Original article

Phytochemical standardization and biological activities of certain desert plants growing in Saudi Arabia

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A R T I C L E  I N F O

Article history:
Received 12 November 2017
Accepted 12 December 2017
Available online 13 December 2017

Keywords:
- Antitumor
- Anti-microbial
- Sub-chronic toxicity
- Phytochemical contents
- Medicinal plants

A B S T R A C T

The phytochemical screening, antimicrobial and antitumor activities of Calendula tripterocarpa, Centarea sinaica, Centaurea pseudosinosa, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum determined. The best antibacterial activity; 41.8 ± 0.23 mm, 39.7 ± 0.25 mm, 35.8 ± 0.58 mm, 34.7 ± 0.51 mm and 32.7 ± 0.25 mm was obtained by Plectranthus arabicus against Klebsiella pneumonia, Tripleurospermum auriculatum against Bacillus subtilis, Centaurea pseudosinosa against Bacillus subtilis, Centaurea pseudosinosa against Staphylococcus pyogenes and Plectranthus arabicus against Staphylococcus epidermidis, respectively. While the highest antifungal activity; 35.9 ± 1.15 mm, 34.6 ± 0.34, 30.6 ± 0.26 mm and 29.9 ± 0.63 mm was obtained by Tripleurospermum auriculatum against Geotrichum candidum, Candida albicans, C. tropicalis and Aspergillus fumigatus, respectively. The antitumor activity (IC50) obtained by Centarea sinaica; 3.1 ± 6.9 μg/ml, 14.3 ± 3.1 μg/ml and 22.7 ± 4.1 μg/ml was better than activity of vinblastine sulphate; 5.9 ± 0.4 μg/ml, 59.7 ± 2.1 μg/ml and 30.3 ± 1.4 μg/ml against breast carcinoma (MCF-7), cervical carcinoma (Hela) and colorectal carcinoma (CACO), respectively. Plectranthus arabicus alcoholic extract showed higher antitumor activity; 15.3 ± 5.3 μg/ml, 28.6 ± 3.6 μg/ml and 24.3 ± 4.1 μg/ml than vinblastine; 21.2 ± 0.9 μg/ml, 59.7 ± 2.1 μg/ml and 30.3 ± 1.4 μg/ml against prostate carcinoma (PC3), cervical carcinoma (Hela) and colorectal carcinoma (CACO), respectively. Also, the antitumor activity of Plectranthus asirensis against cervical carcinoma (Hela) (37.1 ± 2.6 μg/ml) was potent than vinblastine sulphate (59.7 ± 2.1 μg/ml). The obtained results of LD50 and sub-chronic toxicity revealed that the plants have no toxicity.

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1. Introduction

Plants contain a wide variety of secondary metabolite including tannins, terpenoids, alkaloids, and flavonoids which have been proved to have anti-microbial, antioxidant, antitumor and other biological activities and can be of great significance in therapeutic treatments (Gislene et al., 2000). Among about 7,000 species of medicinal plants, the medicinal value of plants is due to the chemical substances that produce a definite physiologic action on the human body (Sivarajan and Balachandean, 1999).

The Asteraceae (commonly known as sunflower) is a large and widespread family which contain many genera (Vinesh and Devendra, 2013). Calendula, Centaurea and Tripleurospermum are the most important genera of the family Asteraceae due to the huge number and medicinal use of their species. Species belonging to these genera are herbaceous, annual or perennial which is widespread all over the world (Baciu et al., 2010). Members of the genera are characterized by the presence of volatile oils in addition to other chemical constituents such as triterpenoids, flavonoids (Mouffok et al., 2012), coumarines, quinines, tannins (Erel et al., 2011), carotenoids, phenolic compounds (Astari et al., 2013) and amino acids (Disha et al., 2013). In folk medicine, the plants belonging to Calendula species are used as anti-inflammatory, and antipyretic (Abbasi et al., 2010), disinfectant, antispasmodic, diuretic (Tiwari, 2008), treatment of kidney and gall stones,
emmenagogue, diaphoretic, sedative (Dall’Acqua et al., 2008), healing properties (Abassi et al., 2010), hepatoprotective (Muley et al., 2009), and for treating burns (Passalacqua et al., 2007).

