Chemical Composition and Active Properties Evaluation of Wild Oregano (*Origanum Vulgare*) and Ginger (*Zingiber Officinale-Roscoe*) Essential Oils

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The compounds in Ginger (Zingiber officinale-Roscoe) essential oil provenience China and wild oregano (Origanum vulgare) essential oil of Romanian origin were identified by GC/MS and their antioxidant and antifungal properties were evaluated. Wild oregano oil was characterized by high content of oxygenated monoterpenes hydrocarbons (84.05%) of which carvacrol was the most abundant (73.85%) followed by β-linalool (3.46%) and thymol (2.29%). Ginger oil had a higher content of sesquiterpene hydrocarbons including zingiberene (31.47%), β-sesquiphellandrene (13.76%), α-curcumene (10.41%), α-farnesene (8.31%) and β-bisabolene (7.55%) but a lower content of oxygenated monoterpenes (7.97%). The high content of oxygenated monoterpens of wild oregano oil is in accordance with total content of polyphenols determined by the Folin–Ciocalteu method (6.71±0.73 mg of gallic acid equivalent per g oil). Ginger oil had only 1.34±0.22 mg gallic acid equivalent per g oil. Wild oregano oils exhibited appreciable in vitro antioxidant activity as assessed by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). The sample concentration required to scavenge 50% of the DPPH free radicals was 0.76±0.13 mg/mL for wild oregano oil compared to 20.22±2.12 mg/mL for ginger oil. Also, wild oregano oils showed significant inhibitory activity against selected pathogenic fungi (Fusarium oxysporum, Aspergillus flavus and Penicillium expansum). 1μL of oregano oil is sufficient for almost 75% growth inhibition of Aspergillus flavus compared to ginger oil which shows antifungal activity at 240μL for 78% growth inhibition. It can be concluded that wild oregano oil could be used as food preservative in some food products in which Fusarium oxysporum, Aspergillus flavus and Penicillium expansum could grow and have potential to produce health hazards mycotoxines.

Keywords: Aspergillus flavus, Fusarium oxysporum, ginger essential oil, GC-MS, Penicillium expansum and wild oregano essential oil

Ginger, the rhizome of Zingiber officinale, is used as a spice all over the world [1-2]. It is a member of the *Zingiberaceae* plant family and is native to Asia, although currently grown in Africa, India and other tropical regions [2]. More than 400 different compounds have been identified in ginger, whereas the quantity and quality of active components of ginger depend on the region of cultivation, the cultivation and processing method used, and whether it is used in fresh or dried form [3]. The odor of ginger depends on the amount of ginger oil present, the levels usually varying between 1% and 3% [2]. Over 50 components have been identified in this oil, most of which are monoterpenoid and sesquiterpenoid compounds. The pungency of fresh ginger is due to the group of phenolic compounds called gingerols, among which 6-gingerol is the most abundant. There are other gingerols in ginger with different length side chains.

The ginger oil have been reported to have strong antimicrobial, antifungal and antioxidant activities [4-5].

Oregano (*Origanum vulgare*) is an aromatic herb belonging to the *Lamiaceae* family, and commonly occurring throughout Asia, Europe, and northern Africa [6]. In folk medicine, wild oregano is used to treat dyspepsia, respiratory disorders, rheumatoid arthritis and urinary tract disorders. It is also used as a culinary herb in gastronomy. Essential oils derived from oregano have valuable pharmacological properties that have been investigated by several groups [7]. Oregano is one of the most used aromatic plants as antimicrobial agent, whose essential oils are notably rich in phenolics mono- and sesquiterpenes [8]. The main chemotype of oregano species are carvacrol and tymol [9].

Previous studies reported the potential of oregano essential oil to preserve foods such as swordfish [10], octopus, [11] and fresh chicken breast meat [12].

In this study we aimed to identify the chemical composition, evaluate the total phenolics and flavonoids content, antioxidant activity and to investigate the antifungal properties of the ginger (*zingiber officinale*) essential oil provenience China and *Origanum. vulgare* essential oil of Romanian origin against three fungi with high incidence in food products, namely *Aspergillus flavus*, *Penicillium expansum* and *Fusarium oxysporum*.

