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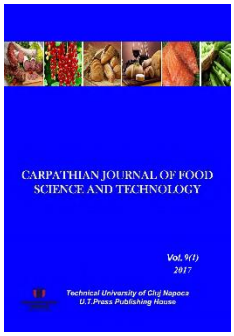

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ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACTS FROM *THYMUS TRANSCAPICUS* AGAINST FOOD PATHOGENIC MICROOGANISMS

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ABSTRACT

Thyme (*Thymus transcapicus*) is a herbaceous perennial plant belonging to the *Lamiaceae* family. Thymus species are considered as medicinal plants due to their pharmacological and biological properties. In the present study, we have investigated the antibacterial capabilities of dichloromethane, ethylacetate and methanol extracts of *Thymus transcapicus*. Antibacterial activities was screening against two Gram-positive bacteria (*Staphylococus aureus*, and *Listeria Monocytogenes)* and two Gram-negative bacteria (*Salmonella enterica*, *Escherichia coli*) by detection of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) method. The highest antibacterial index was observed from methanol extract on *Staphylococus aureus* in MIC-MBC method ($p<0.05$). The results of this investigation indicated that *Thymus transcapicus* was found to possess moderate antibacterial activities. Further research is required to identify the active photochemical responsible for these biological activities.

1. Introduction

Treatment of infectious diseases continues to be problematic in modern time. With the available antiviral and antimicrobial drugs, the treatment often leads to the problem of resistance (Vijayan et al. 2004; Mehrgana et al. 2008). There is little likelihood that available orthodox antiviral drugs can eliminate all or even most viral diseases (Jassim & Naji 2003). The search for new antimicrobial substances exhibiting minimal side effects is warranted because of the severe side effects of some drugs currently in use (Mehrgana et al. 2008; Kandil et al. 1994). So, there is an increasing need for new substances with antiviral and antimicrobial activity. Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases (Vijayan et al. 2004). Therefore, the development of new medicinal plant products is vital in controlling the threats posed by some pathogenic microorganisms (Jassim & Naji 2003).

Lamiaceae (formerly Labiatae) is one of the most important plant families in which *Thymus* with about 215 species, is a significant genus (Mehrgana et al. 2008). *Thymus* species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, the extracts of different species of *Thymus* have been widely used for the treatment of gastritis, diarrhea and enuresis in children, bronchitis and whooping cough (Pertussis) (Ebadi 2002).

Several studies have been shown that *Thymus* species have antibacterial (Mehrgana et al. 2008; Figueiredo et al. 2008; Tohidpour et al. 2010), antifungal (Figueiredo et al. 2008. Bonjar 2004; Sokovic et al. 2009), cytotoxic (Goncalvesa et al. 2010), analgesic (Sokovic et al. 2009), antiparasitic (Goncalvesa et al. 2010), topical anti-inflammatory (Ismaili et al.

2002), antispasmodic (Begrow et al. 2009), mosquitocidal (Pavela et al. 2009) and antioxidant (Zamani et al. 2009; Soares et al. 1997) activities. Antiviral effect of extracts from some plants of the Lamiaceae family against HSV-1 (Vijayan et al. 2004; Nolkemper et al. 2006) and HSV-2 (Nolkemper et al. 2006) has been reported and the extract of *Thymus vulgaris* has been shown antiviral activity (Nolkemper et al. 2006).

Antioxidant (Zamani et al. 2009) and antiemetic (Moallem et al. 2009) effects of *Thymus transcaspicus* have been reported in some studies.

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

2. Materials and methods

2.1. Plant material

The Plant material was collected in May 2016 from North Khorasan Province Mountains in Iran. Then, the plant was identified and confirmed by Natural Products & Medicinal Plants Research Centre, North Khorasan University of Medical Sciences (Iran) and Voucher specimen (No: MP 32/4) was deposited in herbarium of the Natural Products & Medicinal Plants Research Centre.

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 ethylacetate 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 (Boozari et al. 2015).

2.3. Antimicrobial Activity

Determination of the minimum inhibitory concentrations (MIC) antimicrobial activities of methanol, dichloromethane, and ethylacetate extracts of the aerial part of the plant were determined against two Gram-positive bacteria*: S. aureus* (ATCC 6538p), and *L. monocytogenes* (ATCC 35152), two Gram-negative bacteria: *S.enterica* (ATCC 53648), *Escherichia coli* (ATCC 10536).

