

Chemical composition and Antioxidant Properties of Essential Oils (*lippia citriodora*, *thymus daenensis*)

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Abstract

The aerial material of *thymus daenensis* (*t. daenensis*) and *Lippia citriodora* (*l.citriodora*) collected from Iran were extracted by hydrodistillation. The chemical composition was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

The antioxidative properties and total phenolic contents of *L. citriodora* and *T. daenensis* were examined. Antioxidant activity of *t.daenensis* and *l.citriodora* by two complementary test systems, namely DPPH free radical-scavenging and β -carotene/linoleic acid. Were examined . The antioxidant activity is compared with synthetic antioxidant, namely BHT. The antioxidant activity of *t.daenensis* is more than *l.citriodora*.

Keyword: antioxidant , *thymus* , *lippia* , GC / MS , hydrodistillation

1. Introduction

Thymus daenensis is an ancient herb used in medicine by the Greeks [1]. Essential oil of this plant is a rich source of thymol and carvacrol which has been reported to possess the highest antioxidant activity [2]. In Iran, it is predominantly found in the north of the country. It is used as a food ingredient, as a tea, as an herbal drug for its reputed medicinal properties [3].

The genus *Lippia*, (Verbenaceae), comprises approximately 200 species indigenous to southern and central America and Africa. These compounds have wide-spread application in the food, cosmetics and household product industries [3,4.].

Different conditions such as stress, aging and pollution produce high level of free radicals in the body, which these radicals damage DNA, causes heart-diseases, cancer and stroke [5]. Substances like vitamin E, C, and beta carotene act as antioxidant in the body. Vitamin E and beta-carotene protect cell membranes and vitamin C removes free radicals from inside the cell. In recent years, there is a wide

interest in finding natural compounds that could replace synthetic antioxidants such as BHT and BHA which are commonly used in foods, packaging for foods, plastics and medicines because of its possible toxicity and due to a suspected action as promoters of carcinogenesis [6].

The aims of this research were as follow: (i) to identify chemical composition of essential oils. (ii) to evaluate the antioxidant activities of these essential oils by two in vitro assay models (DPPH and β -carotene bleaching assays).

2. Materials and methods

Essential oils were extracted (by a Clevenger system) by mixing 50 g of *L. citriodora* and *T. daenensis* with 600 ml of distilled water for 3h at 100 °C. All reagents used were of the analytical grade with highest purity available.

2.1. Gas chromatography

Samples of 0.1 μ L and in split mode 1: 50 were subjected to analysis by capillary GC (Hewlett-Packard 5890), equipped with a flame ionization detector (FID) and a 30 m \times 0.25 mm HP-5 column with 0.25 μ m film thickness (Hewlett-Packard), was used for this study. The FID and the injector were maintained at 280 °C and 250 °C, respectively. The oven temperature was programmed from 60 °C (held for 5 min) to 250 °C at 5 °C/min and held for 8min Helium was used as carrier gas, the flow rate carrier gas 1.1 ml/min. For the identification of the compounds, retention times and retention indices of standards were used in external standard method.

2.3. Mass spectrometry analysis

GC-MS model Thermoquest trace Ms emission mass selective detector (GC/MS) was used for mass spectral identification of the GC components at MS ionization voltage of 70 eV. Emission current 150 mA was used for the identification of volatile components in (*t. daenensis*, *L. citriodora*) essential oils. All of conditions are the same as above.

2.4. Determination of total phenolic content

The total phenolic content of the essential oils determined according to the method described by Makkar, et al., (1997)[7]. The total phenolic content was expressed as meq gallic acid /g of extract (Table 1)

3 Antioxidant activity

3.1.DPPH assay

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple-colored methanol solution DPPH (Burits et al. 2000)[8]. The IC₅₀ of essential oils are reported in Table 3.

3.2. β -carotene bleaching method

The antioxidant activity of the plant extracts and pure compounds was evaluated in terms of bleaching of β -carotene using the method of Barriere et al., 2001 [9].

4. Statistical analysis

Data were analyzed by the Student's t-test for six data (free radical-scavenging activity and chemical analysis). A statistically different significance was accepted when $P < 0.05$.

5. Results and discussion

The chemical compositions of essential oils were analyzed by GC/MS. The chemical compositions are reported in Table 1. *t.daenensis* has thymol (54.68%) and gamma-terpinene (12.9%) , p-cymene(11.25%) more than other components. *l.citriodora* has R-curcumene (14.11%) more than others.

The total phenolic contents of *l.citriodora* (385.36 mg/l) and *t.daenensis* (307.96 mg/l) are determined. The extractable total phenolics the leaves and stem of *L.citriodora* were significantly higher than *t.daenensis*. The antioxidant activities of essential oils are studied by two methods such as β -carotene linoleic acid and DPPH assays. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a color change from purple to yellow. A lower value of EC₅₀ indicates higher antioxidant activity. Free radical-scavenging activity of *t.daenensis* ($0.8 \pm 0.06 \mu\text{g/ml}$) was higher than *l.citriodora* studied and synthetic antioxidants used as positive controls. On the other hand, *l.citriodora* extract showed the weak antioxidant activity in this system ($3.2 \pm 0.15 \mu\text{g/ml}$).