Aspergillus flavus is a saprophytic fungus which infects various fatty acid-rich food and feed crops and produces toxic secondary metabolites known as aflatoxins. Contamination of food with aflatoxin represents a serious threat to human health in addition to reducing the crop value leading to a substantial economic loss [13]. *Penicillium expansum* is a blue mold which is a wide-spread postharvest disease of fruits, resulting in great economic losses during a long-term storage of harvested fruits [14]. *Fusarium oxysporum* is an ascomycete fungus which infects plants either by mycelia or by germinating spores penetrating the plant roots preventing the plant from transporting nutrients [15].

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Experimental part

Materials

Two commercial essential oils, ginger oil provenience China (*Zingiber officinale*) from ADAMS and wild oregano (*Origanum vulgare)* produced by SC BIONOVATIV Brasov, Romania used in this study were purchased from green pharmacy.

The Folin–Ciocalteu phenol reagent 2N, anhydrous sodium carbonate, methanol, ethanol, gallic acid (>97%), quercetin (>95%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (purity 98% by HPLC), were purchased from Sigma-Aldrich, Darmsadt, Germany). Potassium persulfate (KPS), ACS reagent (purity 99.0%) was purchased from Fluka, Buchs, Switzerland.

Aspergillus flavus, *Penicillium expansum* and *Fusarium oxysporum* were provided from the collection of Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine Bucharest.

Determination of Total Phenolic Content in eseential oil by the Folin-Ciocalteu Method

The amount of total phenols was determined by the Folin-Ciocalteu reagent method as described by Nagendra et al. 2013 [16] with some modifications.

200 μL of diluted essential oil in ethanol (10:100) or standard solutions gallic acid (0-500 μg/mL) was mixed with 1.0 mL of Folin–Ciocalteu phenol reagent (1:10 dil). After 4 min, 0.8 mL of 7.5% sodium carbonate was added to the mixture. After 2 h in darkness the samples were centrifuged and absorbance was measured at 740 nm with a multimode microplate reader Infinite M200 PRO, TECAN against a blank sample that was concomitantly prepared. The concentration of total phenols was expressed as mg/ g essential oil (exp gallic acid).

Determination of total flavonoids

The total flavonoids content was determined according to the Dowd method with some modifications [17]. Briefly, 100 μL of 2% aluminium trichloride ($AlCl_3$) in 80% ethanol was mixed with the same volume of the diluted sample in ethanol. Absorbance was measured at 430 nm after 10 min against a blank sample consisting of ginger oil solution with 100 μL of ethanol without $AlCl_3$. The total flavonoids content was determined using a standard curve with quercetine in the range 5-100μg/mL. Total flavonoids content is expressed as μg /g essential oil (exp quercetine equivalents).

Gas Chromatography-Mass Spectrometry and Flame-Ionized Analysis (GC-MSD/FID)

The essential oils were characterized by gas chromatography coupled with mass spectrometry detector (GC-MSD) for qualitative analysis and with a flame ionized detector (GC-FID) (Agilent, Santa Clara, CA, USA) for quantitative analysis.

The GC analysis was performed on GC 7890A Agilent Technologies chromatograph using ZEBRON (ZB)-5 ms plus (30 m× 0.25 i.d. mm, 0.25 film thickness ìm) from Phenomenex capillary column, a 0.8 mL/min helium flow, an inlet at 250°C, and a 50:1 split ratio.

The temperature program starting at 50°C (1min) with a heating rate of 8°C/min up to 100°C (2 min) then 2 °C/min to 110 °C (2 min), 5 °C/min to 185°C, 30 °C/min to 280 °C (10 min). The MS detection was performed on a 7000A triple Quad Agilent Technologies detector (Agilent, Santa Clara, CA, USA), at 70 eV. Transfer line temperature was 280 °C, source 230 °C and quadrupole 150 °C. Identification of chromatographic peaks was made by reference to Institute of Standards and Technology (NIST) database [5].

Antioxidant Activity Evaluation

The inhibition concentrations of essential oils were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical methods.

Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH)

DPPH scavenging activity was measured according to Stoilova et al. [18] with some modifications. Briefly, 125 μL of the diluted oils were mixed with 50μL DPPH ethanol solution 0.3 mM in a 96-well clear microplate. The plate was read after 30 min incubation in dark at room temperature at 518 nm against a blank of sample with ethanol, with a multimode microplate reader Infinite M200 PRO, TECAN. The antiradical activity (AA) was determined by the following formula:

AA%= 100-{[Abs sample with DPPH - Abs sample without DPPH]x100}/Abs control for determination of absorbance control 50 μL DPPH were mixed with 125 μL ethanol.

$ABTS^+$ (2,2'-Azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) Radical Cation Scavenging Assay

The $ABTS^+$ scavenging test was conducted according to Vital et al. [19] with small modifications.

$ABTS^{\cdot+}$ is generated by mixing 2.5 mL of 7 mM ABTS with 88 μL of 140 mM potassium persulfate (KPS) water solutions and stored in the dark at room temperature for 16 h. The reaction between $ABTS^{\cdot+}$ and potassium persulfate directly generates the blue green $ABTS^{\cdot+}$ chromophore. The $ABTS^{\cdot+}$ activated radical was diluted with ethanol to an absorbance of 0.7±0.02 which was measured at 734 nm. Essential oils diluted in hexane (20 μL) were mixed with $ABTS^{\cdot+}$ solution (980μL) and absorbance was measured after 6 min.

The antioxidant capacity is expressed as percentage inhibition, calculated using the following formula:

$$\text{Inhibition}(\%) = [A_{control} - A_{sample}]/A_{control} \times 100$$

where $A_{control}$ is the absorbance of the ABTS radical in ethanol at t_0, and As_{ample} is the absorbance of an $ABTS^{\cdot+}$ radical solution mixed with the sample after 6 min.

Antifungal activity evaluation

The evaluation of the antifungal activity was performed by testing oregano (*Origanum vulgare*) and ginger (*Zingiber officinale*) essential oils against three food degradation fungi: *Aspergillus flavus*, *Penicillium expansum* and *Fusarium oxysporum*, using the puncture inoculation method described by Ziani et al. [20] with small modifications.

To evaluate the antifungal activity of the studied essential oils, *Aspergillus flavus*, *Penicillium expansum* and *Fusarium oxysporum* were grown on MEA (Malt Extract Agar) medium in 90 mm Petri dishes for 7-9 days and stored at 25°C. Fungal spore suspension was obtained in aseptic conditions, in laminar flow hood. Spore population was counted using a haemocytometer and the concentration of spore suspensions was of 10^6 spores/mL.

The culture media was prepared as directed on the package and sterilized at 121°C for 20 min. After sterilization, the culture media was cooled at a temperature of 40-45 °C and then poured into sterile Petri

dishes (≈15 mL/dish). After solidifying, the culture media was inoculated with 2μL of spore suspension in the center of the plate. The essential oils were then applied on Whatman paper discs (Φ= 8 mm), in different quantities, specific for each essential oil. All determinations were performed in two replicates. Control samples were prepared by the same method, without the oils.

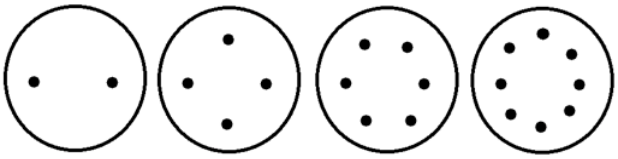

Fig. 1. Distribution of diffusion disks Whatman (Φ = 8 mm) in Petri dishes

The Petri dishes were isolated with parafilm and incubated at 25°C for 7 days. The determination of radial extension growth of the colony was measured in the 5th, 6th and 7th day of incubation and compared with the growth rate of the control plates. Radial inhibition percentages were calculated using the following equation:

$$\text{Radial inhibition} = \frac{M_c - M_i}{M_c}$$

where M_c is the average of the colony diameters of the control plates, and M_i is the average of the colony diameters of the plates treated with essential oils.