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 (Rios et al. 1988; Duffy & Power 2001).

Initial concentration of each extract was prepared with the aid of bath sonicator (0.8 g extract with 4 ml solvent and 30% dimethyl sulphoxide 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; Hi Media, 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 (Rios et al. 1988). 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. All experiments were done in triplicates.

2.7. Statistical analysis

Data for each test were statistically analyzed. The statistical analysis of the data was performed using SPSS statistical software version 18 (SPSS Inc., Chicago, IL). Data recorded as means ± standard deviation of three replicate measurements.

3. Results and Discussion

The highest yield of extract was for methanol extract with 2.92 %, dichloromethane and ethylacetate extracts had 1.78 % and 1.58 % yields.

To our knowledge, this is the first report on the antimicrobial activity of *Thymus transcapicus.* Experimental studies carried out in species of *Thymus* have identified phenols and flavonoids as phytochemicals with antimicrobial properties. The *Thymus transcapicus* extracts were tested for antimicrobial activity against 4 different pathogenic microorganisms, including 2 Gram-positive and 2 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 (Inouye et al. 2001).

The antibacterial activity of flavonoids and polyphenols has been attributed to inhibition of synthesis of RNA and DNA (Arora et al. 2013). Thus, the antibacterial activity of ethylacetate and methanol extracts of *Thymus transcapicus* could be attributed to the high polyphenolic compounds present in the extract.

The negative control (DMSO) is showed activity against all the microbial strains tested. The positive control was showed activity against all the microbial strains. The extracts can inhibit the growth of microbial strains the growth inhibitory effects of the *Thymus transcapicus* extract were concentration dependent (He et al. 2010).

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 (12.5- 100 mg/ml). The methanol and ethylacetate extracts exhibited antibacterial properties against Gram-positive tested bacteria.

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.

As shown in Figs 1 and 2, the results indicated that among the three extracts, ethylacetate extract has greater antimicrobial activity against the tested microorganisms compared to dichloromethane and methanol extract.

Methanol extract showed good activity against S. aureus, showing the inhibition the lowest MIC values (12.5 mg/ml) and the lowest MBC values (25 mg/ml) ($p<0.05$).

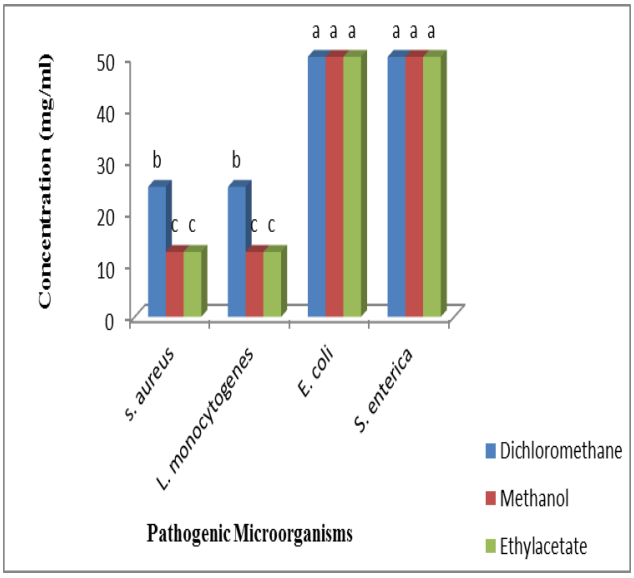

Figure 1. Comparison of minimum inhibitory concentration (MIC, mg/ml) of methanol, dichloromethane and ethyl acetate extracts of *Thymus transcapicus.*
(Each experiment was tested in triplicate.)

