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In vitro antimicrobial activity of *Carum carvi* L. seed essential oil against pink potato spoilage flora

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ABSTRACT

The objective of this research is to assess the ability of using *Carum carvi* L. seeds essential oil as a biological substance for controlling spoilage germs growth in potatoes during storage. The chemical composition of caraway seeds essential oil, analyzed by GC-MS and by gas chromatography with flame ionization detector (GC-FID), led to the identification of twelve compounds, where carvone was the main one with a percentage of 75.64 % of the total oil. The comparison of the microbial profiles of different potatoes samples showed the presence of the genus Citrobacter and three distinct fungi genera: *Aspergillus*, *Phytophthora* and *Fusarium* only for contaminated potato tubers with internal pink pigmentation. Thus, the antimicrobial activity of caraway seeds essential oil was studied against these strains. The antimicrobial activity of the oil against the isolated strains was evaluated through the agar diffusion method using different volumes (10, 20, 50 and 100 µl). All tested strains were inhibited by caraway seeds essential oil in a dose-

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dependent manner. The obtained results suggest the use of *Carum carvi* L. as a promising natural substance for the preservation of potatoes by contact vapor method.

Keywords: potato, Essential oil, Carum carvi L. seeds, antimicrobial activity, Enterobacteria, fungi

Introduction

Solanum tuberosum, commonly known as potato, is a tuberous herbaceous plant native to Latin America, belongs to the family of *Solanaceae* (Sharma et al., 2014). The potato is a globally important crop, with an estimated 377 million tons harvested in 2016, only falling short of the other starch staples, maize, wheat, and rice (FAO, 2016).

In addition to this high starch content, potatoes are an important source of micronutrients, such as vitamin C, vitamin B6, potassium, folate, and iron and contribute a significant amount of fiber to the diet (Robertson et al., 2018).

However, the cultivation of harvested potatoes and tubers is subject to numerous bacterial, fungal, or viral attacks causing several diseases which are responsible for significant economic losses (Abd El-Azeim, 2020). Resorting to conservation is therefore essential to preserve the organoleptic and nutritional qualities of potatoes while strongly inhibiting the development of spoilage flora. The pesticides used in the past to improve the quality and productivity of agricultural products often remain as residues in agricultural soils, a portion of which may be taken up by plant crops. This may pose a major concern with respect to the safety of these products for animal and human consumption (Hwang et al., 2018).

Currently, consumers are increasingly concerned about the harmful effects of pesticides and are more demanding on the treatment used on fruits and vegetables. Hence the use of biotechnology, based on biological substances and biocontrol formulation is gaining more attention among the scientific community in order to avoid most of the negative impact of chemical compounds on food systems and therefore on human health (Larkin, 2016).

Some essential oils extracted from aromatic and medicinal plants are candidates for exploiting their full preservation potential. In fact, studies have shown that these essential oils are effective in controlling the growth of a wide variety of microorganisms, including filamentous fungi, yeasts, and bacteria (Essaidi et al., 2014; Lasrem et al., 2019). Such antimicrobial activities make the use of these oils recommended in food industries (Karameşe and Özgür, 2020).

Caraway (*Carum carvi* L.) is a condiment species belonging to the *Apiaceae* family and it is mainly cultivated for its aromatic seeds. It is one of the common well-known herbs, naturally found in Northern and Central Europe, Siberia, Turkey, Iran, India and North Africa (Saghir et al., 2012; Kazemipoor et al., 2016). In Tunisia, it is the most cultivated condiment species after coriander. It is widely used as a culinary, aromatic, and medicinal plant (Agrahari and Singh, 2014). Additionally, *C. carvi* seeds have antispasmodic, antiflatulence, antibacterial, anticancer, lactiferous, expectorant, and energizing effects; also, they can improve menstruation and appetite (Agrahari and Singh, 2014; Al-Snafi, 2015).

The aim of this research was to extract and determine the chemical composition of caraway seed essential oil in addition to examine the antibacterial and antifungal activity towards isolated strains of contaminated potato tubers with internal pink pigmentation.

