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Seasonal Variations in the Metabolic Profile of Some Selected Medicinal Plant Species from the Saudi Arabian Flora

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ABSTRACT

In this study, four perennial medicinal plant species, namely *Convolvulus hystrix* Vahl. (*C. hystrix*), *Maerua oblongifolia* Forssk. A. Rich. (*M. oblongifolia*), *Cadaba glandulosa* Forssk. (*C. glandulosa*), and *Fagonia indica* Burm. f. (*F. indica*), were analyzed for their primary metabolites (carbohydrates, amino acids, proteins, and proline) and secondary metabolites (phenolic compounds, flavonoids, saponins, alkaloids, and terpenoids) during the summer and winter seasons in Jeddah, Kingdom of Saudi Arabia (KSA). Additionally, the phytochemical composition of the plant species was analyzed using gas chromatography-mass spectrometry (GC-MS) in both seasons. *M. oblongifolia* exhibited higher levels of proline and amino acids in the summer but showed lower concentrations of soluble sugars, phenols, and saponins in the winter. During the summer, *C. glandulosa* displayed high levels of amino acids and alkaloids, whereas in winter, it recorded the lowest levels of soluble proteins. Meanwhile, *F. indica* exhibited its highest levels of proline and alkaloids during the winter season, while sugars, saponins, amino acids, and terpenoids peaked during the summer. Additionally, *C. hystrix* showed elevated concentrations of phenols, flavonoids, alkaloids, and terpenoids in the summer. GC-MS analysis revealed that fatty acids were the main constituents in the plant species, with the highest concentrations observed in *M. oblongifolia*, *C. glandulosa*, and F. indica during the summer, and in C. hystrix during the winter. Furthermore, *C. glandulosa* exhibited the persistence of therapeutic compounds like caryophyllene and vanillin in the winter. Overall, when collecting medicinal plants, it is essential to conduct a comprehensive investigation considering critical factors such as timing, developmental stage, fertilization techniques, protective measures, and irrigation practices to maximize yield.

Keywords: Medicinal plant; Phytochemical composition; PCA; Seasonal fluctuations; Secondary metabolities.

INTRODUCTION

Saudi Arabia is primarily characterized by a desert environment, except in its southwestern region, which experiences a semi-arid climate. Summertime in the central region is hot and dry, with inland temperatures ranging from 27°C to 43°C and coastal temperatures from 27°C to 38°C. Saudi Arabia is susceptible to the consequences of global warming, such as rising temperatures and decreased precipitation. For most of the nation, summers are high temperatures with little precipitation, while winters are mild with lower nighttime temperatures and more precipitation (Almazroui 2020). Summer is defined by temperatures that are over 100 °F (38 °C) in most parts of the kingdom, and it lasts from June to August. In the summer, temperatures in the desert often rise above 130 °F (55 °C). In Saudi Arabia, the winter is comparatively less hot because there is usually some rainfall at this time. The winter season in the Kingdom of Saudi Arabia reaches its peak from December until the end of January (Jayagopal *et al*. 2022).

Plants are constantly interacting with an external environment that is constantly changing and potentially harmful. Abiotic stresses come in several forms and have an impact on plant physiology. Plants under abiotic stress undergo several physiological changes that could be responses to the environment that are adaptive (Fadiji *et al*. 2023). Plants contain a diverse array of chemical metabolites as part of their intricate defensive mechanisms to counteract stress. The potential of plants to adapt to changing environmental conditions is mostly dependent upon their secondary metabolites, which they have a relatively unconstrained potential to synthesize. Nevertheless, the presence of active compounds in medicinal plants is influenced by seasonal variations, consequently impacting their therapeutic effectiveness (Liebelt *et al*. 2019). Plants develop these defense mechanisms in response to both biotic and abiotic stimuli, including factors like temperature, light intensity, herbicides, and microbial assaults (Al-Saleem *et al*. 2018). These environmental stimuli can cause alterations in the overall phytochemical profiles that are essential for the

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synthesis of bioactive compounds. Plant secondary metabolism refers to the capacity of the plant to adjust and endure in reaction to external stimuli across its lifespan, hence forming ecological associations with other organisms (Musilova *et al*. 2016). The production of secondary metabolites is typically highly manipulated, confined to particular plant tissues or developmental phases, or triggered by external stimuli. Environmental anxieties, including non-living factors (abiotic) and living factors (biotic), can impact the production of secondary metabolites by modifying plant metabolism. Those stresses frequently lead to diminished morphological attributes in plants, such as decreased length, leaf number and area, shoot branching, root size, and other related attributes, ultimately resulting in a decline in biomass production (Pradhan *et al.* 2017).

However, plant secondary metabolites were reported to provide a range of protective roles, including anti-toxicity, antimicrobial activity, photoprotection, structural and molecular stabilization, antioxidant properties, and shielding against light/UV radiation, in addition to the role they play in signal transduction (Vasconsuelo and Boland 2007). Consequently, extensive research has been conducted on the impact of seasonal fluctuations on the synthesis of secondary metabolites in plants, revealing distinct differences in the production of specific phytochemicals throughout various seasons. The discrepancies in the synthesis of these phytochemical compounds contribute to significant alterations in the chemical composition of the plant, thereby impacting the quantity of the bioactive substances (Lemos *et al*. 2015). Medicinal plants have served as the foundation of alternative medicine and have emerged as the primary avenue for developing new medications *(Newman et al*. 2000). At the beginning of the nineteenth century, over 80% of medicinal substances were derived from plants.

Particularly following the scientific revolution, the field of herbal medicine played a significant role in the development of the pharmaceutical sector, where the use of synthetic drug products became prominent (Shinwari and Qaiser 2011). The increased utilization of medicinal plants in illness treatment can be attributed to the fact that plants or their derivatives are regarded as safe and efficacious pharmaceuticals, with minimal adverse effects and a relatively affordable cost (Odhav *et al*. 2010).

The concept of folk medicine emerged in the Kingdom of Saudi Arabia around 1940, when there was a decreased demand for conventional medicine. Since 1990, there has been a shift in the Saudi people's perception of traditional medicine, leading to an increased adoption of its practices in everyday life (Ullah *et al*. 2020). A multitude of ethnobotanical questionnaires conducted in Saudi Arabia have revealed that a substantial proportion of the Saudi population adheres to traditional medicine, either exclusively or in conjunction with modern medicine (Bodeker 2005). In their study, Aati *et al*. (2019) concentrated on the ethnomedical uses of native plants in Saudi Arabia. He found that 89 plant families, comprising 309 genera and 471 species, are being utilized in traditional medicine. Some studies have recorded the fluctuation of plant bioactive phyto-chemicals with the growth season. Therefore, it is necessary to conduct regular phytochemical screenings, even on plants with well-known secondary metabolites (Nalawade and Tsay 2004, Alqethami and Aldhebiani 2021). Regrettably, there has been a lack of focus on the investigation of medicinal plants in the Jeddah region, despite the widespread and extensive existence of such plants. The objective of this study was to investigate the seasonal fluctuations in the primary and secondary phytochemical components of four studied plant species naturally grown in Jeddah region, Saudi Arabia, throughout a wet and dry season. Furthermore, the study investigated the variations in the bioactive phytochemical components of the plant species across the two seasons using the GC/MS technique.