Results and discussions

Chemical composition

Total Phenolic content (TPC) and Total Flavonoides content (TPC)

TPC and TFC are the two key indicators widely employed to represent the overall antioxidant activity in many plants. In the present study the TPC of the ethanolic ginger oil solution calculated from the calibration curve (R^2= 0.9994) was 1.34 mg gallic acid equivalents/g oil and for oregano oil was 6.71 mg GAE/g oil respectively. The TFC calculated from calibration curve (R^2= 0.9983) was 73.7 μg quercetin equivalents/g oil for ginger oil and 46.5 μg QE/g oil for oregano oil (table 1). TPC in ginger oil was 5 times lower compared to wild oregano oil but unexpectedly, TFC was 2 times higher.

GC-MSD and GC-FID Analysis

The composition of the essential oils varies depend on the region of cultivation, the cultivation and processing method used, and whether used in fresh or dried form [3]. Additionally, their biological activities depend on the combination and concentrations of their numerous different components. Gas chromatography coupled with mass spectrometry (GC-MSD) and flame ionization (GC-FID) detectors was used to determine the quality and quantity chemical compounds in the essential oils. The GC-MSD chromatograms of studied essential oils are shown in figures 2-3. Table 2 presents the concentrations and retention time of compounds present in ginger and wild oregano oil.

Table 1
TPC IN AND TFC IN GINGER AND WILD OREGANO ESSENTIAL OIL

	Total phenolic content (mg GAE/g oil)*	Total flavonoides (μg QE/g oil)#
Ginger oil	1.34±0.22	73.7±4.7
Wild Oregano oil	6.71±0.73	46.5±4.8

*mg GAE/g oil (mg of gallic acid equivalent per g oil)
#μg QE/g (μg of quercetine equivalent per g oil)

Table 2
THE CHEMICAL COMPOSITION OF GINGER AND WILD OREGANO ESSENTIAL OILS

	Compound	RT	% in ginger oil	% in wild oregano oil
1	hexanal	5.39	0.26	-
2	Tricyclene	7.67	0.28	0.09
3	α-Pinene	7.87	3.34	0.15
4	Camphene	8.25	7.95	0.17
5	1-octen-3-ol	8.72	-	0.36
6	β-Pinene	8.86	0.64	0.8
7	Sabinen	8.98	0.73	0.14
8	α-Thujene	9.50	0.23	-
9	α-Terpinene	9.74	-	0.45
10	Cymene	9.95	-	4.40
11	D-Limonene	10.08	0.61	0.72
12	Eucalyptol	10.19	6.55	1.25

13	γ-Terpinene	10.85		3.44
14	Terpinolene	11.76	0.18	-
15	β-Linalool	12.01	-	3.46
16	D-Camphor	13.95	-	0.95
17	Cinerone	14.62	0.15	
18	endo-Borneol	14.93	0.75	1.30
19	α-Terpineol	15.79	0.4	0.95
20	Isothymol methyl ether	17.35	-	0.22
21	cis-Geraniol	17.73	0.27	-
22	Thymol	19.16	-	2.29
23	Carvacrol	19.64	-	73.85
24	α-Terpineol acetate	21.12	-	0.05
25	Eugenol	21.29	-	0.19
26	α-Copaene	22.14	0.41	0.05
27	β-Elemen	22.52	0.77	-
28	α-Bergamotene	22.85	0.23	-
29	Caryophyllene	23.50	-	2.99
30	Elixene	23.78	0.22	-
31	Humulene	24.52	-	0.50
32	α-Curcumene	25.13	10.41	-
33	Zingiberene	25.54	31.47	-
34	α-Farnesene	25.69	8.31	-
35	β-Bisabolene	25.85	7.55	-
36	β-Sesquiphellandrene	26.29	13.76	-
37	Elemol	26.92	0.33	-
38	Caryophyllene oxide	27.87	-	0.37
	Total monoterpenes hydrocarbons	-	14.11	10.36
	Total sesquiterpene hydrocarbons	-	73.13	3.54
	Total oxygenated monoterpenes hydrocarbons	-	7.97	84.05
	Others		0.59	1.19
	Total	-	95.8	99.14

