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CYTOTOXIC AND ANTIBACTERIAL EFFECTS OF *ANTHEMIS GAYANA* FLOWERS

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ABSTRACT

Herbal drugs are used for prevent and treat diseases and they are known as the oldest form of human health care. The *Anthemis* genus belongs to Asteraceae family that sesquiterpene lactones, flavonoids and polyacetylenes are the main secondary metabolites of this genus. According to the effects of medicinal plants to treatment of diseases, the aim of this study was investigated cytotoxic and antibacterial effects of *Anthemis gayana* flowers.

A. gayana was collected from Isfahan, Iran then flower powder was extracted with ethanol and methanol and the (1-100 µg/ml) concentrations was tested on HT-29 cell line for cytotoxic effect, moreover essential oil was tested invitro against 4 bateria in dilution of (1%, 2%, 5%, 10%, 15%, 20%, 40%, 80%). Antibacterial activity was tested by using microdilution MIC determination and agar well diffusion assay. Data were analyzed by ANOVA test.

The results illustrated that essential oil in dilution 80% has more inhibitor activity and most activity were shown against *Staphylococcus aureus* and *Acinetobacter baumannii*. The *A.*

gayana flowers inhibited bacteria growth but their effectiveness varied. Moreover, the ethanolic and methanolic extracts were significantly effective on cells growth at different concentrations. The antibacterial activities are due to the presence of the terpenes in the essential oil. Also, cytotoxic activity of *A. gayana* is due to the extraction of flavonoids, phenolic compounds and anthocyanins. However, more studies are needed in this regard.

Keywords: *Anthemis gayana*, Antibacterial, Cytotoxic, Extract, Essential oil

INTRODUCTION

Herbal drugs are used for years to prevent and treat diseases over the world. Moreover, they are known as the oldest form of human health care [1]. Natural resources are used to treat 87% of human diseases including bacterial infection, immunological disorders and prevention of cancer due to the presence of the anticancer compounds. Moreover, the components of many plants have antiseptic, antibiotic and antioxidant properties [2,3]. The secondary metabolites of plants are protected them from microbial pathogens. However, the phytochemical compounds are used as antimicrobial agents to inhibit the pathogenic fungi [4].

The genus *Anthemis* belongs to Asteraceae family consists of 39 species that distributed all over of Iran and are used in Turkish folk medicine for treatment the disorders of inflammatory, hepatic, gastrointestinal, hemorrhoid, stomachache, abdominal pain, and kidney stones. Sesquiterpene lactones, flavonoids and polyacetylenes are the main secondary metabolites of this genus. Moreover, several plants belonging to this genus have been shown the important anti-inflammatory, anti- *Helicobacter pylori*, antiprotozoal, and antimicrobial properties [4,5]. *A. gayana* Boiss is an annual native plant of this genus. This plant grows in Isfahan province, Iran. Essential oil of flower and leaf of this genus were investigated and more than 45 and 34 compounds have been isolated, respectively [4]. According to the effects of medicinal plants to treatment of diseases, the aim of this study was investigated cytotoxic and anti-bacterial effects of *Anthemis gayana* flowers.

MATERIALS AND METHOD

Collection of plant

Anthemis gayana Boiss. was collected from around area of Isfahan city (Iran) in the June of 2012. The voucher specimen was incorporated at the herbarium of Research Institute of Isfahan Forests and Rangelands.

Preparation of ethanolic and methanolic extracts

The flowers were dried under shade and ground into fine powder using electric blender, then, 20 g of powder were extracted with 270 ml ethanol and methanol (CH3OH) separately, by Soxhlet extraction for 6h. The residue was dried and then evaporated by using a rotary. The dried extract was stored at 4°C until used.

Essential oil preparation

50 g of flowers powder were obtained from hydro-distillation (500 ml distillate water) for 4h using a Clevenger-type apparatus. Oil sample was stored at 5°C in dark until each experiment.

Study of cytotoxic activity

Cell line HT-29 was purchased from Pasteur Institute of Iran. Cells were cultured in RPMI-1640 (PAA- Austria) medium enriched with 10% fetal bovine serum (FBS), (Gibco-Scotland) and Streptomycin (50 µg/ml) and penicillin (50 IU/ml) antibiotics, in an incubator with 5% COR2R, 37ºC and humidity 95%. After 5 or 6 times passages, cells went to logarithmic phase. In order to investigate the effects of *A. gayana* ethanolic, methanolic extracts on cell death rate, methyl thiazol tetrazolium (MTT) assay was used. To this purpose, 180 µl cell suspension contained 3×10P4P cells was poured into 96 wells of a microplate and incubated 24 h in mentioned conditions. Afterwards, 20 µl of ethanolic and methanolic flower extracts (1, 2, 12.5, 25, 50 and 100 µg/ml) were added to wells. The microplate was then incubated for 48 h and after this time, 180 µl culture medium of each well was exchanged with new medium and 20 µl MTT (Sigma-Germany) was added to each well and incubated for 3h. Then, 180 µl of the old culture medium contained MTT was exchanged with 150 µl DMSO to dissolve pramosone crystals and absorbance was read in 540 nm by ELISA plate reader (Statefax-USA) [6]. Each concentration of sample was repeated in 3 separate experiments. The growth rate in negative control samples considered 100% and doxorubicin was used as positive control.

