RESEARCH ARTICLE

Phytochemical analysis and anticancer screening of some indigenous plants grown in Saudi Arabia

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ABSTRACT

This study was conducted to evaluate the phytochemical analysis, and *in vitro* anticancer screening of four wild plants grown in the northern region of the Kingdom of Saudi Arabia (KSA), namely: *Convolvulus oxyphyllus*, *Rhazya stricta*, *Astragalus kahircus* and *Teucrium polium*. Total phenolics content, flavonoids, anthocyanins, saponins, total antioxidant capacity (TAC) and 2,2-diphenyl-1picrylhydrazyl (DPPH) scavenging activity were assessed in their extracts. *In vitro* anticancer screening of the hydro-alcohol extracts was also assessed using human hepatocellular carcinoma (HepG-2) and breast adenocarcinoma (MCF-7) cell lines. The plant species revealed different metabolomic profiling. *C. oxyphyllus* showed the highest phenolic and flavonoids contents compared to other plant extracts. While, among these plant extracts, *T. polium* showed the highest level of TAC, saponins and anthocyanins contents. *C. oxyphyllus* showed the highest inhibition concentration 50% (IC_{50}) against HepG-2 (18.8 µg/ml) and MCF-7 (4.1 µg/ml). The high-performance liquid chromatography analysis of *C. oxyphyllus* extract revealed the presence of high content of benzoic acid and vanillic acid (phenolics) along with hesperidin (flavonoids). In conclusion, among the screened plants, *C. oxyphyllus* has the most potent anticancer activity against HepG-2 and MCF-7 cell lines *in vitro.*

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INTRODUCTION

Despite the breakthrough in the treatment of cancer either by surgery, chemotherapy or radiotherapy, the outcomes are still limited. Finding a successful treatment is still a potential challenge. Although, the treatment with chemotherapy is used to halt the growing tumor, the normal cells are also being affected (El-Naggar *et al.,* 2014). Recently, studies have been carried out to find either a new and safe targeted therapy against cancer or to ameliorate the side effects after treatments (El-Naggar *et al*., 2014; Singh *et al*., 2018). For instance, some antioxidant agents are considered useful to alleviate oxidative stress, which resulted from chemotherapy. Chemotherapy treatment along with potent antioxidants could be desirable approach to ameliorate toxicity (Kumar and Kuttan, 2005; Sudharsan *et al.,* 2005; Jalali *et al.,* 2012).

Kingdom of Saudi Arabia (KSA) flora is rich with wild medicinal plants commonly used to treat several human diseases (Aboul-Enein *et al.,*2012; Kuete *et al.,* 2013). The metabolic, antioxidant and anticancer activity of some wild growing medicinal plants in KSA were reported (El-Naggar *et al.,* 2015). The recent studies have directed to screen and evaluate

the new compounds naturally present especially in the medicinal plants as anticancer agents (Patra *et al.,* 2002; Moustafa *et al.,* 2014). For instance, the methanolic extracts of some 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 *Sesbani agrandflora* extract had a potent *in vitro* anticancer activity (Jeyaraj *et al.,* 2013). The methanol extracts of *Gingko biloba, Ipomoea carnea* leaves and *Lonchocarpus speciosus* bark exhibited *in vitro* anticancer activity against colon cancer cell line (Moustafa *et al.,* 2014). In our recent study, we have found that the hydro-alcoholic extract of *Plucaria crispa* has a potent antitumor activity *in vitro* against HepG-2 and MCF-7 cell lines (El-Naggar *et al.,* 2015).

Convolvulus oxyphyllus Boiss (Vernacular name: Rukhama) belong to family Convolvulaceae. This plant is ascending, rounded perennial shrublet up to 70 cm high, bearing numerous straight lateral spiny branchlets (Fahad and Al-Hemaid, 1998). *Rhazya stricta* Decne is belonging to family Apocynaceae. This plant is a small glaborous erect shrub with a smooth central stem and dense semi-erect branches which grows commonly in the Arabian Peninsula and the Indian subcontinent (Western, 1989). In the traditional medicine, this plant is used to treat diabetes mellitus, inflammation and helminthiasis (Amal *et al.,* 2015; Aisha *et al.,* 2016). Some selected alkaloids and flavonoids isolated from *R. stricta* showed antimicrobial and anticancer properties (Nabih *et al.,* 2012). Mohamed et al. (2009) declared that *Astragalus kahiricus* DC., a herb highly toxic in livestock with very promising hepatoprotective effect against ethanol-induced liver apoptosis (Allam *et al.,* 2013).

