Saudi Pharmaceutical Journal 26 (2018) 198–204

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

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

Muneera S. Al-Saleem [a], Amani S. Awaad [b,*], Monerah R. Alothman [c], Saleh I. Alqasoumi [d]

[a] Chemistry Department, College of Science, Princess Nora bint Abdul Rahman University, Riyadh, Saudi Arabia
[b] Pharmacognosy Department, College of Pharmacy, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, Saudi Arabia
[c] Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia
[d] Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

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

ABSTRACT

The phytochemical screening, antimicrobial and antitumor activities of *Calendula tripterocarpa*, *Centarea sinaica*, *Centaurea pseudosinaica*, *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 pseudosinaica* against *Bacillus subtilis*, *Centaurea pseudosinaica* against *Stroptococcus 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 *Geotricum candidum*, *Candida albicans*, *C. tropicalis* and *Aspergillus fumigatus*, respectively. The antitumor activity (IC_{50}) 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 LD_{50} and sub-chronic toxicity revealed that the plants have no toxicity.

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 *Tripleeurospermum* 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,

* Corresponding author.
E-mail address: amaniawaad@hotmail.com (A.S. Awaad).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2017.12.011
1319-0164/

emmenagogue, diaphoretic, sedative (Dall'Acqua et al., 2008), healing properties (Abbasi 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 (Kozlowska et al., 2015). From the previous studies, the present study was carried out to determine the phytochemical contents, antimicrobial and antitumor activities of *Calendula 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* dried powder were carried out 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 pharmacopoeial 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), *Proteous 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), *Stroptococcus pyogenes* (RCMB 0100174-2) and; and fungal strains; *Aspergillus fumigatus* (RCMB 02568), *Candida albicans* (RCMB 05036), *C. tropicalis* (RCMB 05239), *Geotricum 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 using few drops of Tween 80 as emulsifying agent (El-Meligy et al., 2017).

2.5.3. Acute toxicity (LD_{50}) test

Dried alcohol-extract of each plant was orally given to the animal for median lethal dose (LD_{50}) 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 p50.05 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*, *Centarea 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, anthraquinones, 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*, *Centarea 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*, *Centarea sinaica*, *Plectranthus arabicus*, *Plectranthus asirensis*, and *Centarea 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

Table 1
Qualitative phytochemical analysis of the investigated plants.

Test	Plant						
	Calendula tripterocarpa	*Centarea sinaica*	*Centarea pseudosinaica*	*Koelpinia linearis*	*Plectranthus arabicus*	*Plectranthus asirensis*	*Tripleuro-spermum auriculatum*
Sterols and/or triterpenes	+	+	+	+	+	+	+
Cardinolides	–	–	–	–	–	–	–
Carbohydrates and/or glycosides	+	+	+	+	+	+	+
Flavonoides	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Saponins	–	–	–	–	–	–	–
Anthraquinones	+	+	+	+	+	+	+
Alkaloides and/or nitrogenous bases	+	+	+	+	±	±	+
Protein and/or amino acids	+	+	+	+	+	+	+

(+) present, (–) absence, (±) trace.

Table 2
Quantitative phytochemical analysis of the investigated plants.

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
Centarea sinaica	17.34 ± 1.56	7.14 ± 1.11	8.14 ± 1.37	3.11 ± 1.32	5.21 ± 1.15
Centarea 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.

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

Plant	Chemical group%						
	Carbohydrate	Lipid	Protein	Alkaloids	Flavonoids	Phenolic compounds	Tannins
Calendula tripterocarpa	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	0.11 ± 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 ± 0.4	5.19 ± 1.4	13.11 ± 1.7	8.15 ± 0.7
Plectranthus asirensis	8.92 ± 1.2	10.43 ± 1.9	6.16 ± 1.9	0.09 ± 0.2	4.89 ± 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.