MATERIALS AND METHODS

Sampling of plant material

Four medicinal plant species were selected from the southern region of the Jeddah governorate, based on their documented therapeutic properties. Leaf samples of *Cadaba glandulosa* (Forssk.) and *Maerua oblong-gifolia* (Forssk., A. Rich.), as well as the aerial parts of *Convolvulus hystrix* (Vahl.) and *Fagonia indica* Burm. f., were harvested during the summer (August) and winter (February) seasons of 2021 (Table 1, Figure 1).

The plant materials were collected from their natural wild habitat (50 individuals per species). The samples were immediately transferred to the laboratory and washed several times with running tap water to remove any surface impurities. Subsequently, the samples were rinsed with distilled water to eliminate any remaining contaminants. Following the washing procedure, the plant material was left to air dry in a shaded place until a constant weight was achieved. The plant samples were then ground into a fine powder and sieved through a 2 mm sieve to obtain a consistent particle size and remove any large debris or unwanted materials. To preserve the quality and integrity of the samples, they were maintained in paper bags until the time of analysis.

Climatic data.

The monthly average temperature, relative humidity and rainfall at Jeddah province during the plant sampling period in 2021 are shown in Figure (2). The highest average temperatures (°C) were recorded in July and August, while the lowest temperatures were observed in January and February. September and October experienced the highest relative humidity (%), whereas May and July had the lowest values. Rainfall levels (mm) were highest in November and January, while the lowest amounts were recorded in September and April.

Quantification of the primary metabolites

Total soluble proteins

Total soluble protein content of the stdied plants was

Table (1): Taxonomic classification, growth forms, lifestyle strategy, and utilized plant parts of selected species in Capparaceae, Convolvulaceae, and Zygophyllaceae Families.

Scientific name	Family	Growth form	Life form	Used plant part
Cadaba glandulosa Forssk.	Capparaceae	Shrub	Phanerophyte	Leaves
Maerua oblongifolia (Forssk.) A.Rich.	Capparaceae	Under-shrub	Phanerophyte	Leaves
Convolvulus hystrix Vahl	Convolvulaceae	Sub-shrub	Chamaephyte	Aerial parts
Fagonia indica Burm. f.	Zygophyllaceae	Herb	Chamaephyte	Aerial parts

Figure (1): Perennial medicinal plant species chosen to carry out the study. A, *Cadaba glandulosa* Forssk; a, A magnified view of the flower. B, *Maerua oblongifolia* Forssk; b, A magnified view of the flower. C, *Convolvulus hystrix* Vahl.; c, A magnified view of the flower. D, *Fagonia indica* Burm. f.; d, A magnified view of the flower.

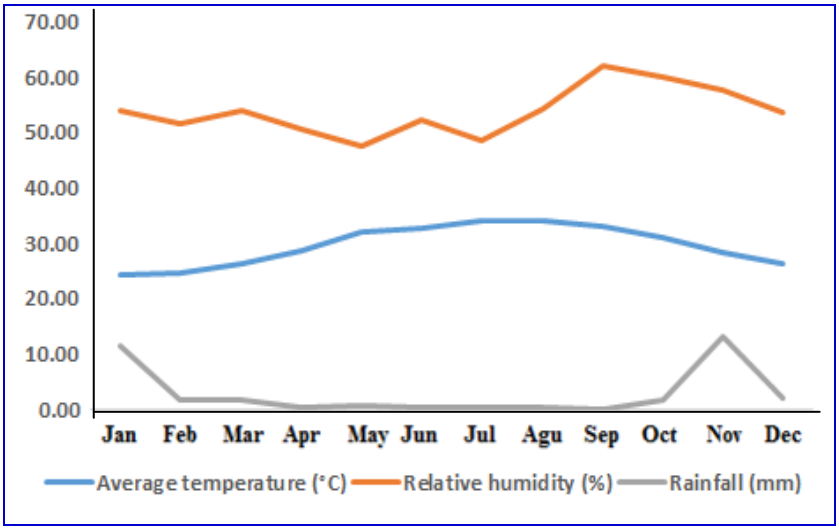

Figure (2): The average monthly variation in temperature, humidity, and rainfall in Saudi Arabia during the year 2021.

determined following the Bradford method (1976). An aliquot of 0.1 mL of borate buffer extract was mixed with 3 mL of Coomassie Brilliant Blue reagent, and the absorbance of the resulting mixture was measured at 595 nm after 30 minutes. Protein content was determined using a standard calibration curve, with Bovine Serum Albumin (BSA) as the reference protein.

Free Proline

The free proline was quantified by applying Bates *et al.* (1973) approach. A sample of 0.1 g powder was extracted with 5 mL of 3% aqueous sulfosalicylic acid. Then 1 mL of the supernatant was combined with 2 mL of acidic ninhydrin reagent and 2 mL of acetic acid. The mixture was subsequently boiled at 100 °C for 1 h, then the reaction mixture was subjected to extraction using 4 mL of toluene. The optical density of the resulting chromophore was measured at 520 nm. Proline was calculated using a calibration curve constructed by proline.

Free amino acids

The quantification of free amino acids was conducted using the ninhydrin reagent, following the method described by Lee and Takahashi (1966). The finely powdered dry materials were extracted in 95% ethanol. A 0.1 mL extract was combined with 1.9 mL of ninhydrin-citrate buffer and glycerol mixture. The mixture was agitated vigorously and boiled in a water bath for 12 min. After cooling, the absorbance was recorded at 570 nm. The amount of free amino acids was calculated using a calibration curve established with glycine.

Soluble carbohydrates

Following the method of Dubois *et al*. (1965), the amount of total soluble carbohydrates in borate extracts was calculated. A 0.1 mL extract was mixed with 0.1 mL 5% phenol and 1 mL H_2SO_4 and heated at 60 °C for 1 h. After cooling, the total soluble carbohydrates fraction was quantified by measuring the absorbance at 495 nm and using glucose as a standard sugar.

Quantification of secondary metabolites

Phenolic compounds

The quantification of total phenolic content was conducted using the methodology outlined by Jindal and Singh (1975). The ethanolic extract (1 mL) was combined with 0.1 mL of foline reagent and 1 ml of Na_2CO_3 (20%). The mixture was then brought to a total volume of 5 ml with distilled water. Subsequently, absorption was assessed at 650 nm after 30 min. The quantification of total phenolic content was conducted by employing a standard curve established with gallic acid.

Flavonoids

The measurement of flavonoids was conducted using the aluminum chloride colorimetric approach, as described by Chang *et al.* (2002). A 0.5 mL ethanol extract was combined with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, and 0.1 mL of 1 M potassium acetate. The solution absorption was measured at 415 nm after 30 min. A calibration curve constructed using quercetin was used to quantify flavonoids content.

Saponins

The quantification of saponins was conducted using the method outlined by Hiai *et al.* (1975). A 0.5 mL sample of ethanol extract was combined with 0.5 mL of 8% vanillin and 5 ml of 72% H_2SO_4. The mixture was thereafter heated in a water bath at 60 °C for 10 min. The absorbance was measured at 544 nm after being cooled in an ice-cold water bath. A standard curve by cholesterol was then utilized to determine the amount of saponins.

