Exploring the Phytopharmacological Potential of Plants from Cholistan Desert of Pakistan as their Metabolites Assay

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ABSTRACT

Significance: Secondary metabolites are mainly resourced from stress-adapted plants and have medicinal properties on one hand and toxicity potential on the other hand. Hence, the practical utilization of these plants as food or medicines need to explore the exact nature and actions of their metabolites. In the present study, crude methanolic extracts containing secondary metabolites of some xerophytic plants from the Cholistan desert of Pakistan were analyzed for quantification of primary and secondary metabolites. Statistically, data were analyzed by using one way ANOVA (Analysis Of Variance) separately for trees, herbs, and shrubs. Data were represented as means and standard deviations (Mean ± SD) of each parameter and means separated by Duncan's multiple range tests. **Findings:** The plants endemic to desert environment were evaluated to have secondary metabolites in quantity higher than that of primary metabolites owing to cope with adverse environmental conditions of the desert. The order of explored metabolites in term of their quantity was as alkaloid> flavonoids> total sugar.

Key Words: Phytopharmacological, potential, Cholistan, Desert, Pakistan, Metabolites.

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INTRODUCTION

The environmentally resourced inorganic contents are metabolized by the plant in synthesizing primary and secondary metabolites [1]. In addition to primary metabolites, the plants store secondary metabolites as flavonoids and alkaloids which are used in medicine and are also known to contain important therapeutic agents [2-4]. Flavonoids and similar compounds are a diverse group of phenolic compounds and the most bioactive plant secondary metabolites [1] commonly present in both edible and non-edible plants. Phenolics are of great interest in the food industry because they slow down the oxidative degradation of lipids and improve the quality and nutritional value of food [5, 6]. The pharmacological effects of flavonoids are antiviral, antimicrobial, anti-inflammatory, and antihepatotoxic [7]. Similarly, alkaloids are basic (i.e. high pH), cyclic compounds containing nitrogen, and are well-known for their pharmacological effects [8].

Moreover, Plants of the adverse environmental conditions such as deserts are regarded to synthesize these secondary metabolites as antioxidants for scavenging reactive oxygen species (ROS). Medicinal potential, antioxidant status, and toxicity nature of secondary metabolites need to be assessed prior to the use of such plants and their products. The use of herbal preparations without any proper scientific studies on their safety can raise concerns about their toxicity. There are two main strategies for the selection of plants for assessing the potential: random screening and ethnomedical knowledge. Secondary metabolites are derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc., i.e. any part of the plant may contain active components.

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Secondary metabolites protect plants against biotic and abiotic stresses. The diet containing secondary metabolites of the plant can affect the metabolism and health status of herbivores including human beings [9]. Secondary metabolites are synthesized in plant tissues or organs in a specific way by specific biosynthetic enzymes [10, 11] transported through xylem and phloem and are accumulated and stored in various plant organs. These compounds have been reported to be used as food additives to improve the quality of nutrition [12]. Some of the secondary metabolites showed negative effects on the consumer also because of being the harmful toxins [13] while others have been shown to have positive effects [14, 15].

The aim was to explore the methanol soluble primary and secondary metabolites [16] as phytopharmacological components of plants from the Cholistan desert of Pakistan. These findings will discriminate the ethnopharmacological and toxicological potential of plants by assessing their secondary metabolites [17]. The selection of plants is based on arid environmental conditions of habitat which enables the plants to synthesize and store secondary metabolites of pharmaceutical importance for adaptation to stressful environments [18].

MATERIALS AND METHODS

Field survey and plant sample collections

Plants of the Din Garh area of the Cholistan desert of Pakistan were selected for metabolites extraction. Methanol was used as a solvent for the extraction procedure [19, 20]. A preliminary survey of the area was conducted. During this survey, meetings with local peoples were arranged to know the geographical status of the area and local plant names. Identification of the plant was performed by matching them with the labeled herbarium specimens lying in the departmental herbarium (Dr.Mumtaz Bukhari herbarium) of Botany Department Bahauddine Zakariya University, Multan Pakistan and/or the literature [21]. Comprehensive field survey and plant specimen collection, after the preliminary visit, was done. Data and specimens were collected according to an appropriate methodology keeping in view the uniformity among age and size of plants and their parts [22-24]. Further processing of collected specimens was carried out in the laboratory of the department.

Crude herbal extract preparation

The collected specimens were first washed with water and 2% methanol to remove surface contaminants. Samples were dried and were ground to a fine powder. Crude Methanolic extract was prepared according to the method as described by Afolayan [25] with slight modification. The extract was filtered and the material was stored at -4°C.