potentiality to be used as therapeutic agent agents against diseases (Pane et al., 2000), due to their ability to act as free radical scavenging agents which conceders 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*, *Centaurea pseudosinaica*, *Koelpinia linearis*, *Plectranthus arabicus*, *Plectranthus asirensis* 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 (0.48 μg/ml), 39.7 ± 0.25 mm (0.03 μg/ml), 35.8 ± 0.58 mm (0.03 μg/ml), 34.7 ± 0.51 mm (0.03 μg/ml) and 32.7 ± 0.25 mm (0.98 μg/ml) was obtained by *Plectranthus arabicus* against Klebsiella pneumonia, *Tripleurospermum auriculatum* against Bacillus subtilis, *Centaurea pseudosinaica* against Bacillus subtilis, *Centaurea pseudosinaica* against Stroptococcus 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*, *Centaurea pseudosinaica*, *Koelpinia linearis*, *Plectranthus arabicus*, *Plectranthus asirensis* 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 4
Antimicrobial activity of ethanol extracts of the investigated plants.

Test organism	Plant Diameter of the inhibition zone (mm)							
	Calendula triptero-carpa	*Centarea sinaica*	*Centarea pseudo-sinaica*	*Koelpinia linearis*	*Plectran-thus arabicus*	*Plectran-thus asirensis*	*Tripleuro-spermum auriculatum*	*Standard antibiotic*
Bacteria								**Gentamycin**
Gram negative								
Escherichia coli	24.2 ± 0.13	17.4 ± 0.17	23.6 ± 0.19	17.2 ± 0.33	22.9 ± 0.25	20.4 ± 0.25	24.3 ± 0.58	25.9 ± 0.3
Klebsiella pneumoniae	15.3 ± 0.14	24.8 ± 0.03	17.6 ± 0.29	15.1 ± 0.44	41.8 ± 0.23	27.7 ± 0.25	16.1 ± 0.35	32.3 ± 0.15
Proteous vulgaris	14.8 ± 0.13	21.2 ± 0.14	16.1 ± 0.26	13.4 ± 0.13	28.9 ± 0.44	24.9 ± 0.44	17.2 ± 0.12	26.3 ± 0.3
Pseudomonas aeruginosa	21.7 ± 0.36	16.3 ± 0.32	18.3 ± 0.25	12.2 ± 0.32	18.3 ± 0.32	11.7 ± 0.43	19.1 ± 0.32	23.3 ± 0.1
Salmonella typhimurium	17.2 ± 0.16	19.4 ± 0.14	11.9 ± 0.17	19.7 ± 0.57	29.6 ± 0.44	21.4 ± 0.39	14.1 ± 0.22	33.6 ± 0.15
Gram positive								**Ampicillin**
Bacillus subtilis	14.4 ± 0.19	25.2 ± 0.27	35.8 ± 0.58	23.4 ± 0.53	31.6 ± 0.63	29.7 ± 0.63	39.7 ± 0.25	38.4 ± 0.3
Staphylococcus epidermidis	12.1 ± 0.19	17.7 ± 0.26	25.5 ± 0.44	21.0 ± 0.43	32.7 ± 0.25	26.2 ± 0.55	14.1 ± 0.24	31.2 ± 0.18
Streptococcus mutans	31.0 ± 0.53	15.7 ± 0.36	19.5 ± 0.64	19.0 ± 1.14	23.3 ± 0.44	28.4 ± 0.58	15.8 ± 0.14	34.3 ± 0.32
Streptococcus pneumoniae	10.8 ± 1.21	22.7 ± 0.36	25.5 ± 0.44	20.0 ± 0.32	26.3 ± 0.17	25.6 ± 0.44	26.6 ± 0.34	29.8 ± 0.2
Streptococcus pyogenes	13.4 ± 0.13	16.2 ± 0.23	34.7 ± 0.51	23.4 ± 0.21	27.6 ± 0.36	22.3 ± 0.25	19.2 ± 0.21	32.4 ± 0.31
Fungi								**Amphotericin B**
Aspergillus fumigatus	21.7 ± 0.36	22.8 ± 0.39	28.3 ± 0.15	17.3 ± 0.34	27.3 ± 0.39	26.4 ± 0.39	29.9 ± 0.63	29.7 ± 1.11
Candida albicans	19.3 ± 0.13	21.9 ± 0.43	29.5 ± 0.32	14.1 ± 1.21	27.6 ± 0.58	25.1 ± 0.23	34.6 ± 0.34	31.4 ± 0.38
Candida tropicalis	26.6 ± 0.54	22.7 ± 0.33	28.4 ± 0.36	17.6 ± 0.58	26.4 ± 0.53	21.6 ± 1.31	30.6 ± 0.26	28.2 ± 0.34
Geotricum candidum	23.3 ± 0.48	25.6 ± 0.19	31.8 ± 0.68	20.3 ± 0.38	28.6 ± 0.58	27.4 ± 1.28	35.9 ± 1.15	34.7 ± 0.27
Syncephalastrum racemosum	17.2 ± 0.33	19.4 ± 0.58	22.5 ± 0.27	18.1 ± 1.25	20.2 ± 0.16	17.3 ± 0.58	24.6 ± 1.27	25.7 ± 0.12

