

## Solvent Based Extraction of Non-enzymatic Antioxidants of Some Plants from Cholistan Desert, Pakistan

Muzamal Hussain<sup>a</sup>, Ghulam Yasin<sup>a\*</sup>, Aqsa Ahmad<sup>a</sup>, Shafaq Sohail<sup>a</sup> and Adeela Altaf<sup>b</sup>

<sup>a</sup>Department of Botany, Bahauddin Zakariya University, Multan, Pakistan

<sup>b</sup>Department of Environmental Sciences, Bahauddin Zakariya University, Multan, Pakistan

(received May 15, 2023; revised March 3, 2024; accepted March 8, 2024)

**Abstract.** Present studies were conducted on some trees and herbs of the Cholistan desert, Pakistan based on solvent dependent extraction of non-enzymatic antioxidants *i.e.* alkaloids, flavonoids and saponins. Three different extraction solvents *i.e.* ethanol, methanol and acetone were used. The results revealed that ethanol has maximum extraction potential followed by methanol and then acetone. In trees, alkaloid contents were in order *Acacia jacquemontii* > *Tamarix aphylla* > *Acacia nilotica* > *Capparis decidua* > *Prosopis cinerari*. Among herbs, *Citrullus colocynthis* gave more alkaloids than *Tribulus longipetalus*. Flavonoids ratios were as methanol > ethanol > acetone. Flavonoids in trees were as *Acacia jacquemontii* > *Capparis decidua* > *Prosopis cineraria* > *Tamarix aphylla* > *Acacia nilotica*. Among herbs, Flavonoids were obtained maximally from *Solanum surattense* and the lowest in *Launaea nudicauli*. For saponin, methanol was found to be the most powerful extraction solvent. The maximum concentrations of saponins were found in *Acacia jacquemontii* and *Tribulus longipetalus* of trees and herbs respectively. These findings provide valuable insights into solvent dependent extraction methods for non-enzymatic antioxidants present in the Cholistan desert flora, offering potential avenues for further exploration in pharmaceutical and nutritional applications.

**Keywords:** antioxidants, inorganic solvent, trees, herbs, Cholistan desert

### Introduction

The desert, Cholistan, covers a region of twenty-six thousand kilometer square (Km<sup>2</sup>) on the southern side of Bahawalpur, Punjab. It stretches out among the Nara and Thar deserts in Sindh somewhere around 27°42'N and 29°45'N and 69°52'E and 75°24' E (Akhter and Arshad, 2006) which was elevated around 111.99 meters above level of sea (Ali *et al.*, 2009). The atmosphere of the Cholistan desert is sub-tropical, dry, facing storms, with low precipitation and long dry seasons. Absolute humidity in the air expressed in %age, is low (Arshad *et al.*, 2006). The mean precipitation of the year ranges from 100 to 250 millimetres. The average temperature of the summer season is 34.1 °C to 38.0 °C and the average temperature of the winter season lies between 15.0 °C to 20.0 °C (Arshad *et al.*, 2007).

Geographically, the region can be partitioned into two geomorphic areas in light of soil types, geology, composition and vegetation pattern. Lesser Cholistan, the northern region, covers around 7770.0 kilometer square (Km<sup>2</sup>) of desert corners with the irrigation of canal water and greater Cholistan is situated in the south

locale of around 1,8130 kilometer square (Km<sup>2</sup>) with sand formed through the wind (Ahmad *et al.*, 2008).

The floral biodiversity of the Cholistan desert is revealed in the form of diverse plants in terms of morphology and genetic pools. Plants and animals of different zones have characteristic ecological niches endemic to specific regions. Natural flora includes approximately 152 species belonging to 38 families and 106 genera. Some plant species bear high fiscal values and are used for various purposes as a whole or as their parts (Hameed *et al.*, 2011). Plants of medicinal importance have significant contributions in the discovery of new drugs and pharmaceutical products. About 70% of allopathic medicines are being derived directly or indirectly from plant resources (Verma *et al.*, 2014). The extraction of phytochemicals depends on the dissolution and dispersion of a specific solvent. Solvent employed for the extraction must be selected wisely. Variable to take under consideration for the phytochemicals extraction solvent selection is that solvent employed for the particular purpose must be safe and must be active enough to bring about the extraction of all undesirable molecules diligently (Tiwari *et al.*, 2013; Seidel, 2012).

