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The Determination of Antibacterial Activities of Herbal-Infused Syrups Recommended for Use as Food Supplements

Gıda Takviyesi Olarak Kullanımı Tavsiye Edilen Bitkisel İçerikli Şurupların Antibakteriyel Aktivitelerinin Belirlenmesi

Abstract

Aim: The study aimed to determine the antibacterial activities of herbal syrups sold as dietary supplements in pharmacies on various Gram-positive and Gram-negative bacteria.

Materials and Methods: In the study, the well diffusion and broth microdilution methods were used to determine the antibacterial activities of 3 different herbal syrups recommended for use in adults against *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 14579, while 3 different herbal syrups recommended for use as dietary supplements in children between the ages of 4-11 were examined against *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Results: According to the results of the study, all three of the herbal syrups recommended for adults and only one of the herbal syrups recommended for children showed antibacterial activity against the bacteria used in the study. When the data obtained from the methods used to determine antibacterial activity in the study were compared, it was determined that antimicrobial activity could not be detected with the well diffusion method, while syrups containing different herbal ingredients had antibacterial activity in various dilutions on different bacteria with the broth microdilution method.

Conclusion: Study results indicate that herbal syrups recommended for use as dietary supplements have antibacterial activities on Gram-positive and Gram-negative bacteria that can cause various infectious diseases. In addition, the findings obtained from the study reveal the importance of the method used to determine the antibacterial activity. According to the study results, it was determined that the well diffusion method was not sufficient to detect antibacterial activity. The broth microdilution method has been determined to be a more sensitive method than the well diffusion method, as it can detect the antibacterial activities of pharmaceutical products in different concentrations.

Keywords: Antibacterial activity, herbal dietary supplements, well diffusion method, broth microdilution method

Özet

Amaç: Çalışmada eczanelerde gıda takviyesi olarak satılan bitkisel içerikli şurupların çeşitli Gram pozitif ve Gram negatif bakteriler üzerindeki antibakteriyel aktivitelerinin tespit edilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışma kuyu difüzyon ve sıvı mikrodilüsyon yöntemleri ile yetişkinlerde kullanımı tavsiye edilen 3 farklı bitkisel içerikli şurubun *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 29213 ve *Bacillus cereus* ATCC 14579 bakterileri üzerindeki antibakteriyel aktiviteleri incelenirken, 4-11 yaş arası çocuklarda kullanımı tavsiye edilen 3 farklı bitkisel içerikli şurubun ise *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 29213 ve *Enterococcus faecalis* ATCC 29212 bakterileri üzerindeki antibakteriyel aktiviteleri incelenmiştir.

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Bulgular: Çalışma sonuçlarına göre yetişkinlerde kullanımı tavsiye edilen bitkisel içerikli şurupların hepsinin, çocuklarda kullanımı tavsiye edilen bitkisel şurupların ise yalnızca bir tanesinin çalışmada kullanılan bakteriler üzerinde antibakteriyel aktiviteye sahip olduğu tespit edilmiştir. Çalışmada antibakteriyel aktivitenin belirlenmesinde kullanılan yöntemlerden elde edilen veriler karşılaştırıldığında kuyu difüzyon yöntemi ile antimikrobiyal aktivite tespit edilemezken sıvı mikrodilüsyon yöntemi ile farklı bitkisel içerikli şurupların farklı bakteriler üzerinde çeşitli konsantrasyonlarda antibakteriyel aktiviteye sahip oldukları belirlenmiştir.

Sonuç: Çalışma sonuçları gıda takviyesi olarak kullanımı tavsiye edilen bitkisel şurupların çeşitli enfeksiyon hastalıklarına neden olabilecek Gram pozitif ve Gram negatif bakteriler üzerinde antibakteriyel aktivitelerinin mevcut olduğunu ortaya çıkarmaktadır. Ayrıca çalışmadan elde edilen bulgular söz konusu antibakteriyel aktivitenin belirlenmesinde kullanılan yöntemin de önemini ortaya koymaktadır. Çalışma sonuçlarına göre kuyu difüzyon yönteminin antibakteriyel aktivitenin tespit edilmesinde yeterli olmadığı tespit edilmiştir. Sıvı mikrodilüsyon yöntemi, farmasötik ürünlerin farklı konsantrasyonlarının antibakteriyel aktivitelerini tespit etmekle birlikte, kuyu difüzyon yöntemine göre daha duyarlı bir yöntem olarak belirlenmiştir.