Table 5
Minimum inhibitory concentration (MIC) of ethanol extracts of the investigated plants.

Plant test organism	Minimum inhibitory concentration (μg/ml)							
	Calendula triptero-carpa	*Centarea sinaica*	*Centarea pseudo-sinaica*	*Koelpinia linearis*	*Plectran-thus arabicus*	*Plectran-thus asirensis*	*Tripleuro-spermum auriculatum*	Standard antibiotic
Bacteria								**Gentamycin**
Gram negative								
Escherichia coli	3.9	31.25	3.9	62.5	3.9	15.63	62.5	0.24
Klebsiella pneumoniae	125	3.9	62.5	250	0.48	0.97	125	0.12
Proteous vulgaris	250	15.63	31.25	500	7.81	7.81	31.25	0.24
Pseudomonas aeruginosa	15.63	31.25	31.25	500	15.63	1000	15.63	1.95
Salmonella typhimurium	250	250	1000	125	15.63	62.5	125	0.24
Gram positive								**Ampicillin**
Bacillus subtilis	62.5	15.63	0.03	31.25	0.48	0.97	0.03	0.007
Staphylococcus epidermidis	31.25	15.63	3.9	3.9	0.98	3.9	31.25	0.24
Streptococcus mutans	0.98	15.63	1.95	1.95	0.98	0.98	31.25	0.24
Streptococcus pneumoniae	500	31.25	7.81	125	7.81	7.81	7.81	0.98
Streptococcus pyogenes	62.5	31.25	0.03	3.9	1.95	3.9	7.8	0.24
Fungi								**Amphotericin B**
Aspergillus fumigatus	15.63	15.63	1.95	62.5	3.9	3.9	1.95	0.98
Candida albicans	62.5	31.25	1.95	250	1.95	3.9	0.98	0.48
Candida tropicalis	1.95	3.9	0.98	31.25	1.95	15.63	0.98	0.24
Geotricum candidum	7.8	7.8	0.98	15.63	0.98	1.95	0.24	0.03
Syncephalastrum racemosum	250	62.5	31.25	62.5	62.5	125	15.63	7.8

Table 6
The IC_{50} values of the investigated plants on different cell lines.

PlantCell line	IC_{50} (μg/ml)						
	A-549 (Lung carcinoma)	CACO (colorectal carcinoma)	HCT-116 (Colon carcinoma)	Hela (Cervical carcinoma)	HepG-2 (Hepatocellular carcinoma)	MCF-7 (Breast carcinoma)	Pc3 (Prostate carcinoma)
Vinblastine Sulphate	24.6 ± 0.7	30.3 ± 1.4	3.5 ± 0.2	59.7 ± 2.1	2.93 ± 0.3	5.9 ± 0.4	21.2 ± 0.9
Calendula tripterocarpa	157 ± 8.2	130 ± 4.2	28.6 ± 2.8	77.6 ± 2.4	56.1 ± 0.3	46.4 ± 0.3	132 ± 1.4
Centarea sinaica	32.6 ± 3.4	22.7 ± 4.1	5.5 ± 1.9	14.3 ± 3.1	3.8 ± 1.6	3.1 ± 6.9	28.5 ± 2.4
Centarea pseudosinaica	56.7 ± 0.5	78.6 ± 3.6	18.6 ± 0.8	87.5 ± 1.7	10.2 ± 2.1	15.3 ± 1.6	52.9 ± 3.2
Koelpinia linearis	68.2 ± 5.9	112 ± 5.4	35.8 ± 3.3	89.3 ± 2.4	24.3 ± 1.5	19.6 ± 1.4	144 ± 4.4
Plectranthus arabicus	30.7 ± 2.4	24.3 ± 4.1	10.6 ± 2.3	28.6 ± 3.6	7.1 ± 2.6	12.7 ± 2.1	15.3 ± 5.3
Plectranthus asirensis	156 ± 5.2	174 ± 4.2	500 <	37.1 ± 2.6	500 <	13.2 ± 0.1	500 <
Tripleuro-spermum auriculatum	42.7 ± 6.2	48.1 ± 1.6	19.9 ± 2.3	85.5 ± 1.2	8.7 ± 1.9	10.3 ± 0.9	48.2 ± 2.9