Experimental

Biological Material

The potato tubers and caraway seeds used in this research were purchased from a local market in Tunis. Two types of potato samples were used: unpigmented potatoes (sound) and potatoes with internal pink pigmentation (infected) for the microbiological characterization.

Essential oil extraction and chemical composition analysis

The caraway essential oil (EO) was obtained by hydrodistillation using a Dean-Stark apparatus. The extraction was carried on until there was no significant increase in the volume of oil collection. The obtained essential oil was dried over anhydrous sodium sulfate and preserved in a sealed vial at 4°C until further analysis.

The chemical composition was analyzed by gas chromatography coupled with mass spectrometry using a GC system apparatus type HP 6890N, connected to a mass spectrometer (MS) with a selective detector HP 59758 N and equipped with a flame-ionization detector (FID). A capillary column type HP5-MS (30m x 0.25mm x 0.25µm) was used for the purpose of separation. The temperature of the column was set from 40 to 280 °C at a rate of 2 $°C.min^{-1}$. Helium was used as carrier gas at a flow rate of 0.9 mL/min and the sample was injected in the split mode (1:10).

The compounds identification was performed according to their GC retention indices, by comparison of their MS spectra by computer matching with the Wiley 238.L mass spectra library, and when possible, co-injection with standard available in our laboratory.

Microbial analysis of potato samples

Enumeration of the microflora of the studied potatoes was carried out on grounded samples which were diluted (10^{-3} to 10^{-7}) and then cultured in selective agar culture media: PCA (total plate count of bacteria), VRBG (total enterobacteria and coliforms), Sabouraud agar (yeasts and molds). The isolated strains were identified by morphological, physiological, and biochemical tests (Gram stain, glucose fermentation test, lactose, gas production and H_2S, oxidative/fermentation test, and IMViC test). The metabolic study of the microorganisms was achieved via API E 20 system (bioMérieux, France) for enterobacteria and API 20C AUX (bioMérieux, France) for yeasts. These tests were read after 24 and 72 hours for enterobacteria and for yeasts, respectively. The analysis results were obtained using Apilab®

identification software. The identification of isolated molds was carried out in the Nabeul health hygiene laboratory (Cape Bon region, Tunisia) through microscopic readings.

Antimicrobial activity

Caraway essential oil was screened for its antimicrobial activity against five strains isolated from the infected potato by agar disk diffusion method defined by Cushine and Lamb (2005). A soft agar, previously sterilized, was inoculated with 50 µl of a fresh culture sample of the isolated microorganisms (10^6 CFU / ml) and poured into petri dishes. After the medium has solidified, sterile disks (6 mm) soaked in essential oil (10, 20, 50 and 100 µl) were placed on the surfaces of the plates. The effectiveness of essential oil was determined after 24-48 hours at 37°C of incubation by the measurement of the inhibition zone diameters (IZDs) around the disk, including the diameter of the disk (in millimeters).

Results and Discussion

Chemical composition of the essential oil of *Carum carvi* L. seed

The analysis of the essential oil of *Carum carvi* L. seeds using the GC-MS and GC-FID approach led to the identification of 12 terpene compounds (Figure 1) which constitute 99.74% of the total oil including 78.44% oxygenated monoterpene derivatives, 21.01% monoterpene hydrocarbons, and 0.29% sesquiterpenes.

