

## PHYTOCHEMICAL AND NUTRITIONAL PROFILING OF *VACHELLIA NILOTICA*, *CAPPARIS DECIDUA*, AND *CALLIGONUM POLYGONOIDES* FROM THE CHOLISTAN DESERT SUPPORT THEIR ETHNOMEDICINAL USES

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### Abstract

Medicinal plants are undoubtedly viable candidates for evaluation, leading to the characterization and isolation of newer, and safer lead compounds. This study aimed to evaluate the metabolite fingerprinting and profiling of *Vachellia nilotica*, *Capparis decidua*, and *Calligonum polygonoides* found in the Cholistan desert. The proximate nutritional analysis of dried, powdered plant materials was performed using approved analytical techniques. The quantity of minerals was measured by atomic absorption spectrometry using a double-beam atomic absorption spectrophotometer, Perkin-Elmer Model 2380. Phenolics and flavonoids were determined by high-performance liquid chromatography (HPLC). Nuclear Magnetic Resonance (NMR) analysis was performed to obtain a <sup>1</sup>H NMR spectrum using a 500 MHz Bruker Avance-500 spectrometer. The results of the present study confirmed that there was a significant difference ( $p < 0.05$ ) in the phytochemical composition among selected species. *C. polygonoides* had exceptionally high magnesium and flavonoid glycosides, with fiber content of 24.5 g/100g. *V. nilotica* was richest in protein, ash, iron, zinc, copper, and flavonoid glycosides whereas. *C. decidua* had the highest fat, carbohydrate, sodium, potassium, and a complex mixture of condensed tannins. Each species showed a distinct phytochemical profile, and these profiles could be explored to isolate phytoconstituents with potential for synthesizing medicines to treat various diseases. The findings provided scientific evidence supporting the traditional use of these plants for treating various ailments, with each species showing unique phytochemical profiles suitable for targeted pharmaceutical applications. Future research should include: (1) bioassay-guided fractionation to isolate and purify individual bioactive compounds responsible for specific therapeutic activities; (2) comprehensive evaluation of seasonal and geographical variations in phytochemical composition to identify optimal harvesting periods and locations; (3) *In vitro* and *In vivo* pharmacological studies to validate the therapeutic potential and establish dose-response relationships.

**Key words:** Medicinal plants; Phytochemical profiling; Metabolite fingerprinting; Scientific validation; Cholistan desert

### Introduction

Plants have been utilized for ages to heal various diseases and disorders, dating back to the dawn of human civilization (Hussain *et al.*, 2018). Plant resources, in addition to providing critical survival supplies and medicinal and forage advantages, help to conserve cultural history and indigenous knowledge (Hameed *et al.*, 2011). Plants serve as a valuable source of novel natural products having medicinal properties (Newman and Cragg, 2020; Tallini *et al.*, 2022). Despite the availability of various research approaches, plants continue to be one of the most promising reservoirs for discovering new phytochemicals to use in making modern drugs (Khalid *et al.*, 2022). Traditional medicine practices play a significant and often underestimated role in healthcare worldwide (Liu, 2021; Haque *et al.*, 2018).

Phytochemical analysis of medicinal plants is fundamental for drug discovery, as it enables the identification, quantification, and characterization of bioactive compounds responsible for therapeutic effects. The major classes of phytochemicals analyzed in this study include flavonoids (such as quercetin), phenolic acids (including gallic acid, ferulic acid, cinnamic acid, and caffeic acid), and minerals (iron, zinc, copper, magnesium), which play crucial roles in human health. Flavonoids exhibit potent antioxidant,

anti-inflammatory, and anti-diabetic properties (Maurya, 2022), while phenolic acids demonstrate antimicrobial, anti-inflammatory, and UV-protective activities (Alam *et al.*, 2016; Pavlikova, 2022). Minerals are essential for various physiological functions, including oxygen transport (iron), immune function (zinc), and enzyme catalysis (copper). Advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) allow precise quantification of individual phenolic compounds, while Nuclear Magnetic Resonance (NMR) spectroscopy provides comprehensive metabolic fingerprints that reveal the overall phytochemical complexity of plant extracts. Understanding the chemical composition of medicinal plants is necessary for: (1) validating traditional medicinal uses through scientific evidence; (2) identifying lead compounds for drug development; (3) establishing quality control parameters for herbal formulations; and (4) discovering novel bioactive molecules with therapeutic potential. The integration of multiple analytical platforms provides a holistic understanding of phytochemical profiles, enabling correlation between chemical composition and biological activities.

The Cholistan desert in Pakistan is a wealth of medicinal plants, and many of them are used by locals as traditional treatments (Ali *et al.*, 2023; Azhar, 2014). *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb.,

*Capparis decidua* (Forsk.) Edgew. and *Calligonum polygonoides* L. are the three important medicinal plants used by the local people (Ali *et al.*, 2023). These plants are used for the treatment of antibacterial, anti-inflammatory, antioxidant, and cancer properties (Dogara, 2024; Hafez *et al.*, 2024; Mohil, 2013), ulcers, male sexual dysfunction, diarrhea, diabetes, hypertension, intermittent fever, obesity, bone fractures, uncomfortable menstruation (Ali *et al.*, 2023; Azhar *et al.*, 2017), typhoid, asthma, cough, and cold (Bewal *et al.*, 2008). Many medically important phytochemicals, such as flavonoids, quinones, saponins, sterols, phenols, fatty acids, terpenes, and triterpenoids, have been isolated and identified from the plants of the Cholistan desert. Although climatic parameters vary widely, and due to an unhealthy environment, and a lack of adequate medical services in the underdeveloped desert regions, some of the local population may suffer from infectious diseases. Plants are the most common natural products in the Cholistan desert, and are likely to be considered potential candidates for evaluation, which leads to new safe lead compounds being characterized and isolated (Ali *et al.*, 2023).

The ethnobotanical surveys have documented the traditional uses of *V. nilotica*, *C. decidua* and *C. polygonoides* (Ali *et al.*, 2023; Azhar, 2014), comprehensive chemical profiling using modern analytical techniques has not been performed for these species from the Cholistan desert. Specifically, no comparative study has simultaneously evaluated the nutritional composition, mineral content, phenolic profiles via HPLC, and metabolic fingerprints via <sup>1</sup>H-NMR for these three species from this unique ecosystem. These three species were specifically selected for chemical profiling based on: (1) their high ethnobotanical importance scores from our previous survey (Ali *et al.*, 2023), indicating frequent use by local communities for treating various ailments; (2) their diverse traditional medicinal applications including antibacterial, anti-inflammatory, antioxidant, anti-diabetic, and anti-cancer properties (Dogara, 2024; Hafez *et al.*, 2024; Mohil, 2013); and (3) the lack of comprehensive chemical characterization studies for these species from the Cholistan

desert ecosystem, despite their ecological and medicinal significance. Because of their adaptability to various environmental pressures, such as salt, temperature, and drought, the medicinal plants of the Cholistan desert have a wide variety of compounds. As the most common natural product in the Cholistan desert, plants are undoubtedly considered viable candidates for evaluation, which leads to the characterization and isolation of newer and safer lead compounds. This is the first study to evaluate the metabolic fingerprints and chemical profiles of *V. nilotica*, *C. decidua*, and *C. polygonoides* found in the Cholistan desert. The basis for this claim is supported by a comprehensive literature search (conducted through Web of Science, Scopus, and PubMed) which revealed that: (a) no prior study has simultaneously evaluated nutritional composition, mineral content, phenolic profiles via HPLC, and metabolic fingerprints via <sup>1</sup>H-NMR for these three species from the Cholistan desert ecosystem; (b) previous studies on these species have been limited to either isolated individual species (Berwal *et al.*, 2021 for *C. polygonoides*; Nazar *et al.*, 2020 for *C. decidua*; Dogara *et al.*, 2024 for *V. nilotica*), single analytical techniques (Alrasheid *et al.*, 2018), and (c) no comparative study integrating multiple analytical platforms across these three species from the same collection site has been published to date. The comparative integration of multiple analytical techniques across these three species from the same ecosystem represents the primary novelty of this work. The specific objectives of this study were: (1) to evaluate the nutritional and mineral composition of the three selected medicinal plants; (2) to quantify and compare phenolic and flavonoid compounds using HPLC; (3) to generate metabolic fingerprints using <sup>1</sup>H-NMR spectroscopy; and (4) to correlate the chemical profiles with traditional medicinal uses.

