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DETERMINATION OF CHEMICAL COMPOSITIONS, ANTIFUNGAL, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *THYMBRA SPICATA* L. FROM TURKEY

Yusuf Bayan[1,*], Nusret Genc[2], Mustafa Kusek[3], Fatih Gul[4], Zeynep Imecik[3]

[1]Ahi EvranUniversity, Faculty of Agriculture, Department of Plant Protection, 40200 Kirsehir, Turkey
[2]University Gaziosmanpasa, Department of Chemistry, Faculty of Science and Art, 60240 Tokat, Turkey
[3]Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Plant Protection, 46100 Kahramanmaras, Turkey
[4]Cankiri Karatekin University, Department of Chemistry, Faculty of Natural Sciences, 18100 Cankiri, Turkey

ABSTRACT

The study was to investigate chemical composition, antibacterial, the antioxidant and antifungal activity of essential oil from *Thymbra spicata* L. (*T. spicata* L.). *T. spicata* L. essential oil fourteen components were determined. The basic components of the essential oil were determined carvacrol (78.53%), γ-terpinene (10.42%) and p-Cymene (5.49%). All plant pathogens at a dose of 10 μL $^{-1}$ the of essential oil blocked the of mycelium growth of 100%. The total phenolic and flavonoid contents of the methanolic extract aerial parts are quite rich. The methanolic extract from *T. Spicata* L. was determined a good antioxidant activity. Essential oil exhibited remarkable activity against the selected *Xanthomonas* spp. (ZI365, ZI366, ZI368, ZI370, ZI373, ZI375, ZI376, ZI378) bacterial isolates. Our work showed that *T. spicata* L., has a strong antifungal, antibacterial and antioxidant activity. These studies may be used in the management of plant pathogenic fungi and bacterial diseases control.

KEYWORDS:
T. spicata L., Antifungal, Antibacterial, Antioxidant, Essential oil

INTRODUCTION

For many years, aromatic plants have been used to food, drink and medicine. Lamiaceae family is one of the largest groups in the medical aromatic plants. *T. spicata* L. a species from the Lamiaceae family, which has a broad distribution in Turkey. The plant is locally known as Zahter, Kara kekik, and Karabaş kekik in the Southeastern Anatolia Region in Turkey, used to form of dry or fresh leaves and flowers in the tea. It is also used medically in other areas, in herbal teas for asthma, colic, bronchitis, cough, and rheumatism, or folk medicine as an antiseptic agent [1-2].

The essential oil components of thymbra species have been reported many times by researchers [3-5]. The predominant component in the essential oil is carvacrol. The other main constituents are γ-Terpinene, p-cymene, and the ratio of these three components to oil in the oil is 70%. Thymbra volatile oil has a strong inhibitory effects on weed germination of weed seeds. *Thybra spicata* species has remarkable antifungal, antibacterial, insecticidal and antioxidant [6-8].

The reactive oxygen and derivatives, some oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation. Some of the natural chemicals in plants have started to be taken as natural antioxidants. This natural chemical perhaps used to preserve humans from oxidative stress damage. Natural antioxidants are much more useful than synthetic antioxidants. There is no side effect of natural antioxidants obtained from plants, synthetic antioxidants were found to have an genotoxic effect [9-11].

Based on that, the purpose of this study: (1) the chemical components of essential oils from *T. spicata* L.was determined, (2) antifungal activity of these essential oils was tested against the *Fusarium oxysporum f.* sp. *lycopersici* (FOL) (Sacc.) W.C. Snyder and H. N. Hans, *Alternaria solani* (Ell. and G.Martin) Fr, *Rhizoctonia solani* Kühn, *Verticillium dahliae.* (3) It also was determined antioxidant activity of *T. Spicata* L. (4) antibacterial activity of these essential oils were tested against the *Xanthomonas* spp. (ZI365, ZI366, ZI368, ZI370, ZI373, ZI375, ZI376, ZI378) bacterial isolates.