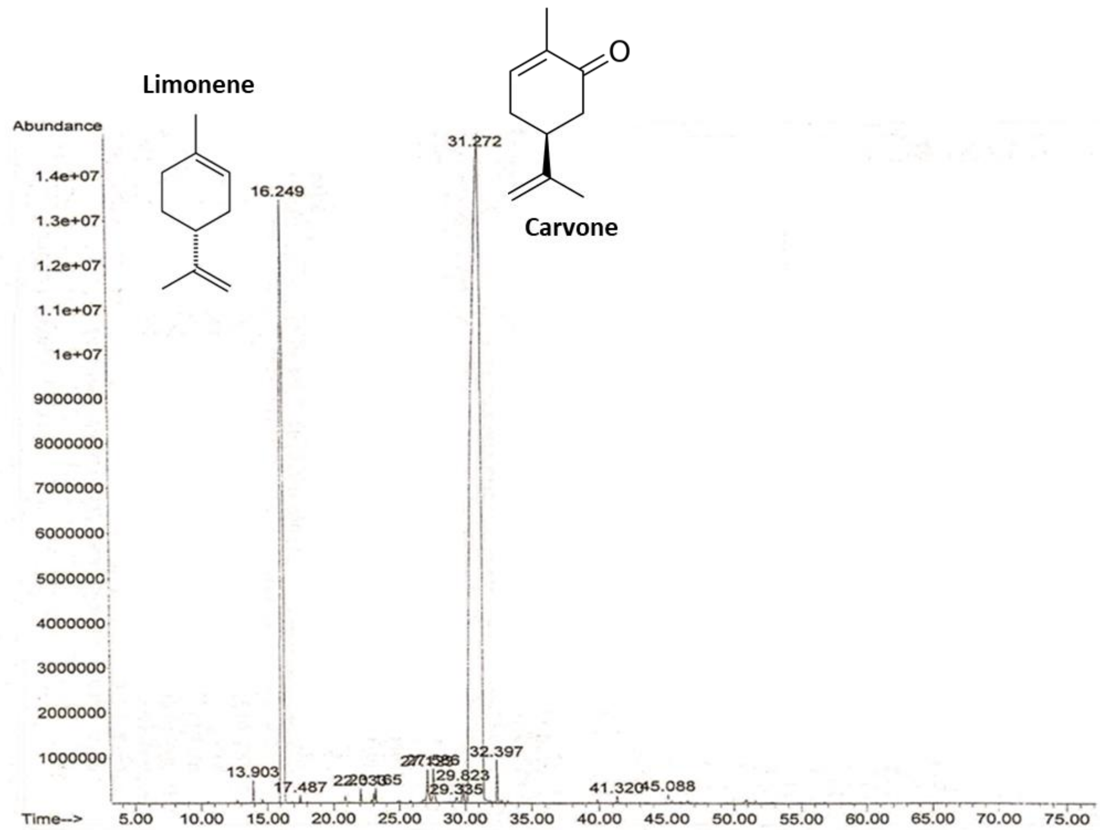

Figure 1. Chromatographic profile of the essential oil of *Carum carvi* L. seed

The two major components of this oil were carvone (75.64%) and limonene (20.70%), representing 96.34% of the essential oil (Table 1**)**. The other compounds were present in smaller percentages.

These results are in agreement with those of Assami et al. (2012) and Laribi et al. (2013) who reported that caraway seeds essential oil consists mainly of carvone and limonene. In addition to their fragrance, these compounds are also known for their antibacterial and antifungal properties (Zhang et al., 2014), antioxidant effects (Afify et al., 2012; De Almeida et al., 2014) as well as ability to inhibit the sprouting of potatoes (Hartmans et al., 1995).

Table 1. Chemical composition of the essential oil of *Carum carvi* L. seed

N	Compound[a]	RI[b]	RI[c]	Identification[e]	Percentage
1	β-Myrcene	991	991	RI, MS, CoI	0.22
2	D-Limonene	1012	1014	RI, MS, CoI	20.70
3	β-Ocimene	1101	1097	RI, MS, CoI	0.09
4	Oxyde de limonene	1132	1133	RI, MS	0.34
5	Cis-dihydrocarvone	1169	1172	RI, MS	0.75
6	Neodihydrocarveol	1170	1177	RI, MS	0.63
7	Trans-dihydrocarvone	1178	1175	RI, MS	0.15
8	Carveol	1203	1197	RI, MS	0.38
9	Carvone	1252	1240	RI, MS, CoI	75.64
10	Perillal	1287	1294	RI, MS	0.55
11	Caryophyllene	1411	1419	RI, MS, CoI	0.09
12	Germacrene D	1508	1477	RI, MS, CoI	0.20
				Monoterpene hydrocarbons	21.01
				Oxygenated monoterpene	78.44
				Sesquiterpenes	0.29
				Total	99.74

[a] Compounds listed in order of elution from HP-5MS column.

[b]Retention indices relative to C8 – C22 n-alkanes on HP-5MS column.

