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Original Article

Autecology, Ethnopharmacology, Phytochemical, Antioxidant and Antimicrobial Activity of *Thymus carmanicus* Jalas. from Golestan Province in North of Iran

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Abstract

This research had been carried out to ecological characters, phytochemical, anti bacterial and antioxidant activity of *Thymus carmanicus* Jalas. in Golestan province .In several field observation, the aerial parts of plant in blooming were collected from Deraznoo Mountain inSeptember 2013 (2700 m). Essential oil was obtained by hydro distillation and analyzed by GC/MS. Phytochemical assay included: TP (total phenol) and TF (Total flavonoid) were determined by spectrophotometrically, antioxidant capacity were obtained by TAC, RP and DPPH methods and the antibacterial by using agar well method and the minimum inhibitory concentration (MIC) assay. Results showed that *T. carmanicus* is the most aromatic mountainous herb, which wild growing in cold dry climate (1800-2750 m) in Golestan province, with annual raining 30.5 mm, annual temperature 17.5 °C , in sandy clay loam soil with pH= 7.5 and Ec=2.1 dc and have long been used in traditional medicines of this region as a strong tonic, anti inflammation, carminative, anti virus, anti infection, anti fungus, anti ulcer and sedative . The carvacrol (41.4%), thymol (27.2%) and β-caryophylene were the major constituents of *T. carmanicus* oil , with total phenols (TP= 81.7±0.3 GAE/ g), total flavonoids (TF= 34.2±0.8 QUE/g), which had good antioxidant activity (IC50 = 21.8±0.1 µg/ml), especially in DPPH method .The maximum activity of the essential oil was observed against *Candida albicans* (35.8±0.6 mm), *S.epidermidis* (33.1± 0.4 mm), *B. subtilus* (29.5± 0.2 mm), *S.aureus* (28.3±0.2 mm), *E.coli* (23.1±0.4 mm), *Enterococcus faecalis* (17.2±0.8 mm) and *Kelebsiella pneumoniae* (16.5±0.2mm) with MIC value in the range of 10 - 132 mg/mL. According to these results, the aerial parts of plant have rich source of terpenoides , flavonoid and phenolic componds and showed good antioxidant and antibacterial activity, which will be confirmed the traditional uses of *T. carmanicus* Jalas. in these regions as an good anti-inflammatory, sedative and anti infection.

Key words: Antioxidant, Autecology, Essential oil, Ethnopharmacology, Golestan province, TF and TP content, Golestan, *Thymus carmanicus* Jalas

Introduction

Global interest in natural antioxidant , antipathogene, anti inflammation and anti mutagen (Poly phenols, terpenoides and flavonoides) in many wild aromatic plants has recently been increased [1]. So present study was carried on phytochemical sources (Essential oil constituents, TF and TP contents), the evaluate of their antioxidant and antibacterial activity which were collected from 2700m in North of Iran .

Free radicals cause the oxidation of biomolecules , cell injury and death In recent decades, so the phytochemical of wild aromatic plants have been great interest as the sources of natural anti oxidant and anti microbial products [2]. The genus *Thymus* (Lamiaceae), native to Southern Europe and Asia , which represented by 215 species in the world and 14 species in Iran flore with four endemic species

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[3,4], which has been used as herbal tea , spicy and medica condiments. The chemical composition , in vitro antioxidant and anti bacterial activities of the essential oils and extracts of several *Thymus* species have recently been reported before ,which has been traditionally used for many inflammation and infection such as respiratory tract infections, colds ,acute and chronic bronchitis , sinusitis and reduced asthma tonic, carminative, anti infection , antispasmodic, anti-inflammatory, antitussive and expectorant to treatment of many Iranian ailments [5-8]

In several studies, the essential oil composition ,antioxidant, antibacterial and antifungal activity of *Thymuscarmanicus* Jalas, *Thymus Pubescens* Boiss. & Kotschy ex Celak., *Thymus Serpyllum* L., *Thymus Kotschyanus* Boiss. & Hohen., *Thymus Persicus* (Ronniger ex Rech.f.) Jalas and *Thymus revolutus* Celak. from Iran and Turkey has been reported, respectively [5,19-11], Which followed the composition and antioxidant activities of *Thymus caespititius* Brot., *Thymus camphoratus* Hoffmanns. & Link and *Thymus mastichina* oils from Portugal have been reported [12].

