



Yüzüncü Yıl Üniversitesi
Tarım Bilimleri Dergisi
(YYU Journal of Agricultural Science)

<http://dergipark.gov.tr/yyutbd>



Araştırma Makalesi (Research Article)

Antifungal and Antibacterial Effect of Dodder (*Cuscuta campestris*) Used for Hepatitis Treatment of Mothers and Newborn Infants in Province Mardin in Turkey

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Article Info

Received: 17.08.2019

Accepted: 11.12.2019

Online Published 31.12.2019

DOI: 10.29133/yyutbd.605970

Keywords

Agar disc diffusion method,
Antimicrobial effect,
Dodder,
Pathogen microorganisms,
The broth microdilution.

Abstract: Dodder is a plant used for the hepatitis treatment in new born infants in and around Mardin. The aim of this research to determine the antifungal and antibacterial effects of traditionally used dooder is known as a regional folk remedy. Thus and so, the efficacy of the drug has been demonstrated by the inclusion of natural therapeutic antimicrobial agents such as anti-inflammatory and wound healing, including infectious diseases. The antimicrobial effects of these plant was analyzed against bacteria, yeast and dermatopyta fungi by the agar disc diffusion method. The nominal concentration of the plant to prevent from the development of microorganisms was detected with the broth microdilutions method. It was determined that it had sensitive and moderately sensitive antimicrobial effect against all of microrganisms (13-19.66 mm/inhibition zone), growth of tested microrganisms were inhibited at 50-6.25 µL.It is though that this natural herbal resource can light the way for treatment of several diseases and direct the next studies because of having the potential for being used as a new antimicrobial agent.

Türkiye'de Mardin İlindeki Annelerin ve Yenidoğan Bebeklerin Sarılık Tedavisinde Kullanılan Küsküt'ün (*Cuscuta campestris*) Antifungal ve Antibakteriyel Etkileri

Makale Bilgileri

Geliş: 17.08.2019

Kabul: 11.12.2019

Online Yayınlanma 31.12.2019

DOI: 10.29133/yyutbd.605970

Anahtar kelimeler

Agar disk diffzyon metod,
Antimikrobiyal etki,
Küsküt,
Patojen mikroorganizmalar,
Broth mikrodilüsyon.

Öz: Küsküt, Mardin ve çevresinde yeni doğan bebeklerde hepatit tedavisi için kullanılan bir bitkidir. Bu araştırmanın amacı yöresel bir halk ilacı olarak bilinen ve geleneksel olarak kullanılan küsküt'ün antifungal ve antibakteriyel etkisini tespit etmektir. Böylece, enfeksiyöz hastalıklar dahil anti-enflamatuar ve yara iyileştirici gibi doğal terapötik antimikrobiyal ajanların dahil edilmesi ile söz konusu ilacın etkinliği ortaya konmuştur. Bu bitkinin antimikrobiyal etkileri agar disk difüzyon yöntemi ile bakteri, maya ve dermatofit funguslara karşı analiz edilmiştir. Bitkinin mikroorganizma gelişimini engellemek için en düşük konsantrasyonu, broth mikrodilüsyon metodu ile tespit edilmiştir. Küsküt bitkisinin; tüm mikroorganizmalara karşı güçlü ve orta düzeyde antimikrobiyal etkiye sahip olduğu (13-19.66 mm/inhibisyon zonu), test mikroorganizmalarının büyümesini 50-6.25 µL de inhibe ettiği tespit edilmiştir. Bu doğal bitkisel kaynağın, yeni bir antimikrobiyal ajan olarak kullanılma potansiyeline sahip olması nedeniyle daha sonraki çalışmaları yönlendirmesi ve çeşitli hastalıkların tedavisine ışık tutacağı düşünülmektedir.

1. Introduction

Medicinal plants in rural areas of developing countries, continued to be used as a primary source of medicine for human diseases (Palombo, 2009). While the various plant species are called by similar local names in different regions of Turkey, the same plant species are entitled by the different names. “KÜSKÜT (IKSUT)” or “Dodder” plant that has become like a part of the culture in and around Mardin is one of the quintessence.

This plant drug is used for liver diseases and hepatitis treatment of mothers and newborn infants; it also has identified by the culture and region mentioned. Much as the source of this drug that is benefited with intent to healing by herbalists and spice-sellers has become a research object for a long while, it is impossible to define the plant whose a powder dried part can be able to be obtained only. It was found at the end of the studies that this related fabulous plant is the Dodder (*Cuscuta* spp.) which is a parasite plant (Şekeroğlu and Koca, 2012).