MATERIALS AND METHODS

Materials. Plant material of *T. spicata* L. collected from the Turkoglu region (Kahramanmaraş) Turkey, in Jul 2016. The plant material was air-dried at room temperature in shady conditions.

Extraction of essential oils. The air-dried plant material was exposed to hydrodistillation for 2 h using a Neos essential oils system. The essential oil from aerial parts samples were preserved in a sealed vial at 4 °C until further analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis. GC/MS analyses were applied on an Agilent Technologies 7890A GC System, 5975C by Triple-Axis Detector mass spectrometer with a built-in- Autosampler formed with the used of the HP-5MS capillary column (30 m x 0.25 mm x 0.25 mm). For GC/MS detection, electron ionisation system and ionisation energy of 70 eV was used. Helium was the transporter gas at a flow rate of 1 mL min-1. The column temperature program was the same as defined upstairs. As in the gas chromatography, 1.0 L split/splitles (10:1) of the sample diluted with hexane were transferred to the clone. Identification of oil components was successful by comparison of their mass spectral fragmentation model by the available mass library (WILLEY and NIST).

Total phenolic contents. The total phenolic compound was determined by Slinkard and Singleton, (1977) Folin-Ciocalteus reagent with a small modification. Plant extract 0.1 ml was mixed with distilled water 4.6 ml. On 0.3 ml of Na2CO3 solution (2%) and 0.1 ml of Folin-Ciocalteus reagent was added to the mixed. It was incubated for 2 hours at room conditions. After the absorbance was measured at 760 nm. As result of gallic acid equivalents (GAE)/g of the extract was calculated.

Determinations of total flavonoid assay. The plant sample total flavonoid contents were determined by Chang at al (2002) [12]. Plant extract 0.1 ml was mixed with methanol 4.8 ml. 0.1 ml Al (NO_3) (10%) and 0.1 ml NH_4CH_3COO solution (1M) was added to the mixed. It was incubated for 45 minute at room conditions. After the absorbance was measured at 415 nm. As result of quercetin equivalents (QE), g^{-1} of the extract was calculated.

DPPH· free radical-scavenging activity. The free radical scavenging activity was determined by Liana-Pathirana made a few changes in the method [13]. Plant methanol extracts were different amounts stock solutions by placing the test tube and volume was completed to 3 ml with ethyl alcohol. Over them 1 ml of DPPH· solution (0.26 mM) was added and stirred with the help of vortex. After standing for 30 minutes in a dark ambient, the absorbance was read at 517 nm. The data obtained were expressed as IC_{50}. The radical scavenging activity was calculated from the equations.

$I_{DPPH}=100.\ [A_{control}-(A_{sample}-A_{blank})]\ /\ A_{control}$

Iron ion reducing antioxidant power (FRAP). The FRAP analysis method applied by Oyaiz (1986) was performed through amendments. 0.25 ml plant extract of 0.2 M phosphate buffer (pH 6.6) with 1.25 mL complete. On 1.25 ml of potassium ferricyanide $[K_3\ Fe\ (CN)_6]$ solution (1%) was added. The mixture was incubated at 50 ° C for 20 minutes. The mixture cooling to room temperature TCA (1.25 mL, 10%) and FeCl3 (0.25 mL, 0.1%) solution were added. After stirring vortex was measured absorbance at 70 nm. The results obtained was Trolox equivalent (TE), calculated.

Cupric reducing antioxidant capacity (CUPRAC). The cupric antioxidant capacity analysis method applied by Chang at al (2002), with slight modifications. Results were compared with used in standard antioxidants BHA and BHT absorbance.

Fungal cultures. The plant pathogenic fungi used were obtained from the stock cultures of the Department of Plant Protection, Faculty of Agriculture, University of Ahi Evran, Turkey.