## Materials and Methods

**Collection of plant samples:** Based on our previous ethnobotanical survey (Ali *et al.*, 2023), three important medicinal plants, i.e., *V. nilotica*, *C. decidua*, and *C. polygonoides*, were selected for chemical profiling (Fig. 1).



Fig. 1. (a) *Calligonum polygonoides*, (b) *Capparis decidua*, (c) *Vachellia nilotica*.

Samples were collected during the flowering/fruiting season (March-April) from the Cholistan desert, Pakistan (coordinates: 28.42°N, 70.13°E). The collection sites were characterized by arid climatic conditions with temperatures ranging from 25–42°C, annual rainfall of approximately 120 mm, and sandy-loam soils with high salinity. For each

species, 15–20 individual plants were sampled, and plant parts were pooled to ensure a representative sample. For *V. nilotica*, leaves and young pods were collected; for *C. decidua*, fruits and aerial parts were collected; and for *C. polygonoides*, aerial parts, including stems and leaves, were collected. All collections were made during the morning

hours (8:00-11:00 AM) to minimize diurnal variation in secondary metabolite content. Collected plant materials were immediately transported to the laboratory in clean polythene bags. All the plant parts were thoroughly washed with distilled water to remove dust, soil particles, and other contaminants, followed by surface drying with a sterile cotton cloth. The cleaned plant materials were then shade-dried at 25-30°C for 7-10 days with periodic turning to prevent fungal growth. The dried plant parts were ground into a fine powder using a West Point coffee grinder (model WF 9222) operated at medium speed for 3 minutes. The resulting powder was sieved using a 0.25 mm sieve to obtain uniform particle size and stored in opaque, airtight containers with screw caps at 4°C until further analysis. Voucher specimens were deposited at the Herbarium of the Department of Forestry and Range Management, Bahauddin Zakariya University, Multan, Pakistan (Table 1) (Voucher

numbers: FRW-2025-31 for *V. nilotica*, FRW-2025-32 for *C. decidua*, and FRW-2025-33 for *C. polygonoides*).

**Preparation of plant extracts:** The ground plant samples were soaked in methanol for 15 days. Afterward, the soluble fractions in methanol were filtered, and the filtrate was concentrated using a rotary evaporator at 40°C, resulting in a crude extract weighing 150 g. This crude extract was then dissolved in 150 mL of methanol. The concentrated methanolic extract was transferred to sample vials and stored at a temperature of -4°C for subsequent analysis.

**Proximate analysis:** The proximate nutritional analysis of dried, powdered plant materials was performed using approved techniques of analysis (Anon., 2006) of selected WEPs. The used formulas are:

1. Moisture content (%) =  $\frac{\text{Fresh sample weight (g)} - \text{Dried sample weight (g)}}{\text{Fresh sample weight (g)}} \times 100$
2. Crude fat (%) =  $\frac{\text{Dried sample weight (g)} - \text{Dried and defatted sample weight (g)}}{\text{Dried sample weight (g)}} \times 100$
3. Crude fiber (%) =  $\frac{\text{Dried sample weight after digestion } W_1 \text{ (g)} - \text{Ash weight } W_2 \text{ (g)}}{\text{Dried sample and defatted sample (g)}} \times 100$
4. Nitrogen (%) =  $\frac{\text{Used volume of H}_2\text{SO}_4 \times \text{Dilution volume (250ml)} \times 0.0014}{\text{Sample weight} \times \text{Diluted sample volume}}$
- Crude protein (%) = Nitrogen (%)  $\times 6.25$
5. Total ash (%) =  $\frac{\text{Weight of white ash grey residues after ignition (g)}}{\text{Initial weight of dried sample}} \times 100$
6. Carbohydrate (%) =  $(100 - \% (\text{Moisture} + \text{Crude fat} + \text{Crude Fiber} + \text{Crude protein} + \text{Ash}))$

**Secondary metabolites:** The quantities of Na, K, Mg, Fe, Zn, Cu, Ca, and Mn of plant samples were measured by atomic absorption spectrometry using a double-beam atomic absorption spectrophotometer, PerkinElmer Model 2380, following Anwar *et al.*, (2022) and Tafere & Teshager (2022).

**Total phenolic content:** The total phenolic content of the methanolic extract was determined using the Folin-Ciocalteu approach, following Jagadish *et al.*, (2009). For every single extract, 1 mL was mixed with 7.5 mL of double-distilled deionized water, 500 µL of Folin-Ciocalteu reagent, and 1 mL of 5% Na<sub>2</sub>CO<sub>3</sub>. The resultant liquid underwent incubation at the ambient temperature for a period of 90 min. before it was measured at 760 nm with an ultraviolet (UV)-visible spectrophotometer (Perkin Elmer Lambda EZ 201, USA). The quantity of phenolic substances was calculated as µg gallic acid equivalents (GAEs) per mg extract.

**Analysis of phenolic compounds:** The process of hydrolysis of extracts from selected medicinal plants followed the procedure outlined by Pak-Dek *et al.*, (2011, 50 mg of extract was dissolved in 24 mL of methanol and mixed. The resulting solution was subsequently diluted with 16 mL of distilled water and

10 mL of 6M HCl and heated to 95°C for two hours. The resulting solution was filtered using a 0.45 µm nylon membrane filter (Biotech, Germany) and evaluated via HPLC. The chromatographic separation was carried out using a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of (A) 0.1% formic acid in water and (B) methanol, with the following gradient program: 0-5 min (10% B), 5-25 min (10-50% B), 25-35 min (50-80% B), 35-40 min (80% B), 40-42 min (80-10% B), 42-50 min (10% B). The flow rate was 1.0 mL/min, and the injection volume was 20 µL. Phenolic compounds were detected using a UV-visible detector at 280 nm. Peaks were identified by comparing retention periods and UV-visible spectra to known standards. The chemicals were quantified using external calibration.

**NMR analysis:** *V. nilotica*, *C. decidua*, and *C. polygonoides* were selected for metabolite profiling. The plant material was extracted on the basis of the nature of their phytochemical constituents. The samples were dissolved in deuterated methanol (CD<sub>3</sub>OD). After the preparation of the extracts, the NMR analysis was carried out to obtain the <sup>1</sup>H-NMR spectrum, as described by Sahoo & Umashankar (2023). The NMR spectrum was recorded using a 500 MHz Bruker Avance-500 spectrometer.

## Statistical analysis

The trials were conducted in triplicate, and the findings were reported as mean  $\pm$  standard deviation (SD). Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software version 26.0 and RStudio. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to compare means among species. Principal Component Analysis (PCA) was performed to visualize species-specific clustering patterns. P-values  $<0.05$  were deemed statistically significant.