According to Turkish Dictionary of Plant local names called in our country by dodder plant are as follows: Bostanbozan, Canavarotu, Bağbozan, Cinsaçı, Eftimon, Gelinsaçı, Kızıl sarmaşık, Küşküt and Şeytansaçı (Baytop, 1997).

It is proved by studies that due to its components, *C. campestris* (dodder) is effective on atopic dermatitis (Choopani et al., 2016); also effective on some of plant-pathogen fungus (Sin et al., 2011); has anticancer effect (Behbahani, 2014; Noureen et al., 2019); has analgesic, hypothermic, antiinflammatory, anti proliferative effect (Agha et al., 1996; Ghule et al., 2011; Lee et al., 2011); includes beneficial pharmacologically active markers such as kaempferol, bergenin and gallic acid (Singh and Shailajan, 2016). Moreover, *C. pedicellata* Ledeb has an antibacterial effect against isolates of *Xanthomonas campestris* that is isolated from unhealthy fruits (Amna et al., 2014); 24 different types of *Cuscuta* include different phytochemical components for the development of new herbal medicines (Ahmad et al., 2017).

However, it was determined that *Cuscuta* species had hepatoprotective and antioxidant activities (Koca-Caliskan et al., 2014a; Koca-Caliskan et al., 2014b; Koca-Caliskan et al., 2018).

It is also determined by studies that *C. reflexa* has an antibacterial and antifungal effect on *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis* and *Aspergillus niger*, *Trichoderma reesei* (Anjum and Khan, 2003; Summit et al., 2010). While extracts of *C. racemosa* including ethanol has partially effective on *S. aureus*, the same material has no antibacterial and antifungal impact on *E. coli*, *P. aeruginosa*, *Candida albicans* and *A. niger* (Ferraz et al., 2010). While extracts of *C. australis* are effective on *E. coli*, *S. aureus*, *P. aeruginosa*, the same extracts do not display an antimicrobial effect on *E. coli*, *S. aureus*, *P. aeruginosa* (Okiei et al., 2009).

Due to the development of resistant bacterial strains, there is an increase in the number of papers published on antimicrobial activity of plant extracts or different mixtures of plant extracts (synergistic effect) (Akyüz et al., 2012; Kırbağ et al., 2013; Erecevit and Kırbağ, 2017a; Erecevit and Kırbağ, 2017b; Küçükgül Güleç et al., 2014). Additionally cutaneous fungal infections induced by *Trichophyton*, *Microsporum* and *Epidermophyton* are among the most important unsolved global public health problems. Therefore, there is a need to discover new treatment alternatives (Badali et al., 2015).

In the previous studies, antifungal effects against dermatophyte fungi of *Cuscuta campestris* (dodder) have not been reported. For our knowledge, this is the first investigation on the evaluation of antibacterial and especially antifungal activities against *Trichophyton* sp., *Epidermophyton* sp. on methanolic extracts of this species. Moreover, due to its antimicrobial characteristics, is essential in terms of curing several diseases which threaten the human health including “tinea” disease (Kuta et al., 2008) related to *Trichophyton* sp. and *Epidermophyton* sp.

2. Materials and Methods

2.1. Collection and extraction of plant material

Dodder was collected from were collected during appropriate vegetation as the material from Mardin province in the Eastern Anatolia of Turkey. The taxonomic identification of plant material was

determined by using the Flora of Turkey (Davis, 1970, 1984, 1985); it was performed by Prof Dr. Şemsettin Civelek who is a systematic-botanic specialist from Fırat University. The collected plant was dried and triturated. The grinded plant (5 g) was treated in 20 mL methanol (98.1%) solvent by keeping on a rotary shaker (100 rpm) for 24 h. Thus, the plant extract was obtained. The dodder extracts were filtered by using Whatman filter paper (No1) and stored at 4 °C for further study. Then, 100 µL (25 mg / L) extracts were injected into empty antibiotic paper discs having a diameter of 6 mm (Schleicher & S hüll No: 2668, Germany) to separately try on each of the test microorganisms.