***In vitro* antifungal effect of the essential oils.** The antifungal activities of essential oil were detected according to the agar well diffusion method [14]. The PDA was autoclaved and cooled to 40^0. After were transferred to 60 mm petri dishes (10 ml petri^{-1}). 5 mm diameter wells were opened on the PDA within the petri dishes. The plant essential oils were transferred 3, 5, 7 and 10 µl petri^{-1} into the wells. Mycelium disks of 5 mm were then placed at equal distances to these wells. The fungi transferred petri dishes were incubated at 22±2°C. According to mycelium inhibitions was calculated the formulated.

I=100×(DC -DT)/DC

I: Inhibition percentage compared to the control (mycelium development)

DC: Mycelium development in the control

DT: Mycelium development in essential oils applications.

Data were analysed using One-Way procedure of ANOVA (Windows version of SPSS, release 15.00). Differences among concentrations were compared to using DUNCAN Multiple Range Test of $p<0.05$.

***In vitro* antibacterial activity**. The plant bacterial sample, eight different *Xanthomonas* spp. (ZI365, ZI366, ZI368, ZI370, ZI373, ZI375, ZI376, ZI378), the isolate was isolated from pepper fields Kahramanmaraş. The bacteria cultures were grown in the nutrient glucose agar solid medium at 25°C. After 24 hours of growth, each microorganism, at a concentration of the 0.1 density was set at a spectrophotometer 600 nm. The after was inoculated on the surface of nutrient glucose agar Petri dishes the help of a glass baguette. Later, filter paper discs (1 mm in diameter) essential oil 10ml were placed on the surface of each inoculated petri dishes. The petri dishes were incubated at 25 °C for 48 h. Bacteria were evaluated by measuring the undeveloped zone. The experiment was performed in two repeats and four duplicates.

RESULT AND DISCUSSION

Composition of the essential oil. The chemical composition *T. spicata* L. of the essential oil was analyses by GC/MS (Table 1).*T. spicata* L. essential oil fourteen components were determined. The main components of the essential oil were determined carvacrol (78.53%), γ-terpinene (10.42%) and p-Cymene (5.49%). Carvacrol (78.53%) is the basic component of volatile oil. In many studies, carried out previously on *T. spicata* L. determined that carvacrol, thymol, γ-terpinene and p-cymene was the main compounds [4, 5, 8].

Kiliç [8], were reported *T. spicata* L. in the essential oil main compounds detected carvacrol (60.39%), γ-terpinene (12.95%), and p-cymene (9.61%). Kizil [4], was reporting on the essential oil composition of *T. spicata* from Turkey, carvacrol content changed among 34.5 and 94.2%.

***In vitro* antifungal results.** The application of the antifungal activity of the essential oil tried are given Table 2. The essential oil of *T. spicata* L.were showed strong antifungal activity against plant pathogenic fungi. Compared with the of control, *T. spicata* L. essential oil at a dose of 7 µL/petri reduced the of mycelium growth of *A. Solani* and *F. oxysporum f.* sp *lycopersici* by 100%. R. solani and *V. dahliae* were blocked mycelium growth of 68.61% and 79.18% respectively.

All pathogens at a dose of 10 $\mu L petri^{-1}$ the of *T. spicata* L. essential oil blocked the of mycelium growth of 100% (Table 2). In former works, Muller-Riebau et al (3) were reported that the essential oils of *T. spicata* L., show a significant, antifungal activity the soil-borne such as *Rhizoctonia solani*, *Fusarium moniliforme*, *Phytophthora capsici* and *Sclerotinia sclerotiorum*. Markovic et al (5) have reported that the essential oils of *T. spicata* L. and carvacrol shows a remarkable antifungal activity against *Aspergillus fumigatus* (plant isolate), *Penicillium funiculosum* (ATCC 36839), *Aspergillus niger*(ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Trichoderma viride* (IAM 5061) and *Penicillium ochrochloron* (ATCC 9112).