Determination of Total Flavonoid Contents. (TFC)

Quantification of flavonoid contents of plants was determind by a method used by Quettier-Deleu et al. [26] with little modifications. 50.0g of fine powdered plant material was weighed in 1000ml conical flask and was extracted in 100ml of methanol by shaking at 200 rpm at room temperature overnight. The extract was filtered through Whatman filter paper and the filtrate was evaporated at a rotatory evaporator at 50°C and weighed.

Determination of alkaloids

Total alkaloid contents were determined by Harborne [27] method with little modifications. 50.0g of the fine powdered plant material was weighed into a 1000ml conical flask. The extract was taken by shaking the plant sample in 200ml of 10% acetic acid in ethanol at room temperature at 200rpm for 4h. The extract was passed through the Whatman filter paper and the filtrate was concentrated in a water bath to one-quarter of the original volume at 95°C, and 40% ammonium hydroxide (NH_4OH) was added dropwise for precipitation and allowed to stand overnight to complete precipitation. The whole solution was filtered through Whatman filter paper, and precipitation was oven-dried at 75°C and weighed.

Determination of Total sugars

The total sugars were estimated by following the Phenol-Sulphuric acid method. Extract of the plant material (100 g of oven-dried bark powder) was taken by shaking the plant material in 1000 ml methanol instead of ethanol. For the estimation of total sugar, 0.2 ml of plant extract in a test tube, 1 ml of 5% Phenol was carefully added and mixed thoroughly. Concentrated Sulphuric acid of analytical grade (5 ml) was added very carefully to the above test tube. The mixture was cooled at room temperature and the absorbance was read at 485 nm against a blank containing distilled water. The amount of soluble sugars was estimated with the help of standard glucose (0.1 mg/ml) and expressed in mg/g dry tissue.

Statistical analysis

Data obtained was analyzed by using one way ANOVA (Analysis Of Variance) at 5% level of statistical significance separately for herbs, shrubs, and trees. Means were compared by Duncan's multiple range test [28].

RESULTS

Alkaloid contents in some trees of Cholistan desert

Alkaloid contents in trees (Table 1) showed significant differences among plants and their parts. The maximum concentration of alkaloid contents (181.833 mg/g) was observed in young shoots of *Tamarix aphylla*. Regarding the alkaloid contents and their %age difference from the

maximum value, when summarized revealed the following order in terms of alkaloid contents.
Tamarix aphylla stem > *Capparis decidua* stem > *Tamarix aphylla* shoots > *Capparis decidua* root > *Accacia nilotica* stem

Total Flavonoid Contents in some trees of Cholistan desert

A significant difference has been observed in the flavonoid contents of trees (Table 1). Maximum concentration (252.8 mg/g) was observed in the stem of *Tamarix aphylla* which shows high range difference from other flavonoid contents. Surprisingly low concentration of 28.2 mg/g (88.85%) was observed in young shoots of *Tamarix aphylla*. Regarding the flavonoid contents following is the order:
Tamarix aphylla stem > *Acacia nilotica* stem > *Capparis decidua* root > *Capparis decidua* stem > *Tamarix aphylla* shoots

Total sugar in some trees of Cholistan desert

The maximum concentration of total sugar (1.472 mg/g) was shown in young shoots of *Tamarix aphylla* and a minimum concentration of 0.648 mg/g (55.98%) in the stem of *Capparis deciduas* (Table 1). Different contents of total sugar have been found in different plants and their %age difference from the maximum value revealed the following order:
Tamarix aphylla shoots > *Tamarix aphylla* stem > *Acacia nilotica* stem > *Capparis decidua* root > *Capparis decidua* stem

Alkaloid contents in some shrubs of Cholistan desert

Alkaloid contents in shrubs showed non-significant differences (Table 2). The maximum concentration of alkaloid contents (394.73 mg/g) was observed in the stem of *Leptadenia pyrotechnica*, and a minimum concentration of 63.47 mg/g (83.92%) was observed in the root of *Salsola imbricata*. The order of plants in terms of alkaloid contents was as:
Leptadenia pyrotechnica stem > *Salsola imbricata* stem > *Salsola imbricata* leaves > *Abutilon indicum* leaves > *Calotropis procera* leaves > *Haloxylon salicornicum* leaves > *Pseuda fruticosa* stem > *Aerva javanica* stem > *Calligonum polygonoides* leaves > *Calligonum polygonoides* root > *Haloxylon salicornicum* stem > *Calotropis procera* stem > *Calligonum polygonoides* stem > *Aerva javanica* flower > *Abutilon indicum* stem > *Salsola imbricata* root

