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ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF DIFFERENT PLANT SPECIES COLLECTED FROM HAIL REGION, KINGDOM OF SAUDI ARABIA

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ABSTRACT

The discovery of bioactive compounds from natural sources entails an extremely important process in drug development. It is well known that an enormous molecular diversity and biological functionality are two important features that distinguish plant extracts as a drug source from synthetic chemicals. The six wild plant species collected from the desert of Hail city, (KSA), cleaned, air dried, grinded then extracted with ethanol 70% (crude extract). the crude extracts were tested for their antioxidant (using 2, 2 diphenyl-1-picrylhydrazyl (DPPH), 2, 2'- azino-bis{ethylbenzthiazoline-6-sulfonic acid (ABTS.)} methods) and anticancer activity (using MCF-7 and Hep.G2). in addition to identify the active ingredients using GC-MS. The antioxidant and anticancer activities of the crude extract exhibited the highest effect especially against ABTS radical (as antioxidant) and MCF-7 cell line (as anticancer). The extracts of Tamarix sp, Atriplex farinosa and Artemisia herba-Alba appeared highest activity with (99.56±2.81, 84.61±1.76 and 90.32±2.04 % at 200 ug/ml against ABTS), when compared with BHT and Vit. C as antioxidant standards (90.82±0.68 and 93.2±2.5 at 200 ug/ml against ABTS)) Moreover, these extracts gave (IC 50 with 44.2±1.7, 31.4±9.6 and 48.9±3.9 ug/ml respectively against MCF-7) when compared with DOX as anticancer standard. This activity was concentration dependent. The GC-MS results showed that there are 8 to 21 active compounds found in plant extract (6 samples) which revealed antioxidant and anticancer activities and the highest number of bioactive compounds found in extract no. 5 and 6. Also, the GC-MS exhibited that most of bioactive compounds undergo under three groups of secondary metabolites

(Phenolics, Flavonoids and terpenoids) which responsible for the biological activities of plant species extracts under study.

In conclusion, the study illustrated the potential of Tamarix sp, Atriplex farinosa as a valuable resource for natural compounds of desirable medicinal properties especially as antioxidants and anticancer.

Keywords: Wild plants - Anticancer – Antioxidant -Hail region – GC-MS

INTRODUCTION

In recent years, the development of novel drugs has become increasingly difficult, expensive and time consuming **[1, 2]**. The complexity of plant extract is presence of a complex mixture of uncharacterized compounds, which may mask the bioactivity of target compounds or cause false positive results, is the most common problem. The differences between areas where plants are grown, the methods of production and processing of plants are also important since these might affect the chemical profile of the natural products [NP] **[3]**.

Now NP discovery work considers herbal preparation, which consists of numerous multi components, as one active entity, which affects multiple targets in living organisms **[4]**. For such an approach, the response of the whole system needs to be observed with all possible tools such as clinical trials or experimental animal models, where both classic physiological observations (e.g. blood pressure, analgesic activity, sedative) and modern molecular observation (gene expression, proteome, metabolome) can be used to obtain better insight into different changes in organism.

Plant species are used medicinally in most countries and are a source of many potent drugs **[5]**. Therefore, the role of active ingredients from plants in maintaining human health is well documented **[6]**. The active ingredients of many drugs found in plant species were identified from natural products **[7]**. Amongst these compounds (at least 12,000) about only ten percent were identified **[8]**. The medicinally useful bioactive constituents were belong to secondary metabolites groups such as (plant acids, alkaloids, phenolics , flavonoids, glycosides and terpenoids) **[9]**. These active ingredients known to carry out important medicinal roles. They were also involved in protective role in animals and used as biomedicine **[10]**.

Floristic piece and vegetation examination in Hail district north of focal Saudi Arabia was considered by **[11]** Who observed that, A

sum of 124 animal categories speaking to 34 families were recorded.

Plants create a wide assortment of auxiliary metabolites. As such, just around 10% of the known plant species have been concentrated phytochemically, bringing about the confinement and distinguishing proof of at least one mixes in every animal groups. Right away, 100,000 distinctive optional metabolites are known. In addition, numerous auxiliary metabolites as of late have been perceived as remedial and infection preventive fixings in nourishment, now known as flutraceuticals **[12 and 13]**.

This investigation was carried out on crude ethanolic extract of six plant species collected from Hail region for potential anticancer and antioxidant activities. In addition to identification for active ingredients in promising extracts using chromatographic analysis.