Alkaloids

Total alkaloids in the investigated samples were determined as described by Harborne (1973). The sample powder was extracted using a mixture of 70% ethanol and glacial acetic acid (4:1 v/v). After filtrations, alkaloids were precipitated by gradually adding concentrated ammonia. Alkaloids were then separated by filtering through a pre-weighted filter paper. The separated alkaloids were then dried in an oven at 70 °C until constant weight and the alkaloid content was calculated.

Terpenoids

A 200 µl ethanolic extract aliquot was combined with 1.5 ml chloroform, thoroughly vortexed, and then 100 µL H_2SO_4 was added. The reaction mixture was then left to incubate for 2h in darkness. Following the decantation of the supernatant, 1.5 mL of 95% methanol was added, and the mixture was vortexed until the precipitate being fully dissolved. With methanol serving as a blank, the absorbance was measured at 538 nm, and the total terpenoids content was computed using a standard curve constructed from linalool (Ghorai *et al.* 2012). All the measured primary and secondary metabolites were quantified in mg g^{-1} DM.

Identification of phytochemical constituents using GC-MS technique

The fluctuation in the active ingredients of the four investigated plant species across two growing seasons, wet (winter) and dry (summer) seasons was achieved using the gas chromatography-mass spectroscopy (GC-MS) approach. The chemical composition of the methanolic extract of the examined plants was determined using a Thermo Scientific TRACE 1310 gas chromatograph coupled with an ISQ LT single quadrupole mass spectrometer (Thermo Scientific, Waltham, Massachusetts, USA).

The column used in this investigation was DB5-MS (30 m × 0.25 mm × 0.25 µm film thickness) column (J&W Scientific, Folsom, California, USA), and the oven temperature was first held at 40 °C for 3 min. before being increased by 5 °C min^{-1} to 180 °C withhold for 5 min, followed by an increase to 200 °C withhold for 1 min. by a rate of 10 °C min^{-1}, and finally increased to 290 °C withhold for 1 min by a rate of 7.5 °C min^{-1}. The temperatures of the transfer and input lines were maintained at 250 °C. Helium was utilized as the carrier gas, maintaining a consistent flow rate of 1 mL min^{-1}. A sample of 1 µL was administered automatically using the autosampler AS3000 and GC in split mode (1:20) after a solvent delay of 5 min. In

complete scan mode, EI mass spectra were acquired within the range of 40–650 m/z at an ionization energy of 70 eV. The ionization chamber was maintained at 200°C temperature. All attained components of the extracts were identified by contrasting their retention times and mass spectra with those of WILEY 09, replib, and NIST 11 databases after conducting the analysis three times for each sample (Konappa *et al.* 2020).

Statistical analysis.

The acquired results underwent one-way analysis of variance (ANOVA) followed by the Tukey-HSD post hoc test ($p \leq 0.05$). The data were presented as the mean ± standard deviation (SD), with three replications for each sample. The statistical analyses were conducted using GraphPad version 6.01 software (GraphPad Software, San Diego, CA, USA). Principal component analysis (PCA) was conducted using XLSTAT statistical analysis software (version 5.03) to reduce the dimensionality of the metabolomic data and assess the impact of seasonal fluctuation on the metabolomic composition of the species under investigation.

RESULTS

Seasonal variation in primary metabolites

Total soluble protein.

The seasonal variation in the protein content of the four plant species under investigation: *Convolvulus hystrix, Maerua oblongifolia, Cadaba glandulosa,* and *Fagonia indica*, is displayed in Table 2. Overall, when comparing the species investigated throughout both seasons, *M. oblongifolia* had the highest soluble protein content (16.64 mg g^{-1} DM) in the summer season, whereas *C. glandulosa* had the lowest value (12.55 mg g^{-1} DM) in the same season. There was no discernible difference in the protein content between *C. hystrix* and *M. oblogifolia* throughout both the summer and winter seasons. In the summer, the two plants reached their peak protein levels, with *C. hystrix* giving 15.70 mg g^{-1} DM and *M. oblogifolia* producing 16.64 mg g^{-1} DM. In the winter season, both plant species exhibited an apparent decline in protein levels, culminating in similar values of 14.25 and 14.77 mg g^{-1} DM, respectively. Conversely, *C. glandulosa* and *F. indica* exhibited an inverse pattern in protein accumulation. They displayed high protein content, more specifically 15.02 and 13.66 mg g^{-1} DM, respectively, during the winter season. However, their protein levels declined during the summer season, resulting in 12.55 and 13.66 mg g^{-1} DM, respectively.

Free proline

Table 2 displays the proline levels in the four plant species under investigation during both winter and summer seasons. *F. indica* exhibited the maximum proline concentration (36.14 mg g^{-1} DM) during winter, whereas *C. hystrix* displayed the minimal proline content (2.18 mg g^{-1} DM) during the summer season. Overall, throughout the winter season, the plant species *C. hystrix* and *F. indica* exhibited the highest proline accumulation, with respective amounts of 4.03 and 36.14 mg g^{-1} DM. However, the proline concentration exhibited a decreasing trend in the two species during the summer season, reaching 2.18 and 23.60 mg g^{-1} DM, respectively. On the contrary, throughout the summer season, *M. oblongifolia* and *C. glandulas* accumulated larger amounts of proline (4.88 and 2.82 mg g-1 DM, respectively) than during the winter (2.26 and 2.61 mg g-1 DM, respectively).

Amino acids

Table 2 provides insight into the impact of seasonal variations on the overall concentration of free amino acids in the plant species under investigation. During the winter season, *C. glandulosa* and *M. oblongifolia* possessed the highest reported amino acid pool, with values of 74.06 and 74.00 mg g^{-1} DM, respectively. Both species demonstrated a reduction in amino acid content throughout the summer season, with an insignificant decline in *M. oblongifolia* (71.04 mg g^{-1} DM) and a significant reduction (66.17 mg g^{-1} DM) in *C. glandulosa*. In contrast, *F. indica* exhibited a higher accumulation of amino acids during the summer season (48.24 mg g^{-1} DM) compared to its level in the winter season (40.05 mg g^{-1} DM). Remarkably, the *C. hystrix* exhibited a relatively stable amino acids content in both seasons, recording 66.85 mg g^{-1} DM during summer and 67.16 mg g^{-1} DM during winter.

*Soluble carbohydrates***.**

The variation in total soluble carbohydrates during the two growth seasons for each of the four plant species under investigation is shown in Table 2. According to the findings, *M. oblongifolia* recorded the lowest soluble sugar fraction (19.69 mg g^{-1} DM) during the winter season, while *F. indica* reported the greatest fraction (77.65 mg g^{-1} DM) during the summer.

In general, the concentration of soluble sugars was found to be higher during the summer season in *C. hystrix, M. oblongifolia,* and *F. indica* compared to the winter season. In contrast, *C. glandulosa* exhibited higher sugar accumulation during the winter season in comparison to the summer season.

Table 2. Seasonal variations in primary metabolites (soluble proteins, free proline, free amino acids, and soluble carbohydrates)w in the studied plant species throughout the summer (S) and winter (W) seasons of 2021.