Teucrium polium L. (Lamiaceae) has been used for over 2000 years in traditional medicine. *T. polium* aerial parts are used as antibacterial, anti-inflammatory, antioxidant, antidiabetic and antispasmodic (Sadraei *et al.,* 2001; Yazdanparast *et al.,* 2005; Ljubuncic *et al.,* 2006; Kerbouche *et al.,* 2012; Belmekki *et al.,* 2013). In addition, this plant is used to lowering blood lipid, induction of vascular relaxation, decreasing of blood pressure and protects against acetaminophen-induced hepatotoxicity (Suleiman *et al.,* 1988; Bello *et al.,* 1988; Shahraki *et al.,* 2007; Kalantari *et al.,* 2013). In this study, we aimed to evaluate the phytochemical properties, antioxidant capacity and *in vitro* anticancer activity for the hydro-alcoholic extract of C. *oxyphyllus*, *R. stricta*, *A. kahiricus* and *T. polium* which belonging to different four families, Convolvulaceae, Apocynaceae, Fabaceae and Lamiaceae, respectively.

MATERIALS AND METHODS

Plant materials collection and plant extracts preparation

Convolvulus oxyphyllus (shoots), *R. stricta* (leaves), *A. kahiricus* (shoot system) and *T. polium* (seeds) were collected from desert around Sakaka City, Aljouf region, KSA. The plant materials were identified and authenticated by taxonomist at Camel and Range research center, Sakaka, Aljouf, KSA. The materials were shade dried then ground to powder using electrical mortar. The powder then stored in airtight container until use for further experiments. 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-Ciocalteau reagent with gallic acid as a standard and expressed (mg) as gallic acid equivalents per gram of extract according to Laplaze et al. (1999). Total flavonoids content was determined using the aluminum 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 and anthocyanins content

Saponins content was determined using vanillin solution according to Ebrahimzadeh and Niknam, (1998) and expressed (mg) as saponins equivalents per gram of extract. The anthocyanins content of the plant extracts was determined according to the modified method of Padmavati et al. (1997). One hundred mg of plant materials was dissolved in acidified methanol in well closed tubes covered with aluminum foils and incubated at refrigerator for 24 h. The absorbance was read at 530 nm and 657 nm. The concentration was calculated using the following equation: anthocyanin concentration (µmol/g) = ([A530 - 0.33 x A657]/31.6) X (volume [ml]/weight [g]).

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

Total antioxidant capacity (TAC) was determined using phospho-molybednum method according to Prieto et al. (1999). TAC 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: % radical scavenging activity = (Ac - As/Ac) x 100, where Ac = Absorbance of negative control at 517 nm and As = Absorbance of sample at 517 nm (Wang and Mazza, 2002).

Cancer cell lines

Human breast adenocarcinoma (MCF-7) and hepatocellular carcinoma (HepG-2) were obtained from Vacsira, Egypt. Cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 2mL glutamine, containing 100 units/ml penicillin, 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 as mentioned above under the same conditions and used as 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 1000-2000 cells/well. Cells were exposed to different extracts for 72 h and subsequently fixed with 10% trichloroacetic acid (TCA) for 1 h at 4 ºC. 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. 1% glacial acetic acid was used to wash the plates several times. After drying overnight, Tris-HClwas 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 were 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 the statistical computer programme MINITAB (version 12.21) was used to test the significance of quantitative data of phytochemical analysis and antitumor activity. For SRB assays, the sigma plot program was used to analyze the generated data

RESULTS

Metabolomic profiling and antioxidant capacity of plant extracts were determined. The potentiality of anticarcinongenic activity of the plant extracts depends on the metabolomic profiling of that extract. Metabolomic profiling of *C. oxyphyllus*, *R. stricta, A. kahircus* and *T. polium* was detected through the determination of the total phenolics, flavonoids, anthocyanins, saponins, TAC and DPPH radical scavenging activity. The results showed that each plant extract has its specific metabolomic profiling.

