

Antimicrobial Activity of Different Flower Extracts

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Abstract

In the present study, the antimicrobial activities of methanol extracts obtained from the flowers of *Muscari neglectum*, *Punica granatum*, *Tussilago farfara*, *Taraxacum officinale*, *Wisteria sinensis*, *Syringa vulgaris*, *Cyclamen coum*, *Chaenomeles speciosa*, *Ornithogalum umbellatum*, *Allium sicutum*, *Viburnum tinus* and *Eryngium bithynicum* were evaluated. The flower extracts with methanol were prepared in soxhlet device. The evaluation process of the antimicrobial activities was performed using the disc diffusion method. The highest antimicrobial activity has been detected at the strain of *S. aureus*. The detected inhibition zone diameters of the flower extracts were evaluated to be 15.5 mm for *V. tinus*, 15 mm for *E. bithynicum* and 14.5 mm for *P. granatum*. It has also been found that the flower extracts of *C. coum* and *M. neglectum* only effect the *C. albicans* strain. The flowers of *P. granatum* has been found to be effective on all the test microorganisms. The extracts obtained from *W. sinensis*, *T. farfara*, *T. officinale* and *O. umbellatum* have no antimicrobial effect on any test microorganisms. It was found that 7 of the 12 plant species used in the study showed antimicrobial activity. In our study, it was observed that the extracts obtained from flowers were also effective in inhibiting microorganisms. To our knowledge, this is the first study to elucidate the antimicrobial activity of *E. bithynicum*, *V. tinus* and *A. sicutum*'s flowers and our results how that the flowers of these species have the potential that can be used as antimicrobial natural sources.

Key words: Flowers, methanolic extract, disc diffusion method, antimicrobial effects

1. Introduction

Two hundred years of modern chemistry and biology have described the role of primary metabolites of plant on basic life functions such as cell division and growth, respiration, storage, and reproduction. The concept of secondary metabolite was first defined by Kossel as the opposite of primary metabolites (Bourgand et al., 2001). Secondary metabolites play a significant role in the survival of the plant in its environment. Pigments and aromatic compounds give color and smell to reproductive organs and fruits, thus attracting pollinators and facilitating the distribution of seeds by animals. Human has greatly benefited from plants and their secondary metabolites. Essential oils have an important role throughout the people's life. These oils are complex mixtures of hydrocarbons and oxygenated hydrocarbons originating from the isoprenoid pathways, which are presenting in monoterpenes and sesquiterpenes. Essential oils are produced and secreted by glandular trichomes, specialized secretory tissues diffused onto the surface of the flowers and the leaves of the plant (Verpoorte et al., 2002; Sharifi-Rad, et al., 2017). Owing to their appearance and secondary metabolites, plant flowers are used in medicine, food and garnish in many parts of the world (Pavithra et al., 2013). Especially, flowers of medicinal plants are a subject of interest due to their medicinal properties and their potential for antimicrobial activity (Hsu et al., 2010). There is a constant and urgent

need to discover new antimicrobial compounds with different chemical structures and novel mechanisms of action since there exist a great emergence of infectious diseases, repetition and increase of resistant strains (Rojas et al., 2003). The initial screening tests for plants with antibacterial activity are of importance in determining the complete antibacterial effects of plant extracts or isolated compounds that can be measured (Sharma et al., 2017).

The aim of this study was to determine the antimicrobial effect of methanolic extracts obtained from 12 different flowers on test microorganisms.

2. Material and Methods

Plant Materials: *Allium siculum* was obtained from Atatürk Horticultural Central Research Institute, Yalova, Turkey. Other plants were obtained from Esentepe Campus and its peripheral (Sakarya, Turkey) in 2018. Latin and Turkish names of the plants used in the study are given in Table 1 (Güner, 2012). The microorganism strains used in this study were *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 1029. All strains were provided from Microorganism Culture Collection of Sakarya University, Sakarya, Turkey.