## Results

**Nutritional composition of selected species:** The nutritional composition showed significant differences among species (ANOVA,  $F(2,6) = 12.4-45.8$ ,  $p < 0.05$  for all parameters), with post hoc Tukey's HSD test revealing species-specific variations. *V. nilotica* had the highest moisture content (21.38%), while *C. decidua* had the lowest moisture content (5.12%). *V. nilotica* shows the highest variability ( $\pm 1.69\%$ ). In *C. decidua* has the highest fat content (4.57 g/100g) was present while *C. polygonoides* had the lowest (2.56 g/100g). *C. decidua* shows the highest variability ( $\pm 0.46$ ) as compared to the other two species. *V. nilotica* was significantly richer in protein (25.28 g/100g) while *C. polygonoides* has the lowest protein content (8.6 g/100g) ( $p < 0.001$ ). *V. nilotica* exhibited the highest variability ( $\pm 2.0$ ). *C. polygonoides* had exceptionally high fiber (24.5 g/100g) while *C. decidua* had very low fiber (6.19 g/100g) ( $p < 0.001$ ). *C. polygonoides* shows substantial variability ( $\pm 4.5$ ). *C. decidua* had the highest carbohydrate content (60.83 g/100g) while *C. polygonoides* has the lowest (32.6 g/100g) ( $p < 0.01$ ). was present in *C. polygonoides* shows moderate variability ( $\pm 3.4$ ) as compared to the other two species. *V. nilotica* has the highest ash content (13.08 g/100g) while *C. decidua* had the lowest (6.53 g/100g) ( $p < 0.05$ ). *V. nilotica* showed the highest variability ( $\pm 1.02$ ) (Fig. 2).

**Mineral composition of selected species:** Mineral composition showed significant species-specific differences (ANOVA,  $F(2,6) = 15.7-98.3$ ,  $p < 0.05$  for all minerals). *C. decidua* had extremely high sodium (180.2 mg/100g) while it was negligible in *V. nilotica* (0.04 mg/100g) ( $p < 0.001$ ). *C. decidua* had the highest potassium (236.7 mg/100g), while *V. nilotica* had very low potassium (0.56 mg/100g) ( $p < 0.001$ ). *C. decidua* showed high variability ( $\pm 7.6$ ). *C. polygonoides* had very high magnesium (63.2 mg/100g) while *C. decidua* and *V. nilotica* have had very low magnesium ( $< 0.5$  mg/100g) ( $p < 0.001$ ). *V. nilotica* had exceptionally high iron (203.1 mg/100g) while it was lowest in *C. polygonoides* (20.5 mg/100g) ( $p < 0.001$ ). *V. nilotica* showed high variability ( $\pm 12.2$ ). *V. nilotica* had very high zinc (108.7 mg/100g) while *C. decidua* had negligible zinc (0.3 mg/100g) ( $p < 0.001$ ). *V. nilotica* showed moderate variability ( $\pm 6.52$ ). Extremely high copper was present in *V. nilotica* (322.7 mg/100g) while *C. polygonoides* has low copper (3.1 mg/100g) ( $p < 0.001$ ) (Fig. 3). These results show the significant differences in mineral composition among species, which may have an impact on their potential applications in nutrition, medicine, and other applications.

## Phenolic and flavonoid composition of selected plants:

HPLC analysis was employed for the quantitative and qualitative determination of flavonoids and phenolic compounds in all the three species: *C. polygonoides*, *C. decidua*, and *V. nilotica*. The twelve flavonoids and phenolic compounds analyzed were: Quercetin, Gallic acid, Caffeic acid, Vanillic acid, Benzoic acid, Chlorogenic acid, Syringic acid, p-Coumaric acid, M-Coumaric acid, Ferulic acid, Cinnamic acid, and Sinapinic acid (Table 1). The concentrations of different Flavonoid (Phenol) and phenolic acid were found to be significantly different (ANOVA,  $F(2,6) = 34.2-187.6$ ,  $p < 0.001$  for all compounds) among the three 1 plants. Post-hoc Tukey's HSD test revealed significant pairwise differences ( $p < 0.05$ ) between all species for most compounds, except for caffeic acid between *C. polygonoides* and *C. decidua* ( $p > 0.05$ ). The concentration of quercetin was highest in *C. polygonoides* ( $264.51 \pm 0.64$  mg/g), while *C. decidua* ( $41.94 \pm 0.22$  mg/g) and *V. nilotica* ( $47.16 \pm 0.38$  mg/g) showed substantially lower concentrations. *C. polygonoides* contained the highest concentration of gallic acid ( $58.81 \pm 0.36$  mg/g), followed by *C. decidua* ( $21.59 \pm 0.13$  mg/g), with *V. nilotica* showing the lowest concentration ( $5.43 \pm 0.06$  mg/g). *V. nilotica* showed the highest concentration of caffeic acid ( $5.94 \pm 0.05$  mg/g), while *C. polygonoides* exhibited moderate levels ( $2.21 \pm 0.08$  mg/g). Notably, *C. decidua* completely lacked caffeic acid ( $0.00 \pm 0.00$  mg/g). *C. decidua* contained the highest concentration of vanillic acid ( $13.01 \pm 0.08$  mg/g), followed by *C. polygonoides* ( $5.19 \pm 0.07$  mg/g). *V. nilotica* showed no detectable vanillic acid ( $0.00 \pm 0.00$  mg/g). *C. decidua* showed the highest concentration of benzoic acid ( $18.21 \pm 0.09$  mg/g), followed by *V. nilotica* ( $7.88 \pm 0.05$  mg/g), with *C. polygonoides* containing the lowest concentration ( $4.53 \pm 0.07$  mg/g). *C. decidua* had the highest concentration of chlorogenic acid ( $22.09 \pm 0.13$  mg/g), followed by *C. polygonoides* ( $10.16 \pm 0.05$  mg/g). *V. nilotica* showed no detectable chlorogenic acid ( $0.00 \pm 0.00$  mg/g).

*C. decidua* was the only species having syringic acid ( $7.74 \pm 0.03$  mg/g), while both *C. polygonoides* ( $0.00 \pm 0.00$  mg/g) and *V. nilotica* ( $0.00 \pm 0.00$  mg/g) showed no detectable levels. *C. decidua* showed an exceptionally high concentration of p-coumaric acid ( $66.59 \pm 0.12$  mg/g), while *C. polygonoides* contained minimal amounts ( $0.46 \pm 0.04$  mg/g). *V. nilotica* showed no detectable p-coumaric acid ( $0.00 \pm 0.00$  mg/g). *C. decidua* had the highest concentration of m-coumaric acid ( $9.01 \pm 0.07$  mg/g), followed by *V. nilotica* ( $4.63 \pm 0.04$  mg/g), with *C. polygonoides* showing the lowest concentration ( $0.40 \pm 0.15$  mg/g). *V. nilotica* exhibited the highest concentration of ferulic acid ( $33.53 \pm 0.35$  mg/g), while *C. polygonoides* contained moderate amounts ( $1.35 \pm 0.06$  mg/g). Notably, *C. decidua* completely lacked ferulic acid ( $0.00 \pm 0.00$  mg/g). *V. nilotica* contained the highest concentration of cinnamic acid ( $23.44 \pm 0.06$  mg/g), followed by *C. decidua* ( $7.37 \pm 0.32$  mg/g), with *C. polygonoides* showing the lowest concentration ( $0.53 \pm 0.09$  mg/g). *V. nilotica* had the highest concentration of Sinapinic acid ( $6.78 \pm 0.04$  mg/g), followed by *C. polygonoides* ( $0.87 \pm 0.05$  mg/g). *C. decidua* showed no detectable Sinapinic acid ( $0.00 \pm 0.00$  mg/g). *C. polygonoides* depicted strong antioxidant potential due to the presence of high concentrations of quercetin and gallic acid. *C. decidua* had a broad spectrum of antimicrobial activity due to the presence of a variety of phenolic acids, whereas *V. nilotica* was highly concentrated in Ferulic acid and cinnamic acid and showed anti-inflammatory and UV protection potential.

