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# Antibacterial activity of various extracts from *Dracocephalum kotschyi* against food pathogenic microorganisms

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**Abstract:** The genus *Dracocephalum* belongs to the family labiatae and it has been used in traditional medicine for stomach and liver disorders, headache and congestion. In the present study, we have investigated the antibacterial capabilities of dichloromethane, ethyl acetate and methanol extracts of *Dracocephalum kotschyi*. Antibacterial activities was screening against three Gram-positive bacteria (*Staphylococus aureus*, *Bacillus cereus*, and *Listeria Monocytogenes)* and three Gram-negative bacteria (*Salmonella enterica*, *Escherichia coli and Enterica Aerogenes*) by minimum inhibitory and bactericidal concentration (MIC and MBC) and disc diffusion methods. The highest antibacterial index was observed from ethyl acetate extract on *Bacillus cereus* in both disc diffusion (12 mm) and MIC-MBC methods and the MIC value for extracts ranging between 0.781 and 25 mg/ml. The results of this investigation indicated that *Dracocephalum kotschyi* was found to possess moderate antibacterial activities. Further research is required to identify the active photochemical responsible for these biological activities.
**Keywords:** antibacterial, *Dracocephalum kotschyi*, MIC and MBC, disc diffusion.

## 1. Introduction

*Dracocephalum* is a genus belonging to the Labiate family and it is found in central Asia, and Europe (1, 2). This genus consists of 60 species and eight species in the flora of Iran and this genus is mainly distributed in the northern and central parts of Iran (1). *Dracocephalum kotschyi* is one of three endemic species growing in North Khorasan and it was known in Iran as Badrandjboie- Dennaie and Zarrin-giah (1, 2). *Dracocephalum* genus has been used in traditional medicine for stomach and liver disorders, headache and congestion (3) and its oil has been used in folk medicine as an antispasmodic agent (1). Recent studies have confirmed some medicinal properties from *D. kotschyi* including antihyperlipidemic, immunomodulatory, antinociceptive, cytotoxic (4-7). It is also a source of essential oils, flavonoids, monoterpene glycosides and Rosmarinic acid (8-10). From ethylacetate extract of *Dracocephalum subcapitatum*, five flavonoids such as calycopterin, isokaempferide, xanthomicrol, luteolin and apigenin and five terpenoids such as oleanolic acid, ursolic acid, neral, geranial and limonene-10-al were isolated (11). *Dracocephalum kotschyi Boiss*. (Lamiaceae) is a rich source of flavonoids such as luteolin, apigenin, cirsimaritin, penduletin, xanthomicrol, calycopterin and rosmarinic acid (12). The major constituents of the oil of *D. kotschyi* were oxygenated monoterpenes such as geranial (35.8%), limonene (15.8%) and 1, 1-dimethoxy decane (14.5%) (13). Limonene and α-terpineol are responsible for antinociceptive properties of essential oil of *Dracocephalum kotschyi* (6). Methoxylated

flavones such as apigenin, isokaempferid, crisimaritin, penduletin and xanthomicrol from *D. kotschyi* have been reported to have anticancer effects (7, 14). The essential oil of *Dracocephalum foetidum* exhibited strong antimicrobial activity against most of the pathogenic bacteria by both the agar diffusion method and the minimum inhibitory concentration (MIC) assay (MIC range was 26-2592 μg/ml) (15).

The aim of this study was to evaluate the antimicrobial activities of the dichloromethane, ethyl acetate and methanol extracts of *Dracocephalum kotschyi*. To the best of our knowledge, we are the first to report that *D. kotschyi* extracts showed antimicrobial activity against the pathogenic microorganisms that were tested.

## 2. Experimental

### 2.1. Plant material

The Plant was collected in Jun 2013 from the North Khorasan Province of Iran. The plant was identified by the Research Center of Natural Products and Medicinal Plants, North Khorasan University of medical sciences (Iran) and its Voucher number was 36-1-2.

### 2.2. Preparation of plant extracts

The aerial parts of the plants were dried under shade at room temperature and then cut into small pieces. About 100 g of sample was macerated in methanol, dichloromethane, and ethyl acetate at room temperature for 48 h separately. Each solvent was allowed to remain in contact with plant material for 24 h, and replaced with fresh solvent four times. Removal of the solvents under vacuum at 40 °C gave the crude extracts (16).