The family Lamiaceae, also known as the mint family, is another important family which contains plants distributed all over the world (Raja, 2012). Members of this family are characterized by their phytochemical compositions and its biological activities (Hajimehdipoor et al., 2014). Nevertheless, the most important bioactive constituents of these plants are the alkaloids, tannins, terpenoids (Okach et al., 2013), volatile oil, polyphenols and flavonoids (Oksana et al., 2016). In many countries, numerous species of this family are in use in traditional medicine as smooth and muscle relaxant, cardiac depressant, antioxidant, antiseptic (Ibrahim and Abu-Salem, 2014), immunomodulator, antimicrobial, antimalarial, antiallergic and antidiabetic agents (Kozłowska et al., 2015). From the previous studies, the present study was carried out to determine the phytochemical contents, antimicrobial and antitumor activities of Centaurea tripterocarpa, Centarea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum and evaluate their validity to be used in folk medicine.

2. Material and methods

2.1. Plant materials

The aerial parts of Calendula tripterocarpa, Centarea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum were collected from different localities in the desert of Saudi Arabia during April 2016. The plants were identified by Dr. Jacob Thomas, assistant professor of Taxonomy, Botany and Microbiology Dept., College of Science, King Saud University, and comparison with the published data (Migahid, 2002). Voucher specimens were kept in the herbarium of Botany and Microbiology Dept., College of Science, KSA. The plant samples were air-dried in shade, reduced to fine powder, packed in tightly closed containers, and stored for phytochemical and biological studies.

2.2. Phytochemical analysis

2.2.1. Qualitative phytochemical analysis

The phytochemical screening and determination of chemical constituents of Calendula tripterocarpa, Centarea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum were performed according to the published methods (Tiwari et al., 2011).

2.2.2. Quantitative phytochemical analysis

Two hundred grams powder of the aerial parts of each plant were extracted by percolation in 95% aqueous ethanol (1L) till complete exhaustion (4 times/72 h) (Awaad et al., 2016). The total ethanol extract was concentrated under reduced pressure and low temperature.

The yield percentage of each plant extract was calculated in relation to the dry weight. Some pharmacopeial constants (moisture, total ash, acid insoluble ash and water soluble ash) were carried out for the plant according to published methods (El-Alfy et al., 2012). Quantitative analysis of phytochemical contents was performed according published methods as following: Alkaloids (Seru et al., 2013), anthocyanins (Paula and Paul, 2011) carbohydrates (Santhi and Sengottuve, 2016), flavonoids (Krishnaiah et al., 2009), lipids (Sneh et al., 2013), phenols (Santhi and Sengottuve, 2016), proteins (Santhi and Sengottuve, 2016), and tannins (Krishnaiah et al., 2009).

2.3. Antimicrobial activity

2.3.1. Test organisms

Different microorganisms including Gram-negative bacteria; Escherichia coli (RCMB 010056), Klebsiella pneumonia (RCMB 0010093), Proteus vulgaris (RCMB 010085), Pseudomonas aeruginosa (RCMB 0100243-5), and Salmonella typhimurium (RCMB 006 (1) ATCC 14028), Gram-positive bacteria; Bacillus subtilis (RCMB 015 (1) NRRL B-543), Staphylococcus epidermidis (RCMB 010027), Streptococcus mutans (RCMB 0100172), Streptococcus pneumoniae (RCMB 0100170-3), Strepotococcus pyogenes (RCMB 0100174-2) and; and fungal strains; Aspergillus fumigatus (RCMB 02568), Candida albicans (RCMB 05036), C. tropicalis (RCMB 05239), Geotrichum candidum (RCMB 05097), and Syncephalastrum racemosum (RCMB 09041) were obtained from the Microbiology Laboratory, Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt and used as test organisms.

2.3.2. Antimicrobial assay

The antimicrobial activity of ethanolic extract of Calendula tripterocarpa, Centarea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum were determined using the well diffusion method (Zain et al., 2012).

2.3.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by microdilution method (Golus et al., 2016) using serially diluted (2-fold) of plant extract.

2.4. Antitumor activity

The antitumor activity of the plants in the present study were determined against different cell lines; namely, HEP-2 (Larynx carcinoma), A-549 (Lung carcinoma), HepG-2 (Hepatocellular carcinoma), CACO (colorectal carcinoma), Hela (Cervical carcinoma), HCT-116 (Colon carcinoma), and MCF-7 (Breast carcinoma) using the method described by Kameyama et al. (2005).

