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Research Article

ANTIBACTERIAL ACTIVITY OF METHANOLIC EXRACT FROM *ANTHEMIS GAYANA* FLOWERS AND LEAVES

Zahra Asadi[1], [*]Leila Amjad[2] and Maryam Mohammadi-Sichani[3]
[1]*Falavarjan Branch, Islamic Azad University, Isfahan, Iran*
[2]*Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*
[3]*Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*
[*]*Author for Correspondence*

ABSTRACT
Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi, bacteria and herbivorous mammals. The aim of this study was *In vitro* studding of antibacterial effects of the leaves and flowers methanolic extract of *Anthemis gayana*. In this study extract was obtained by by Soxhlet extraction for 8h. It was tested *In vitro* against 4 bateria. Several dilution of extract (62.5, 125, 250 mg/ml) was prepared. Antibacterial activity was tested by using microdilution MIC determination and agar well diffusion assay. Data were analyzed by ANOVA test in the P-Value< 0.05. The results illustrated that leaves extract in dilution 250 mg/ml has more inhibitor activity and most activity were shown against *Pseudomonas aeruginosa* (clinical). The results of the present project indicate that the *Anthemis gayana* leaves and flowers inhibited bacteria growth but their effectiveness varied. These antibacterial activities are likely due to the presence of the phenolic compound in the extract. Therefore we will be able perform researches with extraction of this plant effective compound for the treatment of infectious disease.

Keywords: *Anthemis gayana, Antimicrobial, Extract, Flower, Leaf*

INTRODUCTION
Plants have been used in the treatment of different diseases including bacterial infections. In the recent years, anti-microbials derived from the plants have been receiving increasing attention, as synthetic antibiotics have shown ineffectiveness against several pathogenic organisms, due to increasing drug resistance. Anti-microbial activity has been reported in different plant constituents such as phenols, quinines, flavones, flavonoids, flavonols, tannins, terpenoids, essential oils and alkaloids, etc (Cowan, 1999; Harborne and Williams, 2000) .
The genus Anthemis is the second largest genus in the Asteraceae family, tribe Anthemideae, distributed all over of Iran (Uzel *et al.,* 2004). The species of the Anthemis genus are widely used in the pharmaceutics, cosmetics and food craft. The flowers of this genus have well-documented use as disinfectant and healing herbs, the main components being natural flavonoids and essential oils (Uzel *et al.,* 2004).
Anthemis gayana Boiss. is an annual endemic plant of Asteraceae family (Sonboli *et al.,* 2005). This plant grows in the Isfahan west region from Iran. Essential oil leaves were first investigated by Sonboli *et al.,* 2005 and more than 34 compounds of the leaves oil representing 92.4% of total oil were identified, germacrene-D (30.2%), geranyl isovalevate (7.4%), bicyclogermacrene (6.7%) and β–caryophyllene (5.5%) as the major compounds (Sonboli *et al.,* 2005).
Amjad *et al.,* (2013) reported that methanolic extract of *Anthemis gayana* flowers and leaves were not active against *Candida glabrata* CBS 2175 and *Candida albicans* ATCC 62061, ATCC 1677. Flowers and leaves methanolic extract had more effect against *Candida parapsilosis* (Amjad *et al.,* 2013), Thus, leaves methanol extract had more effect against *Candida albicans* ATCC 3153 (Amjad *et al.,* 2012). Vakili *et al.,* (2013) reported that essential oil of *Anthemis gayana* leaves in dilution 80% has more inhibitor activity and most activity was shown against *Escherchia coli* and *Acinetobacter baumannii*. Their results indicated that the *Anthemis gayana* leaves inhibited bacteria growth but their effectiveness varied. These antibacterial activities are likely due to the presence of the terpenes in the essential oil. Hosseini *et al.,*

(2013) reported that the *Anthemis gayana* flowers ethanolic extracts reduced significant the inflammation and pain at concentrations of 100, 200, 300 mg/kg ($p<0.001$). The maximum analgesic and anti-Inflammatory effects of the *Anthemis gayana* flowers ethanolic extracts were observed at concentration of 300 mg/kg. The results obtained in this study indicated that ethanolic extracts of *Anthemis gayana* flowers have analgesic and anti-inflammatory effects. It seems to be related to the presence of flavonoids and other terpene compounds. Quarenghi *et al.*, (2000) reported the flavonoid containing total extract from *A. cotula* flowers exhibits antimicrobial activity against both gram-negative and gram-positive microorganisms.

