of Alwar district in Rajasthan, we selected some plant parts which are commonly used to treat various illnesses for determination of primary and secondary metabolites. Results of quantitative analysis of various primary and secondary metabolites in the selected plants are shown in table 1 and table 2 respectively and these results are graphically represented in figure 1 and figure 2 respectively. Total soluble sugars (TSS), proteins, free amino acids, and lipids are primary metabolites which were determined in the present study. Results revealed that the used plant parts are rich in these primary metabolites which are precursors for various secondary metabolites. So, these plant parts were also found to be rich in secondary metabolites (total phenols, flavonoids, alkaloids, saponins, terpenoids, and tannins). The selected plants are used being used to treat inflammation, hyperglycaemia, ulcers, malaria, micribial infections, infertility, diabetes, hypertension, cancer etc. [20 - 22]. Results of the current study revealed the presence of important phytochemicals which are well known for showing medicinal properties [23, 24]. Flavonoids are known to be natural antioxidants.

Table 1: Quantitative determination of primary metabolites in different plants collected from Alwar district of Rajasthan

Name of plant	Name of primary metabolites			
	TSS (mg/g.dw)	Proteins (mg/g.dw)	Free amino acids (mg/g.dw)	Lipids (mg/g.dw)
Cleome viscosa	3.64±0.67	1.15±0.24	0.85±0.16	2.31±0.82
Elytraria acaulis,	1.17±0.22	1.32±0.27	1.25±0.71	2.86±0.53
Tephrosia villosa,	1.67±0.17	1.73±0.16	0.86±0.10	1.53±0.26
Leucas aspera	2.93±0.77	1.35±0.24	0.78±0.13	1.54±0.56
Aerva javanica	2.08±0.24	1.79±0.66	1.14±0.11	2.06±0.14
Tridax procumbens	3.15±0.67	1.22±0.57	0.84±0.28	2.16±0.65

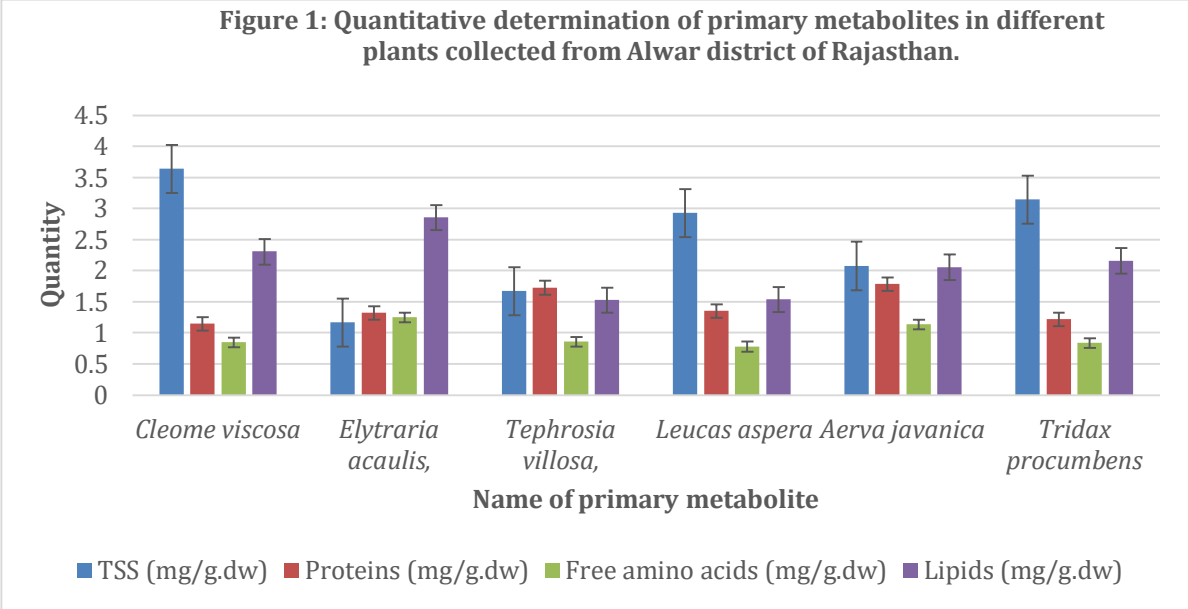
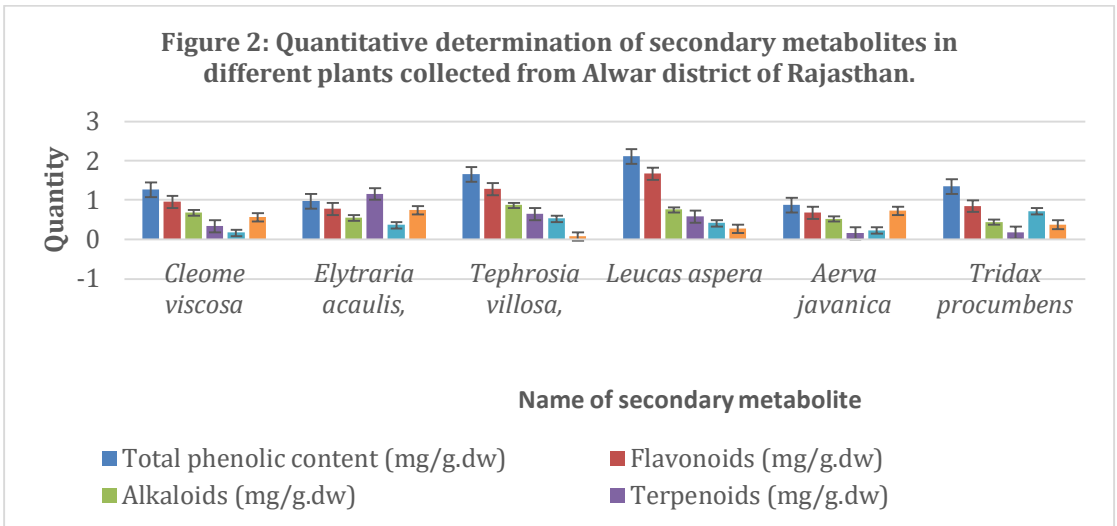


Table 2: Quantitative determination of secondary metabolites in different plants collected from Alwar district of Rajasthan.

Name of plant	Name of Secondary metabolites					
	Total phenolic content (mg/g.dw)	Flavonoids (mg/g.dw)	Alkaloids (mg/g.dw)	Terpenoids (mg/g.dw)	Tannins (mg/g.dw)	Saponins (mg/g.dw)
Cleome viscosa	1.26±0.33	0.96±0.25	0.68±0.17	0.34±0.06	0.17±0.02	0.57±0.11
Elytraria acaulis,	0.97±0.36	0.78±0.33	0.55±0.14	1.16±0.47	0.37±0.08	0.74±0.35
Tephrosia villosa,	1.66±0.88	1.28±0.66	0.87±0.11	0.65±0.21	0.53±0.004	0.08±0.002
Leucas aspera	2.11±1.02	1.67±0.54	0.76±0.10	0.59±0.16	0.42±0.05	0.28±0.03
Aerva javanica	0.87±0.34	0.68±0.11	0.52±0.09	0.16±0.008	0.23±0.001	0.73±0.10
Tridax procumbens	1.35±0.75	0.85±0.10	0.44±0.18	0.18±0.002	0.72±0.03	0.38±0.002



CONCLUSION

From the results of the present investigation, it can be concluded that the selected plants are rich sources of pharmaceutically important phytochemicals. So, these can be used to identify

and isolate active compounds for formulating medicines. Such natural medicines will be affordable with no/lesser side effects.

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