

Full Length Research Paper

Metabolomic profiling, antioxidant capacity and *in vitro* anticancer activity of some compositae plants growing in Saudi Arabia

Sabry A. El-Naggar^{1,2*}, Ibrahim B. Abdel-Farid^{1,3}, Hassan A. Elgebaly¹ and Mousa O. Germoush¹

¹Biology Department, College of Science, Aljouf University, Kingdom of Saudi Arabia.

²Zoology Department, Faculty of Science, Tanta University, Tanta 3111, Egypt.

³Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt.

Received 30 May, 2015; Accepted 26 June, 2015

The present study was conducted to evaluate the metabolic profiling, antioxidant capacity and anticancer activities of some common widely grown plants of the family Compositae. The total phenolics, flavonoids, anthocyanins, saponins, total antioxidant capacity (TAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were determined in the selected plant extracts. *In vitro* anticancer activity was also assessed using human hepatocellular carcinoma (HepG-2) and breast adenocarcinoma (MCF-7) cell lines. The plant species revealed different metabolomic profiling. *Artemisia* showed the highest contents of the detected secondary metabolites compared to other plant extracts. *Pulcaria crispera* showed the highest inhibition concentration 50% (IC₅₀) among the screened extracts against HepG-2 (8.9 µg/ml) and MCF-7 (8.14 µg/ml). The high performance liquid chromatography analysis (HPLC) of *P. crispera* extract revealed the presence of high content of three phenolic compounds, benzoic, chlorogenic acid and vanillic acid, along with two polyphenolic compounds, hesperidin and quercetrin. In summary, among the screened extracts, *P. crispera* has the most potent anti-tumor activity *in vitro* against HepG-2 and MCF-7 cell lines.

Key words: Metabolomic, antioxidant, anticancer, plant, extracts, HepG-2, MCF-7, cell lines.

INTRODUCTION

Kingdom of Saudi Arabia (KSA) flora is rich with wild medicinal plants. In traditional medicine, these plants are used to treat several human diseases (Alyemeni et al., 2010; Aboul-Enein et al., 2012; Kuete et al., 2013). Moreover, there are several studies that reported on the metabolic, antioxidant and anticancer activity of some wild growing medicinal plants in the Kingdom of Saudi

Arabia (Sher et al., 2012). Recently, due to the severe side effect of using the conventional chemotherapy for cancer treatment, several studies have been carried out to find either new and safe targeted therapy against cancer cells only, or to find natural compounds that ameliorate the side effects of the conventional chemotherapy (Patra et al., 2002; Kumar and Kuttan,

*Corresponding author. E-mail: sabry_elnaggar@yahoo.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

2005; Harlev et al., 2012). Therefore, recent studies have been directed to screen, and evaluate the new compounds naturally present especially in the medicinal plants (Patra et al., 2002). For instance, the methanolic extracts of 40 species of plants traditionally used in KSA for the treatment of several diseases were tested for their anticancer activity (Almehdar et al., 2012). By using human breast cancer (MCF-7) and human leukemia (HL-60) tumor cell lines, it has reported that *Sesbania grandiflora* extract has potent *in vitro* cytotoxic activity (Ritesh et al., 2011).

The family *Compositae* is one of the largest families of flowering plants used in traditional medicine. For instance, *Pulicaria crispa* is used to treat inflammation, as an insect repellent, and as a herbal tea (Ross et al., 1997). *Achillea fragrantissima* is used to treat respiratory diseases and gastrointestinal disturbances (Elmann et al., 2011). Therefore, several studies have been done to investigate its anticancer activity. For example, by using human chronic myeloid leukemia (K562), T cell lymphoma (Jurkat) and hepatocellular carcinoma (HepG-2) cell lines, *A. fragrantissima* extract showed a potential anticancer activities *in vitro* (Alenad et al., 2013). In other study, the cytotoxic effect of an aqueous extract of *A. fragrantissima* against HepG-2 cell line was reported (Thoppil et al., 2013). A recent study showed that the anti-proliferative activity of the hydro-alcoholic *A. fragrantissima* and *A. falcata* extracts against the MCF-7 cells did not possess cytotoxic activity up to 200 µg/ml (Hammad et al., 2014). *Artemisia campestris* aerial parts exhibited growth inhibition of HT-29 human colon cancer cells (Akrouf et al., 2011). Aerial parts of *P. crispa* showed cytotoxicity in EJ-38 human bladder carcinoma cell (Stavri et al., 2008). Interestingly, it has been reported that plants grown under desert stress conditions produced a high concentration of secondary metabolites that possess a wide range of pharmacological effects, including anticancer activities (Harlev et al., 2012). The aim of this study was to assess the metabolic profiling, antioxidant and anticancer activity of some plants that belong to family *Compositae* that grow widely in the northern region of KSA.

MATERIALS AND METHODS

Collection of plant materials and preparation of plant extracts

Achillea falcata (leaves- flowers), *A. fragrantissima* (leaves- flowers), *Artemisia judaica* (leaves- flowers and seeds), *Artemisia sieberi* (leaves- flowers and seeds), *P. crispa* (shoot system), *Pulicaria incisa* (shoot system) and *Rhyanteruim eppaposum* (shoot system and flowers) were collected from desert, around Sakaka City, Aljof Region, KSA. The plant materials were identified and authenticated by taxonomist at Camel and Range Research Center, Sakaka, Aljof, KSA. The material was shade dried then ground to powder using an electrical mortar. The powdered material was stored in air tight container until further use. The shade dried (50 g) powder of each plant material was filled in a conical flask containing 80% methanol. After 4 days, the extract was filtered and

concentrated in a rotary evaporator at a temperature not exceeding 50°C.

Spectrophotometrical analysis

Determination of total phenolics and flavonoids

Total concentration of phenolics in the extracts was determined using Folin-Ciocalteu reagent with gallic acid as a standard, and expressed (mg) as gallic acid equivalents per gram of extract, according to Singelton et al. (1999). Total flavonoids content was determined using the aluminium chloride colorimetric method with quercetin as a standard, and expressed (mg) as quercetin equivalent per gram of extract according to Zhishen et al. (1999).

Determination of saponins

Saponins content was determined using vanillin solution, according to Ebrahimzadeh and Niknam (1998), and expressed (mg) as saponins equivalents per gram of extract.