There is no research investigating the antibacterial properties of flowers and leaves methanolic extract of *Anthemis gayana*. For this reason, the study was to evaluate their antibacterial activities against wide variety of bacteria.

MATERIALS AND METHODS

Collection of Plant

Anthemis gayana Boiss. was collected from around area of Aligudarz (Lorestan province, Iran) in the June of 2013. The voucher specimen was incorporated at the herbarium of Research Institute of Isfahan Forests and Rangelands.

Preparation of Ethanolic and Methanolic Extracts

The flowers and leaves were dried under shade and ground into fine powder using electric blender, then, 30 g of powder were extracted with 280 ml methanol (CH3OH), by Soxhlet extraction for 8h. The residue was dried and then evaporated by using a rotary. The dried extract was stored at 4°C until used.

Strains and Growth Conditions

Microorganisms were isolated from patients and recognized as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. One strains of gram-negative bacteria *Pseudomonas aeruginosa*, and one strain of gram-positive bacteria *Staphylococcus aureus* were used. Standard microorganisms were obtained from the Institute of Scientific and Industrial Research, Iran. 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 1.5×10^8 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 62.5, 125, 250, 500 mg/ml dilution of flower and leaf extract were placed onto agar plates. Inhibition diameters (in mm) were measured after incubation at 37°C for 24h (Uzel *et al.*, 2004).

Determination of MIC by Microdilution

Serial dilutions of the extract (250, 125, 62.5, 31.25, 15.62, 7.81 mg/ml) were load in a microdilution plate (96 wells). Then, the inoculums (150µl 1.5×10^8 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 (Chan *et al.*, 2008).

Statistical Analysis

All data were presented as Mean±SD. The statistical comparisons were done with ANOVA test by SPSS 20 software.

RESULTS AND DICUSSION

The antibacterial activity of the extract of *Anthemis gayana* leaf and flower evaluated against two of gram-positive and gram-negative bacteria with a MIC and MBC assay. The mean MICs and MBCs for the extracts are given in Table 1. It should be noted that, stronger activity with MICs of 31.5 mg/ml.

Table 1: Determination of MIC and MBC of *Anthemis gayana* leaves and flowers extract against bacteria

Bacteria	MIC(mg/ml) Flower	MBC(mg/ml) Flower	MIC(mg/ml) Leaf	MBC(mg/ml) Leaf
Pseudomonas aeruginosa (clinical)	62.5	125	125	125
Pseudomonas aeruginosa (standard)	62.5	250	125	250
Staphylococcus aureus (clinical)	62.5	31.5	125	125
Staphylococcus aureus (standard)	125	125	125	125

Extracts were tested for its power of antibacterial activity using a well-diffusion assay which wells were plated onto MHA plates. The methanolic extracts of the flower and leaf exhibited antibacterial activity at against 4 bacteria. In the present study, leaf methanolic extracts in concentration 500 mg/ml have more inhibitory activity against bacteria. Thus, the inhibitory effect of the extracts was compared with standard antibacterial, ciprofloxacin. Maximum inhibition zone (25±0.45 mm) of leaf extract was obtained against *Pseudomonas aeruginosa* (clinical), in 500 mg/ml dilution (Figure 1,2). In other, minimum inhibition zone (0 mm) of leaf and flower extract were obtained against *Staphylococcus aureus* (standard), in 62.5 mg/ml dilution (Figure 3,4). Results showed that, there is direct relationship between dilution and inhibition zone. It is worth mentioning that there are significant changes between the extracts and negative control sample ($P<0.05$) (Figure 2,4).