C. oxyphyllus extract showed the highest phenolics and flavonoids content

As shown in Figure 1A, the phenolics content showed specific pattern of variation among different species. The highest content of phenolics content was found in *C. oxyphyllus* (MPT-55). Significant difference was also detected between the content of phenolics in *C. oxyphyllus* (MPT-55) and the other three plants extracts*, R. stricta* (MPT-53), *A. kahiricus* (MPT-5) and *T. polium* (MPT-16).

Similar to the phenolics profile, *C. oxyphyllus* (MPT-55) showed highest content of flavonoids as shown in Figure 1B. Both of *R. stricta* (MPT-53), *A. kahiricus* (MPT-5) and *T. polium* (MPT-16) showed moderate flavonoids contents. Significant difference was also observed between the content of flavonoids in *C. oxyphyllus* and the other three plants extracts, *R. stricta* (MPT-53), *A. kahiricus* (MPT-5) and *T. polium* (MPT-16). *T. polium* showed the highest level of saponins and anthocyanins contents

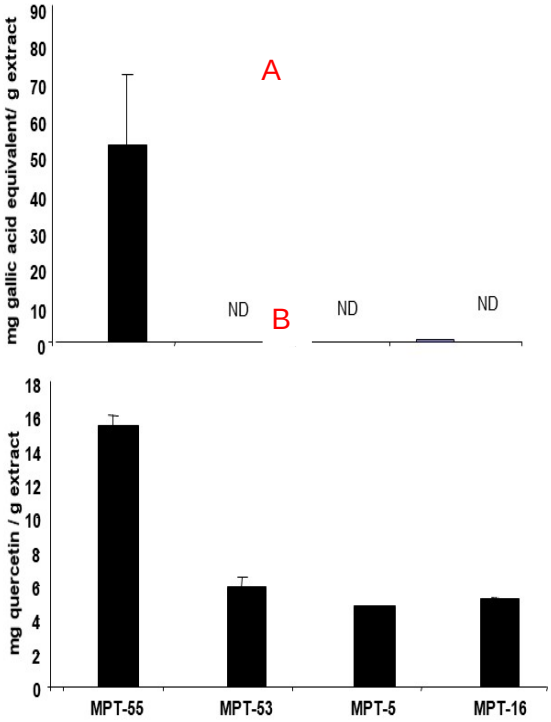

Fig.1. Total phenolics (A) and flavonoids (B) concentrations in the different methanolic plant extracts. MPT-55 (*C. oxyphyllus,* Shoot system), MPT-53 (*R.stricta*, Leaves), MPT-5 (*A.kahircus*, Shoot system), MPT-16 (*T. polium,* seeds).

Among the species under the study, *T. polium* (MPT-16) showed the highest saponins content. As shown in Figure 2A, *R. stricta* (MPT-53), *A. kahiricus* (MPT-5) and *T. polium* (MPT-16) showed low level of saponins content. Similar to the saponins profile, *T. polium* (MPT-16) showed the highest content of anthocyanins (Figure 2B). Both of *C. oxyphyllus* (MPT-55*), R. stricta* (MPT 53) and *A. kahiricus* (MPT-5) showed moderate levels of anthocyanins contents (Figure 2B).

TAC and DPPH radical scavenging activity

Regarding to total antioxidant capacity (TAC), T. polium (MPT-16) showed the highest TAC among other extracts. *C. oxyphyllus* (MPT-55), *R. stricta* (MPT-53) and *A. kahiricus* (MPT-5) showed moderate TAC activities (Figure 3).

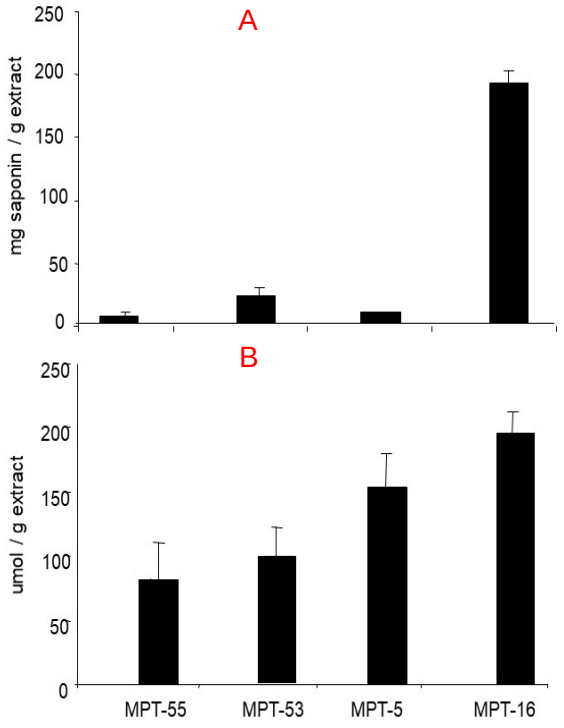

Fig. 2. Saponins (A) and anthocyanins (B) concentrations in the different methanolic plant extracts. Labelling is the same as Fig. 1.