Seasonal variations in secondary metabolites

Phenolic compounds

The variability in the content of phenolic compounds in the investigated plant species throughout the two growing seasons is outlined in Table 3. The results obtained indicated that *C. hystrix* produced the maximum phenol content (15.12 mg g^{-1} DM) during the winter season, whilst *M. oblongifolia* produced the lowest amount (2.68 mg g^{-1} DM) throughout the same season. Overall, there were no significant variations in the concentration of phenolic compounds in *M. oblongifolia, C. glandulosa*, and *F. indica* throughout the summer and winter seasons. In *C. hystrix, C. glan-*

Table (2): Seasonal variations in primary metabolites (soluble proteins, free proline, free amino acids, and soluble carbohydrates) in the studied plant species throughout the summer (S) and winter (W) seasons of 2021.

Plant species	Season	Measured primary metabolites (mg g^{-1} DM)			
		Protein	Proline	Amino acids	Carbohydrates
C. hystrix	**S**	15.70±1.29^{ab}	2.18±0.02^{f}	66.85±2.69^{b}	28.57±2.63^{c}
	W	14.25±1.50^{bcd}	4.03±0.20^{d}	67.16±3.10^{b}	21.29±2.09^{d}
M. oblongifolia	**S**	16.64±0.44^{a}	4.88±0.37^{c}	71.04±0.38^{a}	31.13±2.68^{c}
	W	14.77±1.26^{abc}	2.26±0.10^{f}	74.00±1.05^{a}	19.69±0.84^{d}
C. glandulosa	**S**	12.55±1.77^{d}	2.82±0.12^{e}	66.17±2.90^{b}	22.39±3.24^{d}
	W	15.02±0.82^{ab}	2.61±0.04^{ef}	74.06±0.28^{a}	27.87±1.04^{c}
F. indica	**S**	12.63±1.70^{cd}	23.60±0.57^{b}	48.24±2.54^{c}	77.65±2.18^{b}
	W	13.66±0.53^{bcd}	36.14±0.02^{a}	40.05±1.07^{d}	51.65±2.30^{a}
Statistical analysis	**F**	3.88	7543.6	109.46	233.73
	p	0.0117 *	0.0000 ***	0.0000 ***	0.0000 ***
	LSD at 5%	2.18	0.44	3.58	3.91

Mean clculated value superscript with different letters are significant different based on Tukey-HSD post hoc test ($p \leq 0.05$; $p \leq 0.01$). *, significant; ***, highly significant.

dulosa, and F. indica, the winter content of phenolic compounds was higher compared to the summer level. As opposed to this, *M. oblongifolia* possessed a higher phenolic content during the summer than during the winter.

Flavonoids

In comparison to the overall phenolic component content, the flavonoid content of the studied plant species was typically lower during the two seasons (Table 3). According to the results of this investigation, *C. hystrix* showed the highest flavonoids concentration (1.46 mg g^{-1} DM) in the summer, whereas *F. indica* exhibited the lowest level (0.23 mg g^{-1} DM) in the same season.

The flavonoid content of *C. hystrix* and *F. indica* varied significantly with the growth season, as the maximum content was reported in winter for both species (1.46 and 0.33 mg g^{-1} DM, respectively), but during the summer season, it had decreased to 0.53 and 0.23 mg g^{-1} DM, respectively. According to the results of the two growing seasons, the flavonoids content of the two species, *M. oblongifolia* and *C. glandulosa*, was reasonably the same.

Saponins

In accordance with the results of saponins analysis, it was determined that there was significant variation among the four plant species throughout the two growing seasons (Table 3). *F. indica* was reported to accumulate the highest saponins content (0.79 mg g^{-1} DM) during the summer season, whereas *M. Oblongifolia* was reported to accumulate the lowest saponins content (0.28 mg g^{-1} DM) during the winter season. Except for *C. oblongifolia*, the results showed that the investigated plants demonstrated an increase in saponin accumulation throughout the summer season but experienced a significant reduction during the winter season. Nevertheless, *C. oblongifolia* exhibited relatively a consistent saponin content over both seasons.

Alkaloids

The fluctuations in the total alkaloid level within the plant species examined over the two growth seasons was statistically significant (Table 3). The results indicated that the total alkaloid content varied between the highest level (0.76 mg g^{-1} DM) in *F. indica* during the winter and the lowest level (0.26 mg g^{-1} DM) during the summer, in the same plant. Likewise, *C. glandulosa* and *C. hystrix* exhibited greater alkaloid levels during the winter season compared to the summer season. Nevertheless, *M. oblongifolia* demonstrated no significant difference in its alkaloid content between the summer and winter seasons, with values of 0.60 and 0.58 mg g^{-1} DM, respectively.

Terpenoids

Table (3) displays the fluctuations in the content of terpenoids during two different seasons among the four evaluated plant species. The level of terpenoids varied significantly across the four plant species, with the highest concentration (1.38 mg g^{-1} DM) recorded in *F. indica* during the summer, and the lowest concentration (1.07 mg g^{-1} DM) in the same plant during the winter season. Likewise, *C. hystrix* exhibited a higher accumulation of terpenoids during the summer compared to the winter. Nevertheless, *M. oblongifolia* and *C. glandulosa* did not show any discernible change in their terpenoid content with the alteration in seasons.

Seasonal variation in active phytochemical constituents as determined by GC/MS

Convolvulus hystrix.

The GC-MS technique was employed to analyze the chemical composition of the aerial parts of *C. hystrix*. The results indicated the presence of a variety of chemical compounds, including terpenoids, phenols, fatty acids, esters, and steroids. The concentration of these compounds exhibited fluctuations during both the summer and winter seasons, as illustrated in Table 4. The results indicated that the fatty acids 9-octadecenoic acid, 9,12-octadecenoic acid (Z,Z), hexadecanoic acid, and octadecenoic acid were the predominant constituents in *C. hystrix*. The concentrations of these compounds were relatively higher during the winter

Table (3): Seasonal variations in secondary metabolites (phenols, flavonoids, saponins, alkaloids, and terpenoids) in the studied plant species throughout the summer (S) and winter (W) seasons of 2021.

Plant species	Season	Secondary metabolites (mg g^{-1} DM)				
		Phenolic compounds	Flavonoids	Saponins	Alkaloids	Terpenoids
C. hystrix	**S**	7.44±0.21^{b}	0.53±0.04^{b}	0.69±0.03^{b}	0.35±0.01^{d}	1.08±0.10^{b}
	W	15.12±0.63^{a}	1.46±0.00^{a}	0.49±0.05^{c}	0.66±0.01^{b}	1.31±0.11^{a}
M. oblongifolia	**S**	3.09±0.09^{cd}	0.31±0.01^{cd}	0.39±0.03^{d}	0.60±0.01^{bc}	1.29±0.05^{a}
	W	2.68±0.24^{d}	0.30±0.01^{cd}	0.28±0.03^{e}	0.58±0.04^{c}	1.30±0.07^{a}
C. glandulosa	**S**	2.95±0.04^{cd}	0.30±0.01^{d}	0.34±0.05^{d}	0.54±0.02^{c}	1.37±0.16^{a}
	W	3.15±0.16^{cd}	0.31±0.01^{cd}	0.35±0.05^{d}	0.67±0.02^{b}	1.37±0.05^{a}
F. indica	**S**	3.17±0.20^{c}	0.23±0.00^{e}	0.79±0.03^{a}	0.26±0.08^{e}	1.38±0.04^{a}
	W	3.39±0.16^{c}	0.33±0.01^{c}	0.66±0.04^{b}	0.76±0.01^{a}	1.07±0.04^{b}

Mean clculated value superscript with different letters are significant different based on Tukey-HSD post hoc test ($p \leq 0.05$; $p \leq 0.01$).