MATERIALS AND METHODS

Chemicals and drugs

Pure organic solvents were purchased from E. Merck Co. (Darmstadt, Germany). DPPH and $ABTS^{\cdot+}$ were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Collection and extraction of plant species

Plant materials

Six wild plant species (Plantago sp, Rumex vesicarius, Artemisia herba-Alba, Anthemis sp, Tamarix sp and Atriplex farinosa) were collected from the desert of Hail region, KSA during spring season 2019. The collected plant samples were kindly identified by Prof. Dr. Sanaa M. Shanab, Prof. of Botany, Faculty of Science, Cairo University, Giza, Egypt, the plant was dried under shade and then grinded to fine powder. The dried powder (500g) was extracted with 70% aqueous ethanol by percolation in the solvent with occasional shaking for 48 h and the process was repeated three times. The ethanol extract was combined and concentrated under vacuum at 40 ºC to obtain a dry crude extract (32-38 g). The dry crude extract yield was 6.4 -7.6%.

GC/MS analysis of ethanolic extracts from different plant species

The six ethanolic extracts of different collected plant species were analyzed by GC-MS. The analysis was performed on a Thermoquest- Finnigan Trace GC-MS equipped with a DB-5 (5% (w/v) phenyl) methylpolysiloxane column (60 m \ 0.25 mm i.d., film thickness 0.25 µm). The injection temperature was 220 ºC and the oven temperature was raised from 40 ºC (3 min hold) to 250 ºC at a rate of 5 ºC/min, then held at 250 ºC for 2 min; transfer line temperature was 250 ºC. Exactly 1 µl of sample was injected and helium was used as

the carrier gas at a flow rate of 1.0 ml/min. The mass spectrometer was scanned over the 40 to 500 m/z range with an ionizing voltage of 70 eV and identification was based on standard mass library that National Institute of Standards and Technology (NIST Version 2.0) to detect the possible extract components.

Assessment of antioxidant activity

Two methods were used for antioxidant evaluation of crude extract as follows:

a- DPPH method

The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) tests were carried out for evaluation of antioxidant activity of plant extracts as described by Burits and Bucar **[14]**. One mL of *plant* crude extract at different concentration was mixed with 1mL DPPH reagent [0.002% (w/v)/ methanol solution]. After an incubation period (30 and 60 min), the absorbance was measured at 517 nm. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentages against extract concentrations compared with Butylated hydroxyl toluene (BHT) as synthetic antioxidant and Ascorbic acid (Vit. C) as natural antioxidant standard.

% Antioxidant activity (E) = ((Ac-At)/Ac) x 100

Where: **Ac** was the absorbance of methanolic DPPH solution (control) and At was the absorbance of the extract sample.

b- $ABTS^+$ radical cation scavenging assay

This assay was based on the ability of different fractions to scavenge 2, 2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\cdot+}$) radical cation in comparison to a standard (BHT and Ascorbic acid). The radical cation was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 h until the reaction was completed and the absorbance was stable. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734 nm for measurements. The photometric Assay was conducted on 0.9 mL of $ABTS^+$ solution and 0.1 mL of tested extracts and mixed for 45 s; measurements were taken immediately at 734 nm after 1 min. The antioxidant activity (E) of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

% Antioxidant activity (E) = (Ac-At)/Ac) x 100

Where **At** and **Ac** are the respective absorbance of tested sample and $ABTS^+$, **[15]**. Extracts concentration providing 50% inhibition (IC_{50}) was calculated from the

graph plotting inhibition percentages against extract concentration.

Assessment of cytotoxicity activity

a- Cell culture

Human hepatocellular cancer cell line (HepG-2), breast cancer cell line (MCF-7) were obtained from the Vacsera (Giza, Egypt). Cells were maintained in RPMI-1640 supplemented with 100 µg/mL streptomycin, 100 units/mL penicillin and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37 ºC **[16]**.

b- Cytotoxicity assays

1-Against Solid tumor cell line

The cytotoxicity of crude extracts from different plant species (six plants) were tested against HepG-2 and MCF-7 cells by SRB assay as previously described **[17]**. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to each test compound for 72 h and subsequently fixed with TCA (10%) for 1 h at 4 ºC. After several washings, cells were exposed to 0.4% SRB {sulforhodamine B (SRB), 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfo-benzenesulfonate} solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm. Doxorubicin (DOX) was used as anticancer standard.

Statistical analysis

Data were subjected to an analysis of variance, and the means were compared using the "Least Significant Difference (LSD)" test at 0.01 levels, as recommended by Snedecor and Cochran **[18]**. Data are presented as mean ± SD.

