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Antibacterial Activity Determination of Different Species Belonging to Asteraceae Family

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ABSTRACT

The use of herbs as alternative medicine has increased dramatically in the last years. In recent years, due to the harmful effects of chemical drugs are frequently observed antioxidant, antibacterial and antifungal properties of extracts which derived from plants and they have been used in medicine. In this study, aim was investigated the antibacterial activities of some weed species which distributed to ecologically for different conditions. The antibacterial activities of *Anthemis tinctorial* L., *Matricaria chamomilla* L. and *Achillea biebersteinii* Afan. which belonging to Asteraceae family from Amasya province were determined by using disc diffusion and micro dilution methods. The methanolic extracts of these weeds were prepared with Soxhlet Extractor. The extracts were tested against standard strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella enteritidis*. In this study, it was observed that weed extracts had antibacterial effects when they compared with control antibiotic group. the bacteria to be tested are sensitive to the control group antibiotics. According to the results of disc diffusion method, the highest antibacterial effect was identified *A. tinctorial*, *M. chamomilla* and *A. biebersteinii* respectively. The microdilution method was studied concentration range from 6.25 - to 50 mg/mL. In this context, it can be considered that these weed species are used as an alternative treatment and can lead to later antibacterial studies.

Key Words: Asteraceae, Disc diffusion, Micro dilution

Asteraceae Ailesine Ait Farklı Türlerin Antibakteriyel Aktivitesinin Belirlenmesi

ÖZET

Son yıllarda alternatif tıp uygulamalarında bitkilerin kullanımı artmıştır. Sentetik kimyasalların görülen zararlı etkilerinden dolayı tıbbi bitkilerden elde edilen özütlerin antioksidan, antibakteriyel ve antifungal özellikleri araştırılmaktadır. Bu çalışmada, Amasya ilinde farklı ekolojik şartlarda yayılış gösteren bazı bitki türlerinin antibakteriyel aktiviteleri araştırılmıştır. Asteraceae ailesine ait *Anthemis tinctorial* L., *Matricaria chamomilla* L. ve *Achillea biebersteinii* Afan türlerinin metanolik özütleri sokslet cihazı yardımıyla elde edilmiştir. Elde edilen özütlerin antibakteriyal aktivitesi disk difüzon ve mikrodilüsyon yöntemi ile belirlenmiştir. Antibakteriyel aktivite için *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ve *Salmonella enteritidis* standart suşları kullanılmıştır. Bu çalışmada, bitki özütleri kontrol olarak kullanılan antibiyotik grubuyla karşılaştırıldığında antibakteriyel etkiye sahip olduğu gözlemlenmiştir. Disk difüzyon yönteminin sonuçlarına göre, en yüksek antibakteriyel etki sırasıyla *A. tinctorial*, *M. chamomilla* ve *A. biebersteinii* olarak belirlenmiştir. Mikrodilüsyon yönteminde bitki özütlerinin konsantrasyon aralığı 6,25 - 50 mg/mL olacak şekilde ayarlanmıştır. Sonuç olarak çalışmada kullanılan bitki özütlerinin alternatif tedavi amacıyla kullanılabileceği ve daha sonra yapılacak antibakteriyel çalışmalara yön göstereceği düşünülmektedir.

Anahtar Kelimeler: Asteraceae, Disk difüzyon, Mikro dilüsyon

INTRODUCTION

Botanicals and medicinal plants in their extracts contain some special ingredients which are therapeutic value and healing effect. They have been widely used from the traditional medicine as remedy for human disease and human food in ancient times (Ong et al., 2006).

More recent researches remarked that the medicinal plant based therapy is founded numerous biological effects (Badescu et al., 2015). There are a lot of publications about various medicinal plant species but most of in the world plants are still many under–investigated species. It is known to have medicinal importance of family Asteraceae. Based on literature survey Asteraeceae or Composite (commonly referred to as the aster, daisy, composite or sunflower family) is an exceedingly large and widespread family of flowering plants (Emanuel et al., 2011). The numerous properties reported pharmacological effects of different extracts of Asteraceae family is much best known of the potential antimicrobial, antioxidant, antiviral, anticancer, anti-inflammatory and, antigenotoxic properties (Applequist and Moerman, 2015). Many members belonging to this family has an economically important properties at the same time (Lu and Wang, 2003). *Anthemis tinctoria*, *Achillea biebersteinii*, and *Matricaria chamomilla* species belonging to Asteraceae family which is one of the largest families of flowering plants are widely used in folk medicine of many nations (Nemeth and Bernath, 2008; Si et al., 2006). The aim of this paper was determination of antibacterial effects of methanol extracts of *A. tinctoria*, *A. biebersteinii* and *M. chamomilla* species against Gram-positive and Gram-negative bacteria.