2.2. Test microorganisms

The microorganisms that were used for the present investigation; gram positive bacteria, gram negative bacteria, yeasts and dermatophyta fungi (*Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DMS 50071 SCOTTA, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Trichophyton* sp., *Epidermophyton* sp.). These microorganisms were provided by the Department of Biology, Faculty of Science, Fırat University, Microbiology Laboratory, Elazığ-Turkey.

2.3. Preparation of microorganism cultures

Bacterial strains will be cultivated in to Nutrient Buyyon medium and are incubated at 35 ± 1 °C for 24 hours. The yeast strain sand dermatophyte fungi are respectively incubated on the Yeast Malt extract and Glucose Sabouroud medium at 25 ± 1 °C for 48 hours. Cultures that grow in the broth medium will be transferred to the broth media tubes with pre-sterilized after setting in the Mc Farland (0.5) Standard tube.

2.4. Sensitivity test

The agar disc diffusion method were carried out to determine antimicrobial effect. Mueller Hinton Agar, Yeast Malt Extract Agar and Sabouraud Dextrose Agar sterilized seperately in the erlenmeyer flasks and cooled down to 45-50 °C, with the culture of bacteria, yeast and fungus to be prepared as described above, will be vaccinated at the rate of %1 (10^6 cells / mL of bacteria, 10^4 cells / mL yeast and cells / mL dermatophyta fungi as per Mc Farland standard). After shaking well, 15 ml medium is poured in to sterile petri plates and homogenously distributed. The discs (6 mm diameter) with treated 100 microliters of plant extract were added to the appropriate agar media inoculated with microorganism. Then, petri dishes was stored at 4 °C for 2 h. The inoculated petri dishes were incubated at 37 ± 0.1 °C at 24 h for bacterial strains and also at 25 ± 0.1 °C at 72 h for yeasts and dermatophyta fungi. The antimicrobial sensitivity of plant extract was assesment by measuring the zone of inhibition against the microorganisms (Collins and Lyne, 1989). The positive control; mikostatin and ampicilin sulbactam was used. Methanol injected discs were tested as negative control.

2.5. Detection of minimum inhibition concentration

The minimum inhibitory concentrations (MIC) were determined with Broth dilution assay. The cultures were obtained in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA). The inocula of microorganisms were obtained from 12 h broth cultures and suspensions were setting in 0.5 Mc Farland Standard cloudiness. The plant sample was first rarefied to the maximum concentration 100 µL to be tested, and then serial 2-fold subtilizations were acquired in a concentration serial from 6.25 to 100 µL (1562–25000 µg) in 10 mL sterile test tubes including nutrient broth for bacteria and sabouraud dextrose broth for yeast and dermatophyta fungi. MIC values of this plant against analyzed microorganisms was revealed with a micro-well dilution method (Güllüce et al., 2004). Microorganisms reproduction was detected with optical density quantity at 600 nm using an EL x 800 universal microtiter plate reader. After incubation for 18-24 h at 37 ± 1 °C for bacteria, 25 ± 0.1 °C at 72 h for yeast and dermatophyta fungi. The MIC value was declared as the

nominal concentration of the compounds to obstruction the reproduction of microorganisms or the final tube with no microbial reproduction was saved to symbolize (mg / mL).

2.6. Statistical analysis

Statistical comparisons among extract and control groups (methanol, ampicillin sulbactam, mikostatin) were made with respect to the measurable antimicrobial activity against test microorganisms. SPSS 15.0 software was used for statistical analysis (SPSS Inc., Chicago IL). The results were obtained with analysis of variance (ANOVA) and least significant difference (LSD) tests were given as mean \pm SE. $P < 0.0001$, $p < 0.001$, $p > 0.05$ were used for the differences between the extract and control groups. $P < 0.0001$ and $p < 0.001$ were considered sensitive and moderately sensitive. This study was conducted in three repetitions.

3. Results

Antibacterial and antifungal effects of the plant extract used in the study are given in Table 1-2. National Microbiology Standards and zone diameters comments in Infectious Diseases Laboratory Diagnosis Guide are as follows; if there is an inhibition area around the disc with a diameter of ≥ 14 mm (15-30 mm), the isolates examined are sensitive to the drug. If there is an inhibition area around the disc with a diameter of < 14 ; the isolates examined are moderately sensitive. If there is no zone, the isolates are accepted as resistant (Akbaş, 2014; Okut et al., 2018). In the previous studies, antifungal effects against dermatophyte fungi of *C. campestris* (dodder) have not been reported.