Total Flavonoid Contents in some shrubs of Cholistan desert

Significant differences were observed among flavonoid concentrations (Table 2). The highest concentration (83.67 mg/g) was noted in the stem of *Calligonum polygonoides* and a minimum concentration of 3.8 mg/g (95.46%) was observed in leaves of *Calotropis procera*. Regarding the flavonoid contents following order was observed:
Calligonum polygonoides stem > *Salsola imbricata* leaves > *Calligonum polygonoides* leaves > *Salsola imbricata* root > *Leptadenia pyrotechnica* stem > *Calligonum polygonoides* root > *Haloxylon salicornicum* leaves > *Calotropis procera* stem > *Pseuda fruticosa* stem > *Salsola imbricata* stem > *Aerva javanica* flower > *Haloxylon salicornicum* stem > *Abutilon indicum* stem > *Abutilon indicum* leaves > *Aerva javanica* stem > *Calotropis procera* leaves

Total sugar contents in some shrubs of Cholistan desert

Significant differences have been examined in the total sugar concentration of shrubs (Table 2). The maximum concentration of total sugar was observed in leaves of *Haloxylon salicornicum* (1.60 mg/g) and the minimum concentration was observed in two plant species, leaves of *Calligonum polygonoides* 0.44 mg/g (72.5%) and stem of *Salsola imbricata* 0.44 mg/g (72.5%). When summarized, it revealed the following order in terms of total sugar:
Haloxylon salicornicum leaves > *Calotropis procera* leaves > *Aerva javanica* stem > *Leptadenia pyrotechnica* stem > *Pseuda fruticosa* stem > *Aerva javanica* flower > *Abutilon indicum* leaves > *Calligonum polygonoides* root > *Calligonum polygonoides* stem > *Haloxylon salicornicum* stem > *Salsola imbricata* leaves > *Calotropis procera* stem > *Abutilon indicum* stem > *Salsola imbricata* root > *Salsola imbricata* stem > *Calligonum polygonoides* leaves

Alkaloid contents in some herbs of Cholistan desert

Alkaloid contents in herbs showed significant differences (Table 3). The maximum concentration of 178.83 mg/g was in the shoot of *Cressa cretica* and the minimum concentration of 38.43 mg/g (78.51%) was observed in the root of *Alhagi maurorum*. The results, when summarized, revealed the following order in terms of alkaloid contents:
Cressa cretica shoot > *Polygonium poligaloides* shoot > *Citrullus colocynthis* leaves > *Solanum surattense* leaves > *Citrullus colocynthis* root > *Solanum surattense* root > *Euphorbia granulata* shoot > *Orobanche aegyptiaca* stem > *Orobanche aegyptiaca* leaves > *Citrullus colocynthis* stem > *Euphorbia granulata* root > *Alhagi maurorum* shoot > *Solanum xanthocarpum* root > *Solanum xanthocarpum* leaves > *Alhagi maurorum* root

Total Flavonoid Contents in some herbs of Cholistan desert

A significant difference has been observed in flavonoid contents of herbs (Table 3). The maximum concentration of 84.33 mg/g in the shoot of *Polygonium poligaloides* and minimum concentration of 9.2 mg/g (89.09%) in root of *Citrullus colocynthis* was observed. Summarized results

revealed the following order in terms of flavonoid contents:
Polygonium poligaloides shoot > *Orobanche aegyptiaca* leaves > *Euphorbia granulate* root > *Cressa cretica* shoot > *Alhagi maurorum* root > *Euphorbia granulata* shoot > *Alhagi maurorum* shoot > *Orobanche aegyptiaca* stem > *Solanum xanthocarpum* leaves > *Solanum surattense* root > *Solanum xanthocarpum* root > *Citrullus colocynthis* leaves > *Citrullus colocynthis* stem > *Solanum surattense* leaves > *Citrullus colocynthis* root

Total sugar in some herbs of Cholistan desert

No significant difference has been examined in total sugar contents of herbs (Table 3). The maximum concentration of 0.932 mg/g was found in the shoot of *Cressa cretica* while the minimum concentration of 0.424 mg/g (54.51%) was observed in the root of *Solanum surattense*. All results revealed the following order in terms of total sugar:
Cressa cretica shoot > *Solanum xanthocarpum* leaves > *Solanum xanthocarpum* root > *Orobanche aegyptiaca* stem > *Alhagi maurorum* shoot > *Orobanche aegyptiaca* leaves > *Solanum surattense* leaves > *Citrullus colocynthis* root > *Polygonium poligoloides* shoot > *Euphorbia granulata* root > *Citrullus colocynthis* stem > *Alhagi maurorum* root > *Citrullus colocynthis* leaves > *Euphorbia granulata* shoot > *Solanum surattense* root.