RESULTS AND DISCUSSION

Antioxidant activity

The DPPH and ABTS scavenging assay were performed to test the antioxidant activity (as %) for all six ethanolic extracts of *plant species collected from Hail region* **(Table 1)**. The crude ethanolic extract showed the high and moderate antioxidant activity ranged 43.95±0.75 to 99.56±2.81%. Four of samples tested (36 readings) recorded lower activities (less than 50%). However, two extract (No. 3 and 5) showed very high effect (more than 90%) as antioxidant especially against ABTS radical compared with DPPH radical scavenging result and synthetic (BHT) and natural (Vit. C) Standards.

The highest antioxidant activity was recorded for ethanolic extract of Tamarix sp plant when compared with other extracts in addition to natural and synthetic standards

(99.65, 90.82 and 93.20 % at 200 ug/ml against ABTS respectively). And this results were in agreement with the results reported by Mahfoudhi *et al*. **[19]** who suggest that *T. aphylla* may be considered as a potential sources of new antioxidant drugs especially phenolic compounds, e.g: flavonoids (such as apigenin , quercetin, , kaempferol, isorhamnetin, tamarixetin, quercetin, and kaempferol-7,4'-dimethyl-ether) and phenolic acids (such as gallic acid, caffeic acid, p-coumaric acid and ellagic acid).

The ethanolic extract of Artemisia herba-Alba came in the second level (rank) as antioxidant activity after Tamarix sp with 90.32 % at 200 ug/ml against ABTS. These results were in agreement with Bouzidi *et al*., **[20]** Who found that A. herba alba has promising use as a natural source of antioxidants that can be a valid alternative to replace chemicals.

It was observed that those ethanolic extracts showed a markedly higher ability to scavenge ABTS radicals than DPPH. Crude extract was scavenged the ABTS radical better than the values/results of DPPH radical and in some cases (extracts) more than standard AO compounds (e.g: BHT and ascorbic acid) especially extract no. 5 (with 94.06 and 99.56 % at 100 and 200 ug/ml respectively). This means that, the most extracts were strongly scavenged the ABTS radical than that of DPPH radical (except extracts no. 1 and 4). These results are in agreement with those of Awika *et al*., **[21]** who found that, ABTS radical method is a better choice than DPPH and more sensitive than DPPH radical. The ABTS method has the extra flexibility in that it can be used at different pH degree (unlike DPPH, which is sensitive to acidic pH levels) and thus is useful when evaluate the effect of pH on AO activity of various compounds. It is also useful for measuring antioxidant activity of plant extracted in acidic solvents (low pH). Additionally, ABTS radicalis more soluble in aqueous and organic solvents and is thus useful in studying the antioxidant activity of extracts in different media. Another advantage of ABTS+ radical method was that extracts reacted rapidly with ABTS radical in (PBS) buffer solution reaching during 30 min. to steady state. The DPPH radical reacted slowly with the extracts, approaching, but not reaching, steady state during 8 h.

The potent antioxidant activity make out by the crude extract in comparison to natural and synthetic AO standards may be due to the synergistic action of the biologically active compounds in these extracts as shown in **Table (3)**. This suggestion was previously

confirmed by Aboul-Enein *et al*., **[12]** Who found that the synergistic action of a wide spectrum of antioxidants may be more effective than the activity a single antioxidants compounds.

The antioxidant activity of ethanolic extracts from the promising plant species may be due to the presence of different active compounds **(as shown in Table 3)** and these compounds correlated with the presence of active groups such as hydroxyl group and unsaturated which show high ability for scavenging free radicals and prevent the oxidation processes. These observations were in agreement with the previously published results **[22]**.

Anticancer activity

SRB assay was used to assess the cytotoxicity of the crude extract of six ethanolic extracts from different plan species against two different solid tumor cell lines and. Different cell lines were used according to their origin and morphology as well as sensitivity and receptor sites behavior. The cytotoxicity parameter, IC_{50} was calculated as shown in **Table (2)**. The obtained results of the crude extract showed acceptable potency of extract sample (5 and 6) against HepG2 and MCF-7 cell lines with IC_{50} of 51.6±2.1, 44.2±1.7 and 56.1±12.3 and 31.4±9.6 µg/mL, respectively. However, HepG2 and EACC cell lines showed relatively higher resistance against the crude extract no. 3, 4, 1 and 2. with IC_{50} of 56±1.7, 87.2±3.5, 96.7±1.5 and 470.45±2.7 µg/mL respectively against HepG2, However, most of these extracts showed high activity against MCF-7 with IC50 of 48.9±3.9, 67.3±4.2, 154.7±6.0 and 395±6.3 µg/mL, respectively. when compared with Doxorubicin (DOX) as standard anticancer drug (with IC_{50}= 0.35 ± 0.05 and 0.51 ± 0.05ug/ml against HepG2 and MCF-7 respectively) as shown in **Table 2**. These results indicate that the effect of crude extract on Hep G2 and MCF-7 is concentration dependent through the concentrations tested (1-1000 µg/mL). This effect can be explained as receptor independent for these type of cells **[23-24]**.