Strains and growth conditions

Microorganisms were isolated from patients and recognized as *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Staphylococcus aureus*. Two strains of gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa*, and two strains of gram-positive bacteria *Acinetobacter baumannii* and *Staphylococcus aureus* were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Well-diffusion assay

100 ml of 1.5 × 108 CFU/ml bacteria suspension was spread monotonically onto the Mueller-Hinton Agar (MHA) plate using cotton swabs. Next, wells which arranged on the agar surface impregnated with 1%, 2%, 5%, 10%, 15%, 20%, 40%, 80% dilution of flower essential oil were placed onto agar plates. Inhibition diameters (in mm) were measured after incubation at 37°C for 24h [7].

Determination of MIC by microdilution

Serial dilutions of the essential oil (40%, 20%, 10%, 2.5%, 1.25%, 0.625%, 0.313%, 0.156%, 0.078%) were load in a microdilution plate (96 wells). Then, the inoculums (150µl 1.5×108 CFU/ml) were added to each well. The microplates were incubated at 37°C for 24h. The MIC was defined as a lowest concentration which resulted in inhibition of visual growth absorbance was read in an ELISA plate reader at 630 nm (ELISA, Stit fax 2100, USA). Minimal bactericidal concentrations (MBC) were determined by subculturing 10µl of the culture from each negative well and from the positive control, measured as explained [8].

Statistical analysis

All data were presented as Mean ± SD. The statistical comparisons were done with ANOVA test by SPSS 18 software.

RESULTS

Cytotoxic activity

The results showed that, flower methanolic extract of *A. gayana* has significantly effective on cells growth at different concentrations and days. Moreover, all concentrations of this extract showed significant difference (P= 0) with negative control (**Figure 1**).

A. gayana ethanolic extract was effective on cells growth at different concentrations and days. Whereas, all concentrations of this extract showed significant difference (P= 0) with negative control. Moreover, the extract at 1 µg/ml showed significant difference (P< 0.05) with positive control (**Figure 2**).

So that, minimum and maximum cell survival were 13.87- 52.35% for ethanolic and 8.3-59.1% for methanolic extract. Also, the IC50 of methanolic extract was 7.87 µg/ml and for ethanolic extract was 6.32 µg/ml.

Antibacterial activity

The antibacterial activity of the essential oil of *A. gayana* flower evaluated against four gram-positive and gram-negative bacteria with a MIC assay. The mean MICs and MBCs for the essential oil are given in **Table 1**. It should be noted that, the dilution 80% had strong effect. As well as, it showed strong activity with MICs of 5%.

Essential oil was tested for its power of antibacterial activity using a well-diffusion assay which wells were plated onto MHA

plates. Maximum inhibition zone (20.50±1.91 mm) of essential oil was obtained against *Staphylococcus aureus* in 80% dilution. In other, minimum inhibition zone (3.66±0.57 mm) of that was obtained against *Escherichia coli*, in 20% dilution. Results showed that, there is direct relationship between dilution and inhibition zone (**Table 2**). Moreover, there are significant changes between the essential oil and positive control sample ($P<0.001$).

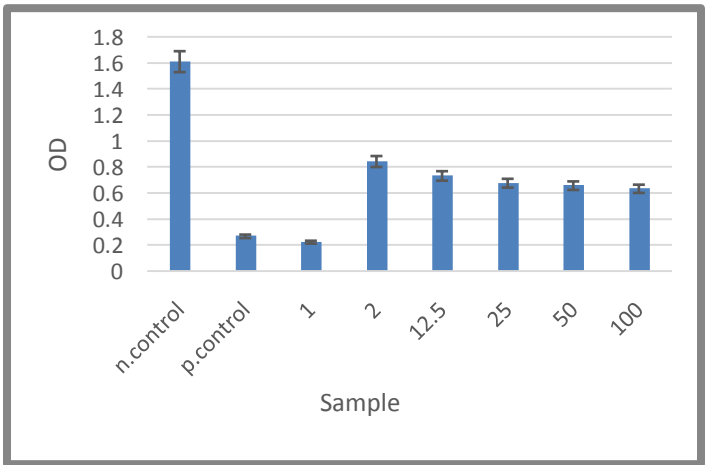

Figure 1: Significant reduction of cell growth at different concentrations of flower methanolic extract in compared with control group

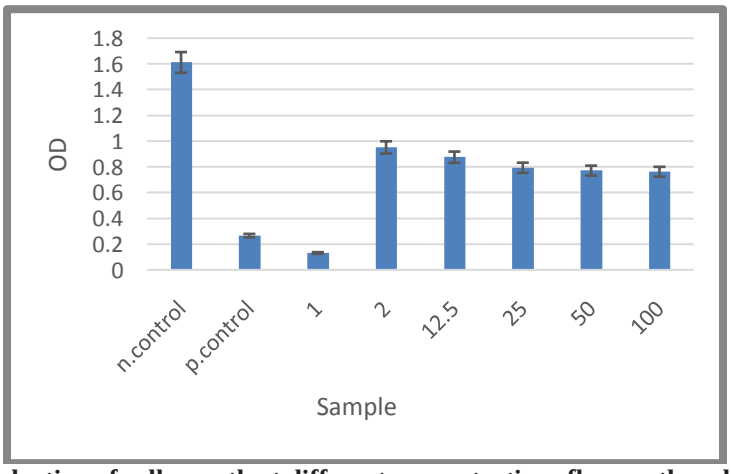

Figure 2: Significant reduction of cell growth at different concentrations flower ethanolic extract in compared with control group

