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DETERMINATION OF VOLATILE COMPONENTS AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OBTAINED FROM KASTAMONU GARLIC BY MICROWAVE-ASSISTED CLEVENGER SYSTEM

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ABSTRACT

In present study, fourteen compounds in *Allium sativum* (garlic, harvested from Kastamonu province) essential oil which was obtained by using a microwave-assisted clevenger system with a solvent-free option in a shorter time than traditional methods were identified by gas chromatography/mass spectrometry (GC/MS). Allyl trisulfide (27.09%), allyl methyl trisulfide (21.26%), allyl disulfide (20.02%), allyl trans-1-propenyl disulfide (7.48%), allyl methyl disulfide (6.08%), 2-vinyl-4H-1,3-dithiine (4.32%) were the most abundant sulphur compounds in oil. Also, total phenolic content was calculated as 2.84 mg gallic acid equivalent (GAE)/g and IC_{50} value which defined the required amount to reduce the 50% of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution and an indicator for antioxidant activity was 63.58 g/L. Butylated hydroxyanisole (BHA) and 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) were accepted as reference synthetic antioxidants and their IC_{50} values were recorded as 0.09 and 0.08 g/L respectively.

Keywords: *Allium sativum*, garlic, volatile compound, phenolics, antioxidant activity

KASTAMONU SARIMSAĞINDAN MİKRODALGA DESTEKLİ CLEVENGER SİSTEMİYLE ELDE EDİLEN YAĞIN UÇUCU BİLEŞENLERİNİN VE ANTİOKSİDAN AKTİVİTESİNİN BELİRLENMESİ

ÖZ

Bu çalışmada, mikrodalga destekli clevenger sistemiyle, geleneksel yöntemlere kıyasla daha kısa sürede çözücüsüz olarak elde edilmiş, *Allium sativum* (sarımsak, Kastamonu bölgesinden toplanmış) uçucu yağında gaz kromatografisi/kütle spektroskopisi (GC/MS) yardımıyla on dört bileşen tespit edilmiştir. Uçucu yağ örneğinde; alil trisülfit (27.09%), allil metil trisülfit (21.26%), alil disülfit (20.02%), allil trans-1-propenil disülfit (7.48%), allil metil disülfit (6.08%) ve 2-vinil-4H-1,3-dithiin (4.32%) en fazla bulunan sülfür bileşikleridir. Ayrıca, uçucu yağın toplam fenolik madde içeriği 2.84 mg gallik asit eşdeğeri/g ve 2,2-difenil-1-pikrilhydrazil (DPPH) çözeltisinin %50'sini indirgemek için gerekli olan miktarın bir ifadesi ve antioksidan aktivitenin bir indikatörü olan IC_{50} değeri ise 63.58 g/L olarak hesaplanmıştır. Bütillendirilmiş hidroksianisol (BHA) ve 6-Hidroksi-2,5,7,8-tetrametilkroman-2-karbosilik asit (troloks) çalışmada referans olarak kabul edilmiş sentetik antioksidanlardır ve IC_{50} değerleri sırasıyla 0.09 ve 0.08 mg/L olarak bulunmuştur.

Anahtar kelimeler: *Allium sativum*, sarımsak, uçucu bileşen, fenolikler, antioksidan aktivite

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INTRODUCTION

Allium sativum (garlic) which is native to Asia belongs to the family of Alliaceae and some species of the genus *Allium* such as *Allium ursinum*, *A. porum*, *A. ascalonicum*, *A. fistulosum* have significant economic value in Asia, America and Europe (Fenwick and Hanley, 1985). Most of these species are often consumed as food because of their unique aromas (Avato et al., 1998). Total garlic production of Turkey was 121805 tons in 2017 and 21.3% of this amount procured from Taşköprü district in Kastamonu (TÜİK, 2018). Dry matter content of Kastamonu garlic makes it suitable for storing long times without deterioration and processing simply (Taşkın et al., 2013). *Allium sativum* have been used for medical purposes in various diseases such as headache, asthma, rheumatism, intestinal worms and tumors since ancient times (Rivlin, 2001; Corzo-Martinez et al., 2007) and recent biological and pharmacological studies have confirmed that these medical features of garlic are arising from its antioxidant, anti-inflammatory, antibacterial, antifungal, anticarcinogenic, antimutagenic, hypocholesterolemic, hypolipidemic, antihypertensive, antithrombotic, immunomodulatory and prebiotic activities (Lanzotti, 2006; Asdaq and Inamdar, 2010).