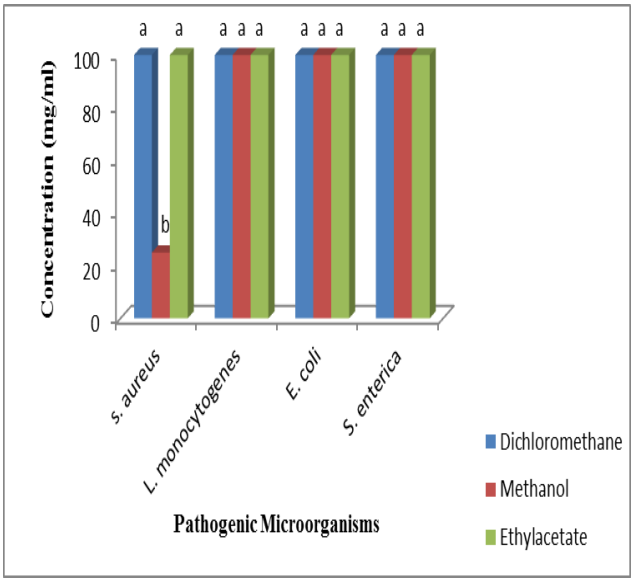

Figure 2. Comparison of minimum bactericidal concentrations (MBC, mg/ml) of methanol, dichloromethane and ethyl acetate extracts of *Thymus transcapicus*
(Each experiment was tested in triplicate.)

Antimicrobial activity of the plants of different areas of the world has been reported (Janovska et al, 2003).

Recent studies have shown that *Thymus* species have antibacterial, antifungal, and antioxidant activities (Rahimmalek *et al.*, 2009; Jordan *et al.*, 2009; Bassam *et al.*, 2004). The anti-bacterial characteristic of *Thymus* spp. is due to the occurrence of thymol in this genus. This substance can be used as a disinfectant. Previous studies showed that the essential oil and extract from *T. daenensis* exhibited antimicrobial activities against *Candida albicans* (Ghasemi Pirbalouti *et al.*, 2009a), *Listeria monocytogenes* (Ghasemi Pirbalouti *et al.*, 2009b), *Campylobacter jejuni* and *Campylobacter coli* (Ghasemi Pirbalouti *et al.*, 2010a), *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Ghasemi Pirbalouti *et al.*, 2010b), *Escherichia coli* O157:H7 (Ghasemi Pirbalouti *et al.*, 2010c), and *Saprolegnia parasitica* (Ghasemi Pirbalouti *et al.*, 2009c).

Antimicrobial activities of some Thymus species have been shown in other previous studies. *Thymus pubescens* and *Thymus vulgaris* extract demonstrated good antibacterial activity against some drug resistant Gram-positive bacteria (Mehrgana et al., 2008; Tohidpour et al., 2010). The essential oil of the Thymus caramanicus showed high inhibitory activity against Helicobacter pylori (Eftekhar et al., 2009). *T. transcaspicus* essential oil was tested for its antibacterial activity against various Gram-positive and Gram-negative bacteria Standard strains. All the bacteria were inhibited by the essential oil but in variable degrees.

Inhibition of *Staphylococcus aureus* (de Oliveira et al., 2010) and antibacterial effects against E. coli (Pei et al., 2009) by thymol and carvacrol have been reported. Carvacrol also has been reported to exhibit a dose dependent inhibitory effect on Vibrio cholerae in food (Rattanachaikunsopon et al., 2010). Thymol, which is the main component of many Thymus spp. and also in the oil of *T. transcaspicus* (64%), is known as an antiseptic agent (Miri et al., 2002).

Several studies have demonstrated the antimicrobial activity the essential oils and/or the extracts of many species of the genus *Thymus* rich in volatile phenols and/or volatile alcohols (Sokovic et al. 2009; Laouer et al.

2009; Mohammed et al. 2009; Reichling et al. 2009; Oh et al. 2009).

The essential oils of *Thymus vulgaris* L., *Thymus tosevii* L. (Sokovic et al. 2009), *Thymus pulegioides* (Pinto et al. 2006) showed antifungal activities against other fungi strains. The most of the species of *Thymus* genus have been investigated for their antimicrobial properties against some bacteria and fungi, depending on thymol and its isomer carvacrol (Laouer et al. 2009; Safaei-Ghomi et al. 2009) and other species depending on thymol as main constituents (Mojab et al. 2008; Cavara et al. 2009).

4. Conclusions

The results of this investigation indicated that *Thymus transcapicus* 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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