DISCUSSION

All of the plant species under study in the Cholistan desert of Pakistan were proved to have secondary metabolites such as alkaloids, flavonoids in addition to primary metabolites as total sugar contents. Quantity of alkaloids was higher in all of the plants and plant parts compared to other metabolites. The quantity of phytochemicals depends also upon its place of synthesis and accumulation [29]. The level of antioxidants was different from plant to plant depending upon variety and their growth conditions [30]. The results of alkaloids showed that the amount of alkaloids in the leaves of some plants was higher as compared to the stem of plants of the same species. Similar results have also been reported previously [31, 32].
Flavonoids belong to the phenolics group. The established health benefits of phenolics, due to their free radical scavenging activities in vitro and in vivo biological systems necessitates their quantification in foods [29]. There is a worldwide interest to identify plants having high levels of phenolics for increasing its functional properties in foods. Phenolics have multiple adaptive roles contributing to the overall fitness of plants. Their primary roles include nontoxic interactions with herbivores and symbiotic organisms. Anthocyanins are also the example of phenolics which are responsible for the beautiful colors that attract insects for pollination [33-35]. Light is a basic factor in the synthesis of phenolic compounds [36]. Our results are also in the same line when the amounts of flavonoids in leaves of the plants are higher as compared to their stem because leaves have a higher amount of chloroplasts in their leaves as compared to the stem. The difference in accumulation and concentrations of different phenolic contents in different plants during their life span is also reported [37]. Flavonoids as polyfunctional compounds in green plastids perform three major functions as they act as substrates in different sorts of biosynthesis by using polyphenols and their catabolic products, they can also be used as a source of energy during transport of electron and proton, the formation of different radicals and exchange of ions through the membrane. The difference in the amount of total flavonoids contents in leaves and stem show that the flavonoids might be converted into secondary phenolic compounds or their degradation may occur by the action of enzymes. Flavonoids' losses during maturation of different plant parts may reflect the conversion of flavonoids into secondary phenolic compounds or they become degraded by the action of enzymes [37, 38].
Phytochemical screenings of plants are used for searching bioactive agents. Plants can provide precursors for starting products for the partial synthesis of some useful drugs. High flavonoid contents of the plant indicate that the plant has a high antioxidant potential [39]. Earlier reporters described that flavonoids have anti-inflammatory, antibacterial, antiallergic, antiviral, antimutagenic, antineoplastic, anti-thrombotic, and vasodilatory properties. The low amount of alkaloid present is also indicative of its harmless effect. There are reports [40] which have pointed out that plants containing high alkaloids contents are not suitable for herbal medicine because they are extremely toxic, yet, they have always been important in allopathic and homeopathy where the dose-rate is so low as to be harmless. However, the 'Solanum alkaloids' from *Solanum* species have been reported to be used in the partial synthesis of drugs [41].

CONCLUSION:

The present work revealed that the studied plants have phenolic, flavonoids, and alkaloids contents. These results, therefore, support the fact that plants due to possessing these important bioactive compounds could be screened for several medicinal purposes. However, it is our opinion that further analysis apart from the studied phytochemicals should be conducted as a whole before their utilization for medicinal purposes.

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Table 1. Quantitative evaluation of Alkaloids, Total Flavonoids, and Total Sugar contents in some trees of Cholistan desert

Species	Parts	Total sugar (mg/g) LSD=(0.405)	%age	alkaloid (mg/g) LSD=(14.83)	%age	flavonoid (mg/g) LSD=(17.78)	%age
Capparis decidua	Root	0.806±0.2 b	45.24	153.533±7.0 b	15.56	79.467±9.18 c	68.57
	Stem	0.648±0.22 b	55.98	166.40±8.05 b	8.49	67.267±12.31 c	73.39
Tamarix aphylla	Stem	1.462±0.20 a	0.67	181.833±7.63 a	000.000	252.8±8.15 a	000.0
	shoots	1.472±0.20 a	0.000	163±7.92 b	10.36	28.2±7.81 d	88.85
Acacia nilotica	Stem	1.073±0.21 ab	27.106	116.4±9.85 c	35.99	170.53±10.70 b	32.54

Mean±standard deviation; Values sharing the same letters in respective column differ non significantly; LSD= least significant difference;n=3:%=%age difference from the highest value

Table 2. Quantitative evaluation of Alkaloids, Total Flavonoids, and Total Sugar contents in some shrubs of Cholistan desert