\*Author for correspondence;  
E-mail: yasingmn\_bzu@yahoo.com

Phytochemicals, which are bioactive compounds found in plants, comprise a diverse array of secondary metabolites crucial for their growth, defense mechanisms and adaptation to environmental stresses. These compounds can be broadly categorized into primary and secondary metabolites. Primary metabolites, such as sugars, lipids and amino acids, serve as fundamental building blocks necessary for plant growth and development. Conversely, secondary metabolites, including phenolics, flavonoids, alkaloids, tannins and saponins which are not directly linked to growth but play significant roles in aiding plant defense mechanisms against various biotic and abiotic stresses (Atanasov *et al.*, 2021).

The Cholistan desert in Pakistan harbours a diverse range of native flora adapted to survive extreme arid conditions. These plants have evolved unique biochemical pathways to synthesize phytochemicals, including antioxidants, as a survival strategy against challenging environmental conditions. Extraction and characterization of non-enzymatic antioxidants from these plants are particularly compelling due to their potential health benefits and promising applications in pharmaceuticals, nutraceuticals and functional foods.

The primary objective of this study is to explore and implement solvent based extraction techniques to isolate non-enzymatic antioxidants present in select plants indigenous to the Cholistan desert in Pakistan. This research endeavors to optimize the extraction process by carefully selecting appropriate solvents and parameters, aiming to effectively isolate and characterize the antioxidants within these plants. Additionally, the study seeks to evaluate the antioxidant activity of the extracted compounds using various assays.

## Materials and Methods

Materials were collected and utilized for the extraction of secondary metabolites (alkaloid contents flavonoid contents and saponin contents).

**Collection of plant material.** The selection of plants for this study involved a meticulous process, carefully evaluating their relative phytochemical content ratios. To ensure precise identification, uniformly aged and sized plant samples were deliberately chosen from diverse desert regions. Identification procedures followed well-established protocols detailed in reputable literature regarding the flora of Pakistan (Khan, 2009; Shafi *et al.*, 2001). Before processing, the plant twigs underwent a thorough cleansing using tap water to

eliminate any extraneous debris. Subsequently, the specimens underwent air-drying, allowing for gradual desiccation and were further dried under shade for 15 days. Once completely dried, the plant material was finely ground into a powder for subsequent analysis.

**Extract preparation.** Plant extracts were prepared with different natural solvents (methanol, acetone and ethanol), 500 g of powder was dissolved in each solvent separately and were kept on the electric shaker for 48 h. Once the shaking was done the solution was filtered firstly with the muslin cloth, followed by its filtration with the whatmans filter paper no.1 and eventually the filtrate was centrifuged for 5 min at 5000 rpm.

**Photochemical analysis.** In quantifying the various phytochemicals, the protocols developed and used by various scientists were used with slight modifications (Ahmed *et al.*, 2022; Gedlu, 2022; Khanal *et al.*, 2022).

For alkaloid content determination, initially finely powdered plant material underwent extraction using an appropriate solvent system to ensure optimal dissolution of alkaloids. The resulting extract was filtered (muslin cloth) and concentrated to yield an alkaloid-rich solution. A specific volume of this solution was then used for quantitative estimation through spectrophotometry (Hitachi Model 2001, Japan). This process relied on a designated alkaloid marker compound and readings were compared against a standard calibration curve, facilitating precise quantification of alkaloid levels in the plant sample.

For evaluating flavonoid content, finely ground plant material was extracted using a solvent system (methanol) known for selectively extracting flavonoids. The resulting extract underwent subsequent purification steps to isolate the flavonoid compounds. Quantitative analysis utilized spectrophotometry at a wavelength corresponding to the maximum absorption of flavonoids (Hitachi Model 2001, Japan). A standard calibration curve, generated from known concentrations of a representative flavonoid compound, enabled the accurate determination of flavonoid content in the plant material.

In the determination of saponin contents the process began with saponin extraction from the plant material using an optimized procedure with a suitable solvent system (ethanol). The resulting extract was treated to eliminate impurities and concentrated to obtain a purified saponin-rich solution. Quantitative estimation involved a colorimetric method where saponin concentration was determined based on specific reaction methods.

Construction of a calibration curve using standard saponin solutions facilitated precise quantification of saponin content in the plant sample, ensuring meticulous measurements and accurate characterization.

**Statistical values.** The data collected from this work was analysed to observe the variance using COSTAT computer package (CoHort software, Berkeley, CA). Comparison was carried out using Duncan's new multiple range test at 5% level of probability (Duncan, 1955). Significant F values were tested by LSD tests at 0.05% significance level by using MSTAT-C computer statistical programme.