**Table 1. Flavanoids and Phenolic acids found in *Calligonum polygonoides*, *Capparis decidua*, and *Vachellia nilotica* of Cholistan Rangeland (mg/g).**

| Chemical compounds | <i>Calligonum polygonoides</i> | <i>Capparis decidua</i> | <i>Vachellia nilotica</i> |
|--------------------|--------------------------------|-------------------------|---------------------------|
| Quercetin          | 264.51 ± 0.64a                 | 41.94 ± 0.22b           | 47.16 ± 0.38b             |
| Gallic acid        | 58.81 ± 0.36a                  | 21.59 ± 0.13b           | 5.43 ± 0.06c              |
| Caffeic acid       | 2.21 ± 0.08a                   | 0.00 ± 0.00b            | 5.94 ± 0.05a              |
| Vanillic acid      | 5.19 ± 0.07b                   | 13.01 ± 0.08a           | 0.00 ± 0.00c              |
| Benzoic acid       | 4.53 ± 0.07c                   | 18.21 ± 0.09a           | 7.88 ± 0.05b              |
| Chlorogenic acid   | 10.16 ± 0.05b                  | 22.09 ± 0.13a           | 0.00 ± 0.00c              |
| Syringic acid      | 0.00 ± 0.00b                   | 7.74 ± 0.03a            | 0.00 ± 0.00b              |
| p-Coumaric acid    | 0.46 ± 0.04b                   | 66.59 ± 0.12a           | 0.00 ± 0.00c              |
| m-Coumaric acid    | 0.40 ± 0.15c                   | 9.01 ± 0.07a            | 4.63 ± 0.04b              |
| Ferulic acid       | 1.35 ± 0.06b                   | 0.00 ± 0.00c            | 33.53 ± 0.35a             |
| Cinnamic acid      | 0.53 ± 0.09c                   | 7.37 ± 0.32b            | 23.44 ± 0.06a             |
| Sinapinic acid     | 0.87 ± 0.05b                   | 0.00 ± 0.00c            | 6.78 ± 0.04a              |

Note: Different alphabets (a, b, c) in the same row indicate significant differences at  $p < 0.05$  (ANOVA followed by Tukey's HSD test)

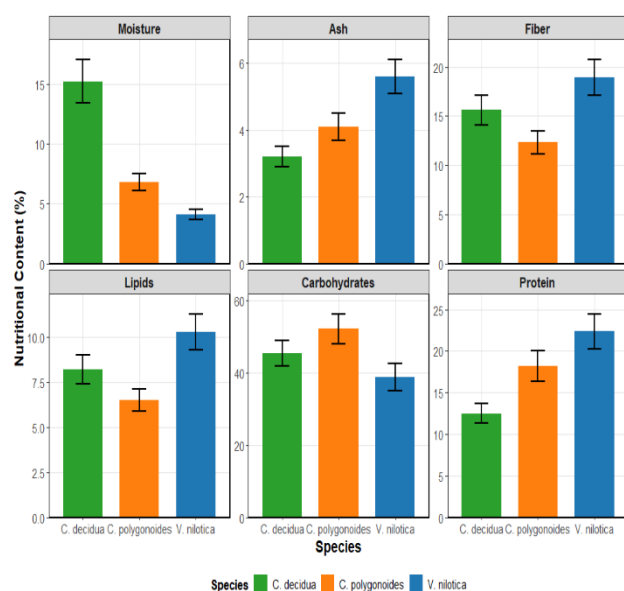


Fig. 2. Nutritional Composition of *Calligonum polygonoides*, *Capparis decidua*, and *Vachellia nilotica*.

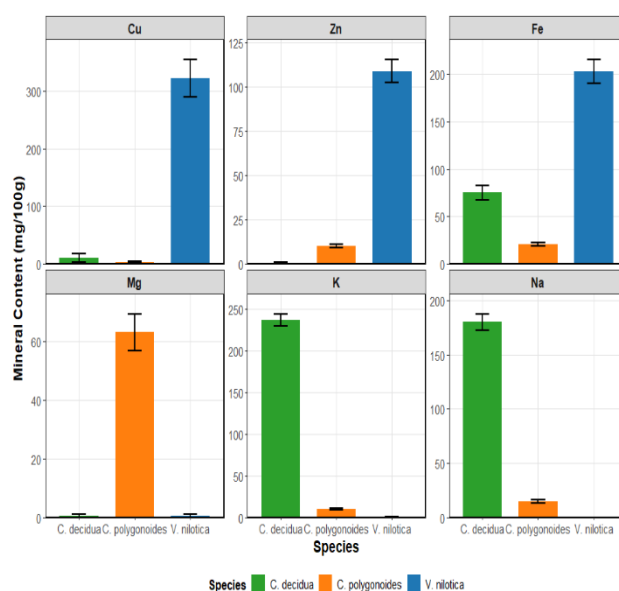


Fig. 3. Mineral composition of *Calligonum polygonoides*, *Capparis decidua*, and *Vachellia nilotica*.

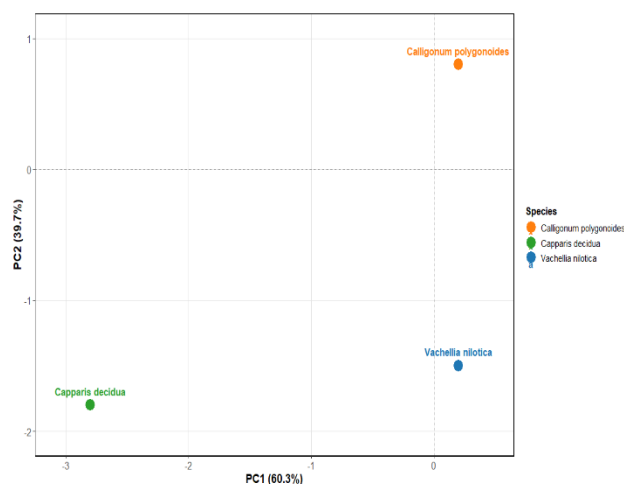


Fig. 4. Principal component analysis (PCA) comparing the nutritional, mineral, phenolic, and flavonoid profiles of *Calligonum polygonoides*, *Capparis decidua*, and *Vachellia nilotica*.