Table 1. Plants used in the current study

Family Name	Plant Scientific Name	Plant Turkish Name
Adoxaceae	<i>Viburnum tinus</i> L.	Kar topu
Apiaceae	<i>Eryngium bithynicum</i> Boiss.	Çakır otu
Asparagaceae	<i>Muscari neglectum</i> Guss. ex Ten.	Arapüzümü
Asparagaceae	<i>Ornithogalum umbellatum</i> L.	Sunbala
Asteraceae	<i>Tussilago farfara</i> L.	Öksürük otu
Asteraceae	<i>Taraxacum officinale</i> F.G.Wigg	Karahindiba
Fabaceae	<i>Wisteria sinensis</i> (Sims) DC.	Mor salkım
Amaryllidaceae	<i>Allium siculum</i> Ucria	Sultan sarımsağı
Lythraceae	<i>Punica granatum</i> L.	Nar
Oleaceae	<i>Syringa vulgaris</i> L.	Leylak
Primulaceae	<i>Cyclamen coum</i> Mill.	Yer somunu
Rosaceae	<i>Chaenomeles speciosa</i> (Sweet) Nakai	Japon ayvası

Preparation of methanolic extracts: Ten grams of powdered flowers were placed in the soxhlet device, with 150 mL of methanol, and subjected to 8 hours of extraction. The solvents in the extracts were evaporated by using rotary evaporator (Heidolph) under vacuum at 45°C for 15 minutes and the dried extracts were used for all investigations. The extract concentrations were adjusted by adding methanol to each extract at the doses of 6400 µg/disc.

Disc diffusion method: The screening of methanolic extracts for the antimicrobial activity was performed by disc diffusion method. To obtain microbial cultures, the test microorganisms were inoculated at Tryptic Soy Broth (Merck) and incubated at 37°C for 24 hours. The overnight cultures were utilized to prepare the microorganisms'

suspensions which were adjusted to 0.5 McFarland by using a densitometer. The prepared microorganism suspension was implanted at the Mueller Hinton agar by using a swab. The sterile discs (6 mm in diameter) were impregnated with the 10 μ L of prepared extracts. The impregnated discs were slightly placed to the inoculated Mueller Hinton Agar. They were then incubated 37°C for 24 h. Methanol-impregnated discs were used as negative controls and the commercial antibiotic discs (Gentamicin and Amphotericin) were used as positive ones. At the end of the incubation, the inhibition zone diameters were measured and averaged.

3. Results and Discussion

In the present study, the antimicrobial activity of the plant flowers against *B. subtilis* ATCC 6633, *E. coli* ATCC 8739, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *S. typhimurium* ATCC 14028, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 1029 were determined. The antimicrobial activity results are given in the Table 2. It has been observed that the methanolic flower extracts of *S. vulgaris*, *T. farfara*, *T. officinal*, *W. sinensis* and *O. umbellatum* have no effect on the test microorganisms. The inhibition zone diameter has been evaluated to be 15.5 mm for *V. tinus*, 15 mm for *E. bithynicum*, 14.5 mm for *P. granatum*, 9.5 mm for *A. siculum* and 8 mm for *C. speciosa*, respectively.

Table 2. Antimicrobial activity results of flowers extracts

Extract (μ g/disc)	Inhibition Zone Diameter (mm)(SD \pm)							
	Ec	Se	Bs	Sa	Ef	St	Pa	Ca
<i>A. siculum</i>	0	9.5 \pm 0.7	8 \pm 0	9.5 \pm 0.3	0	0	0	11 \pm 0
<i>C. coum</i>	0	0	0	0	0	0	0	9.5 \pm 0.7
<i>C. speciosa</i>	0	0	0	8 \pm 0	0	0	0	0
<i>E. bithynicum</i>	0	9.5 \pm 0.7	11 \pm 0	15 \pm 1.0	0	0	0	0
<i>M. neglectum</i>	0	0	0	0	0	0	0	11 \pm 0
<i>O. umbellatum</i>	0	0	0	0	0	0	0	0
<i>P. granatum</i>	11.5 \pm 0.7	12.5 \pm 0.7	11 \pm 0	14.5 \pm 0.5	12 \pm 0	10.6 \pm 0.8	12 \pm 0.2	14 \pm 0.2
<i>S. vulgaris</i>	0	0	0	0	0	0	0	0
<i>T. farfara</i>	0	0	0	0	0	0	0	0
<i>T. officinale</i>	0	0	0	0	0	0	0	0
<i>V. tinus</i>	10 \pm 0	11.3 \pm 0.4	11 \pm 0	15.5 \pm 0.7	0	0	0	0
<i>W. sinensis</i>	0	0	0	0	0	0	0	0
Gentamicin	19	21	17	20	20	21	22	nt
Amphotericin	nt	nt	nt	nt	nt	nt	nt	16

nt-not tested, Ec-*Escherichia coli*, Se-*Staphylococcus epidermidis*, Bs-*Bacillus subtilis*, Sa-*Staphylococcus aureus*, Ef-*Enterococcus faecalis*, St-*Salmonella typhimurium*, Ps-*Pseudomonas aeruginosa*, Ca-*Candida albicans*