Determination of anthocyanins

The anthocyanins content of the plant was determined according to the modified method by Padmavati et al. (1997). 100 mg of plant materials was dissolved in acidified methanol in well closed tubes covered with aluminium foils and incubated in a refrigerator for 24 h. The absorbance was read at 530 nm and 657 nm. The concentration was calculated using the following equation:

$$\text{anthocyanin concentration } (\mu\text{mol/g}) = ([A_{530} - 0.33 \times A_{657}] / 31.6) \times (\text{volume [ml]} / \text{weight [g]})$$

Determination of total antioxidant capacity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays

Total antioxidant capacity (TAC) was determined using phosphomolybdenum method, according to Prieto and Pineda (1999). The antioxidant capacity was expressed as ascorbic acid equivalent. Free radical scavenging activity of the sample extracts was determined spectrophotometrically using the method of Blois (1958), after obtaining crude extracts from the samples through evaporation of the solvent. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\% \text{ radical scavenging activity} = (A_c - A_s / A_c) \times 100, \text{ where } A_c = \text{Absorbance of negative control at 517 nm and } A_s = \text{Absorbance of sample at 517 nm (Wang and Mazza, 2002).}$$

Cancer cell lines and *in vitro* studies

Human breast adenocarcinoma (MCF-7) and hepatocellular carcinoma (HepG-2) were obtained from American type culture collection. Cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 2 ml glutamine, containing 100 units/ml penicillin and 100 units/ml streptomycin at 37°C / 5% CO₂

Plant extracts and doxorubicin preparation for *in vitro* use

Different concentrations of each methanolic extract were prepared

at 6.25, 12.5, 25, 50, and 100 µg/ml dissolved in DMSO (1%). Doxorubicin (Dox.) was also prepared at the same concentrations, 6.25, 12.5, 25, 50, and 100 µg/ml dissolved in DMSO (1%) under the same conditions and used as a positive control.

Determination of inhibition concentration 50% (IC50) for extracts using sulforhodamine B (SRB) colorimetric assay

The cytotoxicity of the plants extracts was tested against MCF-7, HepG-2 cell lines by SRB assay, according to Vichai and Kirtikara (2006). Briefly, the adherent cells were collected after trypsinization using 0.25% Trypsin-EDTA then washed twice and plated in 96-well plates at 1000 to 2000 cells/well. Cells were exposed to different extracts for 72 h and subsequently fixed with 10% trichloroacetic acid (TCA) for 1 h at 4°C. After several washings using distilled water, cells were exposed to 0.4% SRB solution (dissolved in 1% glacial acetic acid) for 10 min in dark place. A 1% glacial acetic acid was used to wash the plates several times. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 570 nm with micro plate reader. The results were linear over a 20-fold range of cell numbers and the sensitivity is comparable to those of fluorometric methods.

Identification and quantification of phenolics and flavonoids by HPLC

Analyses were carried out using a Perkin-Elmer HPLC system (USA) equipped with a binary LC-250 gradient pump and LC-290 UV/Vis detector. Samples were separated on C18 Hypersil ODS column (100 x 4.6 mm) with 5 µm particle size according to Ruiz et al. (2011). The mobile phase consisted of eluent A, 3.0 % acetic acid in water (v/v) and eluent B, methanol. The elution gradient was: at 0 min, 0% B; at 10 min 10% B; at 40 min, 70% B; at 50 min 0% B at a constant flow rate of 1 ml min⁻¹. Phenolic compounds were monitored by absorption at 280 and 330 nm. All measurements were performed in triplicates. Individual phenolic compounds of each sample were identified by comparing their relative retention time with those of the standard mixture chromatogram. A mixture of 17 standard (HPLC grade) phenolic compounds was used for the HPLC analysis. The concentration of each identified compound was calculated by comparing its peak area with that of the comparable standard, then converted to mg phenolic g⁻¹ dried extract. All standards and solvents were HPLC spectral grade.

Statistical analysis

One-way analysis of variance (ANOVA) through MINITAB (version 12.21) was used to test the significance of quantitative data of phytochemical analysis. For SRB assays, the Sigma Plot (version 12) program was used to analyze the generated data.

RESULTS

Metabolomic profiling and antioxidant capacity of plant extracts

The potentiality of anticarcinogenic activity of a plant extract depends on the metabolomic profiling of that extract. Metabolomic profiling of ten extracts of 4 genera belonging to family Compositae were detected through the determination of the TAC and DPPH radical

scavenging activity. The results showed that each genus from Asteraceae has its specific metabolomic profiling.

Artemisia and Pulicaria plants showed the highest content of phenolics

As shown in Figure 1A, the phenolics content showed specific pattern of variation among different genus, different species and organs. The highest content of phenolics content was registered in *Artemisia* and *Pulicaria* plants. *A. sieberi* (MPT-30) and *Pulicaria incisa* (MPT-52) showed higher content of phenolics comparing to *A. judaica* (MPT-34, leaves and flowers) and *P. crispa* (MPT-32), respectively. Although there is no significant difference between the content of phenolics in both *Artemisia* species, there was a significant difference ($p = 0.020$) detected between *P. incisa* and *P. crispa* (MPT-52 and MPT-32). Significant difference was also detected between the content of phenolics in *Achillea falcata* and *A. fragrantissima* (MPT-31 and MPT-33).

Achillea, Artemisia and Pulicaria have a higher content of flavonoids than Rhyanterium

Three genera of Compositae (*Achillea*, *Artemisia* and *Pulicaria*) showed high levels of flavonoids, while the fourth (*Rhyanterium*) showed moderate level of flavonoids (Figure 1B). Both of *A. judaica* and *A. sieberi* (MPT-34 and MPT-30) showed the highest content of flavonoids among other species and organs followed by *A. falcata* (MPT-31) and *P. incisa* (MPT-52). The content of flavonoids showed significant difference between *A. falcata* and *A. fragrantissima* ($p = 0.002$). Significant difference ($p = 0.017$ and 0.005) was also observed between the content of flavonoids in *A. judaica*, *A. sieberi* seeds (MPT-15 and MPT-51) and *R. eppaposum* (MPT-2), respectively.