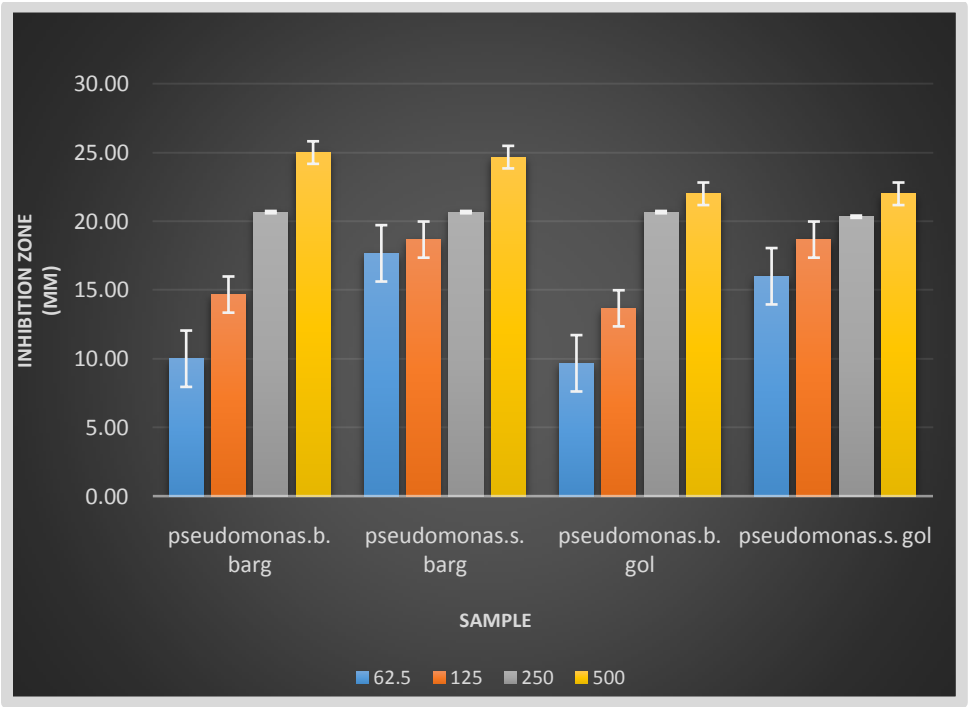

Figure 1: The antibacterial activity of methanolic extract of *Anthemis gayana* leaf and flower on *Pseudomonas aeruginosa* (clinical and standard)

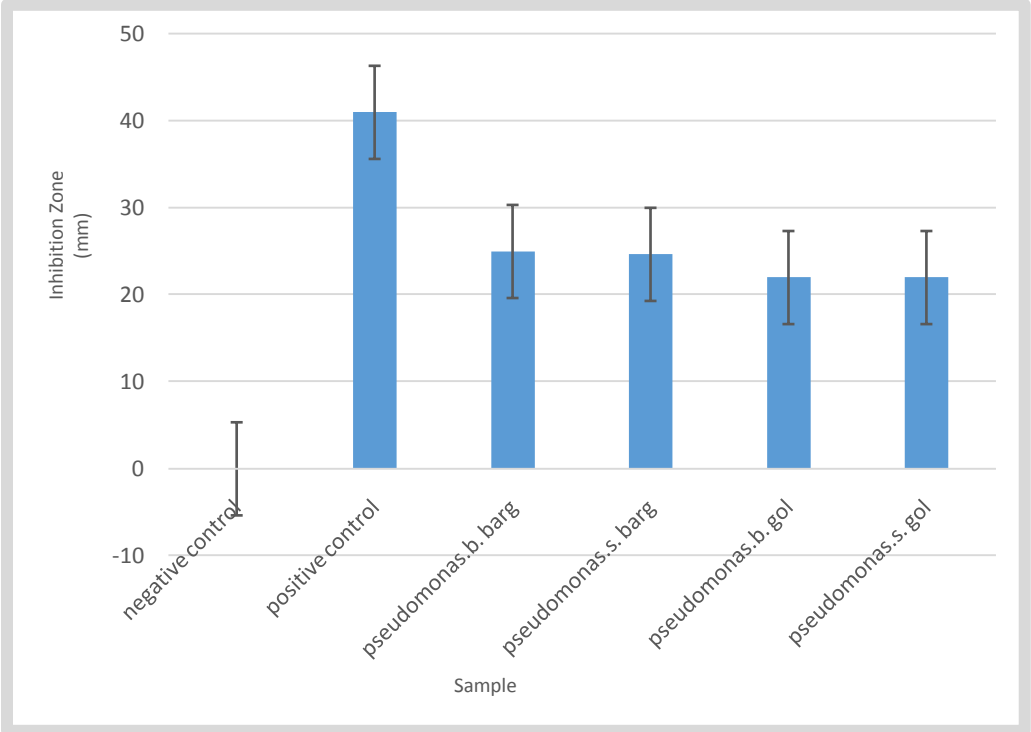

Figure 2: The antibacterial activity of methanolic extract of *Anthemis gayana* leaf and flower on *Pseudomonas aeruginosa* (clinical and standard) in compared with control groups