It has been determined that the flowers of *P. granatum* is effected on all test microorganisms. The flower extract of *V. tinus* produces 10 mm of zone diameter on *E. coli*, whereas it produces the zone diameters of 11.3 mm, 11mm and 15.5 mm for *S. epidermidis*, *B. subtilis* and *S. aureus*, respectively. The flower extracts of *M. neglectum* and *C. coum* are found to be effective only on *C. albicans*, producing the zone diameter of 11 mm. Even though the flowers of the plants are of importance, there are small amount of researches throughout the world since the duration of the blooming of the flowers is limited in time. However, especially in Indian medicine, the flowers are very crucial part especially in the phytotherapy applications in daily, seasonal and cultural activities. There are various studies showing the fact that the flowers show antibacterial and antifungal activities in the literature (Mlcek and Rop, 2011; Jeeva et al., 2011; Ammar et al., 2012). Antimicrobial activity of 12 plants of flowers has been evaluated in vitro against 7 bacterial and one fungus species.

The flower of *P. granatum* shows a wide effect on the test microorganisms. In a study made by Mahboubi et al. (2010) the fractions obtained from the flowers of *P. granatum* L. var. *pleniflora* have antimicrobial activity on *E. coli*, *S. aureus*, *S. dysantriae*, and *B. cereus* strains. There are several studies on the antimicrobial activities of the plant *P. granatum*' pericarp, fruit and leaf parts (Dahham et al., 2010; Tunç et al., 2013), though there is less studies on the leaf part. Therefore, to our knowledge, the results obtained from this study will contribute to the literature. Various parts of *Viburnum* sp (especially the fruit part) display antimicrobial activity (Hızlısoy 2009; Cesoniene et al., 2014; Arslan et al., 2018). There seems to be there is no any study on the flower part of *Viburnum tinus* for their antimicrobial activity. In the current study, we have shown that the flower of *V. tinus* has antibacterial activity on *E. coli*, *S. epidermidis*, *B. subtilis* and *S. aureus* bacteria. It has also been concluded from the study *S. aureus* are more sensitive to *Viburnum* extract than the other microorganisms.

While it has been known that the methanolic extract of the aerial part of *E. campestre* L. plant is only effective on *P. aeruginosa*, its ethanolic extract produces an inhibition zone of the diameter of 11.3 mm, 28.6 mm and 20 mm on *S. epidermidis*, *E. coli*, and *P. aeruginosa*, respectively (Usta et al., 2014). In our study we have measured the inhibition zone diameter for the flower extracts of the *E. bithynicum* as 15 mm, 8.5 mm and 11 mm for *S. aureus*, *B. subtilis* and *S. epidermidis*, respectively. Türker and Usta (2008) have found that the flower extract of *T. farfara* produces an inhibition zone diameter of 8.8 mm on *S. aureus*. In another study the leaf extract of *T. farfara* is said to produce inhibition zone diameters of 10 mm, 12 mm and 10 mm on *E. coli*, *S. aureus* and *C. albicans*, respectively (Dülger and Gönüz, 2004). It has been shown that the methanolic extract of *T. farfara* used in this study has not showed any antimicrobial effect. In general, it can be concluded from the antimicrobial studies that the antimicrobial effect mainly depends upon the type of plant, growing conditions, preserving conditions, the method of extraction, the type of bacteria and the strain.