**Quantitative evaluation of alkaloids in trees.** The mean values of alkaloids are shown in Table 1. Regarding the alkaloids content and their %age difference obtained by using the ethanol extract, it was found different statistically in various plants. 18.9 mg/g (24.35%) of alkaloid was observed in methanol extract in *Acacia jacquemontii*. And this tree showed mean value of 14.46 mg/g of alkaloid concentration in all solvents. *Tamarix aphylla* showed mean amount of 14.46 mg/g. *Capparis*

*decidua* showed 3.0 mg/g in acetone extract and 18.5 mg/g in methanol extract. *Acacia nilotica* showed 19.3 mg/g in ethanol extract. This plant showed 13.30 mg/g mean alkaloid concentration. *Prosopis cineraria* showed mean 7.93 mg/g alkaloid.

Methanol extract has highest mean value of alkaloid. Ethanol extract has lowest mean value. A significant difference was observed in *Capparis decidua* among ethanol extract 18.5 mg/g and acetone extract 3.20 mg/g (82.70%). Non significant differences were observed among the means of alkaloid content in different trees of Cholistan. Mean alkaloid contents in some trees of Cholistan desert was observed in the following order.

*Acacia jacquemontii* > *Tamarix aphylla* > *Acacia nilotica* > *Capparis decidua* > *Prosopis cineraria*

**Quantitative evaluation of flavonoid in trees.** The mean values of flavonoids are shown in Table 2. As far as the flavonoids content and their %age difference in the ethanol extract are concerned, it was found different statistically in various plants.

**Table 1.** Quantitative assessment of alkaloid content in shoot extracts from selected trees of the Cholistan desert using solvent based extraction

Plants name	Ethanol extract mg/g	Methanol extract mg/g	%age	Acetone extract mg/g	%age	Mean mg/g
<i>Acacia jacquemontii</i>	14.4±0.7	18.9±0.7	-31.25	10.1±0.9	29.86	14.46±3.86 <sup>a</sup>
<i>Acacia nilotica</i>	19.3±0.7	14.6±0.6	24.35	6±0.2	68.91	13.3±5.86 <sup>b</sup>
<i>Capparis decidua</i>	18.5±0.5	3±0.3	83.78	3.2±0.3	82.70	8.23±7.71 <sup>c</sup>
<i>Prosopis cineraria</i>	15.30±0.3	4.1±0.25	73.20	4.4±0.4	71.24	7.93±5.53 <sup>c</sup>
<i>Tamarix aphylla</i>	14.4±0.4	17.4±0.4	-20.83	9.5±0.5 <sup>s</sup>	34.03	13.77±3.47 <sup>b</sup>
<b>Means</b>	16.38±2.22 <sup>a</sup>	11.6±6.97 <sup>b</sup>	29.18	6.64±2.86 <sup>c</sup>	59.46	11.54±5.9

Mean ± standard deviation; letters in the column and row show differences; n = 3; % = %age difference from ethanol; - = sign show high value than ethanol.

**Table 2.** Quantitative assessment of flavonoid content in shoot extracts from selected trees of the Cholistan desert using solvent based extraction

Plants name	Ethanol extract mg/g	Methanol extract mg/g	%age	Acetone extract mg/g	%age	Mean mg/g
<i>Acacia jacquemontii</i>	45.8±0.8	99.2±2.1	-116.59	43.57±0.65	4.87	62.85±27.30 <sup>a</sup>
<i>Acacia nilotica</i>	18.4±0.4	22±0.3	-19.57	17.2±0.3	-6.52	19.2±2.18 <sup>c</sup>
<i>Capparis decidua</i>	32.1±0.5	50.1±2.1	-56.07	27.8±0.4	13.40	36.67±10.30 <sup>b</sup>
<i>Prosopis cineraria</i>	28.13±0.6	43.7±2.1	-55.35	24.9±0.5	11.48	33.44±10.54 <sup>c</sup>
<i>Tamarix aphylla</i>	20.4±0.4	21.6±0.6	-5.88	17.5±0.5	14.22	19.83±1.88 <sup>d</sup>
<b>Means</b>	28.97±10.14 <sup>b</sup>	48.04±29.31 <sup>a</sup>	-65.83	26.19±9.96 <sup>c</sup>	9.60	34.4±20.83

Mean ± standard deviation; letters in the column and row show differences; n = 3; % = %age difference from ethanol; - = sign show high value than ethanol.