Saponins content showed highly variation among different species and different organs

Saponins content in plant extracts showed highly variation among not only Compositae genera but also among different species and different organs of each species (Figure 2A). The genus *Artemisia* showed the highest content of saponins comparing to other genera followed by *Achillea*. Among species and plant parts of *Artemisia*, *A. sieberi* (MPT-30) showed the highest content of saponins. Seeds of *A. judaica* (MPT-15) showed higher content of saponins than seeds of *A. sieberi* (MPT-51) (in both cases no significant difference between the content of saponins in both *A. sieberi* and *A. judaica* leaves and flowers or seeds was detected). *A. falcata* showed higher content of saponins than *A. fragrantissima* (MPT-31 and MPT-33). *P. incisa* (MPT-52)

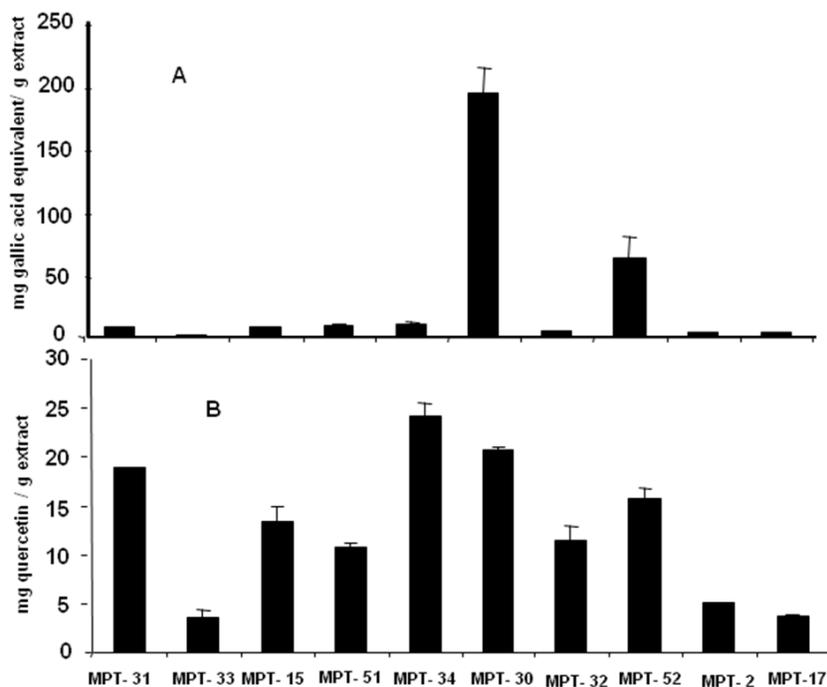


Figure 1. The phenolics (A) and flavonoid (B) concentrations in the different methanolic plant extracts: MPT-31 (*Achillea falcata*, leaves- flowers), MPT-33 (*Achillea fragrantissima*, leaves- flowers), MPT-15 (*Artemisia judaica*, seeds), MPT-51 (*Artemisia sieberi*, seeds), MPT-34 (*Artemisia judaica*, leaves- flowers), MPT-30 (*Artemisia sieberi*, leaves-flowers), MPT-32 (*Pulcaria crisa*, shoot system), MPT-52 (*Pulcaria incisa*, shoot system), MPT-2 (*Rhyanteruim eppaposum*, shoot system) and MPT-17 (*Rhyanteruim eppaposum*, flowers).

showed higher content of saponins than *P. crisa*. In cases of *Achillea* and *falcata*, a significant difference ($p = 0.002, 0.025$) between the content of saponins in the two parts of the two genera was observed.

***Artemisia* and *Rhyanterium* showed the highest content of anthocyanins of all tested genera**

Artemisia (MPT-15 and MPT-51) and *Rhyanterium* (MPT-17) showed the highest content of anthocyanin compare to other extracts but only significant difference was observed between *R. eppaposum* shoot and flowers (MPT-2 and MPT-17) (Figure 2B).

TAC and DPPH radical scavenging activity

Regarding total antioxidant capacity, as shown in Figure 3 and Table 1, the extracts having high content of the detected secondary metabolites showed higher TAC. *Artemisia* (*A. judaica* and *A. sieberi*), *Achillea* (*A. falcata*), *P. crisa* and *R. eppaposum* (shoot) showed higher TAC than other extracts. DPPH radical scavenging activity showed that *R. eppaposum* (MPT-2), *P. incisa* (MPT-52),

R. eppaposum (MPT-17), *A. falcata* (MPT-31) and *A. sieberi* (MPT-30) showed the highest inhibition percentage registering 92, 91, 84, 79 and 76%, respectively. These extracts showed the lowest IC50 among other extracts (54, 55, 59, 63 and 66 $\mu\text{g/ml}$), respectively.

The inhibition concentration 50% (IC50) of different extracts on HepG-2 cell lines

Different concentrations of each extract were prepared at 6.25, 12.5, 25, 50, and 100 $\mu\text{g/ml}$ dissolved in DMSO (1%), versus the conventional chemotherapeutic drug, doxorubicin (Dox.) as a positive control under the same conditions. The HepG-2 line was used to assess the IC50 *in vitro* after 72 h post treatments. The results showed that compared with the positive control Dox. (IC50 3.07 $\mu\text{g/ml}$), the IC50 of *P. crisa* (MPT-32), *P. incisa* (MPT-52), *A. fragrantissima* (MPT-33) and *R. eppaposum* (MPT-2) on HepG-2 cell lines were 8.9, 11.08, 11.04 and 25.21 $\mu\text{g/ml}$, respectively (Table 2 and Figures 4 and 5). The results showed also that the IC50 of *A. falcata* (MPT-31; leaves- flowers), *A. judaica* (MPT-34), *A. sieberi* (MPT-51), *A. sieberi* (MPT-30), *R. eppaposum* (MPT-17)

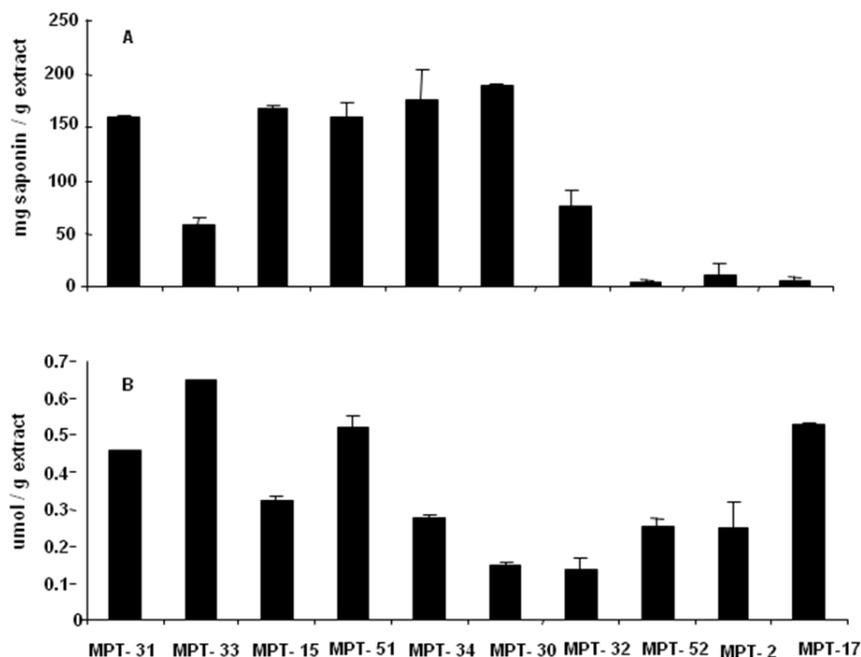


Figure 2. The saponins (A) and anthocyanins (B) concentrations in the different methanolic plant extracts: MPT-31 (*A. falcata*, leaves- flowers), MPT-33 (*A. fragrantissima*, leaves- flowers), MPT-15 (*A. judaica*, seeds), MPT-51 (*A. sieberi*, seeds), MPT-34 (*A. judaica*, leaves- flowers), MPT-30 (*Artemisia sieberi*, leaves- flowers), MPT-32 (*Pulcaria crispera*, shoot system), MPT-52 (*P. incisa*, shoot system), MPT-2 (*R. eppaposum*, shoot system) and MPT-17 (*R. eppaposum*, flowers).

