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MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC ANTIOXIDANT COMPOUNDS AND ANTIBACTERIAL ACTIVITIES OF *THYMUS TRANSCAPICUS* ESSENTIAL OIL FROM NORTH KHORASAN PROVINCE OF IRAN

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ABSTRACT

Thymus is a traditional pharmaceutic plant which is also used as a spice and perfumed plant in different industries. In present study, Microwave Assisted Hydrodistillation (MAHD) and hydrodistillation in a Clevenger-type apparatus methods. After preparation of essential oils, antioxidant properties were measured by two methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). BHT was used as positive control for comparison. Also antibacterial activities were screening against two Gram-positive bacteria (Staphylococus aureus, and Listeria Monocytogenes) and two Gram-negative bacteria (Salmonella enterica, Escherichia coli) by minimum inhibitory and bactericidal concentration (MIC and MBC) and disc and well diffusion method. Comparison between two extraction methods showed that extraction efficiency of antioxidant and antimicrobial activity at, Microwave Assisted Hydrodistillation method is more than hydrodistillation method. Results presented here suggest that the essential oil of Thymus transcapicus possess strong antimicrobial and antioxidant properties, and therefore, they can be used as a natural preservative ingredient in food and/or pharmaceutical industry.

1. Introduction

In the third world and developing countries, due to the increasing consumer demand for more natural foods, the abuse of toxic synthetic food substances and the increasing microbial resistance of pathogenic microorganisms against antibiotics, natural substances isolated from plants are considered as promising sources of food preservatives (Burt, 2004; Peschel et al., 2006; Smith-Palmer et al., 2001). It is clear from these studies that these secondary plant metabolites have potential uses in medical procedures and applications in the cosmetic, pharmaceutical and food industries (Baratta et al., 1998; Baratta et al., 1998).

Among the different groups of plant products, essential oils are especially recommended as one of the most promising groups of natural products for the formulation of safer antimicrobial agents (Varma & Dubey, 2001). Other than antibacterial and antiviral effects, most essential oils investigated possess antiinflammatory, antifungal and antioxidant properties (Sacchetti et al., 2005). Essential oils are also widely used as food flavours and preservatives to prevent growth of food-borne

bacteria and molds, and so extend the shelf life of processed foods (Burt, 2004).

The Thymus genus comprises over 300 species of which, 14 are found in Iran (Rechinger & Hedge, 1982), which grow wiled in many regions and four of them are endemic (Mozaffarian, 1996). Thymus is a well –known medicinal plant which is native to Southern Europe which its essential oil is manufactured commercially for use in cough drops, mouthwashes, liniments, toothpastes, detergents and perfumes. The herb is approved by Commission E in the treatment of bronchitis, whooping cough and upper respiratory inflammation.

In folk medicine, Thymus spp. are used an anthelmintic, antispasmodic, carminative, sedative, diaphoretic usually in form of an infusion, or externally in bath to cure rheumatic and skin disease (Rustaiyan et al., 2000). Thyme oil is also carminative, expectorant and possesses antimicrobial and anthelmintic properties due to concentrated thymol and carvacrol content but it is extremely toxic. Thymus essential oil and extract is a source of aromatic terpens and terpenoids, flavonoids and phenolic acids (Stahl-Biskup & Sáez, 2002). Thymol, which is the main components of many Thymus species is known as an antiseptic agent and is approved for diverse effects like hookworm treatment (Sefidkon et al., 2001; Evans et al., 1998). Also thymol and their salts used about 0.1-1% in formulation of many lotions, creams and ointments. In external used about 0.1-1% in formulation of many lotions, creams and ointments. In external use, thymol is known as a strong antiseptic agent in toothplaste, gargle and mouthwashes (Zargari, 1990).

The other major component of Thymus spp. oil is carvacrol which is used nowadays on a large scale in the food, cosmetic and mouthwashes industries. In addition, it has been shown several activities like antimicrobial, analgesic and antioxidant activities but it is toxic in high concentration (Monzote et al., 2009).

There are many reports of the essential oil composition and biological activity of different Thymus species especially common Thyme (Thymus vulgaris) and wild Thyme (Thymus serpyllum). Hence of the use of Thymus species or their essential oils in the food and traditional medicine of Iran, we were interested in studying on the essential oil contents and chemical composition of all Iranian endemic species. 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.