A significant difference was observed in *Acacia jacquemontii* among ethanol extract 45.80 mg/g, methanol extract 99.2 (116.59%) and acetone extract 43.57 (4.87%); *Capparis decidua* among ethanol extract 32.10 mg/g, in methanol extract 50.10 (56.07%) and in acetone extract 27.80 mg/g (13.40%); *Prosopis cineraria* among ethanol extract 28.13 mg/g; in methanol extract 43.7 (55.33%) and in acetone extract 24.9 mg/g (11.45%). No significant difference was observed in *Tamarix aphylla* among ethanol extract 20.4 mg/g, in methanol extract 21.6 (5.88%) and in acetone extract 17.5 mg/g (14.22%). Mean flavonoid contents in some trees of Cholistan desert is represented by the following order.

*Acacia jacquemontii* > *Capparis decidua* > *Prosopis cineraria* > *Tamarix aphylla* > *Acacia nilotica*

**Quantitative evaluation of saponins in trees.** The mean values of saponins are shown in Table 3. Regarding the saponin content and their %age difference from the ethanol extract, different statistical values were observed in extracts from various plants. Maximum mean concentration of saponins 14.43 mg/g was observed in *Tamarix aphylla*, in ethanol extract 18.5 mg/g, in Methanol extract 15.5 mg/g (66.67%) and in acetone extract 9.30 mg/g (98.92%). Minimum saponins concentration of 4.47 mg/g was observed in *Prosopis cineraria*, in ethanol extract 3.6 mg/g, methanol extract mg/g 4.4 mg/g (18.52%) and acetone extract 5.40 mg/g (33.33%).

Significant difference of saponins content was observed in shoot extract of *Acacia jacquemontii* among ethanol extract 12.6 mg/g, in methanol extract 14.40 mg/g (15%) and in acetone extract 9.60 (31.25%), saponins content observed in shoot extract of *Acacia nilotica* among

ethanol extract 5.60 mg/g, in methanol extract 9.60 mg/g (52.38%) and in acetone extract 6.30 mg/g (11.11%), *Capparis decidua* among ethanol extract 13.50 mg/g, in methanol extract 6.40 mg/g (52.591%) and in acetone extract 3.50 mg/g (74.07%).

#### Quantitative evaluation of alkaloids in some herbs.

Table 4 shows the mean values of alkaloids. Maximum alkaloid contents of 70.6 mg/g were observed in stem of *Solanum surattense* in ethanol extract. Regarding the alkaloid content and their %age difference from the ethanol extract, it was found different statistically in various plants.

It was observed that 65.4 mg/g alkaloid was present in stem of *Citrullus colocynthis* in ethanol extract. There were non significant differences of alkaloid in stem extracts of some herbs of Cholistan desert in extract of ethanol. Minimum alkaloid content was observed in stem of *Tribulus longipetalus* in ethanol extract that was 42.8 mg/g. It was found that 69.4 mg/g (6.12%) alkaloid content was observed in stems of *Citrullus colocynthis* in methanol extract, that was maximum yield of alkaloid in extract of methanol in the studied herbs of Cholistan. Significant difference of alkaloid was observed in stems of *Solanum surattense* among ethanol extract 70.6 mg/g, in extract methanol 60.43 mg/g (14.41%) and in acetone extract 45.6 mg/g (35.41%). No significant difference of alkaloid was observed in *Tribulus longipetalus* among extract of ethanol 42.8 mg/g and in extract of methanol 45.6 mg/g (6.54%) and in extract of acetone 49.4 mg/g (15.42%). The following order of alkaloid in the herbs of Cholista was observed in ethanol extract *Solanum surattense* > *Citrullus colocynthis* > *Euphorbia prostrata* > *Launaea nudicaulis* > *Tribulus terrestris* > *Tribulus longipetalus*

**Table 3.** Quantitative assessment of saponin content in shoot extracts from selected trees of the Cholistan desert using solvent-based extraction

Plants name	Ethanol extract mg/g	Methanol extract mg/g	%age	Acetone extract mg/g	%age	Mean mg/g
<i>Acacia jacquemontii</i>	12.6±0.6	14.4±0.3	-15	9.6±0.4	31.25	12.2±2.14 <sup>b</sup>
<i>Acacia nilotica</i>	5.6±0.3	9.6±0.3	-52.38	6.3±0.3	-11.11	7.17±1.87 <sup>d</sup>
<i>Capparis decidua</i>	13.5±0.3	6.4±0.4	52.59	3.5±0.2	74.07	7.8±4.46 <sup>c</sup>
<i>Prosopis cineraria</i>	3.6±0.2	4.4±0.2	-18.52	5.4±0.4	-33.33	4.47±0.82 <sup>e</sup>
<i>Tamarix aphylla</i>	18.5±0.3	15.5±0.5	-66.67	9.3±0.3	-98.92	14.43±4.08 <sup>a</sup>
<b>Means</b>	10.76±5.65 <sup>a</sup>	10.06±4.50 <sup>b</sup>	-47.51	6.82±2.43 <sup>c</sup>	-57.77	9.21±4.64

Mean ± standard deviation; letters in the column and row show differences; n = 3; % = %age difference from ethanol; - = sign show high value than ethanol.