MATERIAL and METHODS

Plant Material

The plant species were selected based on their biological properties uses reported in available literature (Baytop, 1999). *M. chamomilla*, *A. tinctoria*, *A. biebersteinii* of Asteraceae family were collected from the Amasya province of Turkey in 2015. The parts that used for the study were flowers and leaves these weed species.

Preparation of Extracts

The described extraction was used for the soxhlet methods with slight modifications (Salem et al., 2013). The flowers and leaves of *M. chamomilla*, *A. tinctoria*, *A. biebersteinii* were dried at room temperature, and powdered using a grinder. Methanol (Sigma) were used as solvents. Each powdered plant material (50 g) was extracted with 5000 mL of methanol in a Soxhlet apparatus (Isolab, Turkey) for eight hours. The solvents were removed by rotary evaporator at 40 °C and at low pressure. The evaporated extract were dissolved in dimethyl sulfoxide (DMSO, Sigma) at a of 50 mg/mL concentration then filtered through a sterile filter (0.45 µm) and stored at +4°C until use.

Disc Diffusion Method

The agar well-diffusion method has been used to test the antimicrobial activities of the extracts against reference bacterial strains *S. aureus* ATCC 25923, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603 and *Salmonella enteriditis* ATCC 13076. Isolates were incubated at 37ºC overnight on blood agar plates. The concentration of the microorganisms were adjusted using turbidity measurements (0.5 McFarland) using serum physiologic. These suspensions were inoculated over the entire surface of the Mueller-Hinton Agar (MHA) (Oxoid, Hampshire, England) plate by sterile swabbing. Substances were dissolved in DMSO and 20 µl of these solutions were added to the blank paper discs (Oxoid, UK). The discs were placed on a MHA medium. Later the plates were incubated at 35ºC overnight. After the incubation, the inhibitory zone diameters were measured in millimeters. All the experiments were repeated three times and the averages of the three obtained data points were reported (CLSI, 2010).

Determination of Antibacterial Activity with Microdilution

Antibacterial activities of the weed extracts were determined by broth microdilution method according to Clinical and Laboratory Standards Institute Protocols (CLSI). In this study, *S. aureus* ATCC 25923, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603 and *Salmonella enteriditis* ATCC 13076 standard strains

were tested. Weed extracts were dissolved in DMSO (Sigma, Steinheim, Germany) and sterilized by membrane filter. Extracts were tested at 1.56, 3.125, 6.25, 12.5, 25, 50 mg/mL concentrations. Standard strains were incubated on blood agar plates at 37ºC for 24 h. After incubation, bacterial suspensions were adjusted to a turbidity of 0.5 McFarland and diluted 1:100 in Mueller Hinton Broth (MHB) (Oxoid, Hampshire, England). Inoculum of 100 mL were inoculated into each well. The plates were incubated at 35 ºC for 24 h. All tests were performed on two replicate. Minimum inhibitory concentration were defined as the lowest concentrations of derivatives that inhibited growth of bacteria (CLSI, 2010).

RESULTS and DISCUSSION

Antimicrobial activities of three weed species belonging to Asteraceae family were determinated *in vitro* against five bacterial species. Crude weed extracts were obtained from the flowers and leaves of *A. tinctoria*, *M. chamomilla* and *A. biebersteinii* using methanol as solvent. From the results it was observed that all weed species tested in this study showed antimicrobial activity against some of the test microorganisms, with inhibition zones that ranged from 8 to 14 mm (Table 1).