Anahtar kelimeler: Antibakteriyel aktivite, bitkisel içerikli gıda takviyeleri, kuyu difüzyon yöntemi, sıvı mikrodilüsyon yöntemi

1. Introduction

In recent years, the increasing resistance to antibiotics used in the treatment of infectious diseases has led to the use of alternative products with antibacterial activities. The most important products used for this purpose are pharmaceutical products with herbal ingredients. Various herbal-based pharmaceutical products, including essential oils, have been reported to exhibit antimicrobial activity against bacteria, yeasts, and molds [1,2]. According to the World Health Organisation (WHO), approximately 20,000 medicinal plants have been approved and used for therapeutic purposes. Also, they are classified as Safe Food Assessment (SFA) [3]. The demand for herbal pharmaceutical products is increasing day by day due to their low cost, lack of side effects, low toxicity and natural composition [4]. Medicinal plants contain various bioactive compounds, including flavonoids, alkaloids, terpenoids, tannins, berberine, quinine, and emetines, which are widely utilised in the treatment of infectious diseases [5]. An increase in the number of multi-drug-resistant bacteria causes difficulties in the treatment of infectious diseases. Therefore, the use of medicinal plants with antimicrobial activity is recommended as an alternative therapeutic agent to conventional antimicrobial drugs [6]. Studies have reported that the therapeutic effects of medicinal plants are caused by the synergistic effect of multiple compounds rather than a single active ingredient, and these plants can be used effectively in the treatment of infectious diseases [7,8]. In light of these findings, recent research has focused on identifying inhibitory compounds from natural antimicrobial agents derived from plant extracts [9].

The broth microdilution method, the well diffusion method, and the disk diffusion method are widely used for the evaluation of the antimicrobial activity of herbal pharmaceutical products. These methods are reference methods for antimicrobial susceptibility testing. In dilution-based tests, the lowest concentration of antimicrobial agent that visibly inhibits bacterial growth is determined as the "Minimal Inhibitory Concentration (MIC)", which indicates the concentration required to inhibit microbial growth [10].

The aim of the study was to determine the antibacterial activities of herbal syrups sold as food supplements in pharmacies on various Gram-positive and Gram-negative bacteria. Additionally, the study sought to compare the effectiveness of the broth microdilution method and the well diffusion method in evaluating the antibacterial susceptibility of these herbal products.

2. Materials and methods

The study was conducted in two groups, and in the first group, 3 different herbal syrups recommended for use as food supplements in adults were used. The antibacterial activities of herbal syrups recommended for adults were investigated on *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 14579, bacteria frequently encountered in adults' infections.

Table 1 Herbal contents of syrups recommended for use in adults

Herbal syrup recommended for use in adults	Herbal content
Herbal syrup-A	Echinacea, plantain, grape, thyme
Herbal syrup-B	Thyme, marshmallow, liquorice root, linden flower, ginger
Herbal syrup-C	Thyme, primrose

Table 2 Herbal contents of syrups recommended for use in children

Herbal syrup recommended for use in children	Herbal content
Herbal syrup-D	Black elderberry
Herbal syrup-E	Thyme, linden, marshmallow, carob
Herbal syrup-F	Liquorice root, basil, galania, turmeric, fructus piperis nigri, mint, ginger, grape, white turmeric, black pepper

In the second group, 3 different herbal syrups recommended as food supplements for children aged 4-11 were used. The antibacterial activities of herbal syrups recommended for use in children were investigated on *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212, bacteria frequently encountered in children's infections.

The broth microdilution and the well diffusion methods were used to determine the antibacterial activities of herbal syrups.