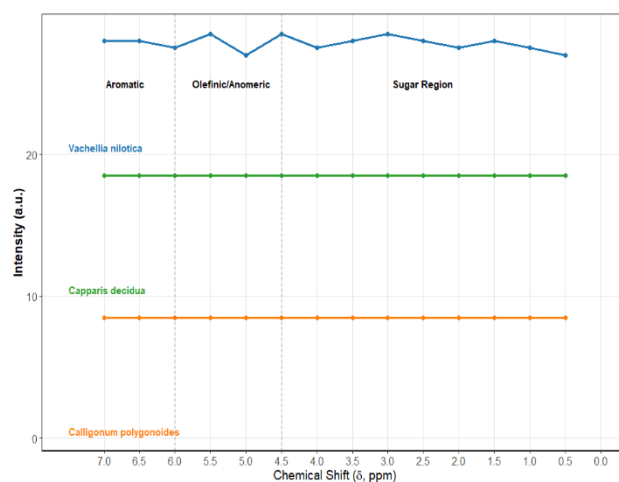
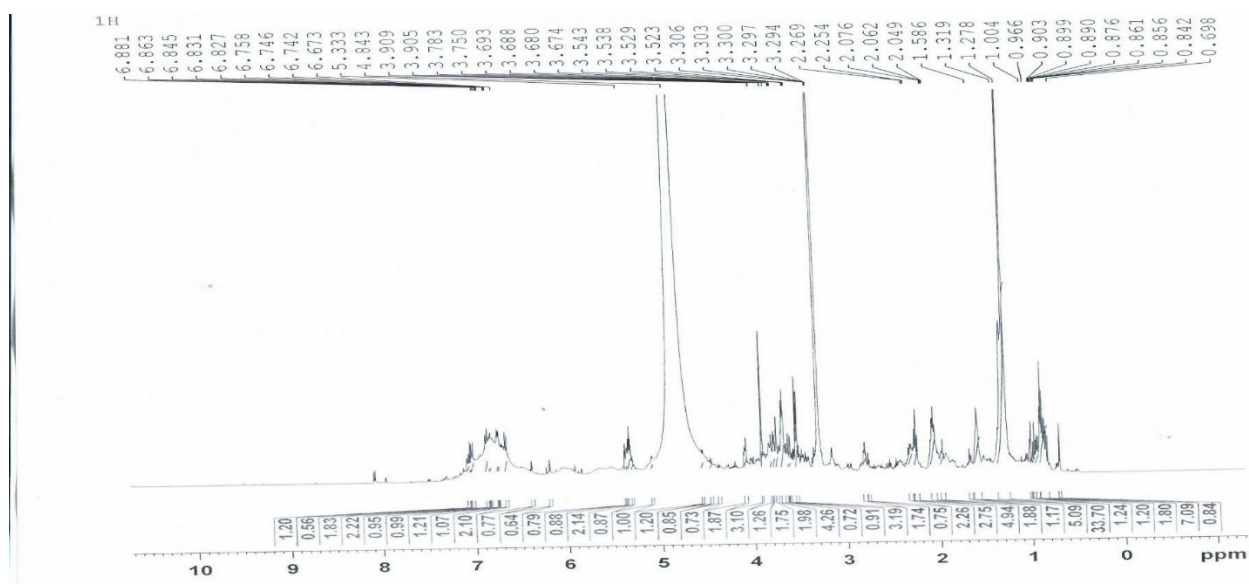
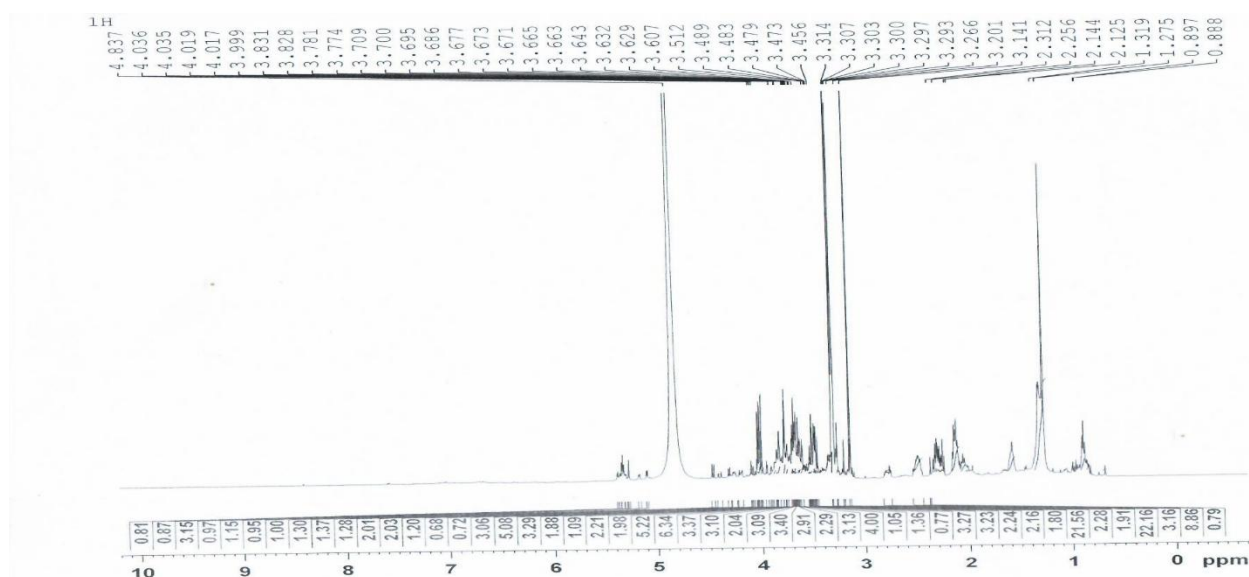
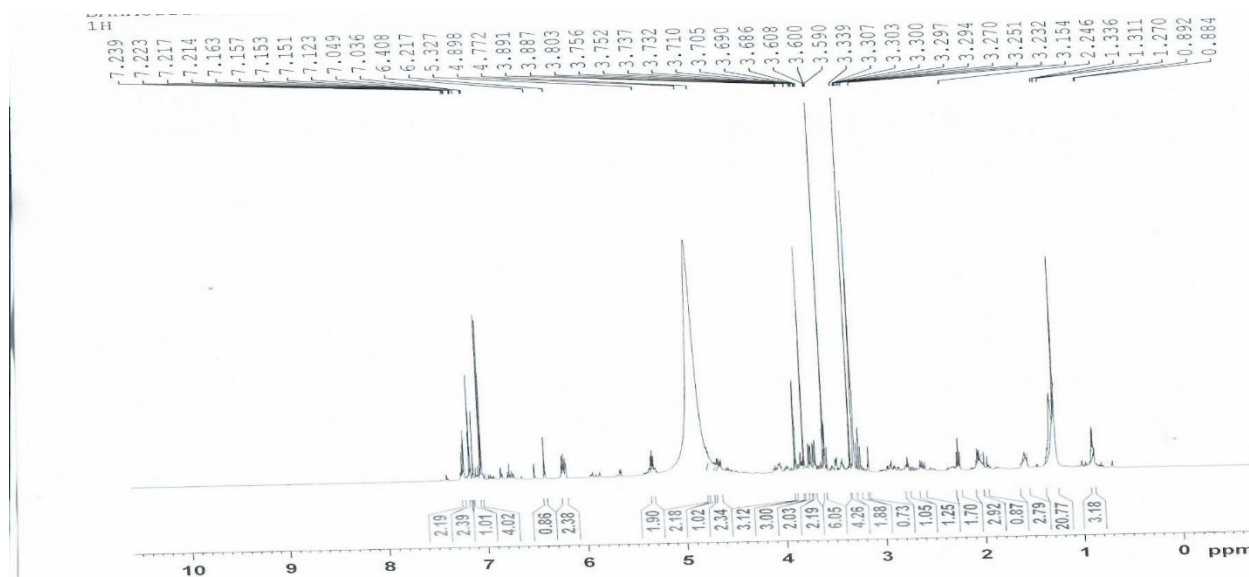


Fig. 5.  $^1\text{H}$ -nuclear magnetic resonance fingerprint of *Calligonum polygonoides*, *Capparis decidua*, and *Vachellia nilotica*.