Ginger oil showed a great chemical homogeneity characterized by relatively high amounts of sesquiterpene hydrocarbons (73.13 %) of the total ginger essential oil while monoterpene hydrocarbons and oxygenated monoterpenes account for only 14.11% and 7.97 % respectively (fig. 2). The major sesquiterpene hydrocarbon detected in the essential oil, was zingiberene in concentration of 31.47% of total compounds. The specific aroma of ginger is predominantly related to Zingiberene. The other main identified sesquiterpene hydrocarbon compounds were β-sesquiphellandrene 13.76%, α-curcumene 10.41%, α-farnesene 8.31% and β-bisabolene 7.55%, while pinene (3.34%) and camphene (7.95%) were the most abundant monoterpene hydrocarbons detected. Eucalyptol was the major oxygenated monoterpene hydrocarbons (6.55%) from a total of (7.97%). Our results are in good agreement with the findings of Noori et al. [21] and Singh et al. [22] who reported that zingiberene and β-sesquiphellandrene are the main constituents of ginger oil. Also, we confirm the findings of El-Baroty et al. and Agarwal et al. [5, 23], who reported that the fresh rhizomes of ginger contained α-curcumene.

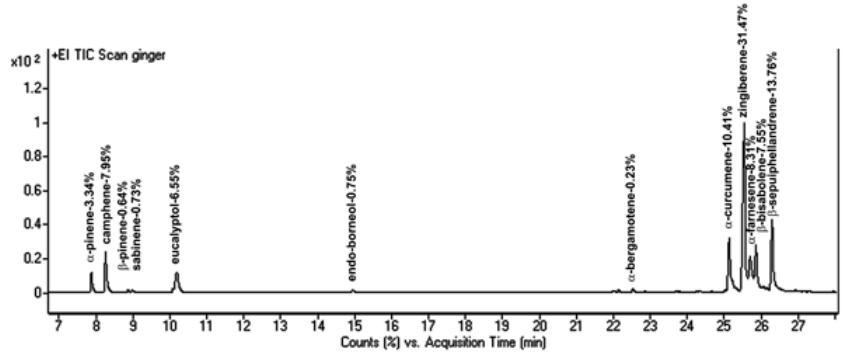

Fig. 2. The Total ion chromatogram (GC-MSD) of ginger essential oil

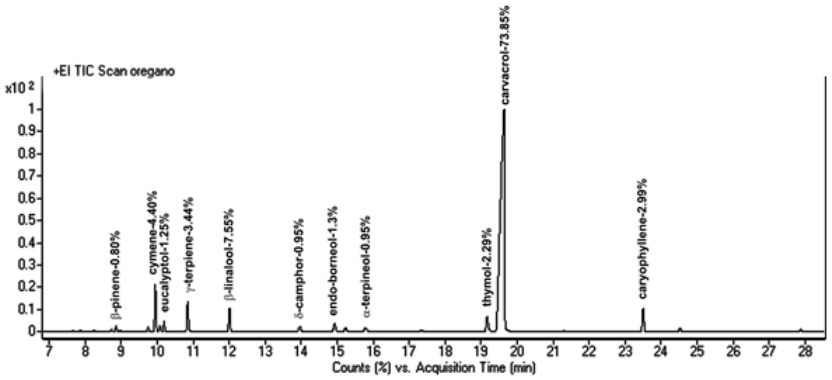

Fig. 3. The Total ion chromatogram (GC-MSD) of wild oregano essential oil

On the other hand, Singh et al. [4] identified geranial (25.9%) as the major constituent in ginger oil, but in our study it was not detected. These differences may be due to age and stage of maturity, weather conditions, soil composition, plant organs, distillation conditions and some other factors [23].

The wild oregano essential oil was mainly composed of oxygenated monoterpenes (84.05%), monoterpene hydrocarbons (10.36%) and sesquiterpene hydrocarbons (only 3.54%). Within oxygenated monoterpenes, carvacrol was the most abundant (73.85%) followed by β-linalool (3.46%) and thymol (2.29%). The most abundant monoterpene hydrocarbons detected were cymene (4.40%) and γ-terpinene (3.44%). Carvacrol, thymol, γ-terpinene and linalool are known to possess strong antioxidant properties [24-26] and carvacrol and thymol also exhibit antibacterial activity against several bacteria [27]. Caryophyllene was the principal sesquiterpene hydrocarbon (2.99%).