It's worth mentioning, that the higher potency of the crude extract against cancer cell lines HepG2 and MCF-7, might be attributed to auto-synergistic effect of bioactive compounds in each extract **[25]**.

The GC-MS results

GC-MS analysis results of six ethanolic extracts from six plant species (includes the retention time (Rt) and percentage of peak of individual compounds) were presented in **Table (3)**. The number of chemical compounds in each extract was as the following: 22, 11, 27, 29, 27 and 18 for extract from 1 to 6 respectively, The major

compounds (compounds with more than 10% as relative percentage) was as the following: Extract no. 1 showed two major compounds i.e. Afromosin 7-O-glucoside (23.43 %) and 8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (10.94 %). Also, Extract no. 2 showed two major compounds i.e: 7,4'-Dimethoxy-3-hydroxyflavone (17.84%) and Isolongifolol (10.66%). Moreover, Extract no. 3 showed two major compounds i.e: Ledane (17.85%) and 2'-Hydroxy-4'-methoxyacetophenone (19.48%). Extract no. 4 showed also two major compounds i.e: 7,4'-Dimethoxy-3-hydroxyflavone (15.42%) and Gardenin (11.14%). However, Extract no. 5 showed three major compounds i.e: β-Sitosterol (10.0%), Vitamin E (14.14%) and 3,6,3',4'-Tetramethoxyflavone (13.98%). Also, Extract no. 6 showed the same number of extract no. 5 as: 6,2',4'-Trimethoxyflavanone (14.19%), Afromosin 7-O-glucoside (23.02%) and Isolongifolol (22.3%).

These compounds (major and minor) responsible for various biological activities as antioxidant, anticancer and antimicrobial activity are reported in different published articles **[26-71]** as shown in **Table (3)**.

From the biological activities mentioned in **Table (3)** for each compound, we can summarize the number and percentage of compounds which responsible for the antioxidant and anticancer activities as shown in **Figure 1**.

The data in **Figure 1** revealed that the highest bioactive compounds percentages were found in extract no. 5 and 6 with (86.75 and 94.05% respectively) and these results went in parallel with the results mentioned in **Tables (1 and 2)** for the antioxidant and anticancer activities followed by extracts no. 1, 4, 2 and 3 as (77.41, 55.88, 53.5 and 50.94% respectively).

Antioxidant-anticancer mechanism

Radical species (ROS and RNS) are products of a normal metabolism and play a vital role in different living organisms in response to any changes in intra- and extracellular environmental conditions (factors) **[72]**. Nucleic acids, Proteins, and lipids were significant targets for radical's attack, any modification/mutations or changes in the chemical structure of these compounds can increase the risk of mutagenesis **[73]**. Therefore, antioxidants ingredients especially (phenolic and flavonoids) are good scavengers for free radicals (ROS and RNS). The antioxidants prevent mutation in nucleic acids, proteins and lipid peroxidation in normal living cells this help for prevention of inflammation and reduce the chance for formation of cancer cells.

The present data shown in **Tables (1 and 2)** illustrated that extracts no. 3, 5 and 6 gave high activities as antioxidant and anticancer activities when compared with BHT, ascorbic acid and DOX as standards. Therefore, there are clear relationship between the antioxidant activity and anticancer potency of tested ethanolic extracts. Also, the data in **Table 3** revealed that most of active compounds identified in extracts no. 3, 5 and 6 had both activities as antioxidant and anticancer such as Terpineol, (-)-Verbenone Caryophyllene oxide, Spathulenol, Elemol, Isovitexin, 5,7,3',4'-Tetramethoxyisoflavone, Genistin in (extract no. 3 of Artemisia herba-Alba), Also, extract no. 5 of Tamarix sp showed these active compounds as antioxidant and anticancer i.e: 6,2',4'-Trimethoxyflavanone, Esculin, 2',5'-Dimethoxyflavone, Luteolin 5,7,3',4'-tetramethylether, Apigenin 8-C-glucoside, Quercetin 3,5,7,3',4'-pentamethyl ether, 6,4'-Dimethoxy-7-hydroxyisoflavone, Lutein, β Carotene, 7,3',4',5'-Tetramethoxyflavanone, Eugenol, Genistin, Moreover, other active ingredients found in extract no. 6 of Atriplex farinose i.e: 6,2',4'-Trimethoxyflavanone, 5-Hydroxyisovanillic acid, Elemol, Apigenin 8-C-glucoside, 5,7-Dimethoxyflavone, Quercetin 3,5,7,3',4'-pentamethyl ether, Afromosin 7-O-glucoside, 7,4'-Dimethoxy-3-hydroxyflavone, 6, 4'- Dimethoxy- 7-hydroxyisoflavone and β Carotene. The chemical nature of most of these active compounds are phenolic, flavonoids then terpenoids.