2.5. Plants toxicity

2.5.1. Animals

Swiss albino mice of both sexes (30–35 g) were purchased from King Saud University animal house, kept in standard polypropylene cages and maintained under standard conditions (Awaad et al., 2016).

2.5.2. Preparation of the extracts for biological studies

Dried alcohol plant-extract of Calendula tripterocarpa, Centarea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum was suspended in distilled water; freshly just before administration and; and fungal strains; Aspergillus fumigatus (RCMB 02568), Candida albicans (RCMB 05036), C. tropicalis (RCMB 05239), Geotrichum candidum (RCMB 05097), and Syncephalastrum racemosum (RCMB 09041) were obtained from the Microbiology Laboratory, Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt and used as test organisms.

2.5.3. Acute toxicity (LD50) test

Dried alcohol-extract of each plant was orally given to the animal for median lethal dose (LD50) as described by El-Meligy et al. (2017).

2.5.4. Sub-chronic toxicity

For determination of the sub-chronic toxicity, rats were divided into 8 groups each of 6 rats. The 1st group was administrated with the vehicle orally and left as a control, while the groups from 2 to 8 were separately administrated the total alcohol extracts in a dose of 200 & 400 mg/kg for 15 days. After the examination period,
the collected sera were used for determination of liver and kidney functions (El-Meligy et al., 2017).

2.6. Statistical analysis

All values were expressed as mean ± S.D. Comparisons between means were carried out using a one-way ANOVA test followed by the Tukey HSD test using SPSS, version 14 (SPSS, Chicago, IL). Differences at p≤0.05 were considered statistically significant (Sabry et al., 2016).

3. Results and discussion

3.1. Phytochemical analysis

Seven plants grown in the desert of Saudi Arabia, namely, Calendula tripterocarpa, Centaurea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum were subjected to standardization in order to determine their constituents. The results exhibited the presence of different active phytochemical groups. Those chemical constituents were qualitatively and quantitatively analyzed using different spectroscopic and analytical techniques; the results are listed in Tables 1–3.

3.1.1. Qualitative phytochemical analysis

Phytochemical screening of the plant extracts showed the presence of the following groups: carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, antraquinones, protein and/or amino acids, alkaloids and tannins and absence of saponin, cardinolides, and oxidase enzyme in all the investigated plants (Table 1). It is believed that the variations in phytochemical content of the plant are due to number of the environmental factors (Kokate et al., 2004).

3.1.2. Quantitative phytochemical analysis

The quantitative yield percentage of Calendula tripterocarpa, Centaurea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum were determined (Table 2). The highest total alcohol percentage: 19.70 ± 1.29, 18.45 ± 1.49, and 18.17 ± 1.34 was detected in Plectranthus arabicus, Tripleurospermum auriculatum, and Centaurea pseudosinaica, respectively. Among all the investigated plants, the total alcohol of Koelpinia linearis (15.37 ± 1.76) was the lowest (Table 2).

The results of the pharmacopoeia constants exhibited a slight variation in the investigated plants. The moisture contents of the plants ranged from 5.17 ± 1.23 to 11.25 ± 2.33 which of course attributed to the desert as the plants habitat. However, the highest percentage of total ash (10.348 ± 1.54), acid insoluble ash (4.19 ± 1.36), and water soluble ash (8.58 ± 1.63) was detected in Koelpinia linearis (Table 2). It is believed that the determination of these constants is significant and indicative to determining the quality of the plant material (Amita and Shalini, 2014).

The results revealed that the carbohydrate, lipid, protein, alkaloids, flavonoids, phenolic compounds and tannins were present in all the investigated plants (Table 3). However, the highest percentage of carbohydrate (9.81 ± 1.5), lipid (11.23 ± 1.4), protein (9.67 ± 1.9), alkaloids (0.11 ± 0.03), flavonoids (5.19 ± 1.4), phenolic compounds (14.13 ± 1.4) and tannins (10.26 ± 0.9) were found in Tripleurospermum auriculatum, Koelpinia linearis, Centaurea pseudosinaica, Centaurea sinaica, Plectranthus arabicus, Plectranthus asirensis, and Centaurea sinaica, respectively (Table 3).