There are several studies showing that the leaves of *T. officinale* have antimicrobial effects on antimicrobial researches (Ghaima Kassim et al., 2013). In this study, which is mainly concentrated on finding the antimicrobial effects of methanolic flower extracts, we have found that the flowers of *T. officinale* have no any antimicrobial effect. Özkan- Eroğlu et al. (2018) have searched the antimicrobial activity of the essential oils produced from the bulb and aerial part of *M. neglectum* (Kahramanmaraş) and found that the bulb essential oil is effective on *C. albicans*, whereas its aerial part has not shown any antifungal activity on *C. albicans*. In the current study, we have observed that the flowers of *M. neglectum* has antifungal activity on *C. albicans*. Xianfei et al. (2007) have studied the essential oils obtained from the fruits of *Chaenomeles speciosa* and searched their antimicrobial effects together with their fatty acids. They found that they have antimicrobial effect on *S. aureus*, *E. coli*, *P. aeruginosa* and *E. faecalis*. In the current study, we have found that the flowers of *C. speciosa* have very little antibacterial effect only on *S. aureus*. The discrepancies on the results can be originated from the differences of the plant collection points and the technique used in preparing the extracts. Türker and Usta (2008) have declared that the flower extracts of *C. coum* have no any antibacterial effect on *E. coli*, *P. aeruginosa*, *S. epidermidis* and *S. aureus* strains. In another work, which is parallel to our study, it has been shown that the *C. coum* extracts Show an antifungal activity on *C. albicans* and the origin of this activity is thought to be originated from the saponins contained within the structure (Sajjadi et al., 2016).

S. vulgaris and *W. sinensis* are preferred not only for their good odours and appearances but also their etheric oils contained within their matrices to be used in cosmetics. In literature, there exists no any study on the antimicrobial activities of the flowers of these species (*S. vulgaris* and *W. sinensis*). Our study has shown us the same truth that they have no any antimicrobial effect on the well known test microorganisms. In addition to that, the most species of *Allium* have shown antimicrobial activities and these activities have been measured to be maximum in fungi (Lazarevic et. al. 2010). In the current study, it has been concluded that the flowers of *A. siculum* is most effective on *C. albicans* amongst the test microorganisms used.

4. Conclusions

There is a growing interest in using natural sources having an antimicrobial potential rather than using the synthetic preservative substances. Therefore, we conclude here that the 7 plant flowers out 12 have antimicrobial activity and those can be used as natural antimicrobial sources.

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References

- Ammar I. Ennouri m. Khemakhem B. Yangui T. Attia H. 2012. Variation in chemical composition and biological activities of two species of *Opuntia* flowers at four stages of flowering. *Industrial Crops and Products* 37 (2012) 34– 40
- Arslan M. Erbil N. Murathan Z.T. 2018. Ardahan ve Çevresinde Yabani Olarak Yetişen Gilaburu Meyve Ekstraktının Antimikrobiyal, Antioksidan ve Antimutajenik Aktivitelerinin Araştırılması, *GÜFBED/GUSTIJ* (2018) 8 (1): 18-25
- Bourgaud F. Gravot A. Milesi S. Gontier E. 2001. Production of plant secondary metabolites: a historical perspective. *Plant science* 161, 839-851.
- Česonienė, L., Daubaras, R., Kraujalyte, V., Venskutonis, P.R. and Šarkinas, A., 2014. Antimicrobial activity of *Viburnum opulus* fruit juices and extracts, *Journal fur Verbraucherschutz und Lebensmittelsicherheit*, 9, 2, 129-132
- Dahham S.S. Naiman-Ali M. Tabassum H. Khan M. 2010. Studies on Antibacterial and Antifungal Activity of Pomegranate (*Punica granatum* L.) *American-Eurasian J. Agric. & Environ. Sci.*, 9 (3): 273-281, 2010
- Dülger B. Gönüz A. 2004. Antimicrobial Activity of certain Plants used in Turkish Traditional medicine. *Asian Journal of plant Sciences*3(1): 104-107
- Eroğlu-Özkan E. Demirci-Kayıran S. Toplan-Gülsoy G. Mataracı-Kara E. Kürkcüoğlu M. 2018. Identification of Volatile Compounds and Antimicrobial Activities of *Muscari neglectum* Growing in Turkey. *Fresenius Environmental Bulletin* 21:7 4674-4678
- Ghaima-Kassim K. Hashim Makie N. Abdarlasool-Ali S. 2013 Antibacterial and antioxidant activities of ethyl acetate extract of nettle (*Urtica dioica*) and dandelion (*Taraxacum officinale*). *Journal of Applied Pharmaceutical Science* Vol. 3 (05), pp. 096-099,
- Güner A., *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*, ANG Vakfı / Nezahat Gökyiğit Botanik Bahçesi, Kasım 2012.
- Hızlısoy, H., 2009. Çeşitli mikroorganizmalar üzerine gilaburunun antimikrobiyal etkisinin incelenmesi. Erciyes Üniversitesi, Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi
- Hsu W.Y. Simonne A. Weşssman A. Kim J.M. 2010. Antimicrobial Activity of Greater Galangal [*Alpinia galanga* (Linn.) Swartz.] Flowers. *Food Sci. Biotechnol.* 19(4): 873-880 (2010)
- Jeeva S. Johnson M. Aparna J.S. Irudayaraj V. 2011. Preliminary Phytochemical and Anti-Bacterial studies on Flowers of selected medicinal plants. *Int. J. Med. Arom. Plants*, Vol. 1, No. 2, pp. 107-114.
- Lazarevic, J.S. Dordevic, A.S., Zlatkovic, B.K., Radulovic, S.N., Palic, R.M. 2010. Chemical composition and antioxidant and antimicrobial activities of essential oil of *Allium sphaerocephalon* L. subsp. *sphaerocephalon* (Liliaceae) inflorescences. *J Sci Food Agric*; 91: 322–329.