T. caramanicus is an endemic Iranian species, which has been used in traditional medicine of Golestan province as sedative , ant infection and ant inflammation in treatment of rheumatism and skin disorders [3]. Nejad ebrahimi was showed that this species is a rich source of antimicrobial and antioxidant activity [5], so the aim of this research was carried out to the essential oil composition , phytochemical , anti oxidant and antibacterial activity in Deraznoo Mountain (Golestan province).

Material and Methods

Study area

The present study was carried out in Deraznoo Mountain region (2700m) , is located in South west of the Golestan province, in of latitude of 36° 37' 24"to 36° 34' 28" and longitude of 54° 35' 26" to 54° 24' 32" with sandy clay loam soils. Its average height is 1100 to 2750 m from sea levels, with dry cool climate, rainfall of about 305 mm/year and a mean temperature -2/8 °C (January to February) and 17/3 °C (July to August).

Ecological requirements and ethno pharmacology

In many field observation, most ecological requirements and traditional pharmaceutical knowledge about *T. carminicus*, were obtained from rural healers and then all obtained data from questionnaires were compared with the findings in vivo and invitro experiments in other similar reports.The aerial parts of *T. caramanicus* were collected at full flowering in september 2014 from its wild habitat in Deraznoo Mountain (2700m). Voucher specimen was deposited at the Herbarium of Research Center of Medicinal Plants (RCMP), in Islamic Azad University of Gorgan branch, Gorgan, Iran.

Isolation of essential oil

The plant samples in blooming stage were dried at room temperature for 3 days. The flowers were hydro distillated in aClevenger-type apparatus for 2 h according to the method recommended in the British Pharmacopoeia (British Pharmacopoeia, 1988). The oil was dried over anhydrous sodium sulphate and deoxygenated under nitrogen gas and the oil obtained was kept refrigerated and protected from direct light until the analysis time.

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil was analyzed by GC-MS. The GC-MS analysis was carried out on a Shimadzu GC-MS (QP5050). The capillary conditions were as follows; carrier gas, helium with a flow rate of 1.7 ml/min; injected 0.1 µL of the essential oil and ionization potential 70 ev. The initial temperature of column was 60 °C (held for 1 min) then heated to 280 °C with a rate of 3 °C/min, then heated to 250 °C and kept constant for 4 min. The same condition of temperature programming was used for n-alkenes mixture to calculate the retention indexes (RI). The identification of each component was studied by mass spectral data, literature and National Institute of Standards and Technology (NIST) computer library. The relative percentage of the oil constituent was calculated.

Plan extract preparation for phytochemical and antioxidant tests

One gram of plant parts with 100 ml (methanol80%) were extracted by maceration. Extracts were filtered with Whatman No. 1 filter paper. The filtrates obtained from extracts were evaporated into dry rotary evaporator at 40 °C and were stored at 4 °C [13].

Chemicals

2,2'-diphenyl-1-picrylhydrazyl (DPPH) and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, Folin-Ciocalteu reagent , BHT (butylated hydroxytoloane), BHA (butylated hydroxyanisole)

and methanol were purchased from Merck Co. (Germany).

Determination of Total Phenolic Content

It was determined using the Folin-Ciocalteu Reagent. Total phenolic content was estimated by the Folin Ciocalteu method, based on the procedure suggested by Pourmorad *et al*. [13]. Then 0.5 ml of plant extracts or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu Reagent (5 ml) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. Gallic acid was used as a standard for calibration curve. Total phenol values were expressed in terms of mg equal gallic acid in 1 gr powder dry plant.