### 2.3. Antimicrobial Activity

Determination of the minimum inhibitory concentrations (MIC) antimicrobial activities of methanol, dichloromethane, and ethyl acetate extracts of the aerial part of the plant were determined against three Gram-positive bacteria: S. aureus (ATCC 6538p), Bacillus cereus (ATCC 10876) and *L. monocytogenes* (ATCC 35152), three Gram-negative bacteria: *S.enterica* (ATCC 53648) , Escherichia coli (ATCC 10536) and *E. aerogenes* (ATCC 13048).

### 2.4. Agar diffusion method

The extracts of *D. kotschyi* were tested for antimicrobial activity using the agar diffusion method on solid media Mueller-Hinton agar (MHA) plates were used for microorganisms. The Sterile paper disc was placed on the agar plate of the appropriate media, which had been surface spread with bacteria at a 108 CFU/ml density. After 20 μl of the extracts were put to each paper disc, the agar plates were incubated for 24 h at 37°C. Antibiotic disc and disc containing DMSO were used as controls. The results were shown by measuring the zones of growth inhibition around the discs (17).

### 2.5. Determination of the minimal inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) were determined by broth macro dilution method in 96-well plates by Rios and Duffy methods (18-19).

Initial concentration of each extract was prepared with the aid of bath sonicator (0.8 g extract with 4 ml solvent and 30% dimethylsulphoxide in sterile distilled water and one drop of Tween 80). 1 ml of diluted extract was infused into macro-plate with 1ml of sterile Mueller-Hinton broth (MHB; HiMedia, India) and then diluted (50% with MHB). 0.5 McFarland standard turbidity for microbial suspension equivalent was prepared by suspensions of the growth from brain-heart infusion medium (HiMedia, India). Suspensions were further diluted to obtain a concentration of $10^7$ colony-forming units (CFU) per ml for the bacteria. Then, 10 μl of diluted inoculums was added to each well of macro-plate. The sterility of the medium was also tested in two wells and Gentamicin was used as the positive control for bacterial strains. Plates were incubated for 24 h at 37 °C for bacteria. The growth of microorganisms was assessed by TTC (2, 3, 5-triphenyl tetrazolium chloride, Sigma, USA) assay. Briefly, 0.5 ml of TTC (5 mg/ml; dissolved in sterile water) was added to each well and the plates were incubated at 37 °C for bacteria. The results were expressed as the lowest concentration of plant

extract that could inhibit any red dye production. MIC values were defined as the lowest concentrations of oil that inhibit bacteria after 24 h. All experiments were done in triplicates.

### 2.6. Determination of minimum bactericidal concentrations (MBC)

The bactericidal effects of extracts were determined according to the method described by Rios (18). 100 µl of clear dilutions in wells of macro-plate were sub cultured on the Mueller- Hinton agar plates and subsequently incubated at 37 °C for 24 h. Minimal bactericidal concentration (MBC) were recorded from the first tube that showed no growth on solid media.

## 3. Results and Discussion

The highest yield of extract was for methanol extract with 11.11%, dichloromethane and ethyl acetate extracts had 2.42 and 0.7 % yields. In the present study, zones of growth inhibition around the discs in agar diffusion method, MIC and MBC of *Dracocephalum kotschyi* extracts were evaluated (Table 1 Table 2 and 3).

**Table 1. The quantitation of antimicrobial activity for *D. kotschyi* extracts measured by the agar diffusion method. The effectiveness of extracts is demonstrated by the size of the microorganism growth inhibition zone around the filter paper disc, which is typically expressed as the diameter of the zone in mm. The strength of the activity is presented as +.**

| Extract | Microorganism | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus*** | ***B. cereus*** | ***L. monocytogenes*** | ***S. enterica*** | ***E. aerogenes*** | ***E. coli*** |
| **Dichloromethane** | + [(a)] | + | + | _ | _ | _ |
| **Methanol** | + | ++ | ++ | _ | _ | _ |
| **Ethyl acetate** | ++ | ++ | ++ | _ | _ | _ |
| **Gentamicyn** | +++ | +++ | +++ | +++ | +++ | +++ |

a)Diameter of theinhibition zone: no inhibition (-) , 8~9.5 mm (+), 10~12 mm (++), >12 mm (+++).