Essential oils of plant origin are one of the important products of industry based on agriculture and these oils are used as flavoring substances in beverages, perfumes, cosmetics and nutritional products (Hussain et al., 2008; Teixeira et al., 2013). Essential oils could be acquired by various techniques such as steam distillation, solvent extraction and novel methods like supercritical fluid extraction (Schaneberg and Khan, 2002), microwaves, ultrasound (Périno-Issartier et al., 2013) and green extraction by solar energy (Yen and Lin, 2017). Solvent-free microwave extraction is accepted as a green and simple technology for the removal of essential oils from aromatic plants which do not require lots of water or an organic solvent (Filly et al., 2014).

The antimicrobial activity of garlic essential oil has been recently studied by Razani Rohani et al. (2011) and Casella et al. (2013). Also, the differential effects of allyl sulfides of *Allium sativum* essential oil in human liver tumor cells were investigated by Wu et al. (2004). To the best of our knowledge, solvent-free microwave extraction of Kastamonu garlic essential oil and its chemical compounds, polyphenol content and antioxidant activity in a comparison with synthetic antioxidants like butylated hydroxyanisole (BHA) and 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) have not been reported to date. This paper will provide valuable information on the suitability of a faster, environment friendly and easier essential oil extraction technique of this aromatic vegetable.

MATERIALS AND METHODS

Extraction and chemical composition of garlic essential oil

Garlic samples (with a moisture content of nearly 69%) were procured from Çetmi Village in Taşköprü, Kastamonu, Turkey and bulbs were extracted in a microwave assisted clevenger system (Fig. 1) without water or an organic solvent. The power was set to the highest level (340 W) in microwave oven (Arçelik, MD 565, Turkey) to reduce processing time and each trial proceeded during 30 minutes. The oil was kept in glass vials and stored at -18°C until analysis. Each extraction was made in triplicate. The yield of essential oil was calculated from the following formula;

$$\text{Yield (\%)} = \frac{\textit{Volume of essential oil (ml)}}{\textit{Weight of sample (g)}} \times 100$$

(Equation 1)

Chemical composition of oil was determined by gas chromatography coupled to mass spectrometry (Agilent 7000 Series Triple Quad GC/MS). A column in the dimensions of 30 m length × 0.25 mm diameter × 0.25 μm film thickness containing 5% phenyl methyl poly siloxane was used. Helium was the carrier gas having a flow rate of 1.0 mL/min; the detector was flame ionization, split mode (1:20), split flow (40 l/min) and the injector at 250°C was employed. Initial column temperature was 50°C followed by increasing to 240°C at 3°C/min. The sample was diluted in dichloromethane (1:100,

v/v). Retention indices were specified according to the Kovats method (Rao et al., 2007) using *n*-alkanes as reference. Kovats indices reported in literature were compared to identified compounds in mass spectra (Adams, 2001).