Determination of Total Flavonoid Content

Total flavonoids content were determined by aluminum chloride method. Extract plants (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. They were kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. Total flavonoid values were expressed in terms of mg equal quercetin in one gram powder dry plant [13].

Antioxidant Activity Tests

Reducing Power assay

This assay is based on Arabshahi-Delouee method. First, The dried extract (12.5–1000 µg) in 1 ml of the corresponding solvent was combined with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (K3Fe(CN) 6; 10 g l-1), after the mixture was incubated at 50 °C for 30 min. Then, 2.5 ml of trichloroacetic acid (100 g l-1) were added and the mixture centrifuged at 1650g for 10 min. Then, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (1 g l-1), and the samples absorbance was measured at 700 nm [14].

1,1-diphenyl-2-picryl hydrazyl radical scavenging capacity Assay

The ability of the extracts for free radical scavenging was assessed by the method suggested by [14]. Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 12.5-1000 µg of dried extract). The mixture was then vortexes vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

DPPH scavenging activity (%) =[(Acontrol – Asample) / Acontrol] ×100

Total Antioxidant Capacity

This experimental procedure was adapted from Arabshahi-Delouee method, which is based on the reduction of Mo (VI) to Mo (V) by the sample and observation of a green phosphate/Mo (V) complex at acidic pH. An aliquot of 0.1 ml of sample solution, containing 12.5-1000µg of dried extract in corresponding solvent, was combined in a tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). They were incubated in a thermal block at 95 °C for 90 min. Then we got cold the samples and measured their absorbance at 695 nm. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent was used for the sample, and was incubated under the same conditions as the rest of the samples [14].

Bacterial Strains

The bacterial strains were obtained from the Microbiology Laboratory, Golestan University of Medical Sciences. The essential oils of plant aerial part was individually tested against two strains of Gram positive and negative bacteria :(*Shigella dysenteria* (PTCC1188), *Pseudomonas aeroginosa* (PTCC1430), *E. coli* (PTCC1399), *Staphylococcus aureous* (PTCC1431), *Bacillus cereus* (PTCC1015), *Salmonella typhimurium* (ATCC1596), *Staphylocuccus epidermidis* (PTCC1114),*Enterococcus faecalis* (PTCC1393), *Kelebsiella pnumonie* (PTCC1291) and one fungal isolate: *Candida albicans* (PTCC5027).

Antimicrobial activity

At a first screening, the plant extract were tested against the above mentioned bacteria. Minimal inhibitory conentrations (MICs) were determined by the agar serial dilution method at concentration ranging from 0.93 to 60 µg/mL. Two fold serial dilutions were made from essential oil in molten Mueller Hinton agar (Pronadisa- Madrid) cooled to 45-50 ^{o}C in a water bath. The essential oil was dispersed in mixture using dimethyl sulfoxide (DMSO). The amount of 0.01 mL of every bacterial suspension, equivalent to McFarland tube No. 0.5 (10^8 CFU/mL), inoculated on the agar of every well. The culture plates were then incubated

at 37 ^{o}C for 24 h. The MIC was defined as the lowest concentration at which no visible growth was observed (15). The Mueller Hinton agar were contained DMSO without essential oil was used a negative control while Gentamycine was used as positive control.

Statistical analysis

For all assays, data were expressed as means ± S.E. and differences at $P<0.05$ were considered statistically significant.

Results

In this study, many field observation showed that the *T. carmanicus* in Deraznoo Mountain is the most Mediteranean wild aromatic perennial chamephyte form plant , which grow to 15-20 cm with small wooden branches, growing spearhead with papil green leaves,which needcool dry weather in North slob and enough light in growing stages. Not heavy soil is suitable soil in Deraznoo Mountain, 57 km far from Gorgan (1800- 2750m) with annual raining 30.5ml, annual temperature 17.5 °C and semi dry cold climate in the sandy clay loam soil (clay=23.8%, silt 32% and sand 53%) with Ec=2.1 desizimence and pH= 7.5 .