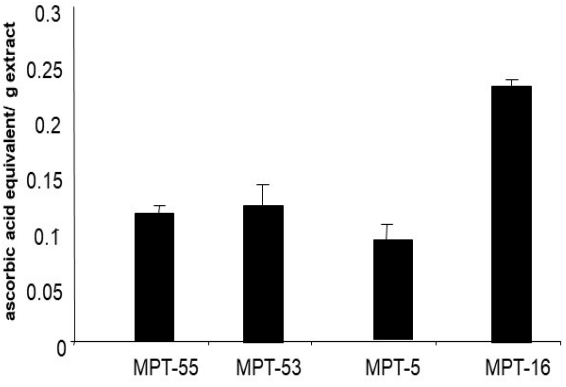

Fig. 3. Total antioxidant capacity (TAC) in the different methanolic plant extracts. MPT-55. **Labelling is the same as Fig. 1 and 2.**

DPPH radical scavenging activity of *C. oxyphyllus* and *T. polium* showed the highest percentage registering 89.4 % and 34.4 %, respectively (Table 1). The inhibition concentration (IC_{50}) of *C. oxyphyllus* and *T. polium* extracts were 55.9 and 145 **µg/ml**, respectively (Table 1). DPPH radical scavenging activity was not detected on *R. stricta* (MPT-53) and *A. kahiricus* (MPT-5) extracts.

Table 1. DPPH radical scavenging activity and the inhibition concentration (IC_{50}) of the selected plant extracts under the study.

Plant species	Code no.	DPPH %	IC_{50} (µg/ml)
C. oxyphyllus	MPT-55	89.4 ±1.9	55.9
R. stricta	MPT-53	ND	ND
A. kahircus	MPT-5	ND	ND
T. polium	MPT-16	34.4 ± 3.9	145

The inhibition concentration 50% (IC50) of different extracts on HepG-2 and MCF-7 cell lines *in vitro*

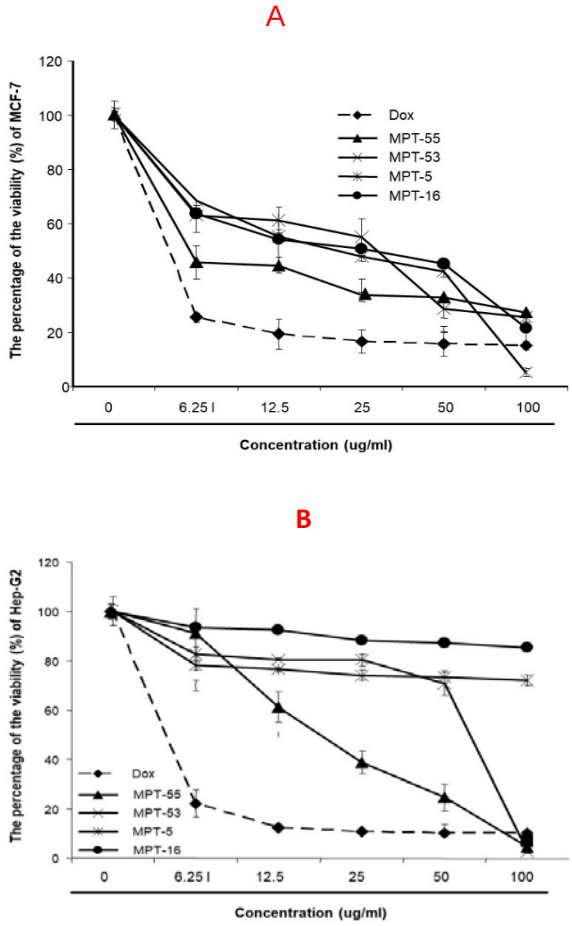

Figure 4. Effect of plants extracts on the viability on MCF-7 and Hep-G2 cell lines. Using 96-well plates, the MCF-7 (A) or Hep-G2 (B) cell lines was cultured in complete RPMI and treated with different concentrations of the methanolic plant extracts (showed in Table 1). The tumor cell line then incubated for 72 hr. the chemotherapeutic drug, doxorubicin (Dox.) was used as a positive control under the same concentrations and conditions. The treated cells were used to determine the viability of the tumor cells after 72hr by MTT assay. The experiment repeated twice