Table 1: Determination of MIC and MBC of *Anthemis gayana* flower essential oil against bacteria

Bacteria	MIC (%)	MBC (%)
Escherichia coli	**10**	**20**
Pseudomonas aeruginosa	**-**	**-**
Acinetobacter baumannii	**10**	**20**
Staphylococcus aureus	**5**	**10**

Table 2: The antibacterial activity of the flower essential oil of *Anthemis gayana* on different bacteria

Concentration	20%	40%	80%	Control +	Control -
Escherichia coli	**3.66±0.57**	**8.33±0.57**	**15± 0.81**	**35**	**0**
Pseudomonas aeruginosa	**-**	**-**	**16.25 ±3.40**	**30**	**0**
Acinetobacter baumannii	**-**	**10.66± 0.57**	**19.75 ± 1.25**	**10**	**0**
Staphylococcus aureus	**-**	**11.33± 0.57**	**20.50 ± 1.91**	**14**	**0**

Inhibition zone: mm, Mean ±SD

DISCUSSION

Recently, The results show that most extracts and essential oils extracted from medicinal plants has anti-fungal, anti-parasitic, anti-bacterial, anti-viral and anti-tumor properties and this is due to the presence of secondary metabolites in plants [9]. So that, sesquiterpene lactones, polyacetylenes and flavonoids are the main secondary metabolites of Anthemis genus [10]. *Artemisia nilagirica* leaf ethanol extract showed the antibacterial activity (200 µg/ml) against *Mycobacterium smegmatis* and cytotoxic activities (500 µg/ml) on mouse macrophage (RAW264.7) cell line. Also, this plant induced apoptosis in cells. This results could be attributed to production of ROS and finally, disruption of membrane and cytoskeletal function [11]. Dichloromethane fraction of *Vernonica scorpio* (5 mg/kg) had cytotoxic activity against Ehrlich ascitic tumor. Plant products such as vinblastine, vincristine, taxol and many other substances are great sources for cancer treatment. *V. scorpio* has a cytotoxic effect due to the presence of sesquiterpene lactones. Moreover, some enzymatic pathways and increased neutrophil by this plant followed by antitumor effect [12]. Cytotoxic effect of *Artemisia turanica* CH2Cl2 extract at (0–200 μg/mL) was reported by Tayarani-Najaran et al. (2013) on K562 and HL-60 cell lines that this effect was dose dependent. Phytochemical investigation of *Centaurea omphalotricha* showed the presence of flavonoid and sesquiterpene lactones (germacranolides, elemanolides, eudesmanolides and guaianolides) that responsible for their pharmacological properties. So that, this plant showed cytotoxic effect against human leukemia HL-60 and U937 cells [13]. Sesquiterpene lactones acts through inhibition of functional proteins and enzymes. Moreover, the properties of this compound might be due to their lipophilicity; however, other factors may affect on activity of sesquiterpene lactones [14]. Hydroalcoholic extract of *Artemisia*

Kopetdaghensis had cytotoxic effects on mouse fibroblast (L929) and Chinese hamster ovary cells that probably due to the presence of camphor [15]. Methanolic extract and essential oils of *Achillea wilhelmsii* due to the presence of phenolic compounds (especially flavonoids) and monoterpenes showed cytotoxic effect against HT-29 cell line. Flavonoids induce apoptosis and inhibit cancer cells [16].

In vitro studies in this research showed antibacterial properties of *Anthemis gayana*. Totally, the gram–positive bacteria are more sensitive than gram–negative bacteria due to the differences in structure of their cell wall. Gram–negative bacteria are resistant due to their outer membrane that act as a barrier against many factors such as antibiotics [17,18,19]. However, the results from Sonboli et al., 2005 reveals the presence of germacrene D (30.2%), geranyl isovalerate (7.4%), bicyclogermacrene (6.7%) and caryophyllene (5.5%) in *Anthemis gayana* leaves essential oil. These compounds showed strong antibacterial effects [20]. Germacrenes are a group of volatile organic hydrocarbons (especially sesquiterpenes) that are produced in a number of plant species and have insecticidal and antimicrobial properties [21].

CONCLUSION

The results indicated the cytotoxic activity of ethanolic and methanolic extracts of *Anthemis gayana* which is due to the extraction of flavonoids, phenolic compounds and anthocyanins that have an inhibitory effect on HT-29 cell line. Moreover, the methanol due to polarization is the best solvent for extraction of flavonoid [22]. On the other hand, antibacterial activity of this plant is likely due to the presence of terpenes in the essential oil. However, more studies are needed in this regard.

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