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Original article

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

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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,

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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,

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