In this concern, various published papers during the past 2 decades has revealed the mechanism by which continued oxidative stress can lead to chronic diseases including cardiovascular, inflammation, cancer, diabetes, and neurological, and pulmonary diseases. Oxidative stress can activate many transcription factors such as HIF-1α, PPAR-γ, NF-κB, AP-1, β-catenin/Wnt, p53 and Nrf2. Activation of these transcription factors can lead to the expression of many various genes **[74]**.

On the other side, the role of antioxidant compounds (list mentioned in **Table no. 3** for each extract) on growth inhibition of cancer cells could be explained as one or more of following suggested mechanisms:

1. The resonance phenomena of double bonds and lone pair atoms (O, N and S) in the chemical structure of the active compound (phenolic, flavonoids and terpenoids). may lead to radical formation and reaction with N- bases of nucleic acids and lead to mutation in DNA of cancer cells and affect cell division (as anticancer agent) **[75 and 12]**.

2. By providing high concentration of oxygen, the antioxidant compounds may be affecting on tumor cells by acting with its free radical's form and in turn affect tumor hypoxia needed for cancer cells (because solid tumors have values less than 10 mmHg with fewer than 10% in the normal range) **[76]**.
3. Through effecting on redox system of cancer cells, the antioxidant compounds have been demonstrated to possess anticancer activity by inhibiting cancer cell initiation and promotion **[77]**.
4. The presence of antioxidant compounds with anticancer medicines enhances the role of chemotherapies **[78]**.
5. Various researchers have investigated that taking food rich with antioxidant compounds can help decrease the risk of developing from cancer in humans **[79]**.

Table 1: Antioxidant activity of six Plant species using DPPH and ABTS radical scavenging activity after (30 and 60 min.) as % compared to standards antioxidant

Conc. (ug/ml)	100			200		
Methods	DPPH		ABTS	DPPH		ABTS
Samples	30 min.	60 min.		30 min.	60 min.	
1	50.1±1.002^{de}	50.49±0.768^{de}	43.95±O.75^{h}	60.39±0.556^{f}	58.91±0.694^{f}	57.8±1.12^{f}
2	54.1±0.819^{d}	54.00±1.206^{c}	59.34±0.59^{f}	63.03±0.747^{e}	61.88±0.621^{e}	77.14±1.50^{e}
3	46.37±0.968^{f}	48.18±0.810^{e}	74.21±1.02^{d}	67.49±0.559^{d}	68.64±0.692^{d}	90.32±2.04^{c}
4	55.77±0.373^{c}	52.97±0.597^{cd}	49.45±0.76^{g}	59.41±0.453^{f}	58.58±0.410^{f}	54.94±1.00^{g}
5	63.20±0.436^{b}	64.20±1.007^{b}	94.06±2.01^{a}	74.75±0.782^{c}	73.93±0.483^{c}	99.56±2.81^{a}
6	50.16±0.535^{e}	51.32±0.741^{d}	61.09±1.34^{e}	59.24±0.506^{f}	59.57±0.570^{f}	84.61±1.76^{d}
BHT	90.3±0.681^{a}	89.02±0.853^{a}	85.4±2.0^{c}	92.63±1.43^{a}	93.5±1.404^{a}	90.82±0.68^{c}
Vit. C	88.8±1.012^{a}	88.3±0.513^{a}	90.2±2.1^{b}	90.3±1.05^{a}	90.9±0.900^{b}	93.2±2.5^{b}

Each value is presented as mean of triple treatments, means within each row with different letters differ significantly at P < 0.01 according to Duncan's multiple range test

1 Plantago sp, 2 Rumex vesicarius, 3 Artemisia herba-Alba, 4 Anthemis sp, 5 Tamarix sp, 6 Atriplex farinosa

Table 2: Cytotoxicity [as IC_{50} (µg/ml)] of plants crude extract on different solid tumor cell lines.

