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Antimicrobial And Antioxidant Activities in Essential Oil of *Juniperus Foetidissima* Willd Berries Growing in Turkey

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Abstract: Aim of this study to identify *in vitro* antioxidant, antimicrobial activity of the essential oils of *Juniperus foetidissima* Willd. (Cupressaceae) (J.F) berries growing in Turkey. Essential oil of J.F showed ineffective antimicrobial activity against to all microorganisms except *Bacillus subtilis* J.F showed antimicrobial activity only against to *Bacillus subtilis.* In addition, J.F was weak antioxidant activity in beta carotene/ linoleic acid assay and was been measured as 19.0%. Because of the essential oil J.F, may be regarded as a potential activity against to *Bacillus subtilis* that can may be suitable addition to animal feed to protect for toxins. But further works is still needed for dose detection.

Keywords: *Juniperus foetidissima*, essential oil, antioxidant activity, antimicrobial activity.

Türkiye'de Yetişen *Juniperus Foetidissima* Willd Meyvelerinin Uçucu Yağlarının Antioksidan ve Antimikrobiyal Aktiviteleri

Özet: Bu çalışmanın amacı, Türkiye'de yetişen Juniperus foetidissima Willd'in (Cupressaceae) (J.F.) meyvelerinin uçucu yağlarının antioksidan, antimikrobiyal aktivitesini in vitro belirlemektir. J. F esansiyel yağı, Bacillus subtilis hariç tüm mikroorganizmalara karşı etkisiz antimikrobiyal aktivite gösterdi. J. F, sadece Bacillus subtilis'e karşı antimikrobiyal aktivite gösterdi. Ek olarak, J. F, beta karoten / linoleik asit tahlilinde zayıf bir antioksidan aktivite gösterdi ve % 19.0 olarak ölçüldü. J. F'nin esansiyel yağı Bacillus subtilis'e karşı aktivite gösterdiğinden hayvan yemlerine ilavesinin toksin üremesini engelleyebileceği uygunluk gösterebileceği düşünülmektedir. Ancak doz tespiti için daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Juniperus foetidissima*, uçucu yağ, antioksidan aktivite, anitmikrobiyal aktivite

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1. INTRODUCTION

Juniperus L. (Cupressaceae) has almost 70 species throughout the world and mostly distributed in the Northern Hemisphere (7). In Turkey, the *Juniperus* genus is represented by 10 taxa under seven species and has been used by Anatolian people since ancient times. The genus *Juniperus* consists of 55 species, eight species of them grow in Turkey (5,12).

Juniperus and Cupressaceae general are mainly used as diuretic, stimulant, antiseptic, for common cold and wound healing, urinary infections, urticarial, dysentery, haemorrhage and relieving menstrual pain in the traditional medicines in Turkish folk medicine and worldwide (1,5,16).

Juniperus foetidissima (J.F) is also a medicinal plant. Previous studies on this plant described the components of its essential oil (2,3,8,13,18,20,22). The main component was determined in studies a few countries as sabinene which have been reported in Turkey (20,22), Greece (1), Iranian (3,8) and Macedonia (17). Lesjak et al (13) reported that was the major component catechinin the J.F of in Zurich although Tayoub et al (18) was reported citronellol in Syria.

Although there are some published reports about phytochemical studies of J.F. growing in different parts of the world (1,3,4,8,13,16,17,18,19,20,21,22).

Some of them concerning about biological activity of the J.F growing in Turkey (4,16,19,20,21,22). They are; extract of J.F about antioxidant and antimicrobial activity (16), essential oil of J.F about antifungal activity (4), wound healing and treatment of tar (19,21), two of them composition of essential oil (20,22). Lesjak et al. (13) and Emami et al. (8) were determined antioxidant activity (3,8) in addition to Asili et al. (3) and Selaa et al (17) were determined antimicrobial activity of JF (3,17).

Aim of this study to work with the purpose of beside of confirming these researches and identify antioxidant, antimicrobial activity of the essential oils of J.F berries growing in Turkey.

2. MATERIALS and METHODS

J.F plants berries were been gathered from Sütçüler /Isparta-Turkey during flowering time in late July. The taxonomic identification was been made by Dr. Erol Dönmez and stored at the herbarium of the Department of Biology, Cumhuriyet University, and Sivas-Turkey (CUFH-Voucher No: ED 11004).

Isolation of the essential oil

The air-dried *berries* of J.F after crashed, subjected for 3h to water distillation using with a Clevenger-type apparatus (yield 1.1% v/w). After filtration, the essential oil obtained was been dried in anhydrous sodium sulphate and storage at +4°C until analysed.