Table 1. Shows the DPPH and inhibition concentration IC50.

Plant species	Code no.	DPPH	IC50
<i>Achillea falcata</i>	MPT-31	79.0±0.5	63.2
<i>Achillea fragrantissima</i>	MPT-33	ND	ND
<i>Artemisia judaica</i>	MPT-15	43.18±8.3	115.7
<i>Artemisia sieberi</i>	MPT-51	33.03 ± 342	151
<i>Artemisia judaica</i>	MPT-34	31.49±4.1	158
<i>Artemisia sieberi</i>	MPT-30	76.0±0.18	65.8
<i>Pulcaria crispera</i>	MPT-32	0.38±2.7	12966
<i>Pulcaria incisa</i>	MPT-52	91.0±1.9	55
<i>Rhyanteruim eppaposum</i>	MPT-2	92.0±9.0	54
<i>Rhyanteruim eppaposum</i>	MPT-17	84.0±1.2	59.2

and *A. judaica* (MPT-15) on HepG-2 cell lines were 38.85, 71.55, 74.04, 94.88, 127.47 and 445.13 ug/ml, respectively (Table 2 and Figures 4 and 5).

The inhibition concentration 50% (IC50) of different extracts on MCF-7 cell lines

The inhibition concentration 50% (IC50) of the previous plant extracts was also determined using MCF-7 cell lines

in vitro after 72 h post treatments. Different concentrations of each plant extracts and Dox. were prepared. The results showed that compared with the Dox. (IC50 2.41 ug/ml), the IC50 of *P. crispera* (MPT-32; shoot system), *P. incisa* (MPT-52), *R. eppaposum* (MPT-2) and *A. sieberi* (MPT-30) were 8.14, 10.25, 9.02 and 9.62, respectively (Table 2 and Figure 2B). The data also showed that *A. judaica* (MPT-34), *Achillea falcata* (MPT-31), *A. sieberi* (MPT-51), *A. fragrantissima* (MPT-33), *A. judaica* (MPT-15) and *R. eppaposum* (MPT-17) were

Table 2. Shows the inhibition concentration 50% (IC50) of extracts versus the positive control, doxorubicin on HepG-2 and MCF-7 cell lines *in vitro* after 72 h post treatments.

Plant species	Code no.	Extracted from	IC50 (HepG-2) (ug/ml)	IC50 (MCF-7) (µg/ml)
<i>Achillea falcata</i>	MPT-31	Leaves- flowers	38.85	38.85
<i>Achillea fragrantissima</i>	MPT-33	Leaves- flowers	11.04	70.07
<i>Artemisia judaica</i>	MPT-15	Seeds	445.13	102.55
<i>Artemisia sieberi</i>	MPT-51	Seeds	74.04	66.71
<i>Artemisia judaica</i>	MPT-34	Leaves- flowers	71.55	32.92
<i>Artemisia sieberi</i>	MPT-30	Leaves-flowers	94.88	9.62
<i>Pulcaria crispa</i>	MPT-32	Shoot system	8.9	8.14
<i>Pulcaria incisa</i>	MPT-52	Shoot system	11.08	10.25
<i>Rhyanteruim eppaposum</i>	MPT-2	Shoot system	25.21	9.02
<i>Rhyanteruim eppaposum</i>	MPT-17	Flowers	127.47	2651.88
Dox. (positive control)			3.07	2.41

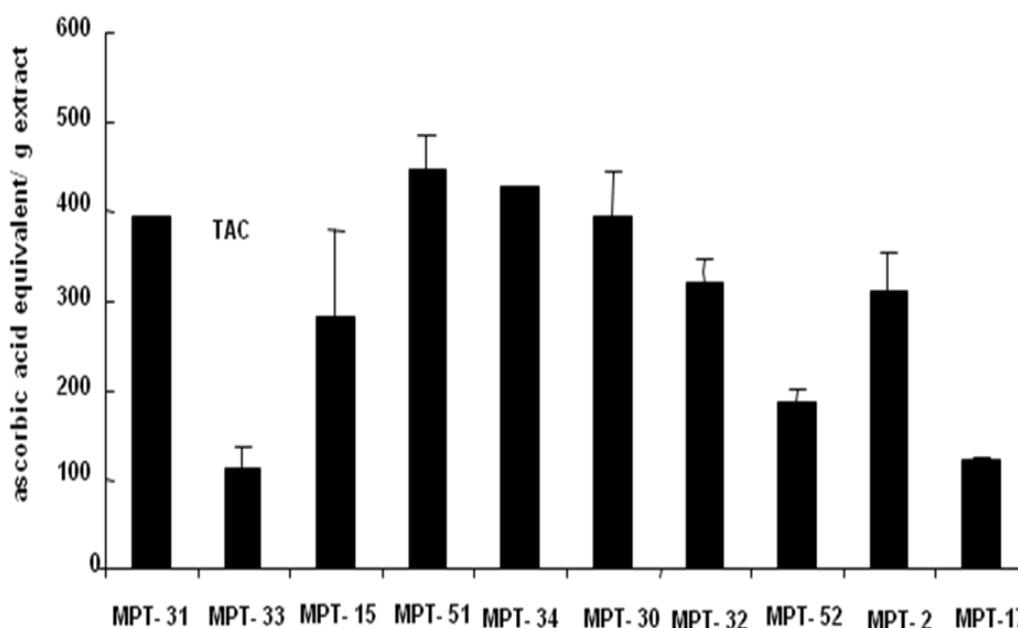


Figure 3. The total antioxidant capacity (TAC) in the different methanolic plant extracts: MPT-31 (*A. falcata*, leaves- flowers), MPT-33 (*A. fragrantissima*, leaves- flowers), MPT-15 (*A. judaica*, seeds), MPT-51 (*A. sieberi*, seeds), MPT-34 (*A. judaica*, leaves- flowers), MPT-30 (*A. sieberi*, leaves-flowers), MPT-32 (*P. crispa*, shoot system), MPT-52 (*P. incisa*, shoot system), MPT-2 (*R. eppaposum*, shoot system) and MPT-17 (*R. eppaposum*, flowers).