Antioxidant activity evaluation

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical was reduced to yellow colored diphenylpicrylhydrazine radical which was measured at 518nm. The substances which are able to perform this reaction can be considered antioxidants and therefore radical scavengers. Essential oils which contain phenolic monoterpenes and/or sesquiterpenes have been recognized for their higher antioxidative capacity [28].

Based on these observations, the higher antioxidant activity of the oregano oil recorded in the present study might be due to its high amounts of total oxygenated monoterpene hydrocarbons (84.05%) compared to ginger oil (7.97%) (table 2). From the two studied essential oils, the wild oregano oil is by far the most radical scavenging active, with an EC50 of 0.76 ±0.13 mg/mL compared to ginger oil of 20.22±2.12 mg/mL (fig. 4B). The antioxidant activity was associated with the presence of phenolic compounds in the composition of essential oils as determined by the Folin-Ciocalteu method and the GC-MSD composition of essential oils (carvacrol 73.85%, linalool 3.46% and thymol 2.29%).

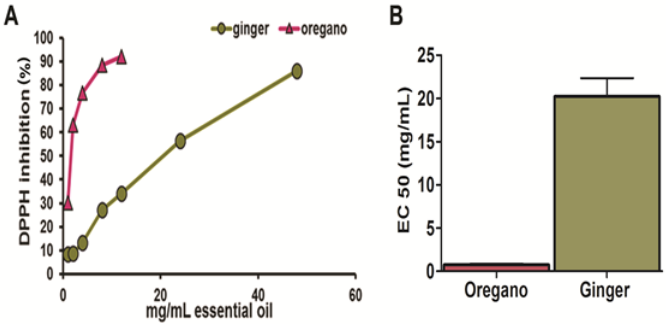

Fig. 4. DPPH radical discoloration of studied oregano and ginger oils (A) and the values of sample concentration required to scavenge 50% of the DPPH radicals (EC50) (B)

The results obtained by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method (fig. 5) are well correlated with those obtained by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) (fig. 4).

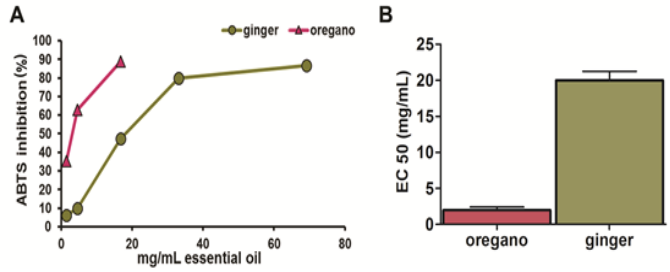

Fig. 5. ABTS radical discoloration of studied oregano and ginger (A) and the values of sample concentration required to scavenge 50% of the ABTS free radicals (EC50) (B.)

Antifungal activity

Fusarium oxysporum

Fusarium oxysporum is a pathogenic fungus common in soils around the world. These *F. oxysporum* strains infect and kill many commercially harvested crops such as tomatoes, peppers, potatoes, eggplant, lettuce, legumes, strawberries and other species. This fungus is resistant to

most available antifungal drugs, these infections are serious and frequently fatal in mammals [15].

Both essential oils were tested against *Fusarium oxysporum*, and the results are presented in figure 6.

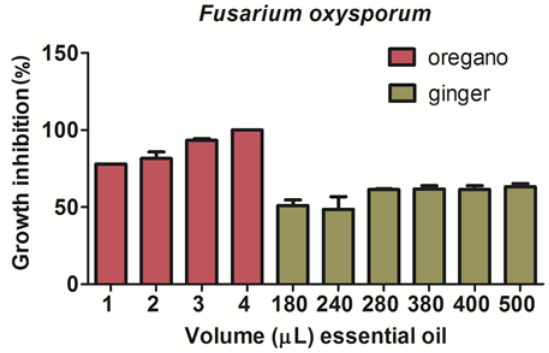

Fig. 6. Inhibition of the Fusarium oxysporum by the wild oregano and ginger essential oils.

