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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-1-picrylhydrazyl (DPPH) scavenging activity were assessed in their extracts. In vitro anticancer screening of the hydro-alcoholic 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.

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
Sesbania agrandflora extract had a potent in vitro anticancer activity (Jeyaraj et al., 2013). The methanol extracts of Ginkgo 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 perennia 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 glabrous erect shrub with a smooth central stem and dense semi-erect branches which grow 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.

**Spectro photometrical 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 Laplaize 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-1-picrylhydrazyl (DPPH) assays**

Total antioxidant capacity (TAC) was determined using phospho-molybdenum 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 trypsination 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 1h 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. 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 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 anticarcinogenic activity of the plant extracts depends on the metabolomic
profiling of that extract. Metabolomic profiling of *C. oxyphyllus*, *R. stricta*, *A. kahiricus* 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. 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). The inhibition concentration 50% (IC50) of different extracts on HepG-2 and MCF-7 cell lines *in vitro* 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 (IC50) 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.

The HepG-2 line was used to assess the IC50 in vitro after 72 hr post treatments. The results showed that compared to the positive control Dox. (IC50 3.07 µg/ml), the IC50 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 µg/ml, respectively (Table 2 and Figure 4A). The inhibition concentration 50% (IC50) 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. (IC50 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).

**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).

**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).
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Figure 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).

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

Table 1. DPPH radical scavenging activity and the inhibition concentration (IC50) of the selected plant extracts under the study.

| Plant species | Code no. | DPPH % | IC50 (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          |

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

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.
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 flavonoids 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 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.,

### Table 2

The inhibition concentration 50% (IC50) 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 | IC50 (Hep-G2) (µg/ml) | IC50 (MCF-7) (µg/ml) |
|---------------|---------|----------------|-----------------------|----------------------|
| *C. oxyphyllus* | MPT-55 | Shoot          | 18.8                  | 4.1                  |
| *R. stricta*   | MPT-53 | Leaves         | 58.4                  | 18.6                 |
| *A. kahiricus* | 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   | Saylilic acid  | 25.89       |
| 5   | Pyrogallol     | 38.37       |
| 6   | Benzoic        | 244.47      |
| 7   | t-P-OH-bensoic | 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

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