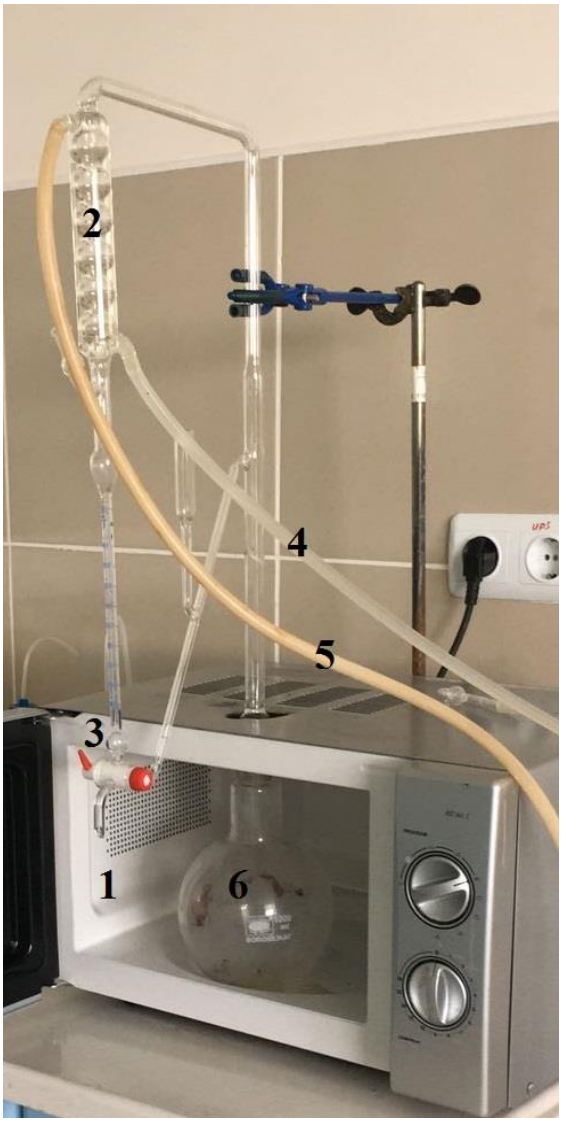

Figure 1. Microwave-assisted clevenger setup (1-Microwave oven, 2-Clevenger unit, 3-Tap, 4-Cooling water inlet, 5-Cooling water outlet, 6-Sample flask

Total phenolic content

The method stated by Li et al. (2015) was used with minor modifications in determining the amounts of polyphenols. 0.5 ml methanolic solution of garlic essential oil (80 g/l) was reacted with 0.5 ml Folin–Ciocalteu's reagent. Then, 3 ml of 10% Na_2CO_3 solution was added to mixture and the tubes were vortexed. After 30 min incubation in dark place, the absorbances were read at 760 nm by a spectrophotometer (Shimadzu, UV 1800, Japan) and compared to a gallic acid curve (R^2=0.9937; y=0.0111x+0.1105). Methanol was used to zero the device. The results were given as mg gallic acid equivalents (GAE)/g oil. The test was carried out in triplicate.

Antioxidant activity (DPPH assay)

Radical scavenging ability of *Allium sativum* essential oil was measured using the stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH). 0.1 ml methanolic solution of essential oil of five different concentrations (8, 16, 32, 40, 80 g/l) was mixed with 2 ml DPPH solution (0.025 g/l) prepared in methanol. Butylated hydroxyanisole (BHA) and 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) were used as reference synthetic antioxidants and their concentrations were 0.025, 0.050, 0.075, 0.100 g/L. The absorbances were read at 517 nm by a spectrophotometer (Shimadzu, UV 1800, Japan) after 30 min keeping in dark. The amount of sample require to scavenge the 50% of DPPH (IC_{50}) was determined graphically. Inhibition (%) of samples was identified by equation 2 (Viuda-Martos et al., 2010).

$$\text{Inhibition (\%)} = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100 \quad \text{(Equation 2)}$$

where A_{DPPH} and A_{sample} were the absorbances of DPPH solution and sample respectively. Methanol was used as blank. The test was carried out in triplicate.

Statistical analysis

Means and standard deviations were estimated using classical statistical methods by Microsoft Office 2010, Excel software. Total phenolic and antioxidant activity data were analyzed using Student's *t*-test and Duncan test (one way analysis of variance, ANOVA) at 95% confidence interval respectively. SPSS version 18 (IBM, USA) was the statistical software.

RESULTS AND DISCUSSION

Extraction and chemical composition of garlic essential oil

The colour of *Allium sativum* essential oil was dark-yellow and it had a characteristic and influential garlic odour. The averaged yield by microwave-assisted solvent free extraction was 0.30% ± 0.00 (0.97% ± 0.00 on dry basis). Rao et al. (2007) determined the essential oil yield of fresh Indian garlic cloves as 0.58% by conventional hydrodistillation on dry matter. This difference may sign that Kastamonu garlic is richer with respect to essential oil. Moreover, the essential oil collection concluded in only 30 minutes at 340 W in this study. But longer extraction times were implied for conventional hydrodistillation (Kimbaris et al., 2009; Romeilah et al., 2010). The reason behind this difference that microwaves make membranes and cell walls of vegetable penetrable because of their high energy and effective heating (Manouchehri et al., 2018). Also, lack of organic solvents and water in microwave assisted extraction step prevented human/environmental toxicity and huge energy consumption respectively (Pavlić et al., 2015).