In the β -carotene/linoleic acid system (Fig. 1), the antioxidant activity of *t.daenensis* is more than *l.citriodora*.

phenolic compounds, thymol and carvacrol) are mainly responsible for the antioxidant potential of the plant oils which contain them [10] . In compare with other study, Bektas Tepe ; 2005 studied

antioxidative activity of the essential oils of *Thymus sipyleus subsp. sipyleus var. sipyleus* and *Thymus sipyleus subsp. sipyleus var. rosulans*. At present study, *t. daenensis* has thymol (54.68%) , At past study , *thymus sipyleus* and *thymus rosulans* have thymol (20.5%). Bektas Tepe was examined antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β -carotene/linoleic acid assays. In the first case, the free radical scavenging activity of the essential oil of *t. rosulans* was superior to *var. sipyleus* oil (IC50 =220 \pm 0.5 and 2670 \pm 0.5 μ g/ml, respectively). The past study shows *thymus sipyleus* and *thymus rosulans* did not show any remarkable antioxidant activity [11].

In conclusion, because of high percentage of oxygenated monoterpenes in *t. daenensis* and *l. citriodora*, they show remarkable antioxidant activity.

Table 1

Chemical components of studied essential oils.

	Components	Component (%)	KI	RT
<i>l. citriodora</i>	limonene	3.82	1052	11.97
	β -caryophyllene	3.93	1462	23.19
	Caryophyllene oxide	6.59	1634	27.51
	Ar-curcumene	14.11	1517	24.61
	carvacrol	1.59	1310	19.17
	E-citral	5.99	1295	18.75
	spathulenol	7.02	1627	27.35
<i>t. daenensis</i>	thymol	54.68	1319	19.39
	gama-terpinene	12.9	1082	12.79
	carvacrol	5.01	1325	19.57
	β -caryophyllene	2.59	1461	23.16
	P-cymene	11.25	1047	11.83

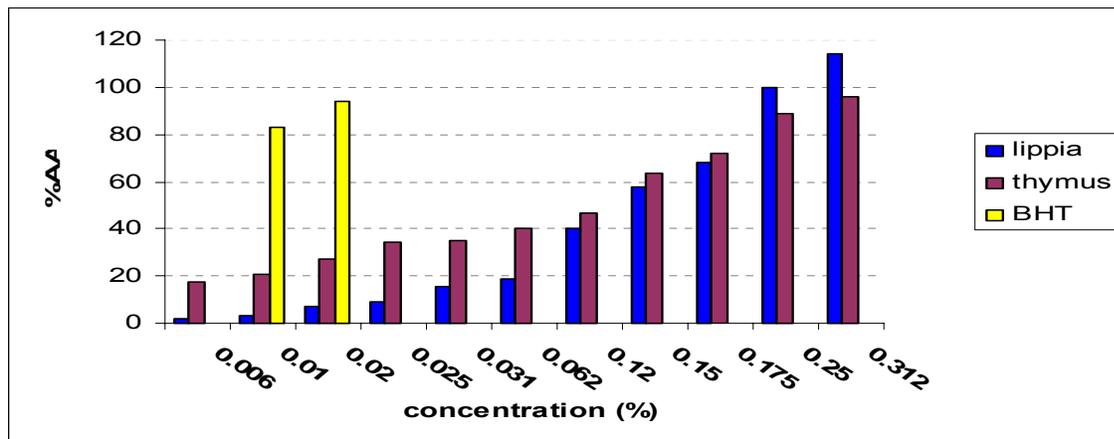


Fig. 1. Comparison of antioxidant activities of studied essential oils (*L.citrodora*, *t.daenensis*) and synthetic antioxidant (BHT) (by β -carotene/linoleic acid method).

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چکیده فارسی:

از ساقه و برگ آویشن برگ باریک و به لیمو به روش تقطیر با آب اسانس این گیاهان دارویی استخراج گردید. ترکیبات شیمیایی این اسانس ها توسط روش کروماتوگرافی گازی و کروماتوگرافی گازی - اسپیکترومتری جرمی آنالیز شدند. غلظت کل ترکیبات فنولیک و فعالیت آنتی اکسیدانتی به دو روش (۱) رادیکال دی فنیل پیکریل هیدرازین (۲) بی رنگ شدن بتا کاروتن اندازه گیری شد. و فعالیت آنتی اکسیدانتی اسانس های مورد مطالعه با فعالیت آنتی اکسیدانت های سنتزی (BHT) مقایسه شد. فعالیت آنتی اکسیدانت اسانس آویشن بیشتر از به لیمو است.

کلیدواژه: آنتی اکسیدانت ، آویشن ، به لیمو ، کروماتوگرافی گازی - اسپیکترومتری جرمی ، کروماتوگرافی گازی