Interestingly, the lipids and phenolic compounds represent the highest chemical percentage in all the investigated plants. The relatively high lipid percentage was correlated to the high temperature in the desert environment (Guowei et al., 2011). While the higher percentage of phenolic compounds increase the possibility of the plant as a resource for bioactive compound(s) and its

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**Table 1**

| Test                        | Plant                  |
|-----------------------------|------------------------|
|                             | Calendula tripterocarpa| Centaurea sinaica | Centaurea pseudosinaica | Koelpinia linearis | Plectranthus arabicus | Plectranthus asirensis | Tripleurospermum auriculatum |
| Sterols and/or triterpenes  | +                      | +                    | +                        | +                     | +                      | +                        | +                        |
| Cardinolides                | –                      | –                    | –                        | –                     | –                      | –                        | –                        |
| Carbohydrates and/or        | +                      | +                    | +                        | +                     | +                      | +                        | +                        |
| glycosides                  |                        |                      |                          |                       |                        |                          |                          |
| Flavonoids                  | +                      | +                    | +                        | +                     | +                      | +                        | +                        |
| Tannins                     | +                      | +                    | +                        | +                     | +                      | +                        | +                        |
| Saponins                    | –                      | –                    | –                        | –                     | –                      | –                        | –                        |
| Anthraquinones              | +                      | +                    | +                        | +                     | +                      | +                        | +                        |
| Alkaloids and/or            | +                      | +                    | +                        | +                     | +                      | +                        | +                        |
| nitrogenous bases           | +                      | +                    | +                        | +                     | +                      | +                        | +                        |
| Protein and/or amino acids  | +                      | +                    | +                        | +                     | +                      | +                        | +                        |

(+) present, (–) absence, (±) trace.

**Table 2**

| Plant                  | Total alcohol | Moisture | Total ash | Acid insoluble ash | Water soluble ash |
|------------------------|---------------|----------|-----------|--------------------|-------------------|
| Calendula tripterocarpa| 16.57 ± 1.11  | 5.17 ± 1.23 | 9.32 ± 1.13 | 2.15 ± 1.27         | 6.12 ± 1.34       |
| Centaurea sinaica      | 17.34 ± 1.56  | 7.14 ± 1.11 | 8.14 ± 1.37 | 3.11 ± 1.32         | 5.21 ± 1.15       |
| Centaurea pseudosinaica| 18.17 ± 1.34  | 6.87 ± 1.99 | 9.65 ± 1.61 | 3.87 ± 1.71         | 6.47 ± 1.78       |
| Koelpinia linearis     | 15.37 ± 1.76  | 8.98 ± 2.19  | 10.348 ± 1.54 | 4.19 ± 1.36         | 8.58 ± 1.63       |
| Plectranthus arabicus  | 19.70 ± 1.29  | 9.77 ± 2.43  | 8.35 ± 1.22  | 3.16 ± 1.23         | 5.22 ± 1.54       |
| Plectranthus asirensis | 17.55 ± 1.41  | 10.15 ± 2.21 | 9.14 ± 1.37  | 3.33 ± 1.34         | 5.28 ± 1.42       |
| Tripleurospermum auriculatum | 18.45 ± 1.49 | 11.25 ± 2.33 | 8.74 ± 1.66  | 2.13 ± 1.57         | 6.18 ± 1.49       |

Values are the mean of triplicates ± standard deviation.
potentiality to be used as therapeutic agent against diseases (Pane et al., 2000), due to their ability to act as free radical scavenging agents which concedes the major cause of daises (Akinyeye et al., 2014; Onanong et al., 2011).

3.2. Antimicrobial activity

The antimicrobial activity of Calendula tripterocarpa, Centarea sinaica, Centarea pseudosinaica, Koelpinia linearis, Plectranthus asirensis, Plectranthus arabicus, Plectranthus asisrensis and Tripleurospermum auriculatum was determined against bacterial and fungal strains (Tables 4 and 5). The results showed that the best antibacterial activity; 41.8 ± 0.23 mm (0.03 μg/ml), 39.7 ± 0.25 mm (0.03 μg/ml), 35.8 ± 0.58 mm (0.02 μg/ml), 34.7 ± 0.51 mm (0.02 μg/ml) and 32.7 ± 0.25 mm (0.02 μg/ml) was obtained by Plectranthus arabicus against Klebsiella pneumonia, Tripleurospermum auriculatum against Bacillus subtilis, Centarea pseudosinaica against Bacillus subtilis, Centarea pseudosinaica against Streplococcus pyogenes and Plectranthus arabicus against Staphylococcus epidermidis, respectively (Tables 4 and 5).