A total of 14 compounds, covering more than 96% of entire chemical composition of sample, were identified successfully with the aid of GC/MS (Figure 2) and allyl trisulfide (synonyms di-2-propenyl trisulfide and diallyl trisulfide) (27.09%), methyl allyl trisulfide (21.26%), allyl disulfide (synonym diallyl disulfide) (20.02%), allyl trans-1-propenyl disulfide (7.48%), allyl methyl disulfide (6.08%) and 2-vinyl-4H-1,3-dithiine (4.32%) were found to be the main components (Table 1). All compounds reported in this paper could be categorized in the group of sulphur components and most of them have already been declared as the dominant volatiles of *Allium sativum* essential oil (Abu-Lafi et al., 2004). On the contrary, allyl trisulfide which was known as its anti-cancer effect (Seki et al., 2008) was not the key constituent in Kastamonu garlic bulb and detected only at the rate of 0.99% by Keleş et al. (2014). Due to applying heat during extraction process, other molecules may transform into allyl trisulfide, thus the amount of related compound may be higher in essential oil than fresh sample.

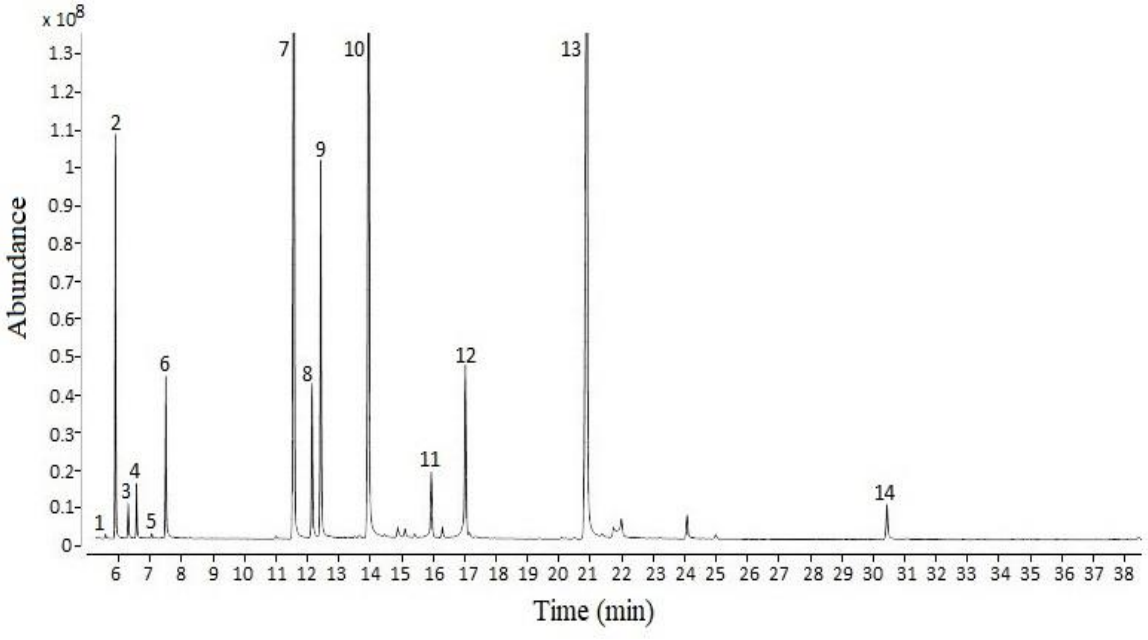

Figure 2. GC/MS chromatogram of garlic essential oil

Table 1. Volatile organic compounds of *Allium sativum* essential oil

No	RI	Compounds	%	ID
1	908	Thiophene, 3,4-dimethyl-	0.08	GC/MS, KI
2	913	Methyl allyl disulfide	6.08	GC/MS, KI
3	931	Methyl cis-propenyl disulfide	0.57	GC/MS, KI
4	940	Methyl trans-propenyl disulfide	0.86	GC/MS, KI
5	945	3H-1,2-Dithiole	0.08	GC/MS, KI
6	972	Dimethyl trisulfide	2.78	GC/MS, KI
7	1071	Allyl disulfide	20.02	GC/MS, KI
8	1097	Allyl cis-1-propenyl disulfide	3.19	GC/MS, KI
9	1103	Allyl trans-1-propenyl disulfide	7.48	GC/MS, KI
10	1130	Methyl allyl trisulfide	21.26	GC/MS, KI
11	1190	3-Vinyl-1,2-dithiacyclohex-4-ene	1.59	GC/MS, KI
12	1195	2-Vinyl-4H-1,3-dithiine	4.32	GC/MS, KI
13	1289	Allyl trisulfide	27.09	GC/MS, KI
14	1555	Diallyl tetrasulfide	1.03	GC/MS, KI
	Sulphur compounds		96.43	
	Total identified		96.43	

RI: Retention indices in the present study, ID: Identification, KI. Kovats index.

Kozan (2012) obtained the essentiol oils from both Kastamonu and Denizli garlics by steam distillation and stated that allyl trisulfide (42.52% and 45.22%, respectively), allyl disulfide (24.48% and 32.60%, respectively) and methyl allyl trisulfide (18.21% and 11.63% respectively) had the highest portions in samples. According to study carried out by Dziri et al. (2014), the predominant volatiles in essential oil of Tunisian garlic which was dried with different methods before hydrodistillation were indicated as allyl trisulfide (37.30, 42.30 and 45.90%), allyl disulfide (29.10, 35.60 and 17.50%), methyl allyl trisulfide (10.40, 8.30 and 7.70%) (for the air, oven and freeze-dried bulbs respectively). In a recent study, the major volatiles of essential oils from two different garlic cultivars (white-skin and purple-skin) were found as allyl trisulfide and allyl disulfide (45.76 and 