Antimicrobial activity

Antimicrobial and antifungal activities of the essential oil was determined against three Gram-positive and five Gram-negative bacteria, one fungus via the disc diffusion method respectively *Staphylococcus aureus* ATCC-25923**,** *Pseudomonas aeruginosa* ATCC-27853, *Escherichia coli* ATCC-35218, *Corynebacterium diphteriae* RSHM-633, *Salmonella thyphi* NCTC-9394, *Proteus vulgaris* RSHM-96022, *Klebsiella pneunomonia* NCTC-5046, *Bacillus subtilis* ATCC-6633 and *Candida albicans* ATCC-10231. Cultures were obtained from the department of Health of Refik Saydam Hygiene Center Contagious Diseases Research Department (Ankara-Turkey).

In Mueller Hinton Agar (MHAOxoid- CM337) at 37°C for bacterial strains and the yeast in Sabouraud Dextrose Agar (SDA-Oxoid-CM41) at 30°C were cultured overnight. All the tests were been repeated in three times. Standard deviation

(SD) and average were been calculated for the inhibition zone diameters.

Disc diffusion method

The evaluation on antimicrobial activities of the essential oil was been used via agar disc diffusion method (14,15). Suspension of a test microorganism (0.1Ml from 108 cells=mL) was spread on the solid medium plates. The filter paper disks (6mm in diameter) were placed on the plate after being treated with 10 µl of oil, incubated at 48°C for 2h and at 37°C for 24 h respectively and at 30°C for 48 h for the yeast. The diameters of the inhibition zones were characterized as millimetres.

Antioxidant activity

The sweeping of free radicals was monitored by two methods with the principle of colour change. Antioxidant activity was been studied with dipeptidylpicrylhydrazyl (DPPH) assay (6) and beta-carotene linoleic acid systems were studied according to method (23). All tests were repeated three times.

3. RESULT AND DISCUSSION

Medicinal plants are the major source of pharmaceuticals in the world, that in recent decades, their use for health and disease prevention has been increased and also to know how to use herbs and how they work, it is necessary to learn about the active ingredients and their effectiveness should be reviewed.

It became much more interested in various plants searching in recent studies about the antioxidant and antimicrobial effects of its essential oil and extracts' (3,8,9,10,11,13,16,17).

Balaban et al. (4) reported that has antifungal activity for J.F, Tunalıer et al. (21) studied in tar of J.F. Tümen et al. (19) determined wound healing property for J.F, Asili et al. (3) reported that inhibition for all bacteria including *Bacillus subtilis* and weak antimicrobial activity. Selaa et al. (17) reported strong antimicrobial activity for leaves of JF. In this study, JF berries have found effective only for against to *Bacillus subtilis*. The results are shown in Table 1.

Essential oil was been assessed for antioxidant activity by two tests which are DPPH radical and β-carotene/linoleic acid.

Table 1. Antimicrobial activity of J.F the essential oils using agar disc diffusion

Microorganisms	**Disc Diffusion Method[a]**	**Gentamicin**	**Nystatin**
Staphylococcus aureus	8±1.52	23±0.54	-
Escherichia coli	8±0.88	16±0.20	-
Proteus vulgaris	9±1.68	22±1.45	-
Salmonella typhi	9±1.16	10±0.18	-
Bacillus subtilis	40±1.35	29±0.80	-
Klebsiellapneumoniae	12±1.18	20±0.40	-
Corynebacteriumdiphteriae	18±1.22	23±0.15	-
Pseudomonas aeruginosa aeruginosa	6±1.01	20±0.28	-
Candida albicans	6±0.15	-	25±0.16

[a]Diameter of inhibition zone (mm) including disc diameter, 6 mm

Antioxidant activities of the essential oil was an ineffective in DPPH and weak activity in β-carotene/linoleic acid system as shown in Table 2. Although Lesjak et al. (13) reported good antioxidant activity for essential oil and extracts. Another study conducted weak antioxidant activity with different parts of the JF in Iranian (8) similar to this study. Changes in results can be explain that obtained different situations of the plant (fruit, seed, and branch), ground soil, climate and periodical differentiation.

Table 2. Effects of J.F essential oil and on the in vitro free radical DPPH and β-carotene linoleic acid system.

SAMPLES	**Inhibition IC_{50} (mg/ml)** (DPPH)	**Inhibition %** (β-carotene/Linoleic acid)
J.F	-	19
BHT	0,0105	100

Although the essential oils from *Juniperus* species have been determined in antimicrobial activity against to numerous microorganisms to date but this is the first study to supply data that essential oil of J.F berries determined against to microorganisms for potential antibacterial and antioxidant activities which grown in Turkey.

4. CONCLUSIONS

The results showed that the essential oil J.F has antimicrobial activity only against to non-pathogenic bacteria of *Bacillus subtilis*, which can contaminate food and rarely result in food poisoning.

Because of the essential oil J.F, may be regarded as a potential activity against to *Bacillus subtilis* that can may be suitable addition to animal feed to protect for toxins. But further works is still needed for dose detection.

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