Table (4):. GC/MS analysis of the phytochemical constituents of *C. hystrix* aerial parts throughout the summer (S) and winter (W) seasons of 2021.

Peak No.	RT (min)	Compound name	Molecular formula	Area %		Chemical group
				S	W	
1	17.27	Benzaldehyde, 4,5-dihydroxy-2-methyl-	$C_8H_8O_3$	1.89	0.77	Terpenoids
2	20.07	1,4-Benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl)-	$C_{13}H_{18}O_2$	0.2	0.2	Phenols
3	25.97	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.17	1.09	Fatty acids
4	26.92	2,6-Diethenyl-1,4-benzenediol	$C_{10}H_{10}O_2$	0.68	0.51	Phenols
5	27.98	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.98	1.21	Fatty acids
6	29.19	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	3.23	-	Fatty acids
7	30.62	Hexadecanoic acid	$C_{16}H_{32}O_2$	9.8	12.94	Fatty acids
8	32.51	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	6.54	-	Fatty acids
9	32.8	Retinol	$C_{20}H_{30}O$	0.39	0.13	Terpenoids
10	33.01	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	3.35	3.98	Fatty acids
11	33.70	9-Octadecenoic acid	$C_{18}H_{34}O_2$	33.39	40.34	Fatty acids
12	34.50	Octadecanoic acid	$C_{18}H_{36}O_2$	8.04	8.09	Fatty acids
13	35.17	9,12-Octadecenoic acid (Z,Z)-	$C_{18}H_{32}O_2$	10.11	10.67	Fatty acids
14	36.79	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	3.16	3.35	Fatty acids
15	38.06	9,12-Octadecadienoic acid (Z,Z)-,2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	4.07	3.75	Fatty acids
16	38.99	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	3.07	1.47	Fatty acids
17	39.72	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	6.3	7.57	Esters
18	41.67	9-Octadecenoic acid (Z)-, tetradecyl ester	$C_{32}H_{62}O_2$	1.04	1.09	Fatty acids
19	47.15	Stigmast-5-en-3-ol, oleate	$C_{47}H_{82}O_2$	2.59	2.84	Steroids

RT = retention time; -, not detected.

season (40.34, 10.67, 12.94, and 8.09%, respectively) compared to the summer season (33.39, 10.11, 9.80, and 8.04%, respectively). On the other hand, some fatty acids that existed during the summer season disappeared during the winter season, like hexadecanoic acid methyl ester and 9-octadecenoic acid (Z) methyl ester. The proportions of the remaining fatty acids varied seasonally, with specific ones increasing during the summer and others increasing during the winter.

The terpenoids contained in *C. hystrix* comprised benzaldehyde, 4,5-dihydroxy-2-methyl, and retinol. The amount of these terpenoids in summer was larger (1.89 and 0.39%, respectively) compared to winter (0.77 and 0.13%, respectively).

The phenolic compound 1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl) exhibited the lowest quantity among the detected phytochemicals (0.20%) without no variation at both seasons. The steroidal compound stigmast-5-en-3-ol, oleate exhibited insignificant fluctuation throughout the two seasons of the investigation.

*Maerua oblongifolia***.**

The chemical composition of *M. oblongifolia* leaves using GC/MS technique identified a diverse array of chemical components, such as terpenoids, phenols, fatty acids, alkaloids, esters, benzothiazoles, and alcohols in this extract (Table 5).

During the summer season, the most prevalent fatty acids were hexadecanoic acid and pentadecanoic acid

14-methyl-, methyl ester, (39.85 and 35.36%, respectively. However, there was a significant decrease in hexadecanoic acid (3.63%) and a complete absence of pentadecanoic acid 14-methyl-, methyl ester during the winter season. The pattern was the opposite for the two fatty acids 10-undecenoic acid, methyl ester, and octadecanoic acid, methyl ester. They exhibited the maximum levels during winter (32.67 and 31.30%, respectively), while their amount reached a minimal value (3.66 and 4.25%, respectively) during the summer season. Similarly, three other fatty acids; 9-octadecenoic acid (Z)-, phenylmethyl ester, 7-hydroxy-2-methyl-octa-3,5-dienoic acid methyl ester, and 7-nonanoic acid, methyl ester, exhibited higher levels in the winter season as opposed to the summer season, however, the fatty acid palmitoyl serotonin was detected in the summer whereas it was completely absent during the winter.

The study findings showed that some compounds like N-(3,4-dimethoxyphenethyl)-2-(3,4-dimethoxy-phenyl) acetamide, benzoic acid, 3,5-dimethyl, 7-hydroxy-8-methylisoflavone, sesquiterpene lactone, olomoucine and 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester were accumulated in *M. oblongifolia* leaves during winter season, though their complete absence during the summer season. On the opposite, the compounds 4'-hydroxy-7-methoxy-flavone, 3-heptyn-1-ol, metipranolol and 1,7-octadien-3-ol, acetate were detected in summer but were not detected during the winter season.

Cadaba glandulosa.

The phytochemical composition of *C. glandulosa* leaf extract exhibited significant fluctuation with respect to the growing season (Table 6). In summer, the three fatty acids 9-octadecenoic acid, n-hexadecanoic acid and 9,12-octadecadienoic acid (Z,Z) were the most abundant compounds (30.83, 21.22, and 10.80%, respectively), though their lower content or complete absence (n-hexadecanoic acid) during the winter season. On the other side, caryophyllene showed higher content (23.01%) in winter, while being infrequent in summer. Nonanoic acid, 9-hydroxy-, methyl ester, and methylprednisolone succinate were also reported in significant concentrations in winter (20.46 and 890%, respectively) despite their absence in the summer season.

Vanillin lactoside and heptadecenoic acid were detected in winter (0.17 and 9.45%, respectively), but not detected in the summer. Oppositely, 2-allyl-5-t-butylhydroquinone and hexadecanoic acid, methyl ester were reported in the summer (0.39 and 1.91%, respectively), while being absent during winter. Some other compounds exhibited higher levels of abundance during summer than in winter like 4-thujanol, 9-octadecenoic acid (Z)-, methyl ester, cis-13-eicosenoic acid, 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester, and stigmast-5-en-3-ol, oleate. Nonetheless, an opposite pattern was observed for for terpinen-4-ol and 8,11-octadecadienoic acid, methyl ester. The compounds thymol, tetradecanoic acid, and octadecanoic

Table (5): GC/MS analysis of the phytochemical constituents of *M. oblongifolia* leaves throughout the summer (S) and winter (W) seasons of 2021.