Values are the mean of triplicates ± standard deviation.

The antitumor activity (IC_{50}) 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 (Table 6). Also, the *Plectranthus arabicus* alcoholic extract showed higher antitumor activity; 15.3 ± 5.3 μg/ml, 28.6 ± 3.6 μg/ml and 2 4.3 ± 4.1 μg/ml with comparison to 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. Moreover, 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) (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 LD_{50} 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 LD_{50} of the tested extracts, up to 5000 mg/kg, is considered safe for human use (Awaad et al., 2013).

Table 7
Effect of the total alcohol extracts of plants under investigations on liver and kidney functions.

Plant	Parameter						
	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Urea (mg/dL)	Creatine (mg/dL)
Control	63.33 ± 2.4	48.90 ± 2.2	1.70 ± 0.1	8.20 ± 0.3	3.7 ± 0	35.06 ± 2.3	0.43 ± 0.7
Calendula tripterocarpa	64.30 ± 2.5	49.40 ± 2.5	1.61 ± 0.3	8.90 ± 0. 4	3.9 ± 0.2	36.13 ± 2.1	0.61 ± 0.6
Centarea sinaica	65.10 ± 2.1	48.62 ± 2.2	1.83 ± 0.4	8.65 ± 0.2	3.5 ± 0.5	34.23 ± 2.1	0.53 ± 0.4
Centarea pseudosinaica	63.30 ± 2.5	50.10 ± 2.2	1.68 ± 0.2	8.87 ± 0.3	3.8 ± 0.7	34.85 ± 2.5	0.52 ± 0.7
Koelpinia linearis	64.30 ± 2.3	47.90 ± 2.8	1.69 ± 0.3	8.32 ± 0. 4	3.9 ± 0.2	36.23 ± 2.1	0.61 ± 0.4
Plectranthus arabicus	64.10 ± 2.6	49.12 ± 2.2	1.71 ± 0.3	8.63 ± 0.5	3.6 ± 0.5	35.98 ± 2.2	0.56 ± 0.4
Plectranthus asirensis	64.30 ± 2.5	50.40 ± 2.5	1.68 ± 0.2	8.54 ± 0.3	3.8 ± 0.8	36.65 ± 2.3	0.52 ± 0.6
Tripleuro-spermum auriculatum	64.30 ± 2.5	50.40 ± 2.5	1.68 ± 0.2	8.54 ± 0.3	3.8 ± 0.8	36.65 ± 2.3	0.51 ± 0.9

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.

References

Abbasi, A.M., Khan, M.A., Ahmad, M., Zafar, M., Jahan, S., Sultana, S., 2010. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province Pakistan. J. Ethnopharmacol. 128, 322–335.

Akinyeye, A., Solanke, E., Adebiyi, I., 2014. Phytochemical and antimicrobial valuation of leaf and seed of Moringa olifera extracts. Int.J. Res. In Med. Health Sci. 4(6):2307–2083.

And, Amita P, Shalini, T., 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J. Pharmacogn. Phytochem. 2 (5), 115–119.

Astari, K.A., Erel, S.B., Bedir, E., Karaalp, C., 2013. Secondary metabolites of *Centaurea cadmea* boiss. Rec. Nat. Prod. 7, 242–244.

Awaad, A.S., Al-Refaie, A., El-Meligy, R.M., Zain, M.E., Soliman, G.H., Marzoke, M.S., El-Sayed, N.H., 2016. Novel compounds with new anti-ulcergenic activity from *Convolvulus pilosellifolius* using bio-guided fractionation. Phytother. Res. 30, 2060–2064.