Ethno pharmacological survey showed that dry Leaves of *T. carmanicus* especially in bloomong, is one of the most wild edible aromatic spicy, which have been used in traditional medicines of Mountainous region of Golestan province as a strong tonic, anti inflammation, carminative, anti virus, anti infection, anti fungus, anti ulcer and sedative to treat of Rheumatism, Ulcer, infection, bronchitis, cold, flu, pain, fever, toothache, backache and digestive infection as a single or in combination with other herbs such as below:

For treat of cold and flu: The tea combination of *T. carmanicus* with, *Satureja mutica, Mentha longifolia*, the flower of *Sambucus nigra, Adiantum cappilus veneris* and *Cynamum zeylanicum* as agood strong tonic, anti inflammation, carminative, anti infection and warm to treat of col, bronchitis and flu.

Mouth Ulcer and aftus: the gargle of *Thymus* infusion with consumption the Dusin (the mixed of *Nigella sativa* extract in honey) .

Finger fungus infection: theextract of *T. carmanicus* with *Mentha longifolia* L. Sedative and anti inflammation: The massage of the oil of *T. carmanicus, Perovskia abrotanoides, Stachys inflata* Benth. and *Capsicum annuum* L. anti spasm, anti inflammation and sedative to treat of rheumatic pain , backache ,sciatic and gout.

Hair tonic: The infiusion of *T. carmanicus, Urtica dioica* L., *Adiantum cappilus–veneris* L., *S. inflata* and *Myrtus communis* L. in Olea oil as an hair tonic, anti dandruff and anti inflammation and promote of hair growth.

Anti helmentic: the consumption of *T. carmanicus* powder with *Ferula gummosa* Boiss. and *peganum harmala* L. in daily.

Toothache: *T. carmanicus* powder with *Cuminum cyminum* L..

Earach and ear infection: the 1 drope of *T. carmanicus* oil in Garlic extract in the ears

Phytochemical and anti oxidant results are presented in Table 1 and showed that , the content of total phenols (TP= 81.7±0.3 GAE/g), total flavonoids (TF= 34.2±0.8 QUE/g) in flowering aerial parts of *T. carmanicus*, which had good antioxidant activity with IC50 = 21.8±0.1 µg/ml especially in DPPH method, which had higher content of IC50 to free radical scavenging .

Essential oil yield of *T. carmanicus* Jalas. was 2.1%. with 31 components were identified, which represented 91.35% of the total detected constituents. The major constituents of the oil were carvacrol (41.4%), thymol (27.2%) and β-caryophylene (11.2%), followed by other components were presents in amounts less than 2% (Fig. 1, Table 2).

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Table 1 The phytochemical analyses of *Thymus carmanicus* Jalas. In Golestan province

Phytocemical	Deraznooregion (2700m)				
	Antioxidant activity			Total phenole	Total flavonoid
	IC50 in RP	IC50 in TAC	IC50 in DPPH		
	67.4±0.6	41.3±0.3	21.8±0.1	81.7±0.3	34.2±0.8

Table 2. Essential oil composition of *Thymus carmanicus* Jalas. In Golestan province (2700m).

No.	Compound	RI	RT	%
1	-	-	3.14	0.3
2	α-thujone	926	4.99	0.1
3	β-pinene	935	5.21	0.2
4	Camphene	949	5.66	0.1
5	Sabinene	965	6.43	0.4
6	β-pinane	976	6.58	0.9
7	α-terpinene	1012	7.61	0.2
8	P-cymene	1014	7.88	0.8
9	Limonene	1018	8.08	0.5
10	β -phelanderene	1021	8.11	0.4
11	1,8-cineole	1024	8.22	0.1
12	β -ocimene	1037	8.58	0.3
13	-terpinene	1052	9.05	0.9
14	Terpinene 4-0l	1213	9.61	0.1
15	Linalylacetat	1224	10.67	0.09
16	Thymol	1237	11.73	27.2
17	Carvacrol	1282	12.46	41.4
18	Bornyl acetate	1284	13.79	0.2
19	Terpinen 4-0l	1288	14.12	0.1
20	α -cucubene	1312	14.06	0.2
21	Benzene	1328	16.57	0.04
22	Naphthalene	1364	17.75	0.2
23	Copaene	1385	18.30	0.5
24	β-burbunene	1404	18.80	0.9
25	β-elemene	1412	19.63	0.5
26	β-caryophylene	1419	21.34	11.2
27	-Bisabolene β	1499	24.13	0.1
28	γ-cadinene	1509	24.46	0.5
29	Caryophylene oxide	1585	24.90	0.3
30	Naphthalene	1589	25.06	1.397
31	Isoledene	1601	25.25	1.255
%Total	91.35			