Peak No.	RT (min)	Compound name	Molecular formula	Area %		Chemical group
				S	W	
1	17.27	Hydrocinnamic acid	$C_9H_{10}O_2$	1.24	1.53	Terpenoids
2	20.07	2,6-Dimethyl-8-oxoocta-2,6-dienoic acid, methyl ester	$C_{11}H_{16}O_3$	0.8	1.04	Phenols
3	16.91	N-(3,4-Dimethoxyphenethyl)-2-(3,4-dimethoxyphenyl)acetamide	$C_{13}H_{19}NO_4$	-	2.02	Alkaloids
4	17.78	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	5.11	4.79	Phenols
5	18.19	4'-Hydroxy-7-methoxyflavone	$C_{16}H_{12}O_4$	0.5	-	Flavonoids
6	18.34	3-Heptyn-1-ol	$C_7H_{12}O$	1.29	-	Alcohols
7	18.65	Metipranolol	$C_{17}H_{27}NO_4$	1.41	-	Phenols
8	18.81	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	0.43	0.77	Fatty acids
9	20.89	Benzoic acid, 3,5-dimethyl-	$C_9H_{10}O_2$	-	2.01	Monoterpenoids
10	21.27	1,7-Octadien-3-ol, acetate	$C_{10}H_{16}O_2$	0.71	-	Terpenoids
11	21.67	7-Hydroxy-8-methylisoflavone	$C_{16}H_{12}O_3$	-	2.99	Flavonoids
12	22.09	7-Hydroxy-2-methyl-octa- 3,5-dienoic acid methyl ester	$C_{10}H_{16}O_3$	0.65	3.24	Fatty acids
13	22.38	Sesquiterpene lactone	$C_{15}H_{24}O_2$	-	3.42	Terpenoids
14	23.91	Olomoucine	$C_{15}H_{18}N_6O$	-	1.69	Alkaloids
15	24.74	7-Nonynoic acid, methyl ester	$C_{10}H_{16}O_2$	1.81	4.93	Fatty acids
16	27.42	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	35.36	-	Fatty acids
17	27.76	Palmitoylserotonin	$C_{26}H_{42}N_2O_2$	2.41	-	Fatty acids
18	29.17	Riluzole	$C_8H_5F_3N_2OS$	0.52	2.76	Benzothiazoles
19	29.31	10-Undecenoic acid, methyl ester	$C_{12}H_{22}O_2$	3.66	32.67	Fatty acids
20	29.78	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	4.25	31.3	Fatty acids
21	30.59	Hexadecanoic acid	$C_{16}H_{32}O_2$	39.85	3.63	Fatty acids
22	36.33	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	-	1.21	Esters

RT = retention time; -, not detected.

Table (6): GC/MS analysis of the phytochemical constituents of *C. glandulosa* leaves throughout the summer (S) and winter (W) seasons of 2021.

Peak No.	RT (min)	Compound name	Molecular formula	Area %		Chemical group
				S	W	
1	9.54	4-thujanol, cis-(.+-.)-	$C_{10}H_{18}O$	0.71	0.57	Terpenoids
2	11.6	Terpinen-4-ol	$C_{10}H_{18}O$	1.43	2.99	Terpenoids
3	14.63	Thymol	$C_{10}H_{14}O$	1.65	1.58	Terpenoids
4	17.24	Vanillin lactoside	$C_{20}H_{28}O_{13}$	-	0.17	Phenols
5	17.8	Caryophyllene	$C_{15}H_{24}$	1.12	23.01	Terpenoids
6	20.06	2-Allyl-5-t-butylhydroquinone	$C_{13}H_{18}O_2$	0.39	-	Phenols
7	25.96	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.94	1.15	Fatty acids
8	26.11	Nonanoic acid, 9-hydroxy-, methyl ester	$C_{10}H_{20}O_3$	-	20.46	Fatty acids
9	27.38	Methylprednisolone succinate	$C_{14}H_{28}O_2$	-	8.9	Steroids
10	29.18	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.91	-	Fatty acids
11	30.53	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	21.22	-	Fatty acids
12	31.98	Heptadecenoic acid	$C_{17}H_{32}O_2$	-	9.45	Fatty acids
13	32.33	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	0.81	2.08	Fatty acids
14	32.49	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	4.01	2.35	Fatty acids
15	33.7	9-Octadecenoic acid	$C_{18}H_{34}O$	30.83	5.59	Fatty acids
16	34.47	Octadecanoic acid	$C_{18}H_{36}O_2$	14.26	15.18	Fatty acids
17	35.15	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	10.8	2.32	Fatty acids
18	37.2	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	2.76	0.59	Fatty acids
19	42.77	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	4.86	2.11	Esters
20	47.15	Stigmast-5-en-3-ol, oleate	$C_{47}H_{82}O_2$	2.3	1.5	Steroids

RT = retention time; -, not detected.

acid showed a nonsignificant fluctuation during the two seasons of the study.

Fagonia indica.

The results of the GC/MS analysis of the phytochemical constituents of *F. indica* aerial parts are presented in Table (7). The study revealed that during the summer season, there were noteworthy amounts (30.65, 14.69, and 12.08%, respectively) of the fatty acids 9-octadecenoic acid (Z), octadecanoic acid, methyl ester, and 9,12-octadecadienoic acid (Z,Z), compared to their abundance in the winter season. In contrast, compared to their abundance throughout the summer season, the fatty acids tetradecanoic acid and eicosanoic acid were reported in significant quantities during the winter (27.16 and 5.59%, respectively). The findings also revealed the absence of some compounds during the summer season, but they were existent during the winter like carbaprostacyclin methyl ester, 2-propenoic acid, 3-(2,3-dimethoxyphenyl)-, (E), andrographolide, 5S,6R-dihydroxy-7E,9E,11Z,14Z-eicosatetraenoic acid and 3,4-dihydroxy-DL-phenyl-alanine. Notwithstanding their absence in the winter, the compounds 1,4-diphenylbut-3-ene-2-ol, terpinen-4-ol, thymol, 9-octadecenoic acid (Z)-, methyl ester, oleic acid, 3-hydroxypropyl ester, and stigmast-5-en-3-ol, oleate were detected during the summer. Some common compounds like cholestan-3-ol, 2-methylene-, (3á,5à) and 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester were reported to be higher in summer than in winter, but the fatty acid hexadecanoic acid showed a nonsignificant variation with the growth season.

Principal component analysis.

Table (8) displays the results of the principal component analysis (PCA), which displays the linear combinations of the original variables used to determine the main components that most effectively account for the variation in a dataset. The biplot diagrams (Figures 3 A-B) depict the correlations between primary and secondary metabolites in four plant species during the summer and winter seasons, based on PC1 and PC2.

These diagrams illustrate both similarities and dissimilarities among the metabolites. The results demonstrated that the investigated plants produced both primary and secondary metabolites during the summer season, as illustrated in Figure (3A). In winter, three plant species *viz.*, *F. indica*, *C. glandulosa*, and *M. oblongifolia* accumulated primary metabolites such as protein, proline, and carbohydrates (Figure 3B). However, secondary metabolites showed limited accumulation during this season, except for phenolic compounds, flavonoids, and alkaloids in *C. hystrix*, which exhibited a high level of accumulation.

DISCUSSION

Medicinal plants are highly valuable to human livelihood and Saudi Arabia is well known for its medicinal plant diversity. Due to their importance in

Table (7):. GC/MS analysis of the phytochemical constituents of *F. indica* leaves throughout the summer (S) and winter (W) seasons of 2021.