On the other hand, the highest antifungal activity; 35.9 ± 1.15 mm (0.24 μg/ml), 34.6 ± 0.34 (0.98 μg/ml), 30.6 ± 0.26 mm (0.98 μg/ml) and 29.9 ± 0.63 mm (1.95 μg/ml) was obtained by Tripleurospermum auriculatum against Geotricum candidum, Candida albicans, C. tropicalis and Aspergillus fumigatus, respectively in addition to the antifungal activity of Centaurea pseudosinaica against Geotricum candidum (31.8 ± 0.68 mm, 0.98 μg/ml) (Tables 4 and 5). The remarkable antifungal and antibacterial activity of the investigated plants is mainly attributed to the phytochemical constituents resulted by the adverse conditions of desert (Akinyeye et al., 2014).

3.3. Antitumor activity

The in vitro antitumor activity of Calendula tripterocarpa, Centarea sinaica, Centarea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asisrensis and Tripleurospermum auriculatum against different types of cell lines was determined (Table 6). The obtained results indicated the direct cytotoxic effect of the investigated plant extracts.

### Table 3
Total percentage of the phytochemical constituents of the investigated plants.

| Plant                     | Carbohydrate | Lipid | Protein | Alkaldoids | Flavonoids | Phenolic compounds | Tannins |
|---------------------------|--------------|-------|---------|------------|------------|--------------------|---------|
| Calendula tripercoarp     | 6.77 ± 1.2   | 10.15 ± 1.2 | 7.21 ± 1.8 | 0.06 ± 0.02 | 3.45 ± 1.2 | 11.22 ± 1.7 | 8.28 ± 1.1 |
| Centarea sinaica          | 8.13 ± 1.4   | 11.15 ± 1.13 | 8.59 ± 1.7 | 0.13 ± 0.03 | 2.35 ± 2.1 | 10.31 ± 1.4 | 10.26 ± 0.9 |
| Centarea pseudosinaica    | 7.63 ± 1.9   | 9.15 ± 1.7   | 9.67 ± 1.9 | 0.04 ± 0.9  | 4.15 ± 1.7 | 12.25 ± 1.6 | 7.56 ± 1.8 |
| Koelpinia linearis        | 6.43 ± 1.3   | 11.23 ± 1.4 | 6.98 ± 1.2 | 0.05 ± 0.1  | 3.18 ± 1.7 | 13.46 ± 1.6 | 9.33 ± 1.4 |
| Plectranthus arabicus     | 6.83 ± 1.9   | 9.21 ± 1.5   | 7.32 ± 1.5 | 0.06 ± 0.6  | 5.19 ± 1.4 | 13.11 ± 1.7 | 8.15 ± 0.7 |
| Plectranthus asisrensis   | 8.92 ± 1.2   | 10.43 ± 1.9 | 6.16 ± 1.9 | 0.09 ± 0.2  | 4.85 ± 1.9 | 14.13 ± 1.4 | 7.22 ± 1.15 |
| Tripleurospermum auriculatum | 9.81 ± 1.5 | 9.81 ± 1.5 | 8.76 ± 1.3 | 0.06 ± 0.7  | 3.16 ± 1.8 | 12.14 ± 1.3 | 9.21 ± 1.15 |

Values are the mean of triplicates ± standard deviation.