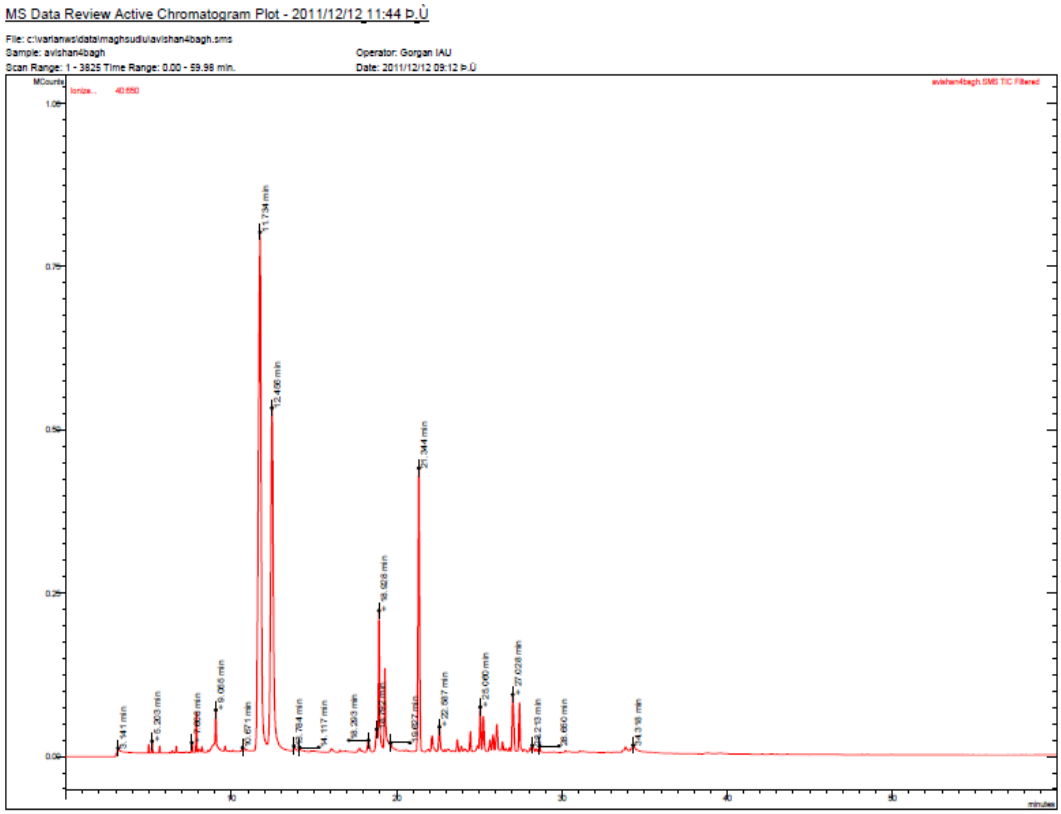

Fig. 1 Essential oil analysis chromatogram of *Thymus carmanicus* Jalas. In South west of Golestan province (2700m).