Awaad, A.S., El-Meligy, R.M., Al-Jaber, N.A., Al-Muteeri, H.S., Zain, M.E., Alqasoumi, S. I., Alafeefy, A.M., Donia, A.M., 2013. Anti-ulcerative colitis activity of the total alcohol extracts and isolated compounds from *Euphorbia granuleta* Forssk. Phytother. Res. 27, 1729–1734.

Baciu, A.D., Mihalte, L., Sestras, A.F., Sestras, R.E., 2010. Variability of decorative traits, response to the Aphis fabae attack and RAPD diversity in different genotypes of *Calendula*. Not. Bot. Hort. Agrobot. Cluj 38, 265–270.

Dall'Acqua, S., Cervellati, R., Loi, M.C., Innocenti, G., 2008. Evaluation of *in vitro* antioxidant properties of some traditional Sardinian medicinal plants: investigation of the high antioxidant capacity of *Rubus ulmifolius*. Food Chem. 106, 745–749.

Disha, A., Anita, R., Anupam, S., 2013. A review on phytochemistry and ethnopharmacological aspects of genus *Calendula*. Pharmacogn. Rev. 7 (14), 179–187.

Edoardo, G., Roberto, T., Vincenzo, S., 2005. Liver enzyme alteration: a guide for clinicians. CMAJ 172 (3), 367–379.

El-Alfy, T., El-Sawi, S., Abdel-Tawab, S., Moawad, D., 2012. Bulletin of Faculty of Pharmacy. Cairo University, 50(1), 17–39.

El-Meligy, R.M., Awaad, A.S., Soliman, G.A., Bacha, A.B., Alafeefy, A.M., Kenawy, S.A., 2017. Prophylactic and curative anti-ulcerative colitis activity and the possible mechanisms of action of some desert plants. J. Enzyme Inhib. Med. Chem. 30 (2), 250–258.

Erel, S.B., Karaalp, C., Bedir, E., Kaehlig, H., Glasl, S., Khan, S., Krenn, L., 2011. Secondary metabolites of *Centaurea calolepis* and evaluation of cnicin for anti-inflammatory, antioxidant, and cytotoxic activities. Pharm. Biol. 49, 840–849.

Gislene, G.F., Nascimento, J.L., Freitas, P.C., Silva, G.L., 2000. Antimicrobial activity of some Brazilian plant. Braz. J. Microbiol. 31, 247–256.

Golus, J., Sawicki, R., Widelski, J., Ginalska, G., 2016. The agar microdilution method – a new method for antimicrobial susceptibility testing for essential oils and plant extracts. J. Appl. Microbiol. 121 (5), 1291–1299.

Guowei, Z., Tian, B., Fujuan, Z., Faqing, T., Weiqi, 2011. Plant adaptation to frequent alterations between high and low temperatures: remodeling of membrane lipids and maintenance of unsaturation levels. Plant Cell Environ. 34 (9), 1431–1442.

Hajimehdipoor, H., Gohari, A.R., Ajani, Y., Saeidnia, S., 2014. Comparative study of the total phenol content and antioxidant activity of some medicinal herbal extracts. Res. J. Pharmacogn. 1 (3), 21–25.

Ibrahim, H.M., Abu-Salem, F.M., 2014. Antibacterial activity of some medicinal plant extracts. World Acad. Sci. Eng. Technol. 8 (10), 1168–1173.

Kameyama, Y., Yamashita, K., Kobayashi, K., Hosokawa, M., Chiba, K., 2005. Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+ C1007G, by using transient expression systems of HeLa and HEK293 cells. Pharmacogenet. Genomics 15, 513–522.

Kokate, C., Purohit, A., Gokhale, S., 2004. Pharmacognosy. Vallabh Prakashan, New Delhi, pp. 466–470.

Kozlowska, M., Laudy, A.E., Przybył, J., Ziarno, M., Majewska, E., 2015. Chemical composition and antibacterial activity of some medicinal plants from Lamiaceae family. Acta Pol. Pharm. 72 (4), 757–767.

Krishnaiah, D., Devi, T., Bono, A., Sarbatly, R., 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plants. Res. 3 (2), 67–72.