Antioxidant activity results. The total phenolic contents of the methanolic extract aerial parts, calculated from the calibration curve was 176.20±1.50 gallic acid equivalents mg (GAE) g^{-1} of extract, and the total flavonoid content was 60.15±3.21 quercetin equivalents mg (QE) g^{-1} of extract Table 3.Phenolic substances are significant plant due to their scavenging ability on free radicals, their hydroxyl groups. For this reason, the phenolic substances of plants are possible to contribute right to their antioxidant activity [15]. The plant secondary metabolites are significant antioxidant and chelating properties. In the presence of the hydroxyl groups of the antioxidant activity, the substituent is attached [16].

Plants plenty including phenolics, carotenoids and flavonoids, have antioxidant activity, because of their redox feature and chemical structures. The showed DPPH· the free radical-scavenging activity of the *T. spicata* L. methanol extract. The methanol extract was found statistically important compared with the control. The DPPH· radical scavenging activity of methanol extract of the *T. spicata*L. Table 3. The methanol extract was found to be near standard BHT, but not BHA and Trolox.

ABTS scavenging activity of methanol extract is shown Table 3. The methanol extract was found to be better than standard BHA, but near BHA and a bit remote Trolox. In previous studies, Öztürk [17], have reported that carvacrol shows a remarkable DPPH and ABTS radical scavenging activity.

The reduction of Fe + 3 ion, indicative of important qualities give electrons for the antioxidant activity of a compound is closely associated with other antioxidant mechanisms. In an analysis of the strong reducing powers of the methanol crude extract (2.18±0.21), compared with a control such as BHT and BHA Table 3.

Cupric reducing antioxidant capacity was showed methanol extract of Table 3. The methanol extract had extract (6.57±0.40) exhibit higher reducing power activity than used standard as BHT and BHA Table 3.

***In vitro* antibacterial results.** The antibacterial activity of the essential oil of *T. spicata* L. was work 10µl against eight plants pathogenic bacterial isolate. Results of the antibacterial potential of the essential oil are presented in table 4. The growth inhibition zone measured ranged from 28 to 39 mm for all the of isolating *Xanthomonas* spp.

T. spicata L. essential oil showed strong antibacterial activity against isolate *Xanthomonas* spp. the of bacterial species tested. The highest effect of essential oil was seen in the ZI373 isolate, by followed (ZL378, ZL375, ZL365), (ZL366), (ZL370, ZL376) and (ZI 368) respectively. Control treatment did not show inhibitory effect on any a bacterial isolate.

Markoviç et al [5], have reported the essential oil of *T. spicata* L. showed strong antibacterial activity; Also, was reported in the carvacrol showed strong antibacterial activity. Dulger et al. [18] have reported that the remarkable antibacterial activity of crude extracts obtained from *T. spicata* var. *Spicata* against the 35 isolates of MRSA.

TABLE 1
The chemical compositions of *T. spicata* L. essential oils.

Compound number	**RT (min)	*RI	Name	Percentage	Methods of identification
1	11,628	902	2-Thujene	0,62	RI, ***MS
2	13,442	962	β-Pinene	0,61	RI, MS
3	14,435	992	α-Terpinene	1,22	RI, MS
4	14,7	999	o-Cymene	5,5	RI, MS
5	15,832	1035	γ-Terpinene	10,42	RI, MS
6	16,178	1045	Sabinene hydrate	0,43	RI, MS
7	20,042	1156	Terpinen-4-ol	0,27	RI, MS
8	23,693	1262	Thymol	0,32	RI, MS
9	24,321	1279	Carvacrol	78,53	RI, MS
10	28,23	1398	Caryophyllene	1,23	RI, MS
11	32,999	1555	Spathulenol	0,16	RI, MS
12	33,224	1563	Caryophylene oxide	0,17	RI, MS
13	40,939	1846	Octadecane	0,26	RI, MS
14	44,086	1973	Octadecane	0,26	RI, MS
Total				100	

*RI, retention indices**RT, retention time***MS, Mass spectra

TABLE 2
Antifungal activity values (Inhibition (%) and Inhibition zone) for *T. spicata* L. essential oil.