2.1.1 The Broth Microdilution Method

Minimum inhibitory concentration (MIC) values of herbal syrups were determined using the broth microdilution method recommended for antibiotics by the Clinical and Laboratory Standards Institute (CLSI) [11]. In the antibacterial activity tests of herbal syrups, firstly, a single colony was taken from 24-hour bacterial cultures and transferred to Mueller Hinton broth and adjusted to the McFarland 0.5 turbidity standard with a spectrophotometer. Then, the bacterial count was standardised to 10^5 bacteria/ml with necessary dilutions. 200 µl of herbal syrup was placed in the first well of the 96-well round-bottom microplate. Subsequently, 100 µl of Mueller Hinton broth was placed in each of the following wells. Serial dilution was performed in the wells by transferring 100 µl of sample taken from the first well to the next well, and herbal syrup was diluted 2-fold in each well. The last well, numbered 12, was not included in the serial dilution process and was used as a negative control. Then, 10 µl of bacterial suspension was added to all wells and incubated for 24 hours at 37°C. At the end of the incubation period, the lowest concentration at which there was no growth was determined as the MIC. Cefepime antibiotic was used as a positive control in the study, and the studies were performed in three replicates.

2.1.2 The Well Diffusion Method

The disc diffusion method was performed with some modifications of previous studies that were conducted to determine the antibacterial activity of herbal products [12,13]. A single colony was taken from 24-hour bacterial cultures and transferred to Mueller Hinton broth and adjusted to the McFarland 0.5 turbidity standard by spectrophotometer. Then, the bacterial count was standardised to 10^5 bacteria/ml with necessary dilutions. 100 µl of the bacterial suspensions were inoculated on Mueller Hinton agar using the spread plate culture method. Wells were opened with glass pipettes on the medium. 20 µl of herbal syrup, 20 µl of antibiotic as a positive control and 20 µl of sterile physiological water as a negative control were added to the wells on the medium. Afterwards, the media were incubated at 37°C for 24 hours. At the end of the incubation period, the inhibition zone diameters around the wells were measured. The studies were carried out in three replicates.

3. Results

According to the study results, it was found that all three herbal syrups recommended for use in adults have antibacterial activity on the bacteria used in the study. Among the syrups recommended for use in adults, the syrup with the highest antibacterial activity at the highest dilution was the herbal syrup-A, and it has shown its antibacterial activity on *E. coli* with 12.5% dilution (Table 3). The study results showed that the only syrup that demonstrated antibacterial activity against all tested bacteria was herbal syrup-C (Table 3). Additionally, it was determined that thyme is the common ingredient in syrups recommended for both adults and children that exhibited antibacterial activity (Tables 3, 4).

According to the results of the study, it was detected that only one of the herbal syrups recommended for use in children has antibacterial activity on the bacteria used in the study. Among these syrups, only herbal syrup-E demonstrated antibacterial effects,

Table 3 MIC values of syrups recommended for use in adults according to broth microdilution method

	Escherichia coli ATCC 25922	*Klebsiella pneumoniae* ATCC 700603	*Staphylococcus aureus* ATCC 29213	*Bacillus cereus* ATCC 14579
Herbal syrup-A	12.5% dilution	Direct	25% dilution	50% dilution
Herbal syrup-B	50% dilution	50% dilution	Direct	25% dilution
Herbal syrup-C	50% dilution	50% dilution	25% dilution	50% dilution

Table 4 MIC values of syrups recommended for use in children according to the broth microdilution method

	Escherichia coli ATCC 25922	*Salmonella typhimurium* ATCC 14028	*Staphylococcus aureus* ATCC 29213	*Enterococcus faecalis* ATCC 29212
Herbal syrup-D	-	-	-	-
Herbal syrup-E	25% dilution	25% dilution	6.25% dilution	0.78% dilution
Herbal syrup-F	-	-	-	-

showing higher dilution efficacy against Gram-positive bacteria compared to Gram-negative bacteria (Table 4). When comparing Gram-positive bacteria, it was observed that herbal syrup-E maintained its antibacterial effect against *E. faecalis* even at higher dilutions (0.78%) compared to *S. aureus* (Table 4).

According to the study results, differences were detected in terms of results among the methods used to determine antibacterial activity. While antibacterial activity could not be detected in any of the herbal syrups with the well diffusion method, antibacterial activity was detected in some herbal syrups analysed with the broth microdilution method.

4. Discussion

Nowadays, the increased side effects of antibiotics and the development of multi-drug-resistant bacteria against pharmaceutical antimicrobial products increased the importance of natural herbal products and food supplements containing these substances in the treatment of infectious diseases. Plants used in traditional medicine have begun to take their place in research as new antimicrobial compounds because they are considered natural, low-cost, lacking ecotoxic side effects, therapeutic and safe [14]. There are various studies showing the antimicrobial activities of these aromatic and medicinal plants [15-17]. Since bacteria develop resistance to antibiotics, it is believed that this global problem can be prevented by the antimicrobial activities of secondary metabolites (such as phenolic compounds, flavonoids, alkaloids) found in plants [18]. For this reason, numerous studies have focused on identifying the antioxidant and antimicrobial components of different herbal products [19-21].