Several previous studies on antimicrobial activity of weed extracts reported that Gram negative bacteria were the more sensitive than Gram positive (Borkataky et al., 2013). These differences may be attributed to the fact that the cell walls of Gram positive bacteria consist of a single layer, whereas those of Gram negative are multilayered structures and are quite complex (Farbood et al., 1976; Ulukanli et al., 2005). The another study reported that the most active extracts were determined as methanol to obtain from plant (Borkataky et al., 2013). Our results showed that especially *A. tinctoria* methanol extracts were mostly active on Gram negative bacteria. Moreover, methanol extracts possess the ability to dissolve and diffuse in wide variety of media. The methanol extracts of the *A. tinctoria, M. chamomilla* and *A. biebersteinii* displayed promising antimicrobial effects on some pathogens tested in this study such as *K. pneumoniae*. The sensitivity of Gram negative bacteria to extract of *A. tinctoria, M. chamomilla* and *A. biebersteinii* was in the order of *K. pneumoniae* > *P. aeruginosa* > *E. coli* > *S. enteritidis. A. biebersteinii* did not exert any antimicrobial activity against *E. coli*, *S. enteritidis* and *P. aeruginosa*. However, it showed antibacterial activity against other test organisms (Table 1). DMSO used as negative control had no antimicrobial effect on the microorganisms tested. Moreover Gentamisin and Ceftriaxone as positive control antibiotics had antimicrobial effect on the microorganisms tested (Kianbakht and Jahaniani, 2003). Determined the MIC value for same bacteria as 50 mg/mL which is similar to that obtained in our study. The microdilution method was studied concentration range from 6.25 - to 50 mg/mL. MIC of the most effective *A. tinctoria* plant extract were 12.5 mg/mL for *K. pneumoniae* and *P. aeruginosa*; 25 mg/mL for *S. aureus* and *E. coli*; 25 mg/ml for *S. enteritidis.* The MIC of the most effective *M. chamomilla* plant extract were 25 mg/mL for *K. pneumoniae, E. coli, S. enteritidis* and *P. aeruginosa*; 50 mg/ml for *S. aureus.* The MIC of the most effective *A. biebersteinii* plant extract were 25 mg/mL for *K. pneumoniae;* ≥ 50 mg/mL for *E. coli and P. aeruginosa*; 12.5 mg/mL for *S. aureus;* 50 mg/mL for *S. enteritidis* (Table 2). On the other hand in our study, methanol extracts of *A. tinctoria, M. chamomilla* and *A. biebersteinii* showed notable antimicrobial activity against two pathogens as *K. pneumoniae* and *P. aeruginosa* (Table 2). MIC results were the same of disc diffusion method. Similar studies confirm this finding (Borkataky et al., 2013).

Table 1. Results of the screening of three weed extracts belonging to asteraceae family by means of the disc diffusion method.

Weed extracts	*Zone of Inhibitions (mm) ** Microorganisms				
	** St. a.	** E. c.	** K. p.	** P. a.	** S. ent.
Anthemis tinctoria	11	9	14	14	-
Matricaria chamomilla	-	8	10	9	11
Achillea biebersteinii	14	-	11	-	-
***Gentamicin**	22	24	17	25	25
***Ceftriaxone**	9	35	25	27	18
****DMSO**	-	-	-	-	-

-: No activity. * Zone of inhibition, including the diameter of the filter paper disc (6 mm, 10 mg of extract in a disc), **Microorganisms: St.a.; *Staphylococcus aureus*, E.c.: *Escherichia coli*, K.p.: *Klebsiella pneumoniae*, P.a.: *Pseudomonas aeruginosa*, S.ent.: *Salmonella enteritidis*, DMSO: Dimethyl sulfoxide, ***30 µg Gentamicin and 30 µg Ceftriaxone as control antibiotics. **** DMSO as solvent.

Table 2. Determination of the minimum inhibitory concentrations of methanolic extracts of weed species belonging to the family asteraceae (MIC mg/mL)

Plant extracts	*Microorganisms ** MIC (mg/mL)				
	St. a.	**E. c.**	**K. p.**	**P. a.**	**S. ent.**
Anthemis tinctoria	25	25	12.5	12.5	50
Matricaria chamomilla	≥50	25	25	25	25
Achillea biebersteinii	12.5	≥50	25	≥50	50

*Microorganisms: St.a.; *Staphylococcus aureus*, E.c.: *Escherichia coli*, K.p.: *Klebsiella pneumoniae*, P.a.: *Pseudomonas aeruginosa*, S.ent.: *Salmonella enteritidis.*

CONCLUSIONS

The present work has shown that most of the used plants are good antimicrobial agents against pathogenic bacteria. But we think it would be more useful to know the chemical composition responsible for the antimicrobial effects of these plants. In this context, it can be considered that these plant species are used as an alternative treatments and can lead to later studies.

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