Doses (µl/petri)	**Plant Pathogens R.s		A.s		FORL		V.d	
	$I^{(\%)}$	$Iz^{(mm)}$	$I^{(\%)}$	$Iz^{(mm)}$	$I^{(\%)}$	$Iz^{(mm)}$	$I^{(\%)}$	$Iz^{(mm)}$
Control	$0.00^{e*}\pm0.0$	60	$0.00^{d}\pm0.0$	60	$0.00^{d}\pm0.0$	60	$0.00^{e}\pm0.0$	60
3	$34.64^{d}\pm0.90$	39.21	$68.36^{c}\pm1.32$	19.98	$68.36^{c}\pm0.79$	25.37	$52.78^{d}\pm1.02$	28.33
5	$40.85^{c}\pm0.89$	35.48	$80.97^{b}\pm0.68$	11.41	$79.70^{b}\pm0.64$	12.17	$63.24^{c}\pm0.77$	22.05
7	$68.61^{b}\pm1.47$	18.83	$100^{a}\pm0.0$	00	$100^{a}\pm0.0$	00	$79.18^{b}\pm0.71$	12.48
10	$100^{a}\pm0.0$	00	$100^{a}\pm0.0$	00	$100^{a}\pm0.0$	00	$100^{a}\pm0.0$	00

* According to DUNCAN, the averages with different letters in the same column are different at the significance level of $p<0.05$
**Plant pathogens; *F. oxysporum f.* sp. *lycopersici* (FOL), *A. Solani*(A.s), V. dalhia (V.d) *R. Solani* (R.s). Negative control (NC)

TABLE 3
Antioxidant activity of *T. spicata* L. of the methanol extract by TP, TF, DPPH, ABTS, Reducing power and CUPRAC[a].

Sample	TP mg GAE g^{-1} extract	TF mg QEg^{-1} Extract	DPPH· IC_{50} (µg mL^{-1})	ABTS IC_{50} (µg mL^{-1})	Reducing power mmol TEAC g^{-1} extract	CUPRAC mmol TEAC g^{-1} extract
Methanol extract	176.20±1.50	60.15±3.21	12.35±0.93	4.96±0.25	2.18±0.21	6.57±0.40
BHA[b]			4.52±0.67	3.30±0.05	6.60±0.16	15.99±1.07
BHT[b]			8.45±0.67	9.40±0.55	2.74±0.35	15.25±0.61
Trolox			3.50±0.03	4.80±0.06		

[an] IC_{50} Rate represents the means the standard deviation of three parallel measure ($p < 0.05$).
[b] Standart compounds

TABLE 4
Antimicrobial activity of essential oil inhibition zone (mm) disk diffusion method.

Xanthomonas spp. isolate	ZI375	ZI368	ZI366	ZI378	ZI376	ZI373	ZI370	ZI365
Inhibition zone (mm)	32	28	31	32	30	39	30	32

CONCLUSIONS

In this study, was determined antibacterial, antifungal, antioxidant activities and chemical composition essential oil from *T. spicata* L. The results of this work have shown essential oil from *T. spicata* L. *strong* antifungal activity against plan pathogenic fungi. Also, was showed strong antibacterial activity against isolate *Xanthomonas* spp. The chemical composition of essential oil from *T. spicata* L. was determined Its main component carvacrol. The methanol extract of *T. spicata* L. was determined total phenolic and flavonoid. *T. spicata* L. has rich in terms of contents of the total phenolics and

flavonoids. These studies can be used in the management of plant pathogenic fungi and bacterial diseases control. As a result of *T. Spicata* L., was showed a high antioxidant activity. Our work showed that *T. Spicata* L., has a strong antifungal, antibacterial and antioxidant activity.

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CORRESPONDING AUTHOR

Yusuf Bayan
Ahi EvranUniversity,
Faculty of Agriculture,
Department of Plant Protection,
40200 Kirsehir – TURKEY

E-mail: yusufbayan@gmail.com

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