Sample	IC_{50} (µg/ml)	
	HepG2 cell line	MCF-7 cell line
1	96.7±1.5b	154.7±6.0b
2	470.45±2.7a	395±6.3a
3	56±1.7d	48.9±3.9d
4	87.2±3.5c	67.3±4.2c
5	51.6±2.1e	44.2±1.7d
6	56.1±12.3d	31.4±9.6e
*DOX	0.35 ± 0.05f	0.51 ± 0.05f

Each value is presented as mean of triple treatments, means within each row with different letters differ significantly at P < 0.01 according to Duncan's multiple range test

***DOX: Doxorubicin (Standard anticancer drug).**

1 Plantago sp 2 Rumex vesicarius 3 Artemisia herba-Alba 4 Anthemis sp 5 Tamarix sp 6 Atriplex farinosa

Table 3: GC-MS results of ethanolic extract of different plant species collected from Hail region

Compounds	Rt	Sample no.						Biological activities	References
		1	2	3	4	5	6		
6,2',4'-Trimethoxyflavanone	3.12	-	-	-	-	4.23	14.19	Antioxidant, anticancer and antibacterial	Liu et al. (26)
Esculin	3.35	5.00	-	-	-	1.82	-	capillary protection, antioxidant and antiinflammation	Joseph et al. (27)
2',5'-Dimethoxyflavone	5.12	-	-	-	-	0.46	-	Antioxidant, anticancer and antibacterial	Liu et al. (26)
Terpineol	5.86	-	-	1.28	-	-	-	antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive, anti-nociceptive	Khaleel et al. (28)
Undecanoic acid	12.39	4.79	-	-	-		-	Antioxidant and anticancer activities	Narra et al. (29)
2-Imidazolecarboxaldehyde	6.68		-	-	-	3.51	-	cytotoxicity and antimicrobial activities	Wang et al. (30)
2'-Hydroxy-2,3,4',6'-tetramethoxychalcone	7.50	4.55	-	-	-	-	-	Anticancer	Hui-Li et al. (31)
(-)-Verbenone	7.99	-	-	2.64	-	-	-	Antioxidant, anticancer, antimicrobial)	Sammaiah et al. (32)
5,7,3',4',5'-Pentahydroxyflavone	10.30	2.94	-	-	5.41	-	-	Antioxidant, anticancer and antibacterial	Liu et al. (26)
Morin	10.31	-	-	-	2.86	-	-	anti-inflammatory, antibacterial, and antiviral activity	Gopal (33)
Caryophyllene oxide	10.92	-	-	1.29	-	-	-	Antioxidant, antimicrobial and cytotoxic activities	Al-Fatimi et al. (34)
5-Hydroxyisovanillic acid	11.17	-	-	-	-	-	3.77	Antioxidant and cytotoxic activities	Khadem and Marles (35)
Spathulenol	11.30	-	-	1.93	2.12	-	-	Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities	do Nascimento et al. (36)
γ-Selinene	11.374	-	-	-	1.56	-	-	Antimicrobial and anticancer	Salleh et al. (37)
Elemol	11.489	-	-	2.84	-	-	-	Antimicrobial, antioxidant and anticancer	Carroll et al. (38)
Hexa-hydro-farnesol	11.46	2.10	-	-	-	-	1.33	Antimicrobial, anticancer	Naser et al. (39)
Luteolin 5,7,3',4'-tetramethylether	11.54	-	-	-	-	1.90	-	antioxidant and anticancer	Harris et al. (40)
β-Santalol	11.702	-	-	0.98	-	-	-	Anticancer	Arasada et al. (41)
cis-Thujopsene	11.731	-	-	1.95	-	-	-	Antimicrobial, anticancer	Chen et al. (42)
Isovitexin	11.842	-	-	1.61	-	-	-	anti-oxidant, anti-cancer, anti-inflammatory, anti-hyperalgesic, and neuroprotective effects	He et al. (43)
4'-Hydroxy-2'-methyl-3,4,5-trimethoxychalcone	12.305	-	-	1.40	1.65		-	Anticancer	Hui-Li et al. (31)
Gossypin	12.82	3.64	-	-	-		-	antidiabetic and antimicrobial	Venkatesan and Sorimuthu Pillai . (44).