32.92, 38.85, 66.71, 70.07, 102.55 and 2651.88 µg/ml (Table 2, Figures 6 and 7), respectively. The 5 most effective extracts of the evaluated plants against carcinogenic cell lines were sorted out, and the ANOVA revealed that the 5 extracts are significantly differed from each other in phenolics, flavonoids, saponins, TAC and DPPH radical scavenging activity. Pearson's correlation showed that the TAC is significantly positive correlated with the content of phenolics in the 5 plant extract ($p = 0.026$).

HPLC analysis for phenolic of *P. crispa*

Based on the phytochemical analysis and anticancer activities of the tested plant extracts, the *P. crispa* methanolic extract showed the highest cytotoxic effect against HepG-2 and MCF-7 cell lines *in vitro*. To this end, the study further analyzed this extract by HPLC to determine the major phenolic and flavonoid compounds. The results showed that the major phenolic compounds were benzoic acid, chlorogenic acid and vanillic acid

Table 3. Assignment of the HPLC data peaks of phenolic of MeOH extracts from *P. crispera* shoot system.

S/N	Phenolic	Conc. µg/ml
1	Chlorogenic acid	55.88
2	Vanillic acid	47.94
3	Catechol	9.48
4	Saylcilic acid	15.79
5	Pyrogallol	18.22
6	Benzoic acid	92.38
7	P-OH-benzoic	5.25
8	Cinnamic acid	5.49
9	Ellagic acid	18.08
10	3-OH- Tyrosol	20.58
11	Epicatechein	7.95

Table 4. Assignment of the HPLC data peaks of flavonoids of MeOH extracts from *P. crispera* shoot system.

S/N	Flavonoids	Conc. µg/ml
1	Hesperidin	614.79
2	Rutin	9.25
3	Naringin	2.22
4	Rosmarinic acid	5.95
5	Quercetin	4.84
6	Kampherol	1.47
7	Hispertin	5.88
8	Apigenin	1.9
9	Naringenin	2.22
10	7-OH flavone	0.59
11	Quercetrin	50.79

(Table 3 and Figure 6). Moreover, two flavonoid compounds namely hesperidin and quercetrin were identified as a major content in the extract (Table 4 and Figure 8).

DISCUSSION

In this study, the 4 genera of family Compositae showed different pattern of variation regarding the secondary metabolites content. The results showed difference in metabolomic profiling among the different species of the same genera, and among different organs of the same species. The 4 genera under evaluation could be arranged as following, regarding their metabolic content: *Artemisia* > *Pulicaria* > *Achillea* > *Rhyanterium*. This finding is in agreement with Abdel-Farid et al. (2014). Also, consistent with this study finding, different species of *Acacia* showed different metabolomic profiling by spectrophotometric analysis by Abdel-Farid et al. (2014).

In this study, was found the extracts which have the highest secondary metabolites content such as those of *A. sieberi*, *P. incisa* and *R. eppaposum* which also showed the highest TAC and DPPH radical scavenging activity. This finding is in agreement with previous reports such as Basar et al. (2013) and Abdel-Farid et al. (2014), which showed that there is a significant positive correlation between various secondary metabolites content such as phenolics, flavonoids, saponins and anthocyanins with the total antioxidant capacity. Abdel-Farid et al. (2014) reported TAC was found to be positively correlated with saponins and flavonoids content. Based on this study data, some plant extracts were found to be rich with some important secondary metabolites which could contribute significantly to the anticancer activity.

In the present study, the cytotoxic effect of 10 alcoholic plant extracts from 4 genera of family Compositae was evaluated by SRB assay using HepG-2 and MCF-7 cell lines *in vitro*. According to the American National Cancer

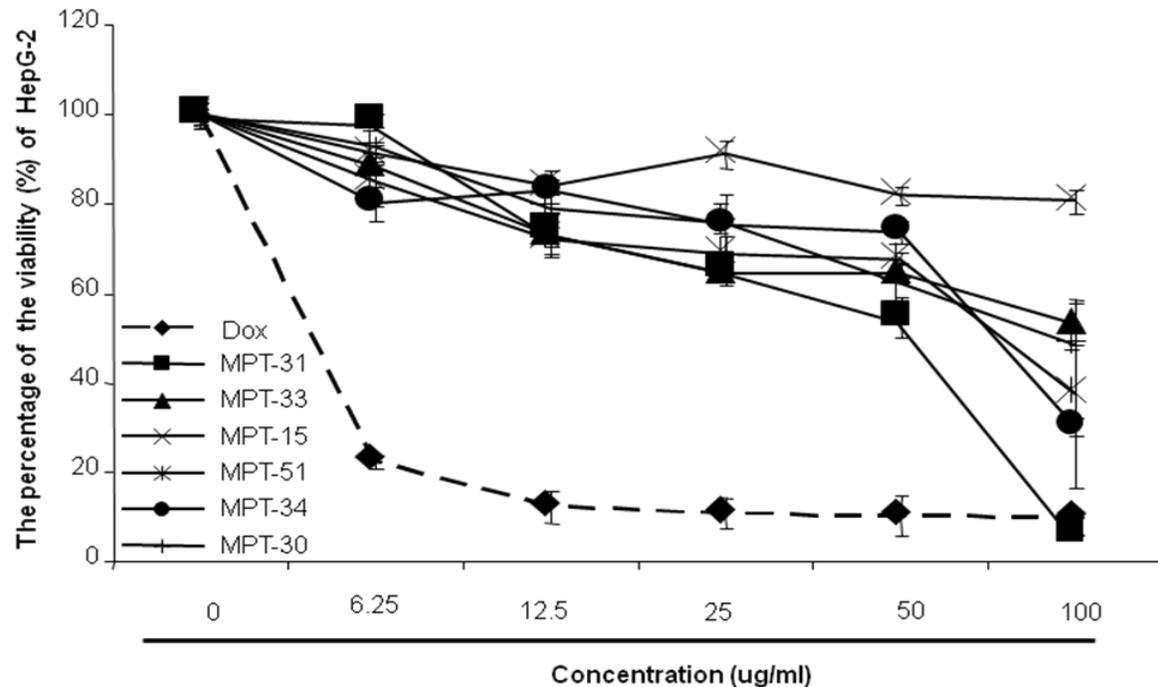


Figure 4 . The viability percentage (%) of different plant extracts against the Hep-G2 cell line. Using 96-well plates, the HepG-2 cell line was cultured in complete RPMI and treated with different concentrations of the methanolic plant extracts. The tumor cell line then incubated for 72 h. The positive control, Dox. and treated cells were used to determine the viability of the tumor cells after 72 h by SRB assay. MPT-31 (*A.falcata*, leaves- flowers), MPT-33 (*A. fragrantissima*, leaves- flowers), MPT-15 (*A. judaica*, seeds), MPT-51 (*A. sieberi*, seeds), MPT-34 (*A. judaica*, leaves- flowers) and MPT-30 (*A. sieberi*, leaves-flowers). The experiment was repeated twice.