The HepG-2 line was used to assess the IC_{50} *in vitro* after 72 hr post treatments. The results showed that compared to the positive control Dox. (IC_{50} 3.07 ug/ml), the IC_{50} of *C. oxyphyllus* (MPT-55), *R. stricta* (MPT-53), A. kahiricus (MPT-5) and T. polium (MPT-16) were 18.8, 58.4, 119.5 and 143.1 ug/ml, respectively (Table 2 and Figure 4A). The inhibition concentration 50% (IC_{50}) of the previous plant extracts was also determined using MCF-7 cell lines *in vitro* after 72 hr post treatments. The results showed that compared with the Dox. (IC_{50} 2.41 µg/ml), the IC50 of *C. oxyphyllus* (MPT-55), *R. stricta* (MPT-53), *A. kahiricus* (MPT-5) and *T. polium* (MPT-16) were 4.1, 18.6, 20.4 and 20.2 µg/ml, respectively. (Table 2 and Figure 4B).

Table 2. The inhibition concentration 50% IC_{50}) of some different plant extracts versus the conventional chemotherapeutic drug, doxorubicin on Hep-G2 and MCF-7 cell lines in vitro after 72 hr post treatments

Plant species	Code no.	Extracted from	IC_{50}(Hep-G2) IC_{50}(Hep-G2) (µg/ml)	IC_{50} (MCF-7) (µg/ml)
C. Coxyphyllus	MPT-55	Shoot	18.8	4.1
R. stricta	MPT-53	Leaves-	58.4	18.6
A. kahircus	MPT-5	Shoot	119.5	20.4
T. polium	MPT-16	Seeds	143.1	20.2
Doxorubicin			3.07	2.41

Table 3 A. Assignment of the HPLC peaks of phenolics in MeOH extract from *C. oxyphyllus* shoot system

No.	Phenolic	Conc. µg/ml
1.	Chlorogenic acid	79.14
2.	e-vanillic acid	118.19
3.	Catechol	76.55
4.	Saylcilic acid	25.89
5.	Pyrogallol	38.37
6.	Benzoic	244.47
7.	`P-OH-benzoic	31.17
8.	Cinnamic	12.15
9.	Ellagic	15.33
10.	3-OH- Tyrosol	16.20
11.	Epicatechein	13.44

Table 3 B. Assignment of the HPLC peaks of flavonoids in MeOH extract from *C. oxyphyllus* shoot system.

Conc. µg/ml	Flavonoids	No.
1717.32	Hesperidin	1
32.64	Rutin	2
77.32	Narginin	3
21.38	Rosmarinic acid	4
---	Quercetin	5
---	Kaempferol	6
5.09	Hispertin	7
1.44	Aspegenin	8
3.06	Narengenin	9
0.47	7-OH flavone	18
1.07	Quercetrin	11

HPLC analysis for phenolic of *C. oxyphyllus*

Based on the phytochemical analysis and anticancer activities of the tested plants extract, the *C. oxyphyllus* methanolic extract (MPT-55), showed the highest cytotoxic effect against HepG-2 and MCF-7 cell lines *in vitro*. To this end, we further analyzed this extract by HPLC to determine the major phenolics and flavonoids compounds. The results showed that the major phenolic compounds were benzoic acid and e-vanillic acid (Table 3A and Figure 5A). Moreover, hesperidin was identified as major flavonoids content in the extract (Table 3B and Figure 5B).