Peak No.	RT (min)	Compound name	Molecular formula	Area %		Chemical group
				S	W	
1	9.17	1,4-Diphenylbut-3-ene-2-ol	$C_{16}H_{16}O$	0.43	-	Alcohol
2	11.8	Terpinen-4-ol	$C_{10}H_{18}O$	0.83	-	Terpenoids
3	14.61	Thymol	$C_{10}H_{14}O$	1.05	-	Terpenoids
4	15	Carbaprostacyclin methyl ester	$C_{22}H_{36}O_4$	-	4.69	Terpenoids
5	17.79	2-Propenoic acid, 3-(2,3-dimethoxyphenyl)-, (E)-	$C_{11}H_{12}O_4$	-	8.01	Phenols
6	19.54	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	12.08	1.57	Fatty acids
7	20.98	Andrographolide	$C_{20}H_{30}O_5$	-	3.9	Terpenoids
8	24.55	5S,6R-Dihydroxy-7E,9E,11Z,14Z-eicosatetraenoic acid	$C_{20}H_{32}O_4$	-	4.42	Fatty acids
9	24.89	3,4-Dihydroxy-DL-phenylalanine	$C_9H_{11}NO_4$	-	6.15	Alkaloids
10	25.96	Tetradecanoic acid	$C14H_{28}O_2$	1.16	27.16	Fatty acids
11	27.37	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	14.69	9.38	Fatty acids
12	29.78	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	-	5.34	Fatty acids
13	30.58	Hexadecanoic acid	$C_{16}H_{32}O_2$	14.84	15.78	Fatty acids
14	32.49	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	6.43	-	Fatty acids
15	33.7	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	30.65	3.47	Fatty acids
16	35.65	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	2.45	1.73	Steroids
17	37.2	Eicosanoic acid	$C_{20}H_{40}O_2$	3.55	5.59	Fatty acids
18	38.06	Oleic acid, 3-hydroxypropyl ester	$C_{21}H_{40}O_3$	3.15	-	Fatty acids
19	42.76	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	4.63	2.81	Esters
20	47.15	Stigmast-5-en-3-ol, oleate	$C_{47}H_{82}O_2$	4.06	-	Steroids

RT = retention time; -, not detected.

Table 8. Principal component analysis (PCA) in the three PCAs for the primary and secondary metabolites in four plant species during the summer and winter seasons.

Measured variables	Summer season			Winter season		
	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
Carbohydrates	-0.41	0.14	0.32	0.38	-0.16	0.26
Amino Acid	0.43	-0.14	0.1	-0.4	-0.01	0.12
Proline	-0.43	0.07	0.27	0.39	-0.09	-0.1
Protein	0.31	0.12	0.84	-0.36	-0.23	0.4
Terpenoids	-0.29	-0.42	-0.07	-0.38	0.06	0.47
Alkaloids	0.28	-0.43	0.18	0.36	0.05	0.71
Saponins	-0.25	0.46	0.11	0.37	0.22	0.16
Flavonoids	0.32	0.38	-0.19	-0.06	0.65	0
Phenolic Compounds	0.21	0.48	-0.17	-0.05	0.66	0.04
Convolvulus hystrix	1.68	2.3	-0.2	-0.6	2.24	0.01
Maerua oblongifolia	1.22	-1.19	1	-1.78	-0.95	-0.71
Cadaba glandulosa	0.38	-1.66	-0.95	-1.33	-0.88	0.78
Fagonia indica	-3.29	0.54	0.16	3.71	-0.41	-0.06
Eigenvalues	5.09	3.25	0.65	6.34	2.29	0.37
Variance %	56.61	36.13	7.26	70.43	25.42	4.14
Cumulative%	56.61	92.74	100	70.43	95.86	100

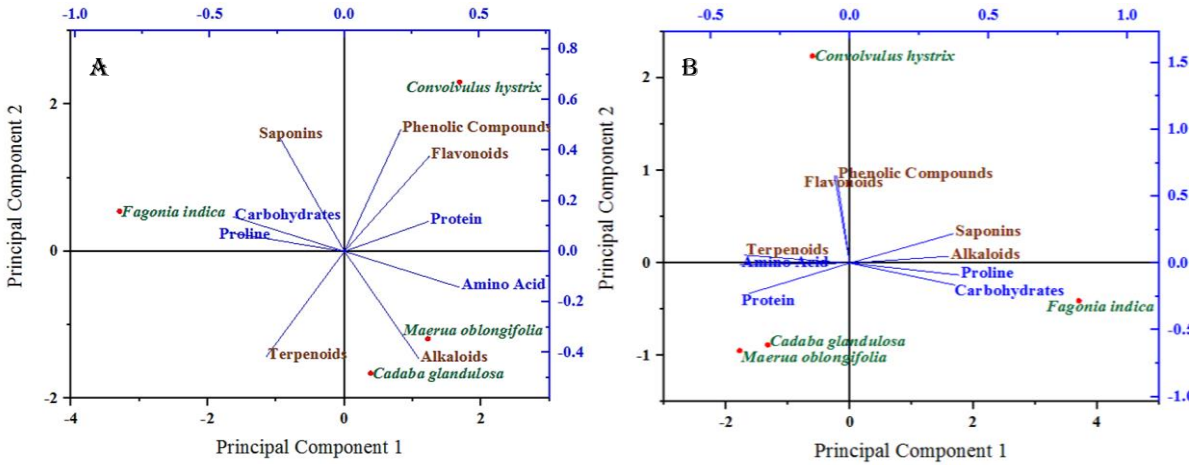

Figure (3): The biplot associations based on PC1 and PC2 between the primary and secondary metabolites in the investigated plant species. A, during the summer of 2021; B, during winter.

traditional medicine and as economically useful plants, studies on the possible effects of climate change on medicinal plants are particularly important. The use of medicinal plants in most developing countries, as a directive source for the maintenance of good health, has been extensively noted (Mahdi *et al*., 2023; Mahesh and Satish 2008, Tanwer and Vijayvergia 2010). Traditionally, investigators have frequently studied the seasonal change of natural product profiles in higher plants to determine the viability of specific plant materials for medical purposes. Furthermore, numerous analyses focus on the seasonal fluctuations of certain categories of plant metabolites in plant species that hold commercial and/or ecological significance (Ganthaler *et al*. 2017; Yang *et al*. 2018; Zidorn 2018; Li *et al*. 2020 and 2022). Our study findings demonstrated considerable variations in the phytochemical composition of the primary and secondary metabolites of the investigated plant species during the summer and winter seasons.

The present study revealed that the primary metabolites analyzed exhibited considerable variations within the plant species under investigation throughout the growing season. During the summer season, it was observed that plant tissues of all species acquired the highest levels of soluble carbohydrates. Nonetheless, the pool of amino acids exhibited no significant change in *C. hystrix* and *M. oblongifolia* with the growing season, however, it showed a higher level in *C. glandulosa* during the summer season and in *F. indica* during the winter season. The accumulation levels of proline and protein differed depending on the plant type and the season of growth. In this context, Obata and Fernie (2012) emphasized the importance of metabolic network reconfiguration in response to stressful situations as a means to restore the metabolic equilibrium and develop essential compounds to alleviate stress (Eissa *et al*.,.2023). In addition, it was reported that this reorganization may not result in a higher concentration of primary metabolites, and that the total quantity of primary metabolites may remain constant during osmotic stress (Quéro *et al*. 2014). Seasonal fluctuations due to the change in weather patterns during both summer and winter seasons could result in modifications in productivity, biomass yield, and accumulation of phytochemical content (Mishra *et al*. 2020).