### Table 4
Antimicrobial activity of ethanol extracts of the investigated plants.

| Test organism            | Plant                                      | Diameter of the inhibition zone (mm) | Standard antibiotic |
|--------------------------|--------------------------------------------|--------------------------------------|---------------------|
|                          | Calendula tripercoarp                      |                                      | Gentamycin          |
| Esherichia coli          | Gram negative                              | 24.2 ± 0.13                          | 24.3 ± 0.58         |
| Klebsiella pneumoniae    | 15.3 ± 0.14                                | 17.4 ± 0.17                          | 25.9 ± 0.3          |
| Proteus vulgaris         | 14.8 ± 0.13                                | 21.2 ± 0.14                          | 26.3 ± 0.3          |
| Pseudomonas aeroginosa   | 21.7 ± 0.36                                | 16.3 ± 0.32                          | 23.3 ± 0.1          |
| Salmonella typhimurium    | 17.2 ± 0.16                                | 19.4 ± 0.14                          | 33.6 ± 0.15         |
| Bacillus subtilis        | 14.4 ± 0.19                                | 25.2 ± 0.27                          | 38.4 ± 0.3          |
| Staphylococcus epidermidis| 12.1 ± 0.19                               | 25.5 ± 0.44                          | 31.2 ± 0.18         |
| Streptococcus mutans     | 31.0 ± 0.53                                | 19.5 ± 0.64                          | 34.3 ± 0.32         |
| Streptococcus pneumoniae | 10.8 ± 1.21                                | 25.5 ± 0.44                          | 29.8 ± 0.2          |
| Streptococcus pyogenes   | 13.4 ± 0.13                                | 34.7 ± 0.51                          | 32.4 ± 0.31         |
| Aspergillus fumigatus    | 21.7 ± 0.36                                | 28.3 ± 0.15                          | 29.7 ± 0.63         |
| Candida albicans         | 19.3 ± 0.13                                | 29.5 ± 0.32                          | 34.6 ± 0.34         |
| Candida tropicalis       | 26.6 ± 0.54                                | 28.4 ± 0.36                          | 31.4 ± 0.38         |
| Geotricum candidum      | 23.3 ± 0.48                                | 31.8 ± 0.68                          | 28.2 ± 0.34         |
| Syncephalanthus racemosum| 17.2 ± 0.33                               | 22.5 ± 0.27                          | 34.7 ± 0.27         |

Values are the mean of triplicates ± standard deviation.
The antitumor activity (IC50) obtained by Centarea sinaica; 3.1 ± 6.9 mg/ml, 14.3 ± 3.1 mg/ml and 22.7 ± 4.1 mg/ml was better than activity of vinblastine sulphate; 5.9 ± 0.4 mg/ml, 59.7 ± 2.1 mg/ml and 30.3 ± 1.4 mg/ml against breast carcinoma (MCF-7), cervical carcinoma (Hela) and colorectal carcinoma (CACO), respectively (Table 6). Also, the Plectranthus arabicus alcoholic extract showed higher antitumor activity; 15.3 ± 5.3 mg/ml, 28.6 ± 3.6 mg/ml and 24.3 ± 4.1 mg/ml with comparison to vinblastine; 21.2 ± 0.9 mg/ml, 59.7 ± 2.1 mg/ml and 30.3 ± 1.4 mg/ml against prostate carcinoma (PC3), cervical carcinoma (Hela) and colorectal carcinoma (CACO), respectively. Moreover, the antitumor activity of Plectranthus asirensis against cervical carcinoma (Hela) (37.1 ± 2.6 mg/ml) was potent than vinblastine sulphate (59.7 ± 2.1 mg/ml) (Table 6).

The obtained results of the antitumor activity of the investigated plants were almost predicted due to the high content of the phenolic compounds and flavonoids which cure the oxidative stress and paraneoplastic symptoms caused by cancer (Loiy et al., 2014).

### 3.4. Plants toxicity

The obtained results of LD50 of Calendula tripterocarpa, Centarea sinaica, Centaurea pseudosinaica, Koelpinia linearis, Plectranthus arabicus, Plectranthus asirensis and Tripleurospermum auriculatum showed no toxicity. Different doses of the alcohol extract, up to 5000 mg/kg, did not produce any sign of acute toxicity or animal death during 24 h of observation. Accordingly, it was suggested that oral LD50 of the tested extracts, up to 5000 mg/kg, is considered safe for human use (Awaad et al., 2013).
The sub-chronic toxicity also supported the nontoxicity of the plant extracts. The oral dosing; which given to rats (400 mg/kg) for 14 days, did not affect the levels of ALT, AST, total bilirubin, total proteins, albumin, urea and creatinine as compared to control (Table 7). The serum transaminase level is used as a measure of hepatic injury; it is useful for the detection of early damage of hepatic tissue (Edoardo et al., 2005). Since the activity of ALT and AST are specific assayable liver enzymes were quit similar to the control after treatment for 14 days, it means that the investigated extracts are not hepatotoxic. Urea and creatinine are used for testing the kidney damage, there will be retention of urea and creatinine in the blood, therefore any increase in serum urea and creatinine will consider as a marker for kidney damage (Salvador and Magdalena, 2015). Considering these indicators, the investigated plant extracts are found to be not nephrotoxic in rats.

**Acknowledgement**

The authors are grateful to Dr. Reham El-Meligy, Assistant Prof. of Pharmacology, Desert Research Center, Cairo, Egypt, for her assistant and help through the pharmacological study.

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