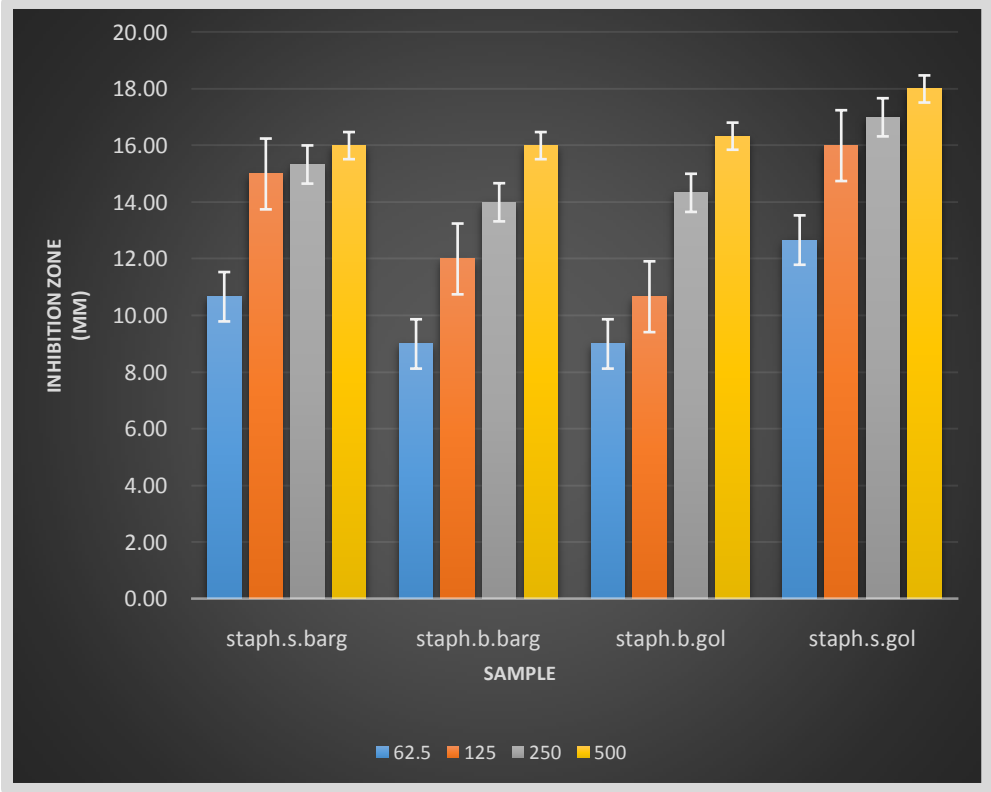

Figure 3: The antibacterial activity of methanolic extract of *Anthemis gayana* leaf and flower on *Staphylococcus aureus* (clinical and standard)

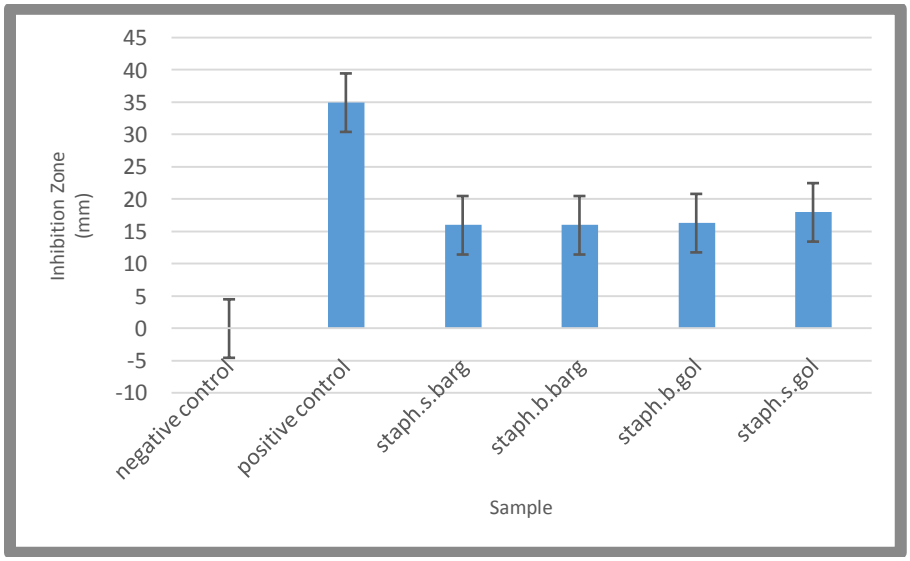

Figure 4: The antibacterial activity of methanolic extract of *Anthemis gayana* leaf and flower on *Staphylococcus aureus* (clinical and standard) in compared with control groups

In vitro studies in this work showed that *Anthemis gayana* leaves and flowers inhibited bacterial growth, but their effectiveness varied. Totally, the gram–positive bacteria are more susceptible than gram–negative bacteria due to the differences in their cell wall structure. Gram–negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics (Afolayan and Ashafa, 2007; Vukovic *et al.*, 2007), but, in our studies the gram–negative bacteria (*Pseudomonas aeruginosa)* is more susceptible than gram–positive bacteria *(Staphylococcus aureus).*

Most useful compounds in plants are secondary metabolites and in many cases, these products serve as plant defense mechanisms against predation by microorganisms, insects, and so forth (Cowan, 1999).