The tested essential oils had antifungal activity against *Fusarium oxysporum* but their efficacy is different. Wild oregano essential oil was the most effective, with inhibition rate of 77.9% at the lowest volume of essential oil used for the growth inhibition of this fungus (1µL). For 4 µL of essential oils the inhibition rate was 100 %. The ginger oil had not antifungal activity under 180µL. This essential oil had an inhibition rate of 61.4% against *Fusarium oxysporum* for 280 µL and for higher volume of ginger oil the inhibition rate is constant (fig. 6.). Similar studies were conducted by Singh et al. [22], who tested the antifungal efficiency of ginger oil and ginger oleoresin on growth inhibition of some important fungals (*Aspergillus terrus*, *Aspergillus niger Aspergillus flavus Trichothecium roseum, Fusarium graminearum*, *Fusarium oxysporum* and *Fusarium monoliforme*). They reported growth inhibition ranging between 31-100 % for 10µL ginger essential oil. As described above, our findings are disagreeing with their results.

Aspergillus flavus

Aspergillus flavus is a saprophytic soil fungus that infects and contaminates preharvest and postharvest seed crops with the carcinogenic metabolite aflatoxin [29].

The inhibition rate of the tested oregano and ginger essential oils against *Aspergillus flavus* is presented in figure 7. The results evidenced that wild oregano oil was effective against *Aspergillus flavus* at a volume of 3µL with a growth inhibition of 100%, while ginger oil shows antifungal activity at a volume of 240µL (with growth inhibition of 78.3%). Like the antioxidant activity, the strong antifungal activity of wild oregano oil is attributed to phenolic compounds carvacrol (73.85%), linalool (3.46%) and thymol (2.29%) which are not present in ginger oil.

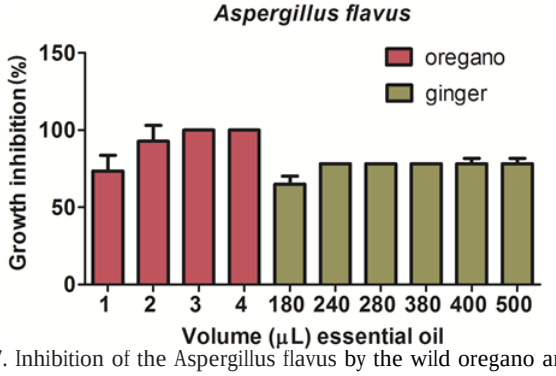

Fig. 7. Inhibition of the Aspergillus flavus by the wild oregano and ginger essential oils.

Penicillium expansum

Penicillium expansum is one of the most important postharvest pathogens of apple fruit worldwide. It causes blue mold, a decay that can lead to significant economic losses during storage, which can also impact fruit destined for processing due to the production of patulin, a carcinogenic mycotoxin [30].

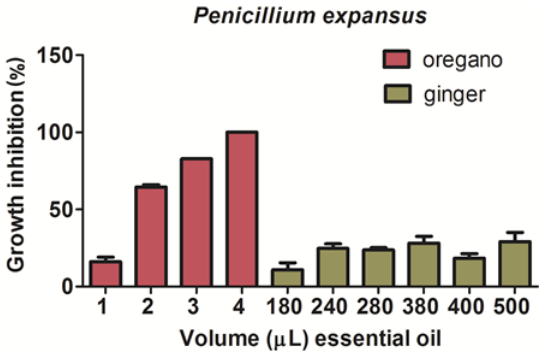

Fig. 8. Inhibition of the Penicillium expansum by the wild oregano and ginger essential oils.

The effectiveness of wild oregano oil as an antifungal agent was showed in data presented in figure 8. The wild oregano essential oil is more active than ginger essential oil, showing an inhibition rate of 100% at 4 µL, while ginger oil did not inhibit the fungal growth of *Penicillium expansum*. For the highest volume used in this study (500 µL), the growth inhibition was below 29%.

Conclusions

The results of our study showed that wild oregano oil was characterized by higher content of oxygenated monoterpene hydrocarbons compared to ginger oil when the major class of compounds was sesquiterpene hydrocarbons.

Concerning the antioxidant activity, the EC50 of the wild oregano essential oils is about 26 times higher compared to ginger oils and this is well correlated with total polyphenolics and oxygenated monoterpenes hydrocarbons content.

The results of this study demonstrate the potential of wild oregano oil for use as naturally antifungal agents in some food products against tested fungi (*Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium expansum*).

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