**Table 2. Minimum inhibitory concentrations (MIC, mg/ml) of the extracts of *D. kotschyi***

| Extract | Microorganism | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus*** | ***B. cereus*** | ***L. monocytogenes*** | ***S. enterica*** | ***E. aerogenes*** | ***E. coli*** |
| **Dichloromethane** | 12.5 | 6.25 | 12.5 | 100 | 50 | 50 |
| **Methanol** | 1.562 | 1.562 | 12.5 | 25 | 25 | 25 |
| **Ethyl acetate** | 1.562 | 0.781 | 6.25 | 25 | 25 | 25 |

**Table 3. Minimum bactericidal concentrations (MBC, mg/ml) of the extracts of *D. kotschyi***

| Extract | Microorganism | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus*** | ***B. cereus*** | ***L. monocytogenes*** | ***S. enterica*** | ***E. aerogenes*** | ***E. coli*** |
| **Dichloromethane** | 100 | 50 | 100 | >100 | >100 | >100 |
| **Methanol** | 50 | 1.562 | 25 | >100 | >100 | 100 |
| **Ethyl acetate** | 3.125 | 1.562 | 12.5 | 100 | 100 | 100 |

To our knowledge, this is the first report on the antimicrobial activity of *Dracocephalum kotschyi*. Experimental studies carried out in species of Dracocephalum have identified phenols and flavonoids as phytochemicals with antimicrobial properties. The *D. kotschyi* extracts were tested for antimicrobial activity against 6 different pathogenic microorganisms, including 3 Gram-positive and 3 Gram-negative bacteria. The Gram-positive strains of bacteria that were tested seemed to be more sensitive to the extracts, which are

attributed to the absence of an outer lipopolysaccharide layer in Gram-negative bacteria that provides a resistant barrier (20). The antibacterial activity of flavonoids and polyphenols has been attributed to inhibition of synthesis of RNA and DNA (21). Thus, the antibacterial activity of ethyl acetate and methanol extracts of D.*kotschyi* could be attributed to the high polyphenolic compounds present in the extract. The disc diffusion method is dependent to the diffusion ability of the substances and in this method antibacterial property was expressed as diameter (mm) of the zone of inhibition (ZOI) caused by the extracts (22). The diameter of inhibition zones (mm) of extracts are represented in Table 1. The ethyl acetate extract are found to have highest antimicrobial activity against *S. aureus* (12mm), *B. cereus* (12 mm) and *L. monocytogenes* (12 mm) and the lesser antimicrobial activity is found against *S.enterica*, *E. aerogenes* and *E. coli* (6 mm) . The negative control (DMSO) is showed activity against all the microbial strains tested. It is no activity was observed against pathogens. The positive control was showed activity against all the microbial strains (18-25 mm). The extracts can inhibit the growth of microbial strains the growth inhibitory effects of the *D.kotschyi* extract were concentration dependent (22). The standard antibiotic was more potent, having lower MIC values against bacteria. The minimum inhibitory concentrations (MIC) of different extracts were determined by preparing solution of varying concentration (0.781 - 100 mg/ml). The methanol and ethyl acetate extracts exhibited antibacterial properties against gram-positive tested bacteria. These two extracts showed antimicrobial activity against *B. cereus*, *L. monocytogenes* and *S. aureus* with zones of inhibition ranging from 8 to 12 mm. According to the present results, these extracts did not show any inhibitory activity against all the gram-negative microorganisms and Gram-positive bacteria were more sensitive to these extracts than Gram-negative bacteria. The MBCs of Gram-positive bacteria were 2-4 times higher than their corresponding MIC. The MIC varied from 0.781 to 12.5 mg/ml and MBC varied from 1.562 to 100 mg/ml, depending on the susceptibility of the test microorganism. As shown in Figs 1 and 2, the results indicated that among the three extracts, ethyl acetate extract has greater antimicrobial activity against the tested microorganisms compared to dichloromethane and methanol extract.