22.38%) in white-skin cultivar, allyl trisulfide and allyl methyl trisulfide (58.53 and 21.94%) in purple-skin cultivar (El-Sayed et al., 2017). Considering these results, it can be said that microwave-assisted extraction presented in this paper may be destructive on aroma constituents (except methyl allyl trisulfide) of garlic essential oil than conventional procedure, however same compounds were detected dominantly in all mentioned researches. The amount of methyl allyl trisulfide in essential oil extracted by microwaves is higher than previous reports. It may probably be more stable compound than both allyl trisulfide and allyl disulfide and needs more processing time for degradation. Kimbaris et al. (2009) prepared two different semi-synthetic garlic essential oils by mixing oil sample with either standard diallyl sulfide (DS) or standard diallyl disulfide (DDS) in a ratio of 1:1 (w/w) after simultaneous hydrodistillation solvent extraction in order to investigate larvicidal activity against mosquitoes. They emphasized that the major constituents were methyl allyl trisulfide (9.40 and 10.00%), diallyl trisulfide (5.10 and 10.90%), 2-vinyl-4H-1,3-dithiin (9.30 and 11.50%) and 3-vinyl-4H-1,2-dithiin (7.40 and 10.20%) (for DS and DDS respectively). The differences in quantitative results of chemical composition may be also arised from various factors like genetic variation, geographical location, climatic conditions and pretreatments (drying etc.) as well as extraction techniques.

Total phenolic content and antioxidant activity

Polyphenols are accepted as strong antioxidants found in several foods throughout the world and the preventive effects of serious diseases such as cancer and neurodegenerative disorders come from adequate intake of phenolics (Arts and

Hollman, 2005; Szychowski et al., 2018). Total phenolic content of *Allium sativum* essential oil was determined as 2.84 ± 0.18 mg GAE/g oil (P<0.05) by Folin–Ciocalteu method. Mnayer et al. (2014) declared the total phenolic content of garlic oil extracted by turbo hydrodistillation as 5.61 mg GAE/g which was higher than onion and Chinese chive. Variation between two results can be expressed by both growing condition and genetic factors (Petropoulos et al., 2018) such in chemical composition. On the other hand, microwaves may possibly influence bioactives because of supplying rapid heating inside material eliminating other genetic or geographical factors. Indeed, Albi et al. (1997) studied the microwave and conventional heating of edible fats and their effects on phenolic compounds and stated that microwave heating of olive oil and virgin olive oil caused 85% and 96% loss of initial polyphenols respectively. However these losses were in level of 64% and 10% in heating in a conventional electric oven. Also, Nieto et al. (2013) attributed the low phenolic content of essential oils when compared to plant extracts to hydrophilicity and poor solubility of bioactives in oil medium.