**Table 4.** Quantitative assessment of alkaloid content in shoot extracts from selected herbs of the Cholistan desert using solvent-based extraction

Plants name	Ethanol extract mg/g	Methanol extract mg/g	%age	Acetone extract mg/g	%age	Mean mg/g
<i>Launaea nudicaulis</i>	55.8±0.4	60.5±0.04	-8.42	40.52±0.04	27.38	52.273±9.04 <sup>c</sup>
<i>Citrullus colocynthis</i>	65.4±0.2	69.4±0.2	-6.12	47.5±0.02	27.37	60.77±10.10 <sup>a</sup>
<i>Solanum surattense</i>	70.6±0.2	60.43±0.01	14.41	45.6±0.2	35.41	58.88±10.89 <sup>b</sup>
<i>Tribulus longipetalus</i>	42.8±0.3	45.6±0.2	-6.54	49.4±0.2	-15.42	45.93±2.88 <sup>f</sup>
<i>Tribulus terrestris</i>	44.7±0.2	47.8±0.3	-6.94	52.4±0.2	-17.22	48.3±3.36 <sup>e</sup>
<i>Euphorbia prostrata</i>	59.7±0.1	48.1±0.3	19.43	43.4±0.2	27.30	50.4±7.26 <sup>d</sup>
<b>Means</b>	56.5±10.42 <sup>a</sup>	55.30±8.96 <sup>b</sup>	2.12	46.47±3.99 <sup>c</sup>	17.75	52.76±9.27

Mean ± standard deviation; letters in the column and row show differences; n = 3; % = %age difference from ethanol; - = sign show high value than ethanol.

#### Quantitative evaluation of flavonoids in some herbs.

The mean values of flavonoids in stems of some herbs of Cholistan desert are represented in Table 5.

It was observed that maximum mean of flavonoids of all the studied plants was observed in ethanol extract. Maximum flavonoids in Ethanol extract was observed in stem of *Citrullus colocynthis* that was 8.4 mg/g. Mean 7.45 mg/g flavonoids concentration was observed in *Solanum surattense* in ethanol extract. It was observed there was non significant difference of flavonoid concentration in ethanol extract between *Tribulus longipetalus* 6.42 mg/g and *Euphorbia prostrata* 5.96 mg/g. And there was non significant differences of flavonoids in extract of ethanol among stem of *Tribulus terrestris* 4.48 mg/g and *Launaea nudicaulis* 4.4 mg/g. Maximum flavonoids in extract of methanol was observed in stem of *Solanum surattense* 6.76 mg/g (9.26%). There was non significant difference of flavonoid in methanol extract among stem of *Tribulus*

*longipetalus* 5.5 mg/g (14.33%) and stem of *Citrullus colocynthis* 5.46 mg/g (35%). No significant difference was observed between *Tribulus longipetalus* 5.5 (14.33%) and *Euphorbia prostrata* 5.01 mg/g (15.94%). Minimum flavonoids contents was observed in *Launaea nudicaulis* 2.60 mg/g (40.90%) in methanol extract. Maximum flavonoids in acetone extract were observed in *Solanum surattense* 6.01 mg/g (19.33%). There was non significant difference of flavonoids in acetone extract among *Tribulus longipetalus* 4.99 mg/g (22.27%) and *Euphorbia prostrata* 4.94 mg/g (17.11%). Minimum flavonoids in *Tribulus terrestris* was observed in acetone extract was 2.99 mg/g (33.26%). Significant difference of flavonoids in stem extract of *Launaea nudicaulis* was observed among in ethanol extract 4.4 mg/g and in methanol extract 2.6 mg/g (40.90%) and in acetone extract 3.4 (22.72%).