Compounds	Rt	Sample no.						Biological activities	References
		1	2	3	4	5	6		
Apigenin 8-C-glucoside	12.87	-		-	-	2.0	1.59	Antimicrobial, antioxidant and anticancer	Al-Enazi et al. (45)
9-cis-Retinoic acid	12.98	-	1.17	-	-	0.87	-	differentiation and proliferation of A spermatogonia	Ingrid et al. (46)
5,7-Dimethoxyflavone	12.98	1.95	-	-	-	-	-	Antioxidant, anticancer and antibacterial	Liu et al. (26)
Neophytadiene	12.98			1.14				Antioxidant and antibacterial	Mallappa et al. (47)
Quercetin 3,5,7,3',4'-pentamethyl ether	13.01	-	-	-	-	0.51	0.72	Antioxidant and antiproliferative	Manthey, and Guthrie, (48).
Phytol	13.11	3.61	1.72	6.50	-	0.18	3.10	Antinociceptive and Antioxidant	Camila et al. (49)
Perillaldehyde	13.413	-	-	4.84	-	-	-	Antioxidant	Malu et al. (50)
Astilbin	13.523	-	-	6.02	-	-	-	Antioxidant	Trinah et al. (51)
4,6-Dimethyl-3-(4-hydroxyphenyl)coumarin	13.556	-	-	5.06	-	-	-	Antioxidant	Anish et al. (52)
Afromosin 7-O-glucoside	13.62	23.43	4.62	-	-	-	23.02	Antioxidant and Anticancer	Konoshima et al. (53)
7,4'-Dimethoxy-3-hydroxyflavone	13.76	3.32	17.84	6.52	15.42	-	5.14	Antioxidant, anticancer and antibacterial	Liu et al. (26)
6,4'-Dimethoxy-7-hydroxyisoflavone	13.76	-	4.32	-	2.99	1.93	1.41	Antioxidant, anticancer and antibacterial	Liu et al. (26)
β-Sitosterol	13.88	-	-	-	1.04	10	-	Antioxidant	Baskar et al. (54)
Lutein	13.92	-	-	-	2.06	2.21	-	Antioxidant, anticancer	Krinsky et al. (55)
Ascorbic acid, permethyl-	14.14	-	-	-	-	6.64	-	bone formation, wound healing and the maintenance of healthy gums and antioxidant	Khalid et al. (56)
β Carotene	14.237	-	-	-	0.38	6.18	0.77	Antioxidant, anticancer	Krinsky et al. (55)
7,3',4',5'-Tetramethoxyflavanone	14.257	-	-	0.40	-	1.1	-	Antioxidant, anticancer and antibacterial	Liu et al. (26)
Geranyl isovalerate	14.53	-	-	-	2.48	0.16	4.21	antileishmanial and cytotoxicity activities	Hamdi et al. (57)
Quinine	14.66	-	-	-	6.39	-	-		
9,12-Octadecadienoic acid (Z,Z)-	14.67	5.93	9.26	2.78	-	-	-	Antioxidant	Kaushik et al. (58)
8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	14.71	10.94	-	-	-	-	-	Antioxidant	
Isolongifolol	14.73	-	10.66	0.30	-	-	22.3	Antimicrobial and antioxidant	Ahmad et al. (59)
5β,7βH,10α-Eudesm-11-en-1α-ol	14.77	-	5.37	-	7.59	-	-		
Linolenic acid, methyl ester	14.81	2.47	3.97	-	-	-	-		
Retinal	14.85	4.32	-	-	1.82	-	-		
Ledane	14.799	-	-	17.85		-	-	Antimicrobial	Asit et al. (60)
Patchoulane	14.823	-	-	5.10	1.22	-	-	Antimicrobial	Bakkali et al. (61)
Vitexin	14.84	0.55	-	-	2.48	-	-	antioxidant activity, anti-Alzheimer's	Layana et al. (62)