- Mahboubi A. Asgarpanah J. Sadaghiyani P.N. Faizi M. Total phenolic and flavonoid content and antibacterial activity of *Punica granatum* L. var. *pleniflora* flowers (Golnar) against bacterial strains causing foodborne diseases. *BMC Complementary and Alternative Medicine* 15:366
- Mlcek J. Rop O. 2011. Fresh edible flowers of ornamental plants- A new source of nutraceutical foods. *Trends in Food Science & Technology* 22 (2011) 561-569.
- Pavithra G.M. Saba S. Abhishktha S.N. Prashith K.T.R. Vinayaka K.S. 2013. Antioxidant and antimicrobial activity of flowers of *Wendlandia thyrsoidea*, *Olea dioica*, *Lagerstroemia speciosa* and *Bombax malabaricum*. *Journal of Applied Pharmaceutical Science* Vol. 3 (06), pp. 114-120
- Rojas R. Bustamante b. Bauer J. Fernandez I. Alban J. Lock O. 2003. Antimicrobial activity of selected Peruvian medicinal plants. *Journal of Ethnopharmacology* 88, 199–204
- Sajjadi S.T. Saboor A. Mohammadi P. 2016. Comparison of aglycon and glycosidic saponin extracts of *Cyclamen coum* tuber against *Candida* spp. *Curr Med Mycol*, 2016 Jun, 2(2): 40-44
- Saratale G.R. Benelli G. Kumar G. Kim Su D. 2018. Bio-fabrication of silver nanoparticles using the leaf extract of an ancient herbal medicine, dandelion (*Taraxacum officinale*), evaluation of their antioxidant, anticancer potential, and antimicrobial activity against phytopathogens. *Environ Sci Pollut Res* (2018) 25:10392–10406.
- Sharifi-Rad J. Sureda A. Tenore G.C. Daglia M. Sharifi-Rad M. et al. 2017. Biological Activities of essential oils: From Plant chemoecology to Traditional Healing Systems. *Molecules*, 22, 70;
- Sharma A. Flores-Vallejo R.C. Cardoso-Taketa A. Villarreal M.L. 2017. Antibacterial activities of medicinal plants used in Mexican traditional medicine. *Journal of Ethnopharmacology*. 208(2017)264–329
- Tunç K. Konca T. Hoş A. 2013. *Punica granatum* Linn. (nar) bitkisinin antibakteriyel etkisinin araştırılması. *SAÜ. Fen Bil. Der.* 17. Cilt, 2. Sayı, s. 167-172,
- Türker A.U. Usta C. Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities. *Natural Product Research*, Vol. 22, No. 2, 20 January 2008, 136–146
- Usta C. Birinci-Yıldırım A. Uçar-Türker A. 2014. Antibacterial and antitumour activities of some plant grown in Turkey. *Biotechnology & Biotechnological Equipment*, Vol. 28, No. 2, 306-315,
- Verpoorte R. Contin A. Memelink J. 2002. Biotechnology for the production of plant secondary metabolites. *Phytochemistry Reviews* 1: 13-25.
- Xianfei X. Xiaoqiang C. Shunying Z. Guolin Z. 2007. Chemical composition and antimicrobial activity of essential oils of *Chaenomeles speciosa* from China. *Food Chemistry* 100 (2007) 1312–1315