Loiy, A., Mohamed, B., Aman, S., Hussein, M., Nahdzatul, S., Zeyad, D., Amin, M., 2014. Correlation of antiangiogenic, antioxidant and cytotoxic activities of some Sudanese medicinal plants with phenolic and flavonoid contents. BMC Complement Altern Med. 14, 406.

Migahid, A.M., 2002. Flora of Saudi Arabia. 4th ed. King Saud University Press., Volume 1, 127.

Mouffok, S., Haba, H., Lavaud, C., Long, C., Benkhaled, M., 2012. Chemical constituents of *Centaurea omphalotricha* Coss. & Durieu ex Batt. & Trab. Rec. Nat. Prod. 6, 292–295.

Muley, B.P., Khadabadi, S.S., Banarase, N.B., 2009. Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae). Trop. J. Pharm. Res. 8 (5), 455–465.

Okach, D., Nyunja, A., Opande, G., 2013. Phytochemical screening of some wild plants from Lamiaceae and their role in traditional medicine in Uriri District – Kenya. Int. J. Herbal Med. 1 (5), 135–143.

Oksana, S., Irene, H., Marek, Z., Cornelia, R., Marian, B., 2016. Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. Saudi J. Biol. Sci. https://doi.org/10.1016/j.sjbs.2016.01.036.

Onanong, K., Sirithon, S., Natthid, W., Naret, M., 2011. Phenolic compounds and antioxidant activities of edible flowers from Thailand. J. Funct. Foods 3 (2), 88–99.

Pane, T., Razdanac, C., Andotraa, A., Kallaac, S., Koulb, C., 2000. Koelpinin-A, B and C—three triterpenoids from Koelpinia linearis. Phytochemistry 53 (2), 305–309.

Passalacqua, N.G., Guarrera, P.M., De Fine, G., 2007. Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). Fitoterapia 78, 52–68.

Paula, N., Paul, R., 2011. Determination of anthocyanins in cranberry fruit and cranberry fruit products by high-performance liquid chromatography with ultraviolet detection: single-laboratory validation. J AOAC 94 (2), 459–466.

Raja, R., 2012. Medicinally potential plants of Labiatae (Lamiaceae) family: an overview. Res. J. Med. Plants 6, 203–213.

Sabry, O.M., El-Sayed, A.M., Slee, A.A., 2016. Potential anti-microbial, anti-inflammatory and anti-oxidant activities of *Haplophyllum tuberculatum* growing in Libya. J. Pharmacogn. Nat. Prod. 2, 1–6.

Salvador, L., Magdalena, M., 2015. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J. Nephrol. 4 (1), 57–73.

Santhi, K., Sengottuve, R., 2016. Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int. J. Curr. Microbiol. App. Sci. 5 (1), 633–640.

Seru G, Maddi R, Kanuri Y, Vijay K, Dibbanti H., 2013. Quantitative phytochemical estimation and evaluation of hepatoprotective activity of methanolic extract of Dendrobium ovatum (L.) Kraenzl. whole plant against CCl4 induced hepatotoxicity. J. Pharmac. Photochem., 2(3), 113–118.

Sivarajan, V.V., Balachandean, I., 1999. Ayurvedic Drugs and their plants sources. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, p. 96–121.

Sneh, V., Tandra, M., Revathy, T., Suthindhiran, K., Jayasri, M.A., 2013. Phytochemical and pharmacological evaluation of selected plants. Am. J. Biochem. Biotechnol. 9 (3), 291–299.

Tiwari, S., 2008. Plants: A rich source of herbal medicine. J. Nat. Prod. 1, 27–33.

Tiwari, S., Bimlesh, K., Mandeep, K., Gurpreet, K., Harleen, K., 2011. Phytochemical screening and extraction: a review. Int. Pharm. Sci., 98–106

Vinesh, K., Devendra, T., 2013. Chemical composition and biological activities of essential oils of genus *Tanacetum* – a review. J. Pharmacogn. Phytochem. 2 (3), 159–163.

Zain, M.E., Awaad, A.S., Al-Outhman, M.R., El-Meligy, R.M., 2012. Antimicrobial activities of Saudi Arabia desert plants. Phytopharmacology 2, 106–113.