Fig. 6. <sup>1</sup>H-nuclear magnetic resonance fingerprint of *Calligonum polygonoides*.Fig. 7. <sup>1</sup>H-nuclear magnetic resonance fingerprint of *Capparis decidua*.Fig. 8. <sup>1</sup>H-nuclear magnetic resonance fingerprint of *Vachellia nilotica*.

**PCA analysis:** Fig. 4 presents a principal component analysis (PCA) comparing the nutritional, mineral, phenolic, and flavonoid profiles of *C. polygonoides*, *C. decidua*, and *V. nilotica*. The first two principal components explain 60.3% and 39.7% of the total variance, respectively, cumulatively accounting for 100% of the variation among species. The PCA biplot clearly separates the three species into distinct clusters based on their chemical signatures. *C. polygonoides* occupies the positive PC1 region, primarily driven by its exceptionally high QE, GA, magnesium, and fiber content. *C. decidua* clusters in the negative PC1 and positive PC2 space, characterized by elevated levels of sodium, potassium, p-CA, CA, fat, and carbohydrates. *V. nilotica* positions distinctly in the negative PC2 region, distinguished by its superior protein, ash, iron, zinc, copper, FA, and CINN concentrations. The wide dispersion of variables along both axes demonstrates the substantial phytochemical diversity among these desert medicinal plants. This clear separation validates the species-specific metabolic specialization and supports their traditional therapeutic applications. The PCA findings align with the ANOVA results, confirming significant differences ( $p < 0.05$ ) in phytochemical composition across all three species.

#### **<sup>1</sup>H-NMR fingerprinting of methanolic plant extracts:**

The <sup>1</sup>H-NMR spectra of the three methanolic extracts, i.e., *C. polygonoides*, *C. decidua*, and *V. nilotica* revealed distinct metabolic profiles, indicating significant differences in their phytochemical composition (Fig. 5).

#### **<sup>1</sup>H-nuclear magnetic resonance fingerprint of *Calligonum polygonoides* methanolic extract:**

The <sup>1</sup>H-NMR (500 MHz; CD<sub>3</sub>OD) spectra of the *C. polygonoides* methanolic extracts included 6.88, 6.86, 6.84, 6.83, 6.82, 6.75, 6.74, 6.67, 5.33, 4.84, 3.90, 3.78, 3.75, 3.69, 3.68, 3.67, 3.54, 3.53, 3.52, 3.30, 3.29, 2.26, 2.25, 2.07, 2.06, 2.04, 1.58, 1.31, 1.27, 1.00, 0.96, 0.90, 0.89, 0.87, 0.86, 0.85, 0.84, 0.69 (Fig. 6). The <sup>1</sup>H-NMR fingerprint of the methanol extract of *C. polygonoides* showed a complex profile rich in polyphenol compounds. The spectrum is dominated by the characteristic signal of flavonol glycosides, as evidenced by an aromatic proton region (6.67-6.88 ppm) and an independent anomeric proton signal (4.84 ppm). The presence of sharp methoxy compounds (3.90 ppm) further suggests the presence of methylated flavonoids, such as isormentin derivatives, a well-known component of the species. In addition, signs of hydrolyzed tannins (gallotannins/ellagitannins) have been observed in the upfield aromatic region. The intense multiplet in the region 3.5-4.0 ppm confirms the high content of glycosylated metabolites. The diverse phenolic composition is consistent with *C. polygonoides* reported antioxidant and biological activity, providing the chemical basis for its ethnopharmacological use.

#### **<sup>1</sup>H-nuclear magnetic resonance fingerprint of *Capparis decidua* methanolic extract:**

The <sup>1</sup>H-NMR (500 MHz; CD<sub>3</sub>OD) spectra of the *C. decidua* methanolic extracts included 4.83, 4.03, 4.01, 3.99, 3.83, 3.82, 3.78, 3.77, 6.70, 6.69, 6.68, 6.67, 6.66, 6.65, 6.64, 6.63, 6.62, 6.61, 6.60, 3.51, 3.48, 3.47, 3.45, 3.31, 3.30, 3.29, 3.26, 3.20, 3.14, 2.31, 2.25, 2.14, 2.12, 1.31, 1.27, 0.89 and 0.88 (Fig. 7). The <sup>1</sup>H-NMR fingerprint of the methanol extract of *C. decidua* revealed a

characteristic phytochemical profile. The spectrum is dominated by dense signal clusters (6.60-6.70 ppm) in the aromatic regions, showing a complex condensed tannin mixture. Further evidence of glycoside metabolites was confirmed by a distinct anomeric proton signal at 4.83 ppm and a complex multiplet at 3.14-4.03 ppm, corresponding to the sugar component of the flavonoid glycoside (kaempferol-3-glucoside). Furthermore, sharp signals of 3.83 ppm indicated the presence of methyl phenol compounds. The signals in the high field region (0.88-2.31 ppm) are consistent with co-extracted fatty acids and sterol compounds.

#### **<sup>1</sup>H-nuclear magnetic resonance fingerprint of *Vachellia nilotica* methanolic extract:**

The <sup>1</sup>H-NMR (500 MHz; CD<sub>3</sub>OD) spectra of the *V. nilotica* methanolic extracts including 7.23, 7.22, 7.21, 7.16, 7.15, 7.12, 7.04, 7.03, 6.40, 6.21, 5.32, 4.89, 4.77, 3.89, 3.88, 3.80, 3.75, 3.73, 3.71, 3.70, 3.69, 3.68, 3.60, 3.59, 3.33, 3.30, 3.29, 3.27, 3.25, 3.23, 3.15, 2.24, 1.33, 1.31, 1.27, 0.89 and 0.88 (Fig. 8). The analysis of <sup>1</sup>H-NMR of the methanol extract of *V. nilotica* revealed a rich profile of a variety of phenolic compounds. The spectrum displayed characteristic signals for flavonoid aglycones, including a pair of meta-coupled doubles 6.21 and 6.40 ppm, which are diagnostic for A-ring protons in the flavonoid-type skeleton. Several signals in the region 7.03-7.23 ppm correspond to the protons in the B-ring of various flavonoids. The presence of anomeric protons at 4.49 and 4.77 ppm, along with complex multipliers at 3.55-3.90 ppm, confirms a significant content of flavonoids. A sharp methoxy signal at 3.89 ppm indicated the presence of methoxylated phenol derivatives.

## **Discussion**

Medicinal plants are considered to be the most valuable and useful natural resources for the development of new drugs. Different substances are well known as the characteristics of the effectiveness of the medicinal plants used to treat different diseases (Davis & Choisy, 2024; Parasuraman, 2018). In the present study, it was found that there were significant differences ( $p < 0.05$ ) in the nutritional composition, including variation in moisture content, fat, protein, fiber, carbohydrate, and ash content of studied species. The high moisture content of *V. nilotica* (21.38%) compared to *C. decidua* (5.12%) may reflect differences in tissue structure and water storage capacity, with succulent tissues retaining more water. *V. nilotica* has the highest moisture content (21.38%), while *C. decidua* has the lowest (5.12%). Alrasheid *et al.*, (2018) also found a low moisture content of 5.18% in *C. decidua*. *C. decidua* has the highest fat content (4.57 g/100g) while *C. polygonoides* has the lowest (2.56 g/100g). *V. nilotica* is significantly richer in protein (25.28 g/100g) while it is lowest in *C. polygonoides* (8.6 g/100g). Different research studies also found that Acacia species are significantly richer in protein, ranging between 11.8 and 21.6% (Abdalla *et al.*, 2014; Zapata-Campos *et al.*, 2020). The high protein content of *V. nilotica* suggests its potential as a dietary protein supplement, particularly in regions with limited protein sources. *C. polygonoides* has exceptionally high fiber (24.5 g/100g) while *C. decidua* has very low fiber (6.19 g/100g). A similar low fiber content value was also