Table 3 Antibacterial activity of aerial parts of *Thymus Carmanicus* Jalas. from Golestan province

Microorganisms	region (2020m)		
	inhibition zone (mm) ±SD	MIC (µg/mL)	Gentamycin
Staphylococcus aureus	28.3±0.2	18.4	20.7
Staphylococcus epidermidis	33.1±0.4	12.3	22.3
Bacillus subtilis	29.5±0.2	19.2	16.5
Entrococcusfaecalis	17.2±0.8	73.8	9.6
Escherichia coli	23.1±0.4	41.6	17
Pseudomonas aeroginosa	13.9±0.2	80.5	9
Klebsiella pneumonia	16.5±0.2	94.2	na
Salmonella typhymorium	11.2±0.1	132	na
Shigelladisentria	12.1±0.5	102.3	na
Candida albicans	35.8±0.6	10	13.9

As can be seen in Table 3, essential oil have good potential antimicrobial activity against all of microorganisms tested. The MIC values were in the range of 10-132mg mL-1. The maximum activity of the essential oil was observed against pathogenic fungus: *Candida albicans* (35.8±0.6mm) ,and two groupe of Gram positive and negative bacteria: *S.epidermidis* (33.1± 0.4 mm), *B.subtilus* (29.5± 0.2mm), *S.aureus* (28.3±0.2mm), *E.coli* (23.1±0.4mm), *Enterococcus faecalis* (17.2±0.8mm) and *Kelebsiella pneumoniae* (16.5±0.2mm), but this oil has poor activity on the growth of *Shigella dysentria* and *Salmonella tyfimoriom*with MIC of 102-132 mg/ mL.

Discussion

Briefly, according to results in Table 1,2,3 , indicated that carvacrol and thymol were the major essential oil terpenoides, with good TP= 81.7±0.3 GAE/ g), total flavonoids content (TF= 34.2±0.8 QUE/g) in flowering aerial parts of *T. carmanicus*, which had good antioxidant activity with IC50 =

21.8±0.1 µg/ml especially in DPPH method , which had higher content of IC50 to free radical scavenging and good anti *Candida* and anti bactrial activity (Table 3).

The natural antioxidants is positively reduces risk of developing infectious, inflammatory and cancer diseases. Therefore, there is a growing interesting to natural antioxidants (Terpenoides, phenolic compounds and flavonoids) search in wild aromatic plants with strong radical scavenging activities [1].

Thymus species the most popular aromatic plants throughout the world, which are commonly has been used as a tonic teas, anti infection, carminative, anti-inflammatory , anti oxidant and antispasmodic to treat of cold, flu, whooping cough, bronchitis, inflammation and rheumatism in Iranian folk medicine and many other [8].

The *S. aureus* and *P. aeruginosa* have been applicable in cases of infectious boils, sores , wounds and are considered as the main pathogens causing hospitalized patients' infections [17] and also, the effectiveness of plant essential oil in growth inhibition against E. coli , which can cause the diarrhea and dysentery in humans and animals [18,19].

In many literatures, it is described that the antibacterial activity of *Thymus* species can be dependent on variation of their terpenoids , phenols and flavonoids in essential oil or extract, their habitat and their period growth (5).According to similar reports, the essential oil of several *Thymus* species (*T. revolutus, T. pubescens ,T. serpyllum , T. kotschyanus, T. persicus , T. caespititius, T. camphorate , T. satureioides, T. mastichina and T. caramanicus)* from Iran , Turkey and another countries(Morroco) have been reported before and were showed differences in quantities of their essential oil components, but their major components were similar and included: thymol, carvacrol, α-pinene, 1,8-cineole, and caryophyllene [5-10].

In similar reports, essential oils rich in phenolic compounds, such as carvacrol and thymol were reported are widely reported to possess high levels of antimicrobial activity , In fact, other constituents, such as camphen , 1,8-cineol , γ-terpinene and terpinene 4-ol have been considered to display relatively good activity due to their possible synergisticor antagonistic effects, anti inflammation , anti infection and sedative to treat of Cold, flu, arthritis, rheumatic pain and UTI [5-20].