#### Quantitative evaluation of saponins in some herbs.

The mean values of saponins in stem extracts of some

**Table 5.** Quantitative assessment of flavonoids content in shoot extracts from selected herbs of the Cholistan desert using solvent-based extraction

Plants name	Ethanol extract mg/g	Methanol extract mg/g	%age	Acetone extract mg/g	%age	Mean mg/g
<i>Launaea nudicaulis</i>	4.4±0.02	2.60±0.02	40.90	3.4±0.03	22.72	3.46±0.77 <sup>f</sup>
<i>Citrullus colocynthis</i>	8.4±0.01	5.46±0.01	35	4.24±0.01	49.52	6.03±1.05 <sup>b</sup>
<i>Solanum surattense</i>	7.45±0.03	6.76±0.01	9.26	6.01±0.01	19.33	6.74±0.626 <sup>a</sup>
<i>Tribulus longipetalus</i>	6.42±0.02	5.5±0.01	14.33	4.99±0.02	22.27	5.64±0.62 <sup>c</sup>
<i>Tribulus terrestris</i>	4.48±0.02	3.71±0.04	17.19	2.99±0.02	33.26	3.73±0.64 <sup>e</sup>
<i>Euphorbia prostrata</i>	5.96±0.01	5.01±0.01	15.94	4.94±0.02	17.11	5.30±0.49 <sup>d</sup>
<b>Mean</b>	6.19±1.49 <sup>a</sup>	4.82±1.30 <sup>b</sup>	22.13	4.43±1.04 <sup>c</sup>	28.43	5.15±1.50

Mean ± standard deviation; letters in the column and row show differences; n = 3; % = %age difference from ethanol; - = sign show high value than ethanol.

herbs of Cholistan desert are shown in Table 6. Non-significant difference of saponins was observed in *Tribulus longipetalus* among ethanol extract 23.7 mg/g, in methanol extract 24.3 mg/g (2.53%) and in extract of acetone 18.35 mg/g (22.57%).

In *Tribulus terrestris*, there was non significant difference of saponins in ethanol extract 20.7 mg/g and in methanol extract 21.76 mg/g. There was significant difference of saponins in ethanol extract and in acetone extract. *Tribulus terrestris* contained maximum saponins in methanol extract 21.76 mg/g (5.12%). Non significant difference of mean saponins was observed in ethanol extract 20.7 mg/g and in acetone extract 19.33 mg/g (6.62%). In *Launaea nudicaulis* maximum concentration of saponins was observed in ethanol extract 17.6 mg/g and minimum was observed in acetone extract 9.8 mg/g (44.32%) it has been observed *Launaea nudicaulis* has 14.6 mg/g (17.04%) concentration of saponins in methanol extract. It was observed that *Citrullus colocynthis* has 20 mg/g of saponins in ethanol extract and 14.7 mg/g (26.5%) in methanol extract and minimum concentration was observed in acetone extract 11.4 mg/g (43%). Significant difference of saponins has been examined in *Euphorbia prostrata* among ethanol extract 17.66 mg/g, in methanol extract 13.66 mg/g (22.65%) and in acetone extract 9.66 mg/g (45.30%). It was observed that the maximum mean saponins was observed in *Tribulus longipetalus* 22.11 mg/g and minimum was observed 9.78 mg/g in *Solanum surattense*. Methanol extract had highest mean of saponins quantity.

## Results and Discussion

Phytochemicals extraction depends on the ability of each compound to get dissolved along with their

dispersion in the external solvents (Shi *et al.*, 2005). In addition to these aspects one other important thing to take in consideration is the nature of extraction solvent employed in this work. Selection of extraction solvent depends on different variables *i.e.* solvent being used must be safe and have potential to extract undesirable molecules, also dissolvability of the objective compound (Tiwari *et al.*, 2013; Seidel, 2012). The plants of desert area face oxidative stress and in response synthesize non-enzymatic antioxidants for defense mechanism. These antioxidants show variability in their solubility in different solvents. The present work has the importance of determining the extraction potential of these solvents as explained earlier (Sarker and Nahar, 2012; Benzie and Wachtel-Galor, 2011).

Herbs and domestic plants contain diverse phytochemicals. These phytochemicals in the domestic plants have strong defensive impact (Gurib-Fakim, 2006). Distinctive phytochemicals are being found to have an extensive variety of uses, which might help in providing protection against infections.