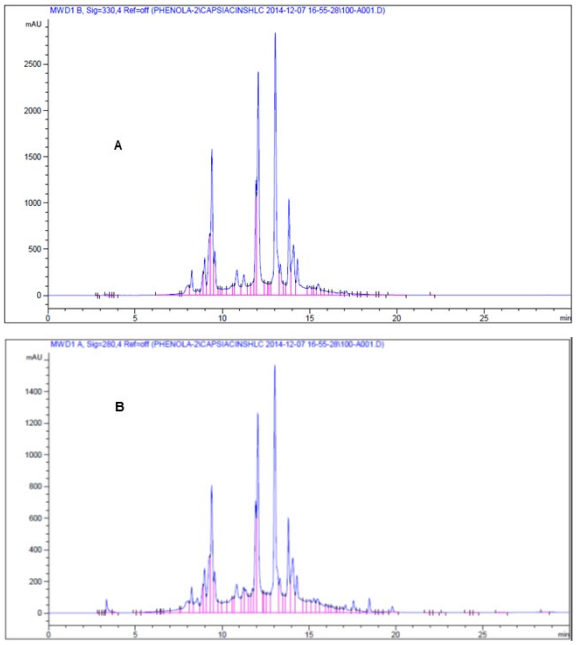

Fig. 5. The phenolics (A) and flavonoids compounds (B) in methanolic extract of *C. oxyphyllus* by HPLC analysis

DISCUSSION

For cancer patients, chemotherapy is recommended for the treatment; however, it has severe side effects on different organs upon its application. So that finding new treatment approaches to fight against cancer are ultimately needed. One of these approaches is to screen the medicinal plants to find new natural compounds that might replace the conventional chemotherapy or at least mitigate its side effects (Kumar and Kuttan, 2005; Sudharsan *et al.,* 2005; Ghasemzadeh *et al.,* 2015). In this study, we evaluated the phytochemical properties, antioxidant capacity and *in vitro* anticancer activity of *C. oxyphyllus, R. stricta, A. kahircus and T. polium*. The phytochemical screening showed that *C. oxyphyllus* has the highest content of phenolics and flavonoids contents among the tested extracts, while the *T. polium* showed the highest saponins and anthocyanins contents. This finding is in agreement with previous reports 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 (Basar *et al.,* 2013; Abdel-Farid *et al.,* 2014). In our previous work, we found that TAC was to be positively correlated with saponins and flavonoids content (Abdel-Farid *et al.,* 2014). In this study, we found that the four tested plant extracts have strong anticancer activity against MCF-7 cell line *in vitro*, however, only the methanolic extract of *C. oxyphyllus* showed the most potent anticancer activity against HepG-2 cell line *in vitro*. The strong anticarcinogenic activities against HepG-2 and MCF-7 cell lines may be attributed to the high content of some secondary metabolites such as phenolics, flavonoids and saponins in these extracts. Consistent with our findings, Elmasri et al. (2015) reported that there were three saponins of *T. polium* completely inhibited the growth of a breast and colon cancer cell line *in vitro*. In other study, Mosadegh et al. (2002) reported that the aerial parts of *T. polium* have saponins and flavonoides with antibacterial but not antifungal effect. According to these studies, saponins in *T. polium* are biologically active compounds. The methanolic extract of *T. polium* was found to increase the cytotoxic and apoptotic effects of the different chemotherapeutic drugs such as vincristine, vinblastine and doxorubicin against a panel of cancerous cell lines. Furthermore, *T. polium* extract showed inhibition of cell proliferation and induced cell cycle arrest of human prostate cancer cells (Rajabalian, 2008; Kandouz *et al.,* 2010), protective effect on hepatotoxicity (Forouzandeh *et al.,* 2013) and anticancer activity on hepatocellular carcinogenic (Movahedi *et al.,* 2014). The leaves, flowers and fruits of *R. stricta* are also used to treat cancer (Khan, 2007). The ethanol extract of *A. kahiricus* roots showed hepatoprotective potentiality against ethanol-induced liver apoptosis (Allam *et al.,* 2013). A novel anticancer effect has been found of *Astragalus* saponins (Auyeung *et al.,* 2009). Consistent with these findings, it could explain the potent activity of *T. polium*, *R. stricta* and *A. kahiricus* extracts as cytotoxic agents against MCF-7 cell lines *in vitro.*

Conclusion

C. oxyphyllus (MPT-55) showed the most potent cytotoxic effect against HepG-2 and MCF-7 cell lines while, *T. polium*, *R. stricta* and *A. kahiricus* showed potential cytotoxic effect against MCF-7 cell line *in vitro.*

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