Species	Parts	alkaloid (mg/g) LSD=(292.467)	%age	flavonoid (mg/g) LSD=(12.94)	%age	sugar (mg/g) LSD=(0.53)	%age
Calligonum polygonoides	Leaves	127.4±7.25	67.72	51.53±11.79 b	38.41	0.44±0.28 e	72.5
	Root	126.23±7.05	68.02	45.27±5.80 b	45.89	0.682±0.23 cde	57.5
	Stem	92.13±10.59	76.66	83.67±17.96 a	00.00	0.60±0.20 cde	62.5
Haloxylon Salicornicum	Stem	115.13±7.77	70.83	26.8±3.08 de	67.97	0.56±0.25 de	65
	Leaves	142.27±13.63	63.96	45.13±8.56 b	46.07	1.60±0.40 a	0.00
Salsola Imbricata	Leaves	209.3±7.15	46.98	74.6±10.81 a	10.84	0.56±0.41 de	65
	Root	63.47±8.15	83.92	50.2±11.98 b	40.00	0.53±0.47 de	66.88
	Stem	393.87±497.23	0.23	28.6±4.73 d	65.82	0.44±0.27 e	72.5
Calotropis procera	Leaves	161.8±7.33	59.01	3.8±0.8 f	95.46	1.54±0.50 a	3.75
	Stem	112.73±6.67	71.44	42.73±4.89 bc	48.93	0.56±0.36 de	65
Leptadenia pyrotechnica	Stem	394.73±496.48	00.00	46.13±2.72 b	44.87	1.19±0.25 abc	25.63
Pseuda fruticosa	Stem	135.33±8.65	65.72	30.4±5.77 cd	63.67	1.18±0.23 abc	26.25
Aerva javanica	Flower	78.2±7.70	80.19	28±5.70 de	66.53	1.09±0.24 abcd	31.88
	Stem	131.7±7.82	63.64	13.8±1.8 ef	83.51	1.30±0.35 ab	18.75
Abutilon Indicum	Leaves	174.3±5.80	55.84	17.47±2.32 de	79.12	0.82±0.27 bcde	48.75
	Stem	75.03±11.80	80.99	18.47±1.40 de	77.93	0.54±0.26 de	66.2

Mean±standard deviation; Values sharing the same letters in respective column differ non significantly; LSD= least significant difference; n=3:%=%age difference from the highest value

Table 3. Quantitative evaluation of Alkaloids, Total Flavonoids, and Total Sugar contents in some herbs of Cholistan desert

Species	Parts	alkaloid (mg/g) LSD=(13.16)	%age	flavonoid (mg/g) LSD =(9.33)	%age	Total sugar (mg/g) LSD =(0.47)	%age
Citrullus colocynthis	Stem	97.8±8.32 g	45.31	15.13±2.21 ef	82.06	0.60±0.23	35.62
	Leaves	171.83±6.55 abc	3.910	18.6±14.86 def	77.9438	0.592±0.29	36.69
	Root	158.27±8.20 cd	11.5	9.2±0.72 f	89.0905	0.651±0.33	30.26
Cressa cretica	Shoot	178.83±7.50 a	00.00	35.33±3.06 c	58.1051	0.932±0.27	0.000
Polygonium poligoloides	Shoot	175.17±7.16 ab	2.05	84.33±8.08 a	0.00	0.609±0.33	35.62
Orobanche aegyptiaca	Leaves	101.33±9.01 g	43.34	75.53±7.91 a	10.4352	0.73±0.30	21.67
	stem	132.3±6.09 f	26.02	20.33±2.01 de	75.8923	0.818±0.16	13.09
Euphorbia granulata	Shoot	142.9±6.54 ef	20.09	29.07±2.72 cd	65.5283	0.565±0.28	39.38
	Root	94.2±5.6 g	47.32	52±5.33 b	38.3375	0.606±0.23	34.98

Alhagi maurorum	Shoot	57.23±8.9 h	67.99	28.53±3.51 cd	66.1686	0.817±0.28	12.34
	Root	38.43±6.35 i	78.51	32.53±5.28 c	61.4254	0.592±0.37	36.48
Solanum xanthocarpus	Leaves	39.27±5.15 i	78.04	19.53±2.30 def	76.841	0.917±0.36	1.61
	Root	51.3±7.18 hi	71.31	18.8±3.27 def	77.7066	0.823±0.33	11.70
Solanum surattense	Leaves	162.47±11.40 bcd	9.15	13.13±2.21 ef	84.4302	0.691±0.26	25.86
	Root	154.5±11.21 de	13.61	19.33± 2.41 def	77.0781	0.424±0.13	54.51

Mean±standard deviation; Values sharing the same letters in respective column differ non significantly; LSD= least significant difference; n=3:%=%age difference from the highest value