To confirming of our ethnopharmacological survey, (5)were showed that the carvacrol (58.9-68.9%), p-cymene (3.0-8.9%), c-terpinene (4.3-8.0%), thymol (2.4-6.0%) and borneol (2.3-4.0%) were the major compounds in *T. carmanicus* oils which have strong antibacterial activity against seven Gram-positive and Gram-negative bacteria by Different ranges inhibition zone (15-36 mm) and MIC = 0.5–15.0 mg/ml, thus, they represent aninexpensive source of natural antibacterial, antifungal, antiviral, antiparasitic and antioxidant activities substances that exhibited potential for use in pathogenicsystems [5,21-23].

In this study, carvacrol and thymolwere the major chemotype of *T. carmanicus* essential oil from Golestan province, but in several before studies, the *Thymus* species has varieties in essential oil composition (geraniol, linalool, γ-terpineol, carvacrol, thymol ,trans-thujan-4-ol and terpinen-4-ol) which grown in Iran or in other countries [8]. The thyme specie samples in Turkey, Spanish and other countries (thymol , alpha terpinene , p-cymene, alpha pinene, 1,8-cineole, terpenyl acetate, borneol, linalool, beta - pinene, alphaterpineol , carvacrol and camphor were found to be the main componenet in the previose report [8]. So comparison between our results and the results of other reports showed differences, probably due to the plant varieties or sites, as well as the time of harvesting.

The before similar antifungal and antibacterial activity exhibited by Thymus genus essential oil has been demonstrated by several researchers , which reporting that the essential oils are relatively more active against Gram+ve than Gram−ve bacteria[11,16].

However, It was often reported that Gram-negative bacteria were more resistant to the *Thymus*essential oils [24]. our results (Table 1,3) antibacterial and antioxidant activity of essential oils depends on their chemical composition, which is determined by the genotype and influenced by environmental and agronomic conditions [16]. According to before obtained , thetymol, carvacrol,camphor , alpha-pinene , beta –pinene,borneoland 1, 8-cineole are mainly responsible for the antimicrobial and anti oxidant activity in many aromatic plants oil, such as: *Thymus species, Perovskia abrotanoides Achillea millefolium* L., *F. gummosa* and *Ditrichia* species) [17,27].

To confirming of our phytochemical and ethnopharmacological survey of *T. carmanicus*, other studies showed that the thymol and carvacrol are the main phenolic constituent of *Thymus* species oil have the biological activity,which in medicinal field, their essential oils has been used as a good anti-inflammatory and anti infection to treat acute and chronic bronchitis , acute sinusitis and reduced asthma [6-8].

It has been recognized that secondary metabolites (terpenoides, polyphenols and flavonoids) in aromatic wild plants have antioxidant agents through scavenging on human health [25]. So in general, recently, interest has increased considerably in finding naturally occurring antioxidants and anti pathogen for use in foods or medicinal materials to replace synthetic antioxidants and anti bacterial drugs [26].

According to above, the extract of aerial parts of *T. carmanicus* have better capacity against free radical, especially in DPPHtest, which can provided and confirmed of the ethnopharmacological survey of this plant in traditional medicine of this region as anti inflammation, sedative and anti infection.

Conclusion

It can be concluded that the phytochemical and essential oil composition of *T. carmanicus* with major constituents(carvacrol and thymol) , possess more antibacterial , antioxidant and anti nflammatory activity. Considering, it can , we believe that the present search together with previous studies provide support of ethnopharmacological results of this study as a tonic, anti infection, antibacterial, anti inflammatory and antioxidant properties of this plant to treat of many infectious and inflammatory current diseases. It can be used as antibacterial supplement towardsthe development of new therapeutic agents. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antimicrobial agent in topical or clinical applications, and further studies are necessary to evaluate the in vivoeffects of active compounds of this plant. In addition,investigations confirm that wild aromatic plants used as anti infective as a natural source for novel antibiotics. Our results demonstrate that the oil of *T. carmanicus* could become potentials for controlling certain important Gram positive and negative bacteriawhich produces many infectious diseases.

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