The extraction of phytochemicals depends on the dissolution of each compound in the plant material and their dispersion in the external solvents (Shi *et al.*, 2005). Along these lines, the decision of extraction solvent is a standout amongst the most important matters to consider for extraction. The variables that should be considered while picking the solvents or solvents for extraction of phytochemicals are safety of the solvents and potential for arrangement or extraction of undesirable molecules lastly dissolvability of the objective compound (Tiwari *et al.*, 2013; Seidel, 2012). Herbs and domestic plants contain diverse phytochemicals. A great part of the defensive impact of domestic

**Table 6.** Quantitative assessment of saponins content in shoot extracts from selected herbs of the Cholistan desert using solvent-based extraction

Plants name	Ethanol extract mg/g	Methanol extract mg/g	%age	Acetone extract mg/g	%age	Mean mg/g
<i>Launaea nudicaulis</i>	17.6±0.2	14.6±0.3	17.04	9.8±0.3	44.32	14±3.42 <sup>d</sup>
<i>Citrullus colocynthis</i>	20±0.3	14.7±0.2	26.5	11.4±0.3	43	15.37±3.76 <sup>c</sup>
<i>Solanum surattense</i>	15.75±0.3	7.69±0.2	51.17	5.9±0.3	62.53	9.78±4.55 <sup>f</sup>
<i>Tribulus longipetalus</i>	23.7±0.3	24.3±0.3	-2.53	18.35±0.3	22.57	22.11±2.85 <sup>a</sup>
<i>Tribulus terrestris</i>	20.7±0.3	21.76±0.3	-5.12	19.33±0.3	6.62	20.60±1.09 <sup>b</sup>
<i>Euphorbia prostrata</i>	17.66±0.3	13.66±0.3	22.65	9.66±0.3	45.30	13.66±3.47 <sup>e</sup>
<b>Mean</b>	19.25±2.67 <sup>c</sup>	16.12±5.64 <sup>a</sup>	-30	12.41±4.99 <sup>b</sup>	-0.08	15.92±5.33

Mean ± standard deviation; letters in the column and row show differences; n = 3; % = %age difference from ethanol; - = sign show high value than ethanol.

plants has been ascribed by phytochemicals, which are the non-supplement mixtures (Gurib-Fakim, 2006). Distinctive phytochemicals have been found to have an extensive variety of uses, which might help in assurance against infections.

Our findings are in agreements with past research of Chatha *et al.* (2006) who reported that great extract yield (g/100 g) from rice grain was acquired with methanol. The difference in the extract yields from the tested plant materials in the present study may be attributed to the diverse accessibility of extractable components and may considered to be because of the various structure of plants (Hsu *et al.*, 2006). The measure of the antioxidant components that can be isolated from a plant material is mostly influenced by the force of the extraction method, which might vary from sample to sample. Amongst other contributing elements, productivity of the extracted solvents to dissolve endogenous mixes may likewise be important (Sultana *et al.*, 2007; Siddhuraju and Becker, 2003).

## Conclusion

The examination of trees and herbs in Pakistan's Cholistan desert, using ethanol, methanol and acetone as extraction solvents for non-enzymatic antioxidants, demonstrated distinct efficiencies based on the solvents used. Ethanol displayed the highest extraction potential, followed by methanol and acetone. Alkaloid levels varied across trees and herbs, notably higher in *Acacia jacquemontii* and *Citrullus colocynthis*. Methanol was most effective in extracting flavonoids, showing significant levels in *Acacia jacquemontii* and *Solanum surattense*. Additionally, methanol emerged as the best solvent for saponin extraction, highlighting *Acacia jacquemontii* and *Tribulus longipetalus* as rich sources. These findings emphasize the critical role of solvent selection in extracting antioxidants from Cholistan desert flora, promising opportunities for pharmaceutical and nutritional applications.