reported by Alrasheid *et al.*, (2018). The high fiber content of *C. polygonoides* indicates its potential value as a dietary fiber source, which may contribute to digestive health. *C. decidua* has the highest carbohydrate content (60.83 g/100g) while *C. polygonoides* has the lowest (32.6 g/100g). Similarly, the high carbohydrate content was found in the *C. decidua* fruit of 73.48 g/100g (Nazar *et al.*, 2020), 61.95 g/100g (Alrasheid *et al.*, 2018), and 63.06 g/100g (Vyas *et al.*, 2009). Carbohydrates are the main components of caper fruits and buds, followed by raw proteins and neutral fibers (Goyal and Grewal, 2003; Kumar *et al.*, 2011). *V. nilotica* has the highest ash content (13.08 g/100g) while *C. decidua* has the lowest (6.53 g/100g). Alrasheid *et al.*, (2018) also reported low ash content in *C. decidua* (6.34 g/100g). Some authors reported that the ash contents of Acacia species could vary between 3.5% to 5.9% (Zapata-Campos *et al.*, 2020); and 3.9 to 5.4% (Hadi *et al.*, 2021). The high ash content of *V. nilotica* indicates substantial mineral accumulation, consistent with its high iron, zinc, and copper concentrations.

In the present study, it was found that there are significant differences ( $p < 0.05$ ) in the mineral composition of *V. nilotica*, *C. decidua*, and *C. polygonoides*. *C. decidua* has extremely high sodium (180.2 mg/100g) while *V. nilotica* has negligible Na (0.04 mg/100g). Iqbal *et al.*, (2014) also reported a high content of Na (60-150 mg/100g). Alrasheid *et al.*, (2018) conducted a study in the semi-arid region of Sudan and reported the Na content in the fruits of *C. decidua* of 26.9 mg/100g. The difference in Na content may be due to the arid and semi-arid soil conditions. The highest accumulation of soluble salts and Na typically occurs in arid regions due to low rainfall and high evaporation rates, which prevent salts from being leached out of the soil (Stavi *et al.*, 2021). The high Na and K accumulation in *C. decidua* reflects the arid soil conditions of the Cholistan desert, where high evaporation rates prevent salt leaching. The species-specific differences in mineral uptake can be attributed to variations in root architecture and ion transport mechanisms. Nazar *et al.*, (2020) found the concentration of Na in *C. decidua* is 160 mg/100g. *C. decidua* has the highest K (236.7 mg/100g), while *V. nilotica* has very low potassium (0.56 mg/100g). Iqbal *et al.*, (2014) also found a similar concentration of K (140-480 mg/100g) in the fruits of *C. decidua*. *C. polygonoides* has very high Mg (63.2 mg/100g) while *C. decidua* and *V. nilotica* have very low Mg ( $< 0.5$  mg/100g). Nazar *et al.*, (2020) found the concentration of Mg in *C. decidua* was 49.16 mg/100g. *V. nilotica* has exceptionally high Fe (203.1 mg/100g) while *C. polygonoides* has the lowest Fe (20.5 mg/100g). *V. nilotica* has very high Zn (108.7 mg/100g) while it is negligible in *C. decidua* (0.3 mg/100g). Vyas *et al.*, (2009) also found negligible Zn (0.22 mg/100g) in the fruits of *C. decidua*. *V. nilotica* has extremely high Cu (322.7 mg/100g) while *C. polygonoides* has low Cu (3.1 mg/100g). The exceptionally high iron, zinc, and copper concentrations in *V. nilotica* suggest its potential as a natural mineral supplement, particularly for addressing micronutrient deficiencies. The distinct mineral profiles likely reflect species-specific metal ion uptake, transport, and sequestration mechanisms, as well as differences in soil chemistry at collection sites. These results show the

significant differences in mineral composition among species, which may have an impact on their potential applications in nutrition, medicine, and other fields.

The unique metabolite profiles observed in this study likely reflect the harsh environmental conditions of the Cholistan desert, including high temperatures (25-42°C), low rainfall (~120 mm annually), high soil salinity, and intense UV radiation. These stress factors are known to trigger the accumulation of specific secondary metabolites as adaptive responses (Tao *et al.*, 2006; Adegbaaju *et al.*, 2020).

In the present study, it was found that there are significant differences ( $p < 0.05$ ) in the phenolics and flavonoids composition, including QE, GA, m-CA, VA, BA, CGA, SA, p-CA, 3-CA, FA, CINN, and SA of selected medicinal plant species, i.e., *V. nilotica*, *C. decidua*, and *C. polygonoides*. The effects of plant chemicals, such as phenols, are pathogen defense mechanisms and can be applied to medical applications (Gálvez *et al.*, 2005; Altemimi *et al.*, 2017). The distinct phenolic profiles likely reflect differences in the shikimate and phenylpropanoid pathways, with each species showing preferential accumulation of specific pathway intermediates. The concentration of QE was highest in *C. polygonoides* ( $264.51 \pm 0.64$  mg/g), while *C. decidua* ( $41.94 \pm 0.22$  mg/g) and *V. nilotica* ( $47.16 \pm 0.38$  mg/g) showed substantially lower concentrations. El-Toumy *et al.*, (2011), Saleh & El-Badry (2013), and David *et al.*, (2016) reported that QE was found in the flowers of *V. nilotica*. QE possesses antioxidant, anti-inflammatory, and anti-diabetic effects (Maurya, 2022). The exceptionally high quercetin content in *C. polygonoides* (264.51 mg/g) is substantially higher than previously reported for any Calligonum species (Berwal *et al.*, 2021 reported 45-85 mg/g), suggesting that the Cholistan desert populations may represent a unique chemotype with enhanced flavonoid production. This exceptional quercetin accumulation may be an adaptive response to the intense UV radiation and oxidative stress characteristic of the Cholistan desert. *C. polygonoides* contained the highest concentration of GA ( $58.81 \pm 0.36$  mg/g), followed by *C. decidua* ( $21.59 \pm 0.13$  mg/g), with *V. nilotica* showing the lowest concentration ( $5.43 \pm 0.06$  mg/g). Tannins, including catechin gallate, GA, and ellagic acid, are the main phytochemicals discovered in various regions of *V. nilotica* (Jigam *et al.*, 2010). The pharmaceutical industry regularly employs GA due to its extraordinary anti-inflammatory and antioxidant properties, which have been proven in several *In vitro* and *In vivo* studies (Cai, 2022). *V. nilotica* contained the highest concentration of CA ( $5.94 \pm 0.05$  mg/g), while *C. polygonoides* showed moderate levels ( $2.21 \pm 0.08$  mg/g). Singh *et al.*, (2010) and Mbatchou & Oumar (2012) reported CA found in the seed pod and bark extracts of *V. nilotica*. The antioxidant effects of CA play an essential role in many beneficial effects on human health (Pavlikova, 2022). *C. decidua* contained the highest concentration of VA ( $13.01 \pm 0.08$  mg/g), followed by *C. polygonoides* ( $5.19 \pm 0.07$  mg/g). VA showed a wide range of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and antidepressant properties (Calixto-Campos *et al.*, 2015). *C. decidua* showed the highest concentration of BA ( $18.21 \pm 0.09$  mg/g), followed