Compounds	Rt	Sample no.						Biological activities	References
		1	2	3	4	5	6		
								and neuroprotective activity	
Kaempferol-7-O-neohesperidoside	14.913	-	-	1.27	-	-	-	Antioxidant	Piccolella et al. (63)
5,7,3',4'-Tetramethoxyflavone	15.00	-	0.87	-	-	-	2.51	Antioxidant, anticancer and antibacterial	Liu et al. (26)
Fisetin	15.307	-	4.21	-	-	-	-	Anti-inflammatory and antioxidant	Funakoshi-Tago et al. (64)
Gardenin	15.811	2.81	-	0.45	11.14	-	-	Antibacterial and antimalarial	Cuca Suarez et al. (65)
Kaur-16-ene	15.97	-	-	-	2.55	-	-		
Isovitexin	16.681	0.84	-	-	-	4.07		antioxidant activity, anti-Alzheimer's and neuroprotective activity	Layana et al. (62)
9-cis-Retinoic acid	16.69	-	-	-	1.76	0.65	0.87		
Vitamin E	17.21	-	-	-		14.14	-	Antioxidant	Leth and Sondergaard H. (66).
α-Bisabolol	17.49	-	-	1.23	7.50	4.92	-	Antimicrobial	Forrer et al. (67)
Eugenol	17.53	-	-	-	-	6.14	-	Antiinflammatory , antioxidant and antimicrobial	Barboza et al. (68)
2'-Hydroxy-4'-methoxyacetophenone	17.895	9.23	-	19.48	1.46	-	-	antimicrobial	Kaithwal et al. (69)
5,7,3',4'-Tetramethoxyisoflavone	18.317	-	-	3.20	1.83	-	-	Antioxidant, anticancer and antibacterial	Liu et al. (26)
3-(3,4-Dimethoxyphenyl)-4-methylcoumarin	18.986	1.73	-	-	-	-	-	Antioxidant	Anish et al. (52)
Genistin	18.998	-	-	0.47	1.69	1.74	-	Antioxidant, anticancer and in osteoporosis, cardiovascular diseases	Polkowski and Mazurek (70)
3,6,3',4'-Tetramethoxyflavone	20.54	2.11	-	-	-	13.98	-	Antioxidant, anticancer and antibacterial	Liu et al. (26)
1-Hexacosanol	21.16	-	-	-	2.56	6.46	4.99	Antimicrobial activity	Rios and Recio (71)
3-(3,4-Dimethoxyphenyl)-7-methyl-4-phenylcoumarin	21.988	3.62	-	-	1.10	1.06	3.52	Antioxidant	Anish et al. (52)
3-Hydroxy-7,8,2'-trimethoxyflavone	22.00	-	-	-	1.37	2.79	3.33	Antioxidant, anticancer and antibacterial	Liu et al. (26)
Stigmasterol	22.25	-	-	-	8.13	-	3.14	Antioxidant	Baskar et al. (54)
5,7-Dimethoxycoumarin	22.75	-	-	-	1.31	-	-	Antioxidant	Anish et al. (52)

1 Plantago sp, 2 Rumex vesicarius, 3 Artemisia herba-Alba, 4 Anthemis sp, 5 Tamarix sp, 6 Atriplex farinosa

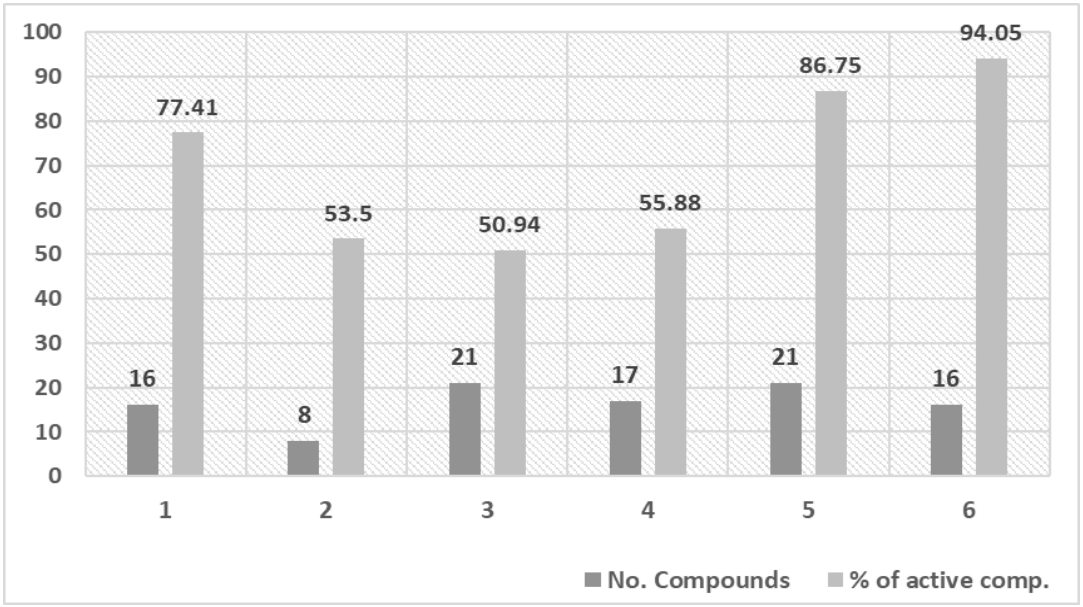

Figure 1: The number of bioactive compounds and the percentage of antioxidant and anticancer compounds presented in plant extracts (6 species) as result of GC-MS analysis

CONCLUSION

It can be concluded that the obtained results/data may encourage a pig project for making a pharmaco-economic value in Hail city, KSA. The present study reveals the high potency of crude extracts of six plant species from Hail region as antioxidants and potential anticancer when comparison to natural and synthetic standards (Vit. C, BHT and DOX). This crude extract represents high economic and industrial value for the production of valuable product. Due to its the contents from the active ingredients (phenolic, flavonoids and terpenoids) this led to potentially used these extracts as natural antioxidant and anticancer formulas.

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Competing interests

The authors declare that they have no competing interest.

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