According to the inhibition effect of *C. campestris* extracts treatment with methanol on the development of test microorganisms, it was detected that they had significant sensitive and moderately sensitive antimicrobial effects against all of the microorganisms. By this way; *E. coli*, *P. aeruginosa* and *C. albicans*, *C. glabrata* (14.66 mm / inhibition zone), *S. aureus* (14.33 mm / inhibition zone), *B. megaterium* (17.66 mm / inhibition zone), *Epidermophyton* sp. (13.00 mm / inhibition zone), *Trichophyton* sp. (19.66 mm / inhibition zone).

Table 1. Antimicrobial effects of extracts of *Cuscuta campestris* (dodder) (mm)

Microorganisms	Inhibition zone (mm)	
	<i>C. campestris</i>	Control
		Methanol Standart antibiotics
<i>E. coli</i>	14.66 \pm 0.33 ^d	- 12.33 \pm 0.3*
<i>S. aureus</i>	14.33 \pm 0.33 ^d	- 10.33 \pm 0.3*
<i>B. megaterium</i>	17.66 \pm 0.33 ^{cd}	- 12.33 \pm 0.3*
<i>P. aeruginosa</i>	14.66 \pm 0.33 ^d	- 12.33 \pm 0.3*
<i>C. albicans</i>	14.66 \pm 0.33 ^d	- 12.66 \pm 0.6 **
<i>C. glabrata</i>	14.66 \pm 0.33 ^d	- 9.33 \pm 0.66 **
<i>Epidermophyton</i> sp.	13.00 \pm 0.57 ^d	- 8.66 \pm 0.66**
<i>Trichophyton</i> sp.	19.66 \pm 0.33 ^{cd}	- 8.66 \pm 0.66**

The positive control; ampicillin sulbactam (*) and mikostatin (**) (120 μ L and 20 μ g/disc), the negative control; methanol. Inhibition zone ≥ 14 mm (sensitive effect; $p < 0.0001$; cd), $14 < \text{mm}$ moderately sensitive effect; $p < 0.001$; d), not inhibited: (a: $p > 0.05$)

The standardized method of the NCCLS for determining susceptibility was used in a study indicating the effect of *C. campestris* on the proliferation of all of the gram-positive bacteria, the gram-negative bacteria, yeasts, and dermatophyte fungi. These microorganisms are among the most commonly found pathogens on the human. Our study showed that the effective dose for dermatophyte fungi was 50-6.25 μ L (about 12 500-6250 μ g).

With the microplate technique, the MIC value was 5 times lower at 1 5625 mg / mL. In experiments up to this stage, 100 µL of extract were used. Also, the efficiency of this method was compared with the standard disc diffusion method technique.

With the Broth dilution assay technique, the lowest inhibitory concentration of *C. campestris* extraction *S. aureus*, *B. megaterium*, *P. aeruginosa*, *C. albicans*, *C. glabrata*, *Trichophyton* sp. and *E. coli*, *Epidermophyton* sp. were determined as 6.25µL (about 1.562mg / mL) and 50µL (about 12.5 mg/ mL) respectively. According to the disc diffusion method; the amount used for sensitivity is 25 mg/ mL on all of the microorganisms (Table No. 2).

When antibacterial, antifungal, antidermatophyta activity of *C. campestris* (iksut) extracts were analyzed to disc diffusion method (Table 1); it was detected that antimicrobial activity in the dodder extracts was present sensitive inhibition with compared to control group methanol and standard antibiotics (ampicillin for bacteria, mikostatin for yeast and dermatophyte fungi).

Table 2. The Minimum inhibition concentration (MIC in 100 µL) of *C. campestris* (dodder) extracts against the microorganisms

Test Microorganisms	MIC values (µL)
	<i>C. campestris</i>
<i>E. coli</i>	50.00
<i>S. aureus</i>	6.25
<i>B. megaterium</i>	6.25
<i>P. aeruginosa</i>	6.25
<i>C. albicans</i>	6.25
<i>C. glabrata</i>	6.25
<i>Epidermophyton</i> sp.	50.00
<i>Trichophyton</i> sp.	6.25