by *V. nilotica* ( $7.88 \pm 0.05$  mg/g), with *C. polygonoides* containing the lowest concentration ( $4.53 \pm 0.07$  mg/g). BA has demonstrated antimicrobial properties against many microbial agents, including mycobacteria (Zhang *et al.*, 2003). *C. decidua* contained the highest concentration of p-CA ( $66.59 \pm 0.12$  mg/g), m-CA ( $9.01 \pm 0.07$  mg/g), SA ( $7.74 \pm 0.03$  mg/g), and CGA ( $22.09 \pm 0.13$  mg/g) as compared to the other two species. Phenolic compounds and flavonoids were detected in the aqueous, n-hexane, and acetone extracts of *C. decidua* leaves (Baghiani *et al.*, 2012). *V. nilotica* showed the highest concentration of FU ( $33.53 \pm 0.35$  mg/g), CINN ( $23.44 \pm 0.06$  mg/g), and SA ( $6.78 \pm 0.04$  mg/g). Hydroxycinnamic acid derivatives are an important class of polyphenolic compounds, including CA, p-CA, FU, CINN, and CGA. These phenolic compounds have strong antioxidant and anti-inflammatory effects (Alam *et al.*, 2016). *C. polygonoides* showed strong antioxidant potential due to the presence of high concentrations of QE and GA. The various portions of *C. polygonoides* are very rich sources of phenolic compounds (13-35% on a dry weight basis), with the majority being gallic acid (CGA, p-CA, VA, and syringic acid (Berwal *et al.*, 2021). *C. decidua* has a broad spectrum of antimicrobial activity due to the presence of a variety of phenolic acids (Gull *et al.*, 2015), whereas *V. nilotica* showed high concentrations of ferulic acid (33.53 mg/g) and cinnamic acid (23.44 mg/g). These compounds have been previously associated with anti-inflammatory and UV protection properties in other studies (Al-Rajhi *et al.*, 2023; Alam *et al.*, 2016). However, direct pharmacological assays are needed to confirm these activities in the present context. More *et al.*, (2021) reported that methyl gallate, GA, vitexin, and CA hexose were found in the ethanol extracts of *V. nilotica* leaves.

In the present study, the  $^1\text{H-NMR}$  spectra of the three methanolic extracts, i.e., *C. polygonoides*, *C. decidua*, and *V. nilotica* revealed distinct metabolic profiles, indicating significant differences ( $p < 0.05$ ) in their phytochemical composition. The different factors like geographic location, growth season, soil type, and mineral condition, have an impact on the level of secondary metabolites (Tao *et al.*, 2006; Adegba *et al.*, 2020). The  $^1\text{H-NMR}$  fingerprint of the methanol extract of *C. polygonoides* shows a complex profile rich in polyphenol compounds. The spectrum is dominated by a characteristic signal of flavonol glycosides, methylated flavonoids, hydrolyzed tannins, and glycosylated metabolites. The diverse phenolic composition is consistent with *C. polygonoides* reported antioxidant and biological activity, providing the chemical basis for its ethnopharmacological use. Ahmed *et al.*, (2020) also confirmed the presence of flavanols, flavanol glycosides, and aglycones in all parts of *C. polygonoides*. The  $^1\text{H-NMR}$  fingerprint of the methanol extract of *C. decidua* revealed a characteristic phytochemical profile. The spectrum is dominated by a characteristic signal of a complex condensed tannin mixture, glycoside metabolites, flavonoid glycosides, methyl phenol compounds, fatty acids, and sterol compounds. *C. decidua* contains a variety of alkaloids, terpenoids, glycosides, flavonoids, sterols, aromatic acids, and fatty acids (Rathee *et al.*, 2010; Soda, 2010; Tlili *et al.*, 2011; Lansky *et al.*, 2013).

The analysis of  $^1\text{H-NMR}$  of the methanol extract of *V. nilotica* in the present study revealed a rich profile of a variety of phenolic compounds, especially flavonoids. The spectrum displayed characteristic signals for flavonoid aglycones, flavonoids, and methoxylated phenol derivatives. *Vachellia* species contain secondary metabolites such as terpenoids, tannins, alkaloids, saponins, glycosides, flavonol and flavone glycosides, which have been shown to positively contribute to *In vitro* biological activities (Hnini *et al.*, 2023; Banso, 2009; Deshpande, 2013; Okoro *et al.*, 2014; More *et al.*, 2021). Okoro *et al.*, (2014) conducted a study and confirmed the presence of sterols in the ethanolic extracts of *V. nilotica*. *V. nilotica* contains various types of glycosides, including cyanogenic glycosides and anthraquinone glycosides. These compounds have been associated with laxative, anti-inflammatory, and antimicrobial properties (Khan *et al.*, 2019).

**Limitations of the study:** This study provides comprehensive chemical profiling; several limitations should be acknowledged. The study did not include pharmacological assays to directly confirm the bioactivities suggested by the chemical profiles. The sampling was limited to a single collection season and location for each species, and seasonal and geographical variations may affect phytochemical composition. Second, the study focused on profiling using  $^1\text{H-NMR}$  rather than full structural elucidation through 2D NMR (COSY, HSQC, HMBC) or LC-MS/MS. While the  $^1\text{H-NMR}$  fingerprints provide valuable metabolic profiles, future studies should employ these advanced techniques for definitive compound identification and validation. Future studies should address these limitations through bioassay-guided fractionation, seasonal sampling, and isolation of bioactive compounds.

## Conclusion

The current study evaluated the metabolite fingerprinting and profiling of *Vachellia nilotica*, *Capparis decidua*, and *Calligonum polygonoides* found in the Cholistan desert. The results confirmed that there was a significant difference ( $p < 0.05$ ) in the phytochemical composition among selected species. *C. polygonoides* had exceptionally high fiber, magnesium, and flavonoid glycosides. *V. nilotica* was richest in protein, ash, iron, zinc, copper, and flavonoid glycosides. *C. decidua* had the highest fat, carbohydrate, sodium, potassium, and a complex mixture of condensed tannins. Each species showed a distinct phytochemical profile, and they can be explored for the isolation of phytoconstituents having the potential to be used for the synthesis of medicine for the treatment of different diseases. Future research should include bioassay-guided fractionation to isolate active compounds, evaluation of seasonal variations in phytochemical composition, and *In vitro* and *In vivo* pharmacological studies to validate the therapeutic potential of these plants. The finding offered scientific proof for the use of these medicinal plants in primary health care by the local people as a remedy for the treatment of various diseases.

**Declarations of Competing Interests:** The authors certify that they have no financial interest in the subject matter discussed in this article.

## Acknowledgments

This article is a part of the Ph.D. thesis of the first author. The Higher Education Commission of Pakistan funds the research study under the National Research Program for Universities (NRPU) Project # 11119.

**Financial Support Statement:** The Higher Education Commission of Pakistan supported this work under the National Research Program for Universities (NRPU) Project # 11119.

**Abbreviations:** HPLC, High-performance liquid chromatography; NMR, Nuclear Magnetic Resonance; QE, Quercetin; GA, Gallic acid; CA, Caffeic acid; VA, Vanillic acid; BA, Benzoic acid; CGA, Chlorogenic acid; SA, Syringic acid; p-CA, p-Coumaric acid; m-CA, m-Coumaric acid; FA, Ferulic acid; CINN, Cinnamic acid; UV, Ultraviolet; <sup>1</sup>H NMR, Proton nuclear magnetic resonance; DMSO, Dimethyl sulfoxide.

**Author's Contributions:** Ehsan Ali: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. Muhammad Farooq Azhar: Supervision, Project administration, Funding acquisition, Writing –review & editing.

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