In the last few decades, various technologies including maceration, mechanical rabbling, heat reflux, ultrasound and ultrahigh pressure have been applied to polysaccharide extraction. However, these techniques have had some drawbacks including low extraction efficiency, high operating costs and abnormal extract quality (Zhao et al., 2013). Recently, microwave-assisted extraction has been widely applied to extract bioactive compounds from various natural resources. The microwave energy penetrates the material structure, producing molecular friction due to the dipolar rotation of polar solvents, and accelerates the mass transfer of target compounds. Compared with traditional extraction processes, microwave-assisted extraction has enhanced the extraction efficiency and is also more environmental friendly in terms of its reduced use of energy and solvents (Chen et al., 2015a).

The aim of this work was to identify of total phenolic and flavonoid contents of extract of T. transcapicus and also antioxidant and antimicrobial activity of the plant. To the best of our knowledge, this is the first report on chemicals and biological activity of T. transcapicus.

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. Extraction of *Thymus transcapicus* oil through Microwave Assisted Hydrodistillation (MAHD)

A domestically modified microwave oven (Samsung MW71E model) was fitted to the Clevenger-type apparatus as describe previously in literature (Golmakani and Rezaei, 2008). For MAHD extraction, 1 L sized reactor (round bottom flask) containing 25 g of powdered *Thymus transcapicus* matrix (pre-soaked in distilled water at 8:1 w/w of water to dried *Thymus transcapicus* powder) was placed within the microwave oven cavity. A Clevenger apparatus which has been set on top, outside the microwave oven, was used to collect the extracted essential oil. Extraction was continued for about 90 min and at microwave power level of 250 W. The extraction parameters were selected based on previous research (Jeyaratnamet al., 2016a). After extraction, the *Thymus transcapicus* oil was dehydrated over anhydrous sodium sulfate to remove excess water, then the concentrated *Thymus transcapicus* oil was weighed and stored in vial at 4°C for further analysis.

2.3. Hydrodistillation

The plant (80 g of dried material) was submitted to hydrodistillation for 3 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (1975). The volatile arrack was accumulated over anhydrous sodium sulphate and refrigerated previous to analysis (Yamini et al., 2008).

2.4. Oil extraction yield

The amount of oil calculated by the following formula:

Oil (%v/w) = Volume of essential oil (ml) / Weight of raw materials (g).

2.5. Preparation of plant extract

The aerial parts of the plants was dried under shade at room temperature and then cut into small pieces. About 100 g of sample was macerated in methanol 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 extract (Boozari et al. 2015).

2.6. Determination of total phenolic and flavonoid contents

The total phenolic content (TPC) in each extract was determined using the Foline–Ciocalteu procedure as described in Ardestani and Yazdanparast (2007) with minor modifications. Briefly, to prepare a sample extract, 10 mL of 80% methanol was added to 250 mg of the dried-milled samples and shaken slowly. The solution thus obtained was filtered, 0.5 mL of the methanolic extract was mixed with 2.5 mL of the Foline-Ciocalteu's reagent (1:10 diluted with distilled water) and 2 mL of 7.5% sodium carbonate solution in a tube test and shaken well. The mixture was maintained at 45 °C in a hot water bath for 15 min. Then, the absorbance of the mixture was measured at 765 nm using a spectrophotometer. A blank sample consisting of water and the reagents was used as the reference. Tannic acid equivalents (TAE) were used as the reference standard and the TPC was expressed as mg of TAE per gram of each extract on a dry basis.

The aluminum chloride colorimetric method was adapted for the determination of total flavonoids (Zhang et al., 2015) with some changes. A volume of 125 µL of the extract was added to 75 µL of a 5% $NaNO_2$ solution. The blend was allowed to remain for 6 min before 150 µL of $AlCl_3$ (10%) was added and incubated

for 5 min. To this was then added 750 µL of NaOH (1 M). The final volume of the solution was made to 2500 µL with distilled water. After 15 min of incubation, the mixture turned pink and the absorbance was measured at 510 nm. The total flavonoid content (TFC) was presented in mg of quercetin equivalents (QE) per gram of the extract.

2.7. Antioxidant Activity Determination

2.7.1. Antioxidant activity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay

The antioxidant activity of the essential oil was measured on the basis of the scavenging activity of the stable radical DPPH according to the method of Wang (Wang et al., 2003). 100µl from essential oil at different concentration range (2.5- 25 mg/ml) were mixed in the freshly prepared 4 mM DPPH in methanol. Absorbance at 517 nm was specified after 30 min. The scavenging activity was calculated using Eq.1.

$$\% \text{ DPPH scavenging activity} = \frac{\text{A 517 of control} - \text{A 517 of sample}}{\text{A 517 of sample}} \quad (1)$$

The percent of scavenging activity was plotted against the sample concentration to obtain EC_{50} (effectual concentration) defined as the concentration of sample necessary to scavenge 50% of the DPPH radicals and it was computed using graphpad prism (version 5.0) software. BHT was used as reference antioxidants.