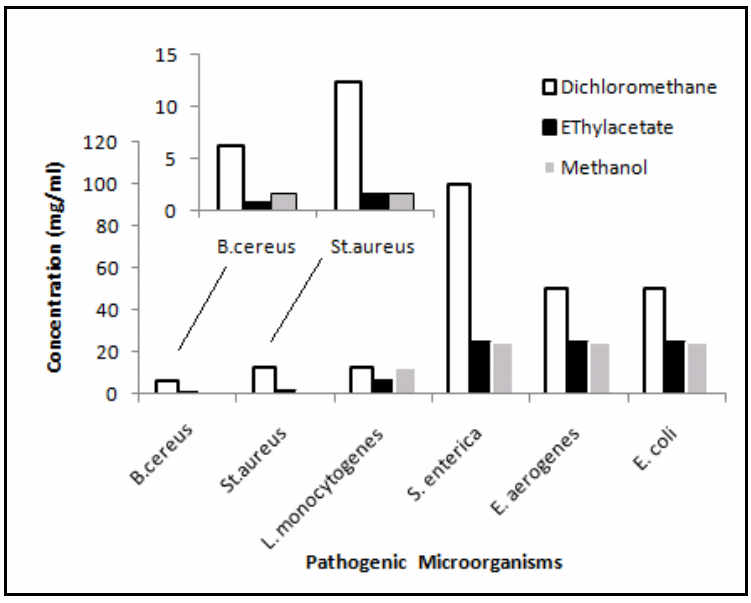


**Figure 1- Comparison of minimum inhibitory concentration of methanol, dichloromethane and ethyl acetate extracts of *D. kotschyi***

Ethyl acetate extract showed good activity against *Bacillus cereus,* showing the inhibition zones (12 mm) and the lowest MIC values (0.781 mg/ml) and the lowest MBC values (1.562 mg/ml). This study showed that agar diffusion method and broth macro dilution method have same results; ethyl acetate extract had highest antimicrobial activity in two methods than methanol and dichloromethane extracts. *Dracocephalum heterophyllum* oils showed a broad spectrum of antibacterial activity with the zones of inhibition ranging from 7.3 to 27.0 mm and Salmonella enterica was the most sensitive strain with minimum inhibitory concentration (MIC) values of 8000 µg/ml (21). Essential oil of *Dracocephalum foetidum* exhibited strong antibacterial activity against Staphylococcus aureus at a very low MIC value (15). In the literature, there are some reports on the presence of phenolic compounds such as caffeic acid, chlorogenic acid, phenylpropanoids and flavonoids in *D.moldavica*, which can be responsible for the observed antioxidant and antibacterial activity (23). The increase

in the content of Rosmarinic acid and the total phenolic compounds contributes to superior antioxidant activity of hairy roots1 of *D. moldavica* (24). The extract of *Dracocephalum moldavica* contained polar compounds such as luteolin-7-*O*-glucoside, rosmarinic acid, luteolin and apigenin and this extract demonstrated antioxidant activity in all the antioxidant assays but it was not as potent as the positive control (25). The most active compounds of this genus are flavonoids, such as Luteolin, quercetin, apigenine and these flavonoids appear to play an important role in the biological activities from Dracocephalum species (10). In addition, these flavonoids possess anti-bacterial properties (26).

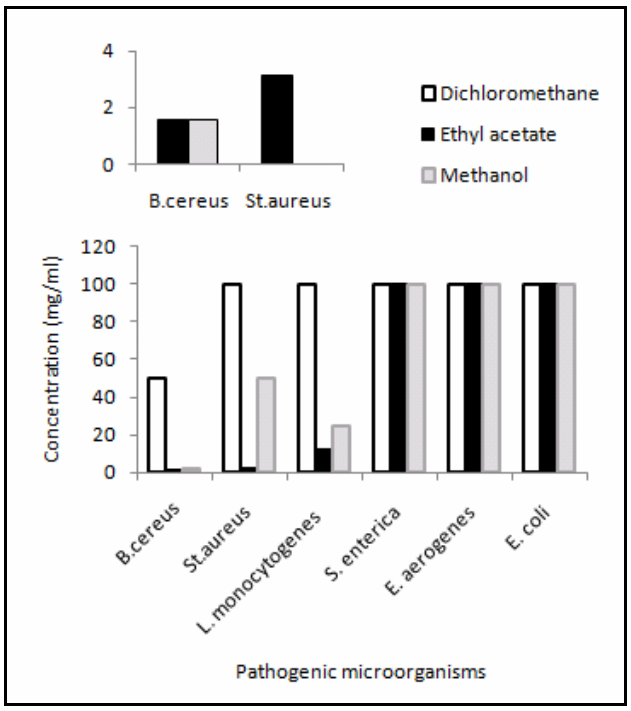


**Figure 2- Comparison of minimum bactericidal concentrations of methanol, dichloromethane and ethyl acetate extracts of *D. kotschyi***

The results of this investigation indicated that *Dracocephalum kotschyi* was found to possess moderate antibacterial activities and its antibacterial activity due to presence of flavonoids. Further research is required to identify the active photochemical responsible for these biological activities.

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