**Conflict of Interest.** The authors declare that they have no conflict of interest.

## References

- Ahmad, K., Khan, Z.I., Ashraf, M., Hussain, M.I., Aleem, E.H. 2008. Status of plant diversity at Kufri (Soone valley) Punjab, Pakistan and prevailing threats there in. *Pakistan Journal of Botany*, **403**: 993-997.
- Ahmed, M., Khan, K.R., Ahmad, S., Aati, H.Y., Sherif, A.E., Ashkan, M.F., Alrahimi, J., Motwali, E.A., Tousif, M.I., Khan, M.A., Hussain, M., Umair, M., Ghallo, B.A., Korma, S.A. 2022. Phytochemical, antioxidant, enzyme inhibitory, thrombolytic, anti-bacterial, antiviral and in silico studies of *Acacia jacquemontii* leaves. *Arabian Journal of Chemistry*, **15**: 104345
- Akhter, R., Arshad, M. 2006. Arid rangelands in the Cholistan desert (Pakistan). *Secheresse*, **17**: 210-217.
- Ali, I., Chaudhry, M.S., Farooq, U. 2009. Camel rearing in Cholistan desert of Pakistan. *Pakistan Veterinary Journal*, **29**: 85-92.
- Arshad, M., Ashraf, M.Y., Ahmad, M., Zaman, F. 2007. Morpho genetic variability potential of *Cenchrus ciliaris* L. from Cholistan desert, Pakistan. *Pakistan Journal of Botany*, **39**: 1481-1488.
- Arshad, M., Ashraf, M., Arif, N. 2006. Morphological variability of (*Prosopis cineraria* L.) Druce, from the Cholistan desert, Pakistan. *Genetic Resources and Crop Evolution*, **53**: 1589-1596.
- Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Orhan, I.E., Banach, M. 2021. Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*, **20**: 200-216.
- Benzie, I.F., Wachtel-Galor, S. 2011. *Herbal Medicine: Biomolecular and Clinical Aspects*, 2<sup>nd</sup> edition, pp. 500, CRC Press, Boca Raton, Florida, USA.
- Chatha, S.A.S., Anwar, F., Manzoor, M., Bajwa, J.R. 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. *Grasas Aceites Sevilla*, **57**: 328-335.
- Duncan, D.B. 1955. Multiple range and multiple *F*-test. *Biometrics*, **11**: 1-42.
- Gedlu, A.M. 2022. Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre*, **46**: 1-22. <https://doi.org/10.1186/s42269-022-00770-8>
- Gurib-Fakim, A. 2006. Medicinal plants: tradition of yesterday and drugs of tomorrow, review article. *Molecular Aspects of Medicines*, **27**: 1-93.
- Hameed, M., Ashraf, M., Al-Quriany, F. Nawaz, T., Ahmad, M.S.A., Younis, A., Naz, N. 2011. Medicinal flora of the Cholistan desert: a review. *Pakistan Journal of Botany*, **43**: 39-50.
- Hsu, B., Coupar, I.M., Nag, K. 2006. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. *Food Chemistry*, **98**: 317-328.

- Khan, F.M. 2009. Ethno-veterinary medicinal usage of flora of greater Cholistan desert (Pakistan). *Pakistan Veterinary Journal*, **29**: 75-80.
- Khanal, L.N., Sharma, K.R., Pokharel, Y.R., Kalauni, S.K. 2022. Phytochemical analysis and *in vitro* antioxidant and antibacterial activity of different solvent extracts of *Beilschmiedia roxburghiana* Nees stem barks. *The Scientific World Journal*, **2022**: 1-7.
- Sarker, S.D., Nahar, L. 2012. An introduction to natural products isolation. *Methods Molecular Biology*, **864**: 1-25.
- Seidel, V. 2012. Initial and bulk extraction of natural products isolation. *Methods in Molecular Biology*, **20**: 27-41.
- Shafi, M.S., Ashraf, M.Y., Sarwar, G. 2001. Wild medicinal plants of Cholistan area of Pakistan. *Pakistan Journal of Biological Science*, **4**: 112-116.
- Shi, J.H., Nawaz, J., Pohorly, G., Mittal, Y., Kakuda, Y., Jiang. 2005. Extraction of polyphenolics from plant material for functional foods engineering and technology. *Food Review International*, **21**: 139-166.
- Siddhuraju, P., Becker, K. 2003. Antioxidant properties of various extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agriculture and Food Chemistry*, **51**: 2144-2155.
- Sultana, B., Anwar, F., Przybylski, R. 2007. Antioxidant activity of phenolic components present in barks of barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* Lam. trees. *Food Chemistry*, **104**: 1106-1114.
- Swain, T. 1979. *Tannins and Lignins*. In *Herbivores, Their Interaction with Secondary Plant Metabolites*. (eds.), Rosenthal, G., Janzen, D.H., pp. 657-682, Academic Press, New York, USA.
- Tiwari, B.K., Brunton, N.P., Brennan, C. 2013. *Hand Book of Plant Food Phytochemicals Sources, Stability and Extraction*, E-Book, pp. 28, Publisher Wiley-Blackwell, UK.
- Verma, N., Jha, K., Chaudhary, S., Singh, O., Kumar, A. 2014. Phytochemistry, pharmacology and traditional uses of *Leptadenia pyrotechnica* - an important medicinal plant. *Indian Journal of Pharmaceutical and Biological Research*, **2**: 128-134.