2.7.2. Total reduction ability by $Fe3^{+}$-Fe^{2+} transformation

The total reduction ability of essential oil was determined by the method of Oyaizu (1986). The capacity of essential oil to reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) was evaluated by measuring the absorbance at 700 nm. To the different concentrations of the essential oils 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%) were added. The mixture was incubated at 50 °C for 20 min. Then 2.5 ml of trichloroacetic acid (10%) were added. The mixtures were revolved at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride. Absorbance was measured at 700 nm on UV spectrophotometer after allowing the solution to stand for 30 min. Butylated hydroxytoluene (BHT) was used as a standard.

2.8. Antimicrobial Activity

Determination of the minimum inhibitory concentrations (MIC) antimicrobial activities of essential oil of the aerial part of the plant was determined against two Gram-positive bacteria*:* *Staphylococus aureus* (ATCC 6538p), and *Listeria monocytogenes* (ATCC 35152), two Gram-negative bacteria: *Salmonella enterica* (ATCC 53648), *Escherichia coli* (ATCC 10536).

2.8.1. 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 essential oil was prepared with the aid of bath sonicator 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^{-1}; 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.

2.8.2. Determination of minimum bactericidal concentrations (MBC)

The bactericidal effects of essential oil 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.

2.8.3. Antimicrobial activity by disc and well diffusion method

The essential oil of the plant was tested for antibacterial activity using the disc and well diffusion methods on solid media Mueller-Hinton agar (MHA) plates. The sterile paper discs and wells of 6 mm diameter were placed on the agar plates with the appropriate media, and the bacteria density was adjusted to approximately 10^7 CFU/ml. Then, 50 µl of the essential oil was applied to test paper disc and well in plates and the agar plates were further incubated for 24 hr at 37°C. Finally, the zones of growth inhibition around the discs were measured. Gentamicin and DMSO were used as positive and negative controls, respectively (Firdaus et al, 2011).

2.9. Statistical analysis

The measurements of antibacterial activity, total phenolic compounds, DPPH radical scavenging activity and FRAP assay were carried out for three replicates. The results are expressed as mean values ± standard deviation (SD).

3. Results and Discussion

3.1. Essential oil yield

Nowadays, microwave treatment is one of the most commonly used methods for solid-liquid extraction due to its power, convenience, and reasonable cost. Many studies have reported about the benefits of microwaves for extracting some active compounds from plant materials such as triterpene, saponins, and antioxidant components etc. (Li et al., 2010; Zhao et al., 2013). These results highlight the ability of microwaves to disrupt hydrogen bond networks. The microwave-induced dipole rotation of molecules, and the migration of ions that enhance the penetration of solvent in to matrix, disrupts the cell wall and releases the intracellular product, allowing for the extraction of different components (Li et al., 2010).

The present study, the extraction yield of essential oil were with microwave assisted hydrodistillation and hydrodistillation, 0.494% and 0.243% (v/w), respectively. Among the samples based on a dry weight. Essential oil content can be highly affected by both environmental factors and plant species (Bahreininejad, Mirza, & Arzani, 2010; Llorens et al., 2014; Yavari et al., 2010). Previous studies reported various ranges for the EO yields in different Thymus species. For instance, *T. fedtschenkoi* had been previously reported to have its maximum EO yield (2.9%) at the flowering stage and its minimum (0.7%) during the seed set stage (Rustaiee et al., 2011). Hazzit, Baaliouamer, Veríssimo, Faleiro, and Miguel (2009) had also reported an EO yield in the range of 4.2–4.6% for T. pallescens in its full flowering stage and a minimum of 0.9–1.3% at the beginning of the vegetative cycle.

3.2.Total phenolics and total flavonoid contents

TPC and TFC are the two key indicators widely employed to represent the overall antioxidant activity in the samples. The results

the amount of phenolic in essential oil were with microwave assisted hydrodistillation and hydrodistillation,11.42 and 9.21 mg tannic acid equivalents (TAE) g^{-1} DW, respectively and flavonoid content 7.65, 4.04 mg quercetin equivalents (QE) g^{-1} DW, respectively.