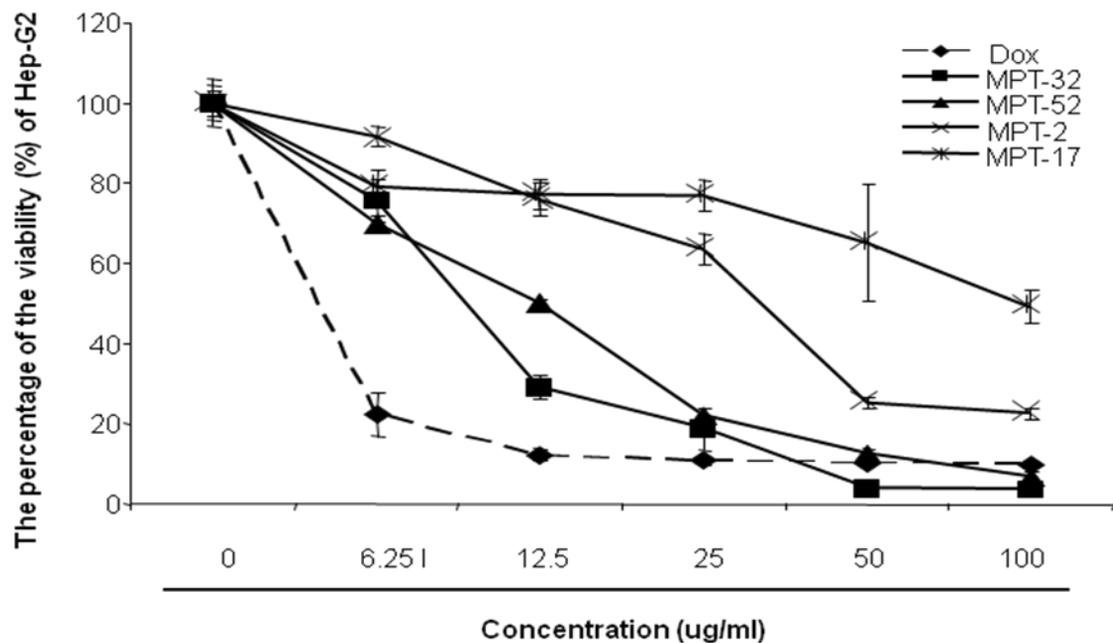


Figure 5. The viability percentage (%) of different plant extracts against the HepG-2 cell line. The treated cells were used to determine the viability of the tumor cells after 72 h by SRB assay: MPT-32 (*P. crista*, shoot system), MPT-52 (*P. incisa*, shoot system), MPT-2 (*R. eppaposum*, shoot system) and MPT-17 (*R. eppaposum*, flowers). The experiment was repeated twice.

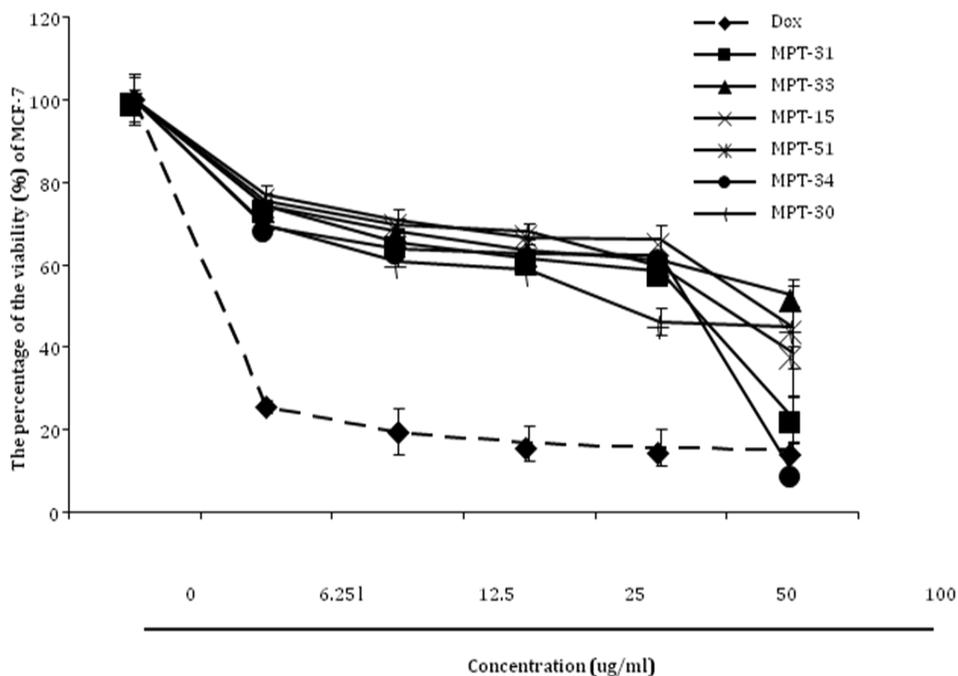


Figure 6. The viability percentage (%) of different plant extracts against the MCF-7 cell line. The treated cells were used to determine the viability of the tumor cells after 72 h by SRB assay: MPT-31 (*A. falcata*, leaves- flowers), MPT-33 (*A. fragrantissima*, leaves- flowers), MPT-15 (*A. judaica*, seeds), MPT-51 (*A. sieberi*, seeds), MPT-34 (*A. judaica*, leaves- flowers) and MPT-30 (*A. sieberi*, leaves-flowers). The experiment was repeated twice.