4. Discussion and Conclusion

Concerning in vitro and in vivo studies performed, *Cuscuta* species have different pharmacological effects. There are many studies on the pharmacological and biological effects of *Cuscuta chinensis* (Nisa et al., 1986; Umehara et al., 2004). It is shown that *C. chinensis* glycosides have effects on aging and *C. chinensis* glycosides strengthen the memory by inducing PC12 cell differentiation (Liu et al., 2003). Subsequent studies revealed the antibacterial effects (Pal et al., 2006) of methanol extracts of *C. reflexa* bodies; antioxidant effects of ethyl acetate and methanol extract of *C. chinensis* (Yen et al., 2008). Moreover, antihypertensive (Oh et al., 2002) impacts of *C. japonica*'s major components. Immunomodulator effect (Stanilova et al., 2000) of *C. europea* C3 binding glycoprotein; liver preventive effect (Yen et al., 2007) of ethanol extract of *C. chinensis* were confirmed. Finally, the methanol extract that is obtained from the *C. reflexa* body suppresses ovarian steroidogenesis in mice (Gupta et al., 20003). We identified that ethanol and aqueous extracts of hexane, chloroform, ethyl acetate, methanol and water extracts that are obtained from *C. arvensis* have analgesic, anti-inflammatory effects (Koca et al., 2011).

There is no phytochemicals research on dodder species in Turkey as well as it is found studies that explain the phytochemicals content of plant extracts for different species of the same plant (Yen, et al., 2007) were found in analyses conducted on *C. chinensis*. Detailed studies revealed 1 trisaccharide, 4 new glycosidic acids, acetic acid, propionic acid, methyl butyric acid, tiglic acid, convolvulinic acid, and jalapinolic acid in a fraction that is similar to resin glycoside that is not dissolved in *C. chinensis* seeds (Du et al., 1998). Studies on *C. japonica* polar extracts showed that active components have caffeoylquinic acid derivatives and caffeoylvinyllate derivatives (Oh et al., 2002).

In a study by Abdullah was found that *Cuscuta europea* (25 mg / ml) was not effective against *E. coli*, but it becomes impactful by 20.5 mm the inhibition zone against *S. aureus* (Abdullah et al., 2016). Another study was determined by Şen et al.; with reference to the results, none of the extracts showed antimicrobial sensitivity against bacteria (*S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Proteus mirabilis*) and from yeasts; *C. albicans*, *C. parapsilosis* and *C. tropicalis* (Şen et

al., 2018). On the other hand, it was determined that *Cuscuta reflexa* showed an inhibition zone from 6 to 17 mm against *S. aureus*, *P. aeruginosa*, *Salmonella typhimurium*, *P. vulgaris* and *Shigella sonnei*, *K. pneumoniae* (Mateen et al., 2011). For Okiei, extracts of *Cuscuta australis* is effective on *E. coli*, *S. aureus*, *P. aeruginosa* while they do not display antimicrobial effect on other bacteria and fungus (Okiei et al., 2009).

Shayanfar conducted a study and reported that *C. campestris* has an antibacterial effect against *Salmonella typhi*, *Micrococcus luteus*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *Streptococcus pyogenes*, *Serratia marcescens*, *B. subtilis*, *E. coli* and *K. pneumoniae* (Shayanfar, 2015). The results of our study show parallelism with other studies conducted. In the previous studies, antifungal effects against dermatophyte fungi of *C. campestris* (dodder) have not been reported.

Compared to our study, different results are due to both different species of the same genus and the amount of useful compounds they contain. Also, genotype, chemotype, geographic origin, environmental and soil conditions are all other parameters that affect the composition of the final natural product (Hacıoğlu, 2005).

According to agar the disc diffusion methods; in this study was detected that methanol extracts of dodder had sensitive and moderately sensitive antimicrobial activity against all of the microorganisms. The MIC values of methanolic extracts were *S. aureus*, *B. megaterium*, *P. aeruginosa*, *C. albicans*, *C. glabrata*, *Trichophyton* sp. and *E. coli*, *Epidermophyton* sp. (respectively; 1 5625 mg / mL and 12500 mg / mL).

It is thought that this natural herbal resource can light the way for the treatment of several diseases and direct the next studies because of having the potential for being used as a new antimicrobial agent. Thus, this study is light about pharmacological for the treatment of bacterial and fungal infections. The results of the study also showed that Dodder can treat cutaneous fungal infections caused by *Epidermophyton* sp. and *Trichophyton* sp.

Acknowledgment

The authors acknowledge that they have no conflict of interests

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