According to the results of the study, it was found that herbal syrups recommended for use as food supplements have antibacterial activities on Gram-positive and Gram-negative bacteria that can cause various infectious diseases. Furthermore, thyme was identified as the common ingredient in the syrups with antibacterial activity for both adults and children. Therefore, it is thought that the detected antibacterial activity is mostly caused by thyme. The study findings align with previous studies on thyme. In the study conducted by Con et al., they tested the antimicrobial activity of essential oils obtained from six different plants against *Listeria monocytogenes, S. aureus, Lactobacillus sake, Lactobacillus plantarum, Yersinia enterocolitica, Pediococcus acidilactici, Pediococcus pentosaceus, Micrococcus luteus* and determined that thyme has the highest antimicrobial activity against the tested bacteria [22]. Sagdic et al. conducted a study on the inhibition of *E. coli* O157:H7 growth by seven plant extracts (cumin, thyme, bay leaf, myrtle leaf, *Helichrysum compactum* Boiss known as everlasting, marjoram, and laurel) and found that thyme and marjoram showed higher antimicrobial activity [23]. Other studies have also demonstrated the antimicrobial effects of thyme oil extracts, with results comparable to those of the study [24-26].

The study results indicate that, among the syrups recommended for use in children, only herbal syrup-E showed antibacterial activity, showing higher dilution efficacy against Gram-positive bacteria than Gram-negative bacteria. Previous studies have shown that Gram-positive bacteria are generally more sensitive to herbal products than Gram-negative bacteria. In a study, the antibacterial effects of 46 medicinal plants and spice extracts were tested on 5 foodborne pathogenic bacteria (*B. cereus, L. monocytogenes, S. aureus, E. coli* and *Salmonella anatum*), and as a result of the study, it was determined that Gram-positive bacteria were more sensitive than Gram-negative bacteria, and the most resistant bacterium was *E. coli* and the most sensitive bacterium was *S. aureus* [27]. In the study conducted by El Astal et al., it was reported that thyme extracts showed antibacterial activity against *S. aureus* and *Enterococcus sp.* [28]. According to the results of the study, it was detected that among the syrups recommended for use in children, herbal syrup-E exhibited the highest antibacterial activity against *E. faecalis* at a 0.78% dilution and against *S. aureus* at a 6.25% dilution. These dilutions are higher than Gram-negative bacteria.

In another study, the antibacterial activities of 16 herbs hydrosols (anise, basil, cumin, dalamagia sage, dill, fennel, laurel, mint, oregano, pickling herb, rosemary, sage, summer savoury, sea fennel, sumac and black thyme were) tested against 15 bacteria and as a result of the study, it was determined that anise, cumin, marjoram, savoury (thyme), thyme hydrosols showed antibacterial activity and the highest antibacterial activity belonged to marjoram and savoury (thyme) [29]. According to the results of the study, considering the dilution ratio of syrup, most effective herbal syrup for adults is herbal syrup-A, and it contains echinacea, plantain, grape, thyme and only one effective herbal syrup for children is herbal syrup-E and it contains thyme, linden, mallow flower, and carob. These results reveal that many different herbal products have antibacterial effects on a wide variety of bacteria.

In another study, they investigated the antimicrobial activity of 16 types of plants against *B. cereus, S. aureus, L. monocytogenes, E. coli* and *Salmonella infantis* using the disk diffusion method and determined that the most

sensitive microorganism was *B. cereus* [30]. According to the results of the study, growth of *B. cereus* was inhibited with all herbal syrups recommended for adults, with herbal syrup-B specifically inhibiting *B. cereus* at a 25% dilution.

When comparing the methods used to determine antibacterial activity, it was found that while the well diffusion method failed to detect antimicrobial activity, the broth microdilution method revealed antibacterial effects of different herbal syrups at various dilutions. It is thought that the reason for this difference is likely due to the amount of herbal syrup and/or antimicrobial substance used in the broth microdilution method as well as the limited diffusion of antimicrobial metabolites from herbal syrups in the well diffusion method [31, 32].

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