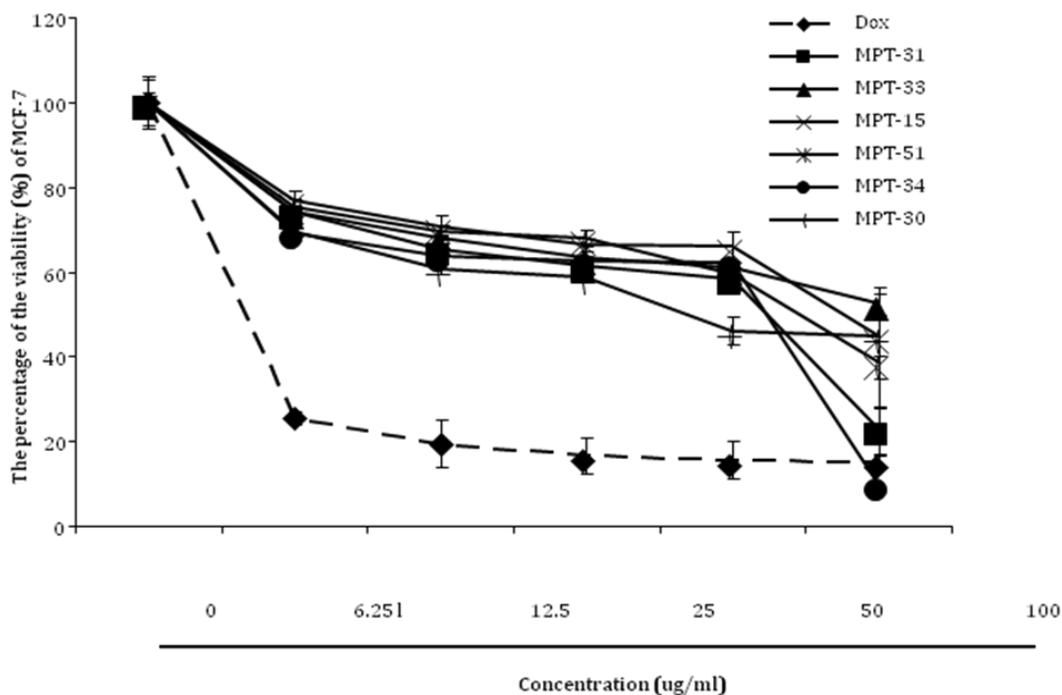


Figure 7. The viability percentage (%) of different plant extracts against the MCF-7 cell line. The treated cells were used to determine the viability of the tumor cells after 72 h by SRB assay: MPT-32 (*P. crispa*, shoot system), MPT-52 (*P. incisa*, shoot system), MPT-2 (*R. eppaposum*, shoot system) and MPT-17 (*R. eppaposum*, flowers). The experiment was repeated twice.

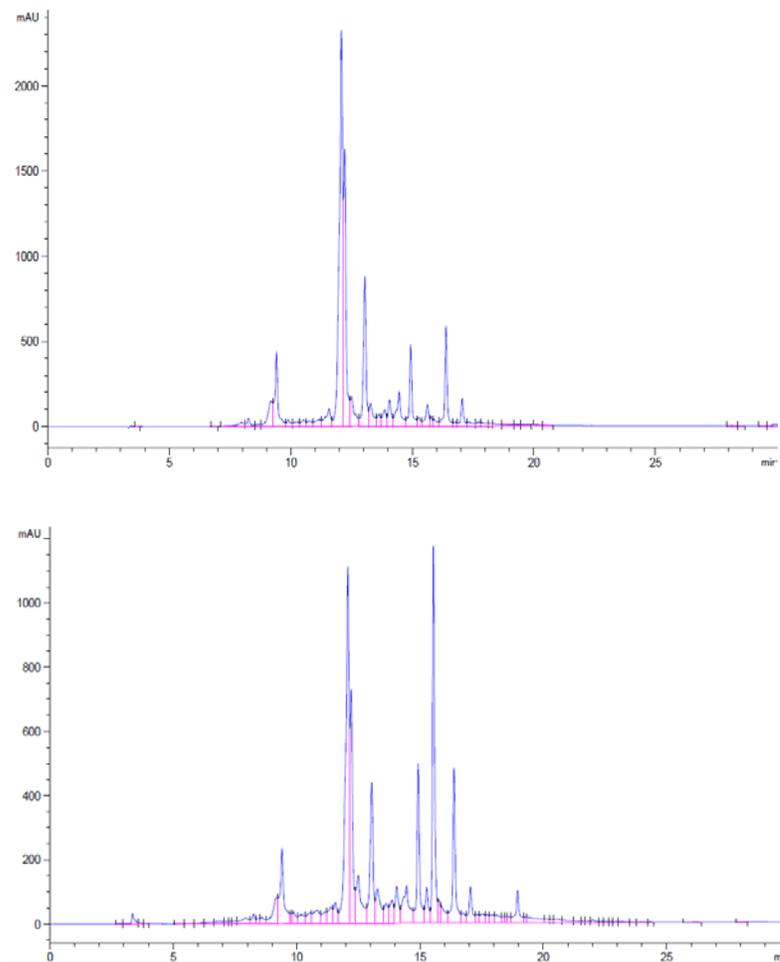


Figure 8. The phenolics (A) and flavonoids compounds (B) on methanolic extract of *P. crispera* by HPLC analysis.

Institute (NCI), the criterion of cytotoxic activity for the crude extracts is an IC₅₀ less than 30 µg/ml (Suffness and Pezzuto, 1990). Based on this fact, this study found that five out of ten extracts showed higher anticarcinogenic potentiality against HepG-2 and/or MCF-7 cell lines. *P. crispera*, *P. incisa* (shoot system), *A. fragrantissima* (leaves and flowers) and *R. eppaposum* (shoot system) showed higher cytotoxic activity against HepG-2 cell lines (less than 30 µg/ml). However, among the tested extracts, only *P. crispera* (shoot), *R. eppaposum* (shoot), *A. sieberi* (leaves and flowers) and *P. incisa* (shoot) showed higher cytotoxic effect against MCF-7 cell lines (less than 30 µg/ml). The strong anticarcinogenic activity of these extracts against HepG-2 and MCF-7 cell lines may be attributed to the high content of some secondary metabolites such phenolics, flavonoids and saponins in these extracts. The study finding is in agreement with Alenad et al. (2013). They found that *A. fragrantissima* extract showed a potential anticancer activity *in vitro* against HepG-2 cell lines. Furthermore, in

other study, the cytotoxic effect of an aqueous extract of *A. fragrantissima* against HepG-2 cell line was reported by Thoppil et al. (2013). In a recent study by Hammad et al. (2014), they showed that the anti-proliferative activity of the aqueous and hydro-alcoholic *A. fragrantissima* and *A. falcata* extracts against the MCF-7 cells did not possess cytotoxic activity up to 200 µg/ml, and this finding is not consistent with this study. Consistent with this study findings, the aerial parts of *P. crispera* showed cytotoxicity in EJ-38 human bladder carcinoma cell (Stavri et al., 2008).