DPPH radical scavenging activity is a common *in vitro* method to determine antioxidative property (Xiong et al., 2018). Table 2 demonstrated the DPPH inhibition levels (%) by the different concentrations of both essential oil and reference antioxidant materials. Because the radical scavenging abilities of natural and synthetic specimens were not inequal, the sample solutions were prepared in different ratios. A concentration-dependent antioxidant activity was observed for all samples. When essential oil/synthetic antioxidant amount was increased in medium, DPPH inhibition (%) was also enhanced (P<0.05). Discolouration from purple to yellow in a great way signed a higher antioxidant behavior and hence a lower IC_{50} value (Mnayer et al., 2014). Trolox was the strongest antioxidant (IC_{50}=0.08 g/L) and BHA (IC_{50}=0.09 g/L) and essential oil sample (IC_{50}=63.58 g/L) followed trolox respectively. S-ally-(L)-cysteine (SAC) is a sulphur compound which has high radical scavenging activity (Jang et al., 2018) and it is thought that one of the antioxidant source of garlic essential oil comes from SAC. Significant statistical differences were specified in all concentrations of BHA and trolox (P<0.05). A higher result was indicated for garlic essential oil (IC_{50}=88.91 μg/ml) obtained by conventional clevenger mechanism by Romeilah et al. (2010) in Egypt and this might prove that Kastamonu garlic showed more antioxidant activity and/or microwave assisted extraction of garlic essential oil was a succesfull technique for procuring antioxidant molecules. Zor (2006) determined the DPPH radical scavenging activity of methanolic extract (40 g/L) of Kastamonu garlic as 65%. This may prove that fresh garlic samples possess more antioxidant ability than its essential oil. Because garlic essential oil in this paper had 31.94% DPPH inhibition at 40 g/L concentration. Furthermore, Kastamonu garlic essential oil obtained by microwave assisted extraction has higher antioxidant activity despite of lower polyphenol level when regarding previous studies in literature. There may not be a relation between phenolic compounds and antioxidant capacity or non-phenolic compounds of essential oil may probably show antioxidant behaviour. Baschieri et al. (2017) emphasized that essential oils which did not have phenolics at an important level possessed significant antioxidant activity, however their mechanism was not clearly understood.

Table 2. Antioxidant activity of garlic essential oil and selected synthetic antioxidants

Sample	DPPH Inhibition (%)					
	8 g/L	*16 g/L*	*32 g/L*	*40 g/L*	*80 g/L*	*IC_{50} (g/L)*
Allium sativum	$8.89^{d} \pm 0.86$	$16.92^{bc} \pm 4.07$	$25.87^{bc} \pm 0.86$	$31.94^{b} \pm 1.72$	$60.61^{a} \pm 15.22$	63.58
	0.025 g/L	*0.050 g/L*	*0.075 g/L*	*0.100 g/L*		
BHA	$22.89^{d} \pm 1.55$	$34.17^{c} \pm 0.00$	$41.81^{b} \pm 1.22$	$54.44^{a} \pm 4.71$		0.09
Trolox	$13.44^{d} \pm 3.54$	$27.38^{c} \pm 0.76$	$49.27^{b} \pm 3.18$	$63.31^{a} \pm 1.46$		0.08

Same small letters in each line show insignificant differences (P>0.05).

CONCLUSION

A promising solvent-free extraction with the aid of microwaves in less time was investigated in order to recover the essential oil of *Allium sativum* (garlic) regarding chemical composition, total polyphenol level and antioxidative activity. Findings indicated that, Kastamonu garlic had a great potential of essential oil and gave higher oil yield with new method, however microwave-assisted extraction caused the loss of some volatile compounds like allyl trisulfide and allyl disulfide when compared to steam distillation studies in literature. Methyl allyl trisulfide was one of the major constituents and microwaves did not show an harmful effect on this molecule as such in allyl trisulfide and allyl disulfide. Although, total polyphenols were lower, the antioxidant activity of essential oil was improved by microwaves. Bioactive molecules might be destroyed by processing, since microwaves caused a quick temperature rise in sample. Hence, lower power levels (<340 W) are ought to be recommended for extraction.

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