Plant extracts have been used in food preservation, pharmaceuticals, alternative medicine and natural therapies (Prabuseenivasan *et al.*, 2006). These are potential sources of novel antimicrobial compounds especially against pathogens. However, the results from this study reveals that the leaves and flowers methanolic extract of *Anthemis gayana* have also been shown to possess good antibacterial activity. The studies carried out showed that the flavonoids and phenolic compounds have also exhibited notable antimicrobial activity as well (Mothana *et al.*, 2009; Eleyinmi, 2007). Flavonoids are phenolic substances and they are known to be synthesized by plants in response to microbial infection. Their activity is probably due to their ability to complex with extracellular and soluble proteins. More lipophilic flavonoids may disrupt the microbial membranes (Dhiman *et al.*, 2011). Tannins have received a great deal of attention in recent years, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cells envelop transport proteins, etc. They also combine with polysaccharides. The mode of action of antibacterial effects of saponins seems to involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium (Dhiman *et al.*, 2011). The results of the present work indicate that the plant assayed possess antibacterial properties. This explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve bacterial infections and underline the importance of the ethnobotanical approach for the selection of plant in the discovery of new bioactive compounds. Further phytochemical research is needed to identify the active principles responsible for the antibacterial effects of this medicinal plant.

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REFERENCES

Amjad L, Madani M and Rezvani Z (2012). Potential activity of the *Anthemis gayana* leaves on fungi, *Proceedings of International Conference on Biological, Ecological and Environmental Sciences, and Engineering (ICBEESE), Dubai, United Arab Emirates* **61** 901-902.

Amjad L, Rezvani Z and Madani M (2013). The effect of methanolic extract of *Anthemis gayana* on Candida spp. *International Journal of Agriculture and Crop Sciences* **5**(10) 1140-1144.

Chan LW, Cheah EL, Saw CL, Weng W and Heng PW (2008). Antimicrobial and antioxidant activities of cortex *Magnoliae officinalis* and some other medicinal plants commonly used in South-East Asia. *Chinense Medicine* **3** 15-21.

Cowan MM (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**(4) 564-582.

Dhiman A, Nanda A, Ahmad S and Narasimhan B (2011). *In vitro* antimicrobial activity of methanolic leaf extract of *Psidium guajava* L. *Journal of Pharmecy and Bioallied Sciences* **3**(2) 226-229.

Eleyinmi AF (2007). Chemical composition and antibacterial of *Gongronema latifolium*. *Journal of Zhejiang University Science B* **8** 352-358.

Harborne JB and Williams CA (2000). Advances in flavonoid research science. *Phytochemistry* **55** 481-504.

Hosseini SH, Amjad L and Monajemi R (2013). Analgesic and anti-inflammatory effects of *Anthemis gayana* ethanolic extract in rat. *Advances in Environmental Biology* **7**(14) 4749-4754.

Mothana R, Lindequist U, Geraenert R and Bednarski P (2009). Studies of the *In vitro* anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinial plants from the island Soqotra. *BMC Complementary Alternative Medicine* **9**(7) 1-11.

Prabuseenivasan S, Jayakumar M and Ignacimuthu S (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary Alternative Medicine* **6**(39) 1-8.

Quarenghi MV, Tereschuk ML, Baigori MD and Abdala LR (2000). Antimicrobial activity of flowers from *Anthemis Cotula*. *Fitoterapia* **71**(6) 710-712.

Sonboli A, Kanani MR and Salehi P (2005). Volatile constituents of the leaves and flowers of *Anthemis Gayana* from Iran, Dig. 1th Sem. Medicinal & Natural Product Chemistry, Iran 74.

Vakili E, Amjad L and Karbasizadeh V (2013). Antibacterial properties of *Anthemis gayana* leaves essential oil. *International Journal of Agriculture and Crop Sciences* **5-13** 1464-1467.