Furthermore, phenolic compounds were capable of scavenging the reactive oxygen intermediates without invoking further oxidative reactions (Al-Abd et al., 2015). Previous research has shown that TPC takes on variable values depending on the Thymus species (Jabri-Karoui, Bettaieb, Msaada, Hammami, & Marzouk, 2012).

Safaei-Ghomi et al. (2009) found TPC values for *T. caramanicus* were higher than those obtained for the accessions investigated in the present study. High TPC values have also been reported for *T. spathulifolius* (Sokmen et al., 2004) and *T. Serpyllum* (Mata et al., 2007).

The studies conducted so far have established that the Thymus species can be considered not only as rich sources of phenolics and flavonoids but as promising sources of natural antioxidants as well. The phenolic content of each plant, however, is a function of a multitude of factors such as the extraction method employed and the phenological stage (Gharibi et al., 2015).

However, it is likely that different species use different mechanisms to distribute flavonoids among their subcellular parts. From a metabolic point of view, plant polyphenols such as flavonoids and phenolics are biosynthesized through several pathways and form a heterogeneous group (Gharibi et al., 2015). Baharfar et al. (2015) reported that the TFC value of *T. kotschyanus* ranged from 32.04–74.60 mg QE g^{-1} of the dry extract. Jabri-Karoui et al. (2012) reported a TFC value of 10.62 ± 0.24 mg CE/g DW for *T. capitatus*. Furthermore, the use of different solvents can affect the flavonoid content of the plants. Hossain et al. (2013) revealed that methanol as a solvent produced higher amount of flavonoid in comparison with four other solvents in *T. vulgaris*. The mechanism underlying the flavonoid functions is based on the scavenging or the chelating process. The composition of these compounds is highly influenced by the location where the sample is collected as well as the dominant climatic and environmental factors (Rahimmalek et al., 2009).

3.3.Essential oil antioxidant activities

DPPH assay and reducing power assay were used to assess antioxidant potential of *Thymus transcapicus* essential oil. The synthetic antioxidant BHT was used as an equivalence parameter for the antioxidant activity of the essential oil.

Reactive oxygen species (ROS), including oxygen radicals and their reaction products, are known to react with biological molecules, leading to cell and tissue damage. Antioxidant activity is a complex process usually occurring through several mechanisms. Due to its complexity, the evaluation of the antioxidant activity for pure compounds or extracts should be carried out by more than one test method (Aruoma, 2003). The lower IC_{50} value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher IC_{50} value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction.

The DPPH scavenging activity of the tested oil was found higher than butylated hydroxytoluene (BHT) as is evident from lower IC_{50} value of essential oils. In reducing power assay, *Thymus transcapicus* essential oil showed comparable ferric reducing power to BHT at the tested concentrations of 20–100 mg/ml (Table 1). Microwave assisted hydrodistillation, which led to higher antioxidant activity, is a more efficient technique than hydrodistillation.

Table 1. Antioxidant activity of *Thymus transcapicus* essential oil measured in term of DPPH radical scavenging capacity and Total Ferric Reducing ability.

Technique applied	Test system	Conc. (µg/ml)	*Thymus transcapicus*	BHT	IC_{50} value, µg/ml *Thymus transcapicus*	IC_{50} value, µg/ml BHT
Microwave	DPPH radical scavenging capacity (%)	20	34.6±0.4	31.3±0.9	31.08±0.4	47.28±0.5
		40	59.87±0.5	43.74±0.8		
		60	69.9±0.3	59.9±0.4		
		80	81.9±0.6	75.9±0.5		
		100	89.8±1.2	80.8±0.3		
	Reducing power (absorbance at 700 nm)	20	1.86±0.8	1.13±0.7		
		40	1.92±0.6	1.19±0.5		
		60	1.99±0.3	1.25±0.3		
		80	2.37±0.4	1.53±0.1		
		100	2.87±0.2	1.73±0.5		
Hydrodistillation	DPPH radical scavenging capacity (%)	20	14.9±0.2		49.8±0.2	47.28±0.5
		40	25.87±0.6			
		60	45.3±0.4			
		80	64.9±0.8			
		100	77.1±0.3			
	Reducing power (absorbance at 700 nm)	20	0.65±0.2			
		40	0.79±0.9			
		60	0.91±0.1			
		80	1.19±0.2			
		100	1.22±0.7			

The antioxidant capacity of Thymus species has been well researched. The most relevant chemotypes of Thymus species have been reported to be rich in phenolic monoterpenes such as thymol and carvacrol (Jabri-Karoui et al., 2012). Species such as *T. carmanicus* (Safaei-Ghomi et al., 2009) and *T. spathulifolius* (Sokmen et al., 2004). In most

such studies, phenolics, due to their chemical structures that allow them to donate hydrogen to free radicals, were introduced as the major factor contributing to the antioxidant activity of the species (Ang et al., 2015). Moreover, essential oil consisting of phenolic monoterpenes and/or sesquiterpenes has been recognized for their higher antioxidative capacity (Mancini et al., 2015).