Conclusion

P. crispera (MPT-32), *P. incisa* (MPT-52), *A. fragrantissima* (MPT-33) and *R. eppaposum* (MPT-2) showed potential cytotoxic effect against HepG-2 cell line while, *P. crispera* (MPT-32), *P. incisa* (MPT-52), *R. eppaposum* (MPT-2) and *Artemisia sieberi* (MPT-30) showed potential

cytotoxic effect against MCF-7 cell. The cytotoxic activities of these active plant extracts are probably due to presence of the secondary metabolites such as phenolics and flavonoids, and due to their antioxidant capacity.

Conflict of Interest

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by the Aljouf University, project No. 34/2018. The authors would like to acknowledge the researchers in the Camel and Range Research Center, Sakaka City, Aljouf Region, KSA, for their assistance in identifying the plant species during this study.

REFERENCES

- Abdel-Farid IB, Sheded MG, Mohamed EA (2014). Metabolomic profiling and antioxidant activity of some *Acacia* species. Saudi J. Biol. Sci. 21:400-408.
- Aboul-Enein AM, Abu El-Ela F, Shalaby EA, El-Shemy HA (2012). Traditional medicinal plants research in Egypt: Studies of antioxidant and anticancer activities. J. Med. Plants Res. 6:689-703.
- Akrout A, Gonzalez LA, El Jani H, Madrid PC (2011). Antioxidant and antitumor activities of *Artemisia campestris* and *Thymelaea hirsuta* from southern Tunisia. Food Chem. Toxicol. 49:342-347.
- Alenad AM, Al-Jaber NA, Krishnaswamy S, Yakout MS, Al-Daghri NM, Alokail, MS (2013). *Achillea fragrantissima* extract exerts its anticancer effect via induction of differentiation, cell cycle arrest and apoptosis in chronic myeloid leukemia (CML) cell line K562. J. Med. Plants Res. 7:1561-1567.
- Almehdar H, Abdallah HM, Osman AM, Abdel-Sattar EA (2012). *In vitro* cytotoxic screening of selected Saudi medicinal plants. J. Nat. Med. 66:406-412.
- Alyemeni MN, Sher H, Wijaya L (2010). Some observations on Saudi medicinal plants of veterinary importance. J. Med. Plants Res. 4:2298-2304.
- Basar MH, Hossain SJ, Sadhu SK, Rahman MH (2013). A comparative study of antioxidant potential of commonly used antidiabetic plants in Bangladesh. Orient. Pharm. Exp. Med. 13:21-28.
- Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200.
- Ebrahimzadeh H, Niknam V (1998). A revised spectrophotometric method for determination of triterpenoid saponins. Ind. Drugs 35:379-381.
- Elmann A, Mordechay S, Erlank H, Telerman A, Rindner M, Ofir R (2011). Anti-neuroinflammatory effects of the extract of *Achillea fragrantissima*. BMC Complement Altern. Med. 11:98.
- Hammad HM, Matar SA, Litescu SC, Abuhamdah S, Al-Jaber HI., Afifi FU (2014a). Biological activities of the hydro-alcoholic and aqueous extracts of *Achillea fragrantissima* (Forssk.) grown in Jordan. Nat. Sci. 6:23-30.
- Hammad HM, Litescu Simona-Carmen, Matar SA, Al-Jaber HI, Afifi FU (2014b). Biological Activities of the Hydro-alcoholic and Aqueous Extracts of *Achillea falcata* L. (Asteraceae) Grown in Jordan. Euro. J. Med. Plants 4:259-270.
- Harlev E, Nevo E, Lansky EP, Lansky S (2012). Anticancer attributes of desert plants: a review. Anticancer Drugs 23:255-271.
- Kuete V, Wiench B, Alsaid MS, Alyahya MA, Fankam AG, Shahat AA (2013). Cytotoxicity, mode of action and antibacterial activities of selected Saudi Arabian medicinal plants. BMC Complement Altern. Med. 13:354.
- Kumar KB, Kuttan R (2005). Chemoprotective activity of an extract of *Phyllanthus amarus* against cyclophosphamide induced toxicity in mice. Phytomedicine 12:494-500
- Padmavati M, Sakthivel N, Thara TV, Reddy AR (1997). Differential sensitivity of rice pathogens to growth inhibition by flavonoids. Phytochem. 46:449-502.
- Patra K, Bose S, Sarkar S, Rakshit J, Jana S, Mukherjee A (2002). Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid. Chem. Biol. Interact. 5:231-239.
- Prieto P, Pineda AM (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E Anal. Biochem. 269:337-341.
- Ritesh J, Sanmati KJ (2011). Screening of *in vitro* cytotoxic activity of some medicinal plants used traditionally to treat cancer in Chhattisgarh state, India. Asian Pacific J. Trop. Biomed. 1:147-150.
- Ross SA, El-Sayed KA, El-Sohly MA, Hamann MT, Abdel-Halim OB, Ahmed AF (1997). Phytochemical analysis of *Geigeria alata* and *Francoeuria crispa* essential oils. Planta Med. 63:479-482.
- Ruiz J, Ascasio JA, Rodriguez R, Morales D, Aguilar CN (2011). Phytochemical screening of extracts from some Mexican plants used in traditional medicine. J. Med. Plant. Res. 5: 27912797.
- Sher H, Aldosari A (2012). Overview on the ecological and geographical appraisal of important medicinal and aromatic plants: An endangered component in the flora of Saudi Arabia. Sci. Res. Essays 7:1639-1646.
- Singelton VR, Orthifer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152-178.
- Stavri M, Mathew K, Gordon T, Shnyder A, Falconer SD, Gibbons RS (2008). Guaianolide sesquiterpenes from *Pulicaria crispa* (Forssk.) Oliv. Phytochemistry 69:1915-1918.
- Suffness M, Pezzuto JM (1990). Assays related to cancer drug discovery. In: Hostettmann, K. (Ed.), Methods in Plant Biochemistry: Assays for Bioactivity, vol. 6. Academic Press, London. pp. 71-133.
- Thoppil RJ, Harlev E, Mandal A, Nevo E, Bi-shayee A (2013). Antitumor activities of extracts from selected desert plants against HepG2 human hepatocellular carcinoma cells. Pharm. Biol. 51:668-674.
- Vichai V, Kirtikara K (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat. Protoc. 1:1112-1116.
- Wang J, Mazza G (2002). Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor- α in LPS/IFN- γ activated RAW 264.7 macrophages. J. Agric. Food. Chem. 50:4183-4189.
- Zhishen J, Mengcheng T, Jianmin g W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. 64:555-559.