The fluctuation in the quantity of primary metabolites in desert plants during summer and winter seasons is a crucial element of these plants' adaptive approaches to cope with changing climatic conditions. Desert plants were shown to undergo substantial changes in their biochemical composition in response to seasonal fluctuations. These plants undergo physiological stress throughout the summer due to elevated temperatures, limited water availability, and heightened solar radiation. As a response, these plants tend to amass elevated quantities of carbohydrates as a source of energy to endure periods of drought. Moreover, proteins and amino acids have pivotal functions in stress tolerance and defense processes, and their concentrations may also escalate in reaction to environmental stresses throughout the summer. In contrast, these plants have colder temperatures and decreased sunshine availability in the winter season. In order to adjust, they may direct additional resources towards the synthesis of proteins and amino acids, which have significant functions in enhancing cold tolerance and safeguarding against freezing damage (Adams 2016, Bechtold 2018, Ouyang *et al*. 2019, Fernández-Marín *et al*. 2020).

The levels of secondary metabolites in this study, including phenols, flavonoids, saponins, alkaloids, and terpenoids, exhibited substantial variability in the four plant species investigated, in response to the seasonal variation. With the exception of *M. oblongifolia*, the accumulation of phenolic compounds and alkaloids was higher during the winter season compared to the summer season. Saponins were shown to accumulate more in the investigated species during the summer season compared to the winter season, with the exception of *C. glandulosa* which showed a consistent level continuously. Flavonoids showed either not changed level or a higher level in the winter season, whilst terpenoids displayed varying patterns of increase, decrease, or no change depending on the plant species. Different investigations have demonstrated that plants produce many secondary metabolites in response to the surrounding stimuli. Plants have immediate responsiveness and adaptability to environmental stress through their metabolic processes, making it possible for them to quickly adapt to their environment (Jakovljevic *et al.* 2013). Hence, the discernible fluctuations in the concentrations of secondary metabolites over the seasons can serve as a reliable indicator for determining the optimal time to harvest medicinal plant material.

The impact of seasons on chemical composition and subsequent bioactivity can be ascribed to climatic variations, including temperature, soil moisture, precipitation, and the various phases of plant metabolic processes (Lemos *et al*. 2015, Ahmed *et al.* 2016). Seasonal variations, typically influenced by the supply of water, reveal a substantial enhancement in the production of secondary metabolites. Abiotic stress typically leads to a decrease in growth, which needs to be balanced against the potential rise in the production of secondary metabolites (Prinsloo and Nogemane 2018). Nevertheless, the reduction or stability of secondary metabolites level has been attributed to consequent processes such as translocation, leakage, or recycling (Covelo and Gallardo 2001). It has been demonstrated that environmental variables can potentially impact the synthesis of secondary metabolites. Tuteja and Sopory (2008) asserted that plants modify their metabolic pathway in accordance with fluctuations and annual oscillations depending on soil conditions, carbon dioxide concentrations, water, and nutrient accessibility, as well as temperature fluctuations. Hence, it is reasonable to anticipate temporal fluctuations in the synthesis of secondary metabolites due to compromises with other biological

processes or in response to biotic relations, both of which exhibit seasonal variations (López-Legentil *et al*. 2006).

The GC-MS analysis of the phytochemical constituents in the studied plant species revealed the existence of diverse chemical compounds, encompassing various chemical classes such as terpenoids, phenols, alkaloids, fatty acids, esters, benzothiazoles, alcohols, and steroids. The presence and the concentration of these compounds displayed variations in the studied species during both the summer and winter seasons. The presence of specific fatty acids during the summer season may be linked to the enhanced activity of synthesizing enzymes in response to heat stress. The analyses identified several 9,12-octadecenoic acid, hexadecanoic acid, tetradecanoic acid, eicosanoic acid, and pentadecanoic acid. The substantial concentration of fatty acids in these plants strongly supports their potential utilization as medicinal plants. Several studies suggested that consuming essential fatty acids in appropriate ratios can potentially prohibit obesity, lower the incidence of cardiovascular disorders, modification cell membrane structure, influence cell protein activities, regulate lipid mediator production, and impact gene expression patterns, ultimately leading to improved health (Marventano *et al.* 2015, Calder 2018, Sande *et al*. 2019, Jóźwiak *et al*. 2020).

Furthermore, the results revealed an increase in terpenoids in summer. The findings of Geron and Arnts (2010) align with our results, as they observed that varying air temperature ranges with similar overall real emission rates might lead to significant variations in terpenoid levels among the year seasons. Moreover, the presence of caryophyllene in *C. glandulosa* was observed throughout the winter season with a higher ratio than its occurrence in the summer. This means harvesting *C. glandulosa* throughout the winter season in order to obtain a higher concentration of caryophyllene. Caryophyllene has been identified as a sesquiterpene compound that possesses anti-inflammatory activities, as demonstrated in many investigations (Rogerio *et al*. 2009, Marques *et al*. 2019). Therefore, the physiological characteristics of plants can be affected by changes in the environment. It is important to consider that plant responses to seasonal fluctuations vary depending on the species.

In summary, the current study found that the plant species that were investigated exhibited a high concentration of specific phytochemicals throughout the summer season. The plants, in response to the elevated temperatures experienced during the summer, demonstrate an adaptive strategy by synthesizing unique secondary metabolites. These metabolites likely play a crucial role in enhancing the plants' resilience to adverse environmental conditions. In contrast, the winter season presents a distinct set of environmental conditions characterized by high levels of moisture. This particular climate encourages the growth and accumulation of different essential metabolites within the plant species. It becomes imperative, therefore, to consider the appropriate season for harvesting medicinal perennial plant species, taking into account their commercial and pharmacological significance. Understanding the seasonal variations in the metabolite profiles of these plants is crucial for optimizing their potential benefits.

Nevertheless, some plants, like *C. hystrix* and *F. indica*, are capable of synthesizing secondary metabolites during both seasons. The two plants in question can be harvested year-round in the Kingdom of Saudi Arabia for their therapeutic properties. By comprehending and harnessing the variations in their metabolite profiles, researchers can effectively optimize the harvest timing of these plants, maximizing their commercial and pharmacological potential. Furthermore, identifying plant species capable of synthesizing secondary metabolites consistently throughout the year contributes to the development of sustainable practices in the utilization of medicinal plants.

CONCLUSION

In conclusion, this study highlights the significant seasonal variations in the primary and secondary metabolite profiles of the four selected analyzed medicinal plant species in Jeddah, KSA. The findings indicate that environmental factors, particularly seasonality, play a crucial role in shaping the phytochemical composition and potential therapeutic properties of these plants. The distinct patterns of metabolite accumulated in response to seasonal changes may suggest that the optimal collection time for these plants which varies depending on the desired metabolic compounds. For example, *M. oblongifolia* is best harvested in the summer for higher proline and amino acids, while *C. glandulosa* and *F. indica* show a seasonal preference for different metabolites. The GC-MS analysis further underscores the prominence of fatty acids and therapeutic compounds like caryophyllene and vanillin. Meanwhile, *M. oblongifolia*, *C. glandulosa*, *F. indica*, and *C. hystrix* each demonstrated unique patterns of accumulated metabolites, suggesting that optimal harvesting times and cultivation practices are essential for enhancing their medicinal value. In addition, these findings reinforce the importance of considering seasonal factors as well as the developmental stages, and agronomic practices to optimize the medicinal value and yield of these plant species for therapeutic applications. Therefore, future research should focus on the implications of these variations for traditional medicine and sustainable cultivation practices, ensuring the effective utilization of these valuable plant resources.

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