The reducing power of the studied *transcapicus* was observed to increase with increasing essential oil concentration. In this model system, *T. transcapicus* showed a more reducing power than BHT. Previous studies had indicated that the high reducing power of the Thymus species was not directly related to its thymol and carvacrol contents but the substitution of hydroxyl group in the aromatic ring might have contributed to its antioxidant activity (Jabri-Karoui et al., 2012).

3.2. Antimicrobial activity

The results presented in table 2, zones of growth inhibition around MIC and MBC of *Thymus transcapicus* essential oil was evaluated.

Table 2. Determination of MIC, MBC of *Thymus transcapicus*

Technique applied	**Test bacteria**	**MIC (mg/ml)**	**MBC (mg/ml)**
Microwave	*Staphylococus aureus* (ATCC 6538p)	25	25
	Listeria monocytogenes (ATCC35152)	25	25
	Salmonella enterica (ATCC 53648)	50	50
	Escherichia coli (ATCC 10536)	50	50
Hydrodistillation	*Staphylococus aureus* (ATCC 6538p)	50	50
	Listeria monocytogenes (ATCC35152)	100	>100
	Salmonella enterica (ATCC 53648)	100	>100
	Escherichia coli (ATCC 10536)	100	>100

Table 3. Antibacterial activity of *Thymus transcapicus* was assessed by disc and well-diffusion methods.

Technique applied	Test system		Microorganism			
Microwave	Diameter of inhibition zones (mm), Disc-diffusion method*		*S. aureus*	*L. monocytogenes*	*S. enterica*	*E. coli*
		Essential oil	30 mm±0.02	31 mm±0.03	29 mm±0.01	28 mm±0.05
		Gentamicin	27 mm±0.05	28 mm±0.02	26 mm±0.04	25 mm±0.02
	Diameter of inhibition zones (mm), Well-diffusion method*	Essential oil	32 mm±0.02	34 mm±0.03	31 mm±0.04	30 mm±0.03
		Gentamicin	32 mm±0.01	33 mm±0.04	29 mm±0.01	28 mm±0.05
Hydrodistillation	Diameter of inhibition zones (mm), Disc-diffusion method*	Essential oil	25 mm±0.07	26 mm±0.01	23 mm±0.04	21 mm±0.02
		Gentamicin	27 mm±0.05	28 mm±0.02	26 mm±0.04	25 mm±0.02
	Diameter of inhibition zones (mm), Well-diffusion method*	Essential oil	26 mm±0.07	28 mm±0.05	27 mm±0.06	25 mm±0.01
		Gentamicin	32 mm±0.01	33 mm±0.04	29 mm±0.01	28 mm±0.05

*Expressed as the size of the growth inhibition zones (mm) as the average of triplicates.

Antimicrobial activity of the plants of different areas of the world has been reported (Janovska et al, 2003). The disc and well-diffusion methods are dependent on the diffusion ability of the substances and in these methods; antibacterial property is expressed as diameter (mm) of the zone of inhibition (He et al, 2010).

Table 3 shows the antibacterial activity of the essential oil of this plant was markedly higher than gentamicin against gram-positive bacteria (*Staphylococus aureus*, and *Listeria Monocytogenes)* and two gram-negative bacteria (*Salmonella enterica*, *Escherichia coli*). 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). The antimicrobial activity of *T. transcaspicus* EO was, therefore, attributed to the presence of Thymol. Other constituents of the essential oil such as gammaterpinene and p-cymene, could be also taken into account for their possible synergistic or antagonistic effects.

The effectiveness of essential oil 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.

4.Conclusions

The results showed that the activity of the oil can be attributed, to a considerable degree. MAE, which led to higher antioxidant and antimicrobial activity, is a more efficient technique than hydrodistillation. The results of this study suggest the possibility of using *Thymus transcapicus* essential oil as a natural food preservative, because the oil found to possess strong antibacterial activity.

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