[c] Retention indices according to Adams (1995)

[d] Percentage (mean of three analyses) based on FID peak area

[e] RI: Retention indices relative to C8 – C22 n-alkanes on HP-5MS column, MS: mass spectrum, CoI: co-injection with authentic compounds (Fluka Chemika).

Identification of the spoilage microflora of potato

The identification of microorganisms from different samples of the healthy potatoes and those pigmented in pink allowed us to highlight two categories of microorganisms: bacteria and fungi. The characterization of this microflora made it possible to detect a difference between the samples of tested potatoes (Table 2). Among the isolated bacteria, *Enterobacter cloacae* was identified in the two types of potato but with different percentages: 100% (sound potato), 10% (infected potato). The genus *Citrobacter* was present only in the pigmented potato tubers with 80% relating to the species *Citrobacter freundii* and 10% to the species *Citrobacter braakii*.

Table 2. Sprouts isolated from samples of sound and pink pigmented potatoes.

Families	Sound potato	Pink pigmented potato
Enterobacteria	*Enterobacter cloacae* (100 %)	*Enterobacter cloacae* (10%) *Citrobacter braakii* (10%) *Citrobacter freundii* (80%)
Yeasts and molds	*Rhodotorula minuta*	*Aspergillus clavatus* *Phytophthora infestans* *Fusarium oxysporium*

With regard to yeasts and molds, *Rhodotorula minuta* species was identified only in sound potato tubers and three genera of molds were identified in pigmented potatoes: *Aspergillus*, *Phytophthora* and *Fusarium.* Microscopic analysis of the molds allowed us to identify mold species that corresponds to *Aspergillus clavatus*, *Phytophthora infestans* and *Fusarium oxysporum*. The presence of these pathogens in infected tubers during storage may be linked to the contamination of potato seeds and soil (Powelson and Rowe, 2008).

Antimicrobial activity of the essential oil of *Carum carvi* L. seed

The antimicrobial activity of the caraway essential oil was tested against two Gram-negative bacteria strains: *Citrobacter Freundii*, *Citrobacter braakii,* and three fungal species: *Aspergillus clavatus*, *Phytophthora infestans* and *Fusarium oxysporum*, isolated from the infected potatoes. The caraway essential oil shows notable inhibitory effects against all the studied microorganisms (Table 3). We note that this effect depends on the strain and the dose of used essential oil. According to Hassan et al. (2006) an inhibition zone diameter more than 10 mm means that an antimicrobial effect is proved.

Table 3. Diameters of inhibition zones (mm) for the different tested strains

	Volume of essential oil (µl)			
	10	**20**	**50**	**100**
Citrobacter freundii	12.0	14.0	18.0	22.0
Citrobacter braakii	6.0	6.0	10.0	14.0
Aspergillus clavatus	15.0	16.0	20.0	22.0
Phytophthora infestans	10.0	12.0	14.0	18.0
Fusarium oxysporium	6.0	8.0	9.0	13.0

The *Citrobacter freundii* strain shows the important inhibition zone diameters than those of *Citrobacter braakii.* In fact, *C. braakii* resisted to caraway essential oil action up to a dose of 50 µl (D = 10 mm), dissimilar to *C. freundii* which was inhibited from the lowest dose of EO which was 10 µl (D = 12 mm). The founded results are consistent with those of Roy et al. (2010) who showed that caraway EO has antibacterial activity against Gram- and Gram+ bacteria and more particularly against *enterobacteria*.

Among fungal species, *Aspergillus clavatus* was the most sensitive strain, followed by *Phytophthora infestans* and *Fusarium oxysporium* (Table 3). At a maximum dose of 100 µl caraway EO, the samples show inhibition zone diameters of 22, 18 and 13 mm, respectively.

This results confirm those obtained by Gómez-Castillo et al. (2013), who founded that the treatment of potato tubers with essential oils, including *Carum carvi* L. oil, reduce the proliferation of pathogenic microorganisms during storage, particularly molds. Moreover, this oil has been selected as a potent inhibitor of potatoes sprouts (Afify et al., 2012; Gómez-Castillo et al., 2013; Şanlı and Karadoğan, 2019).

The antimicrobial activity of caraway seed essential oil may be attributed to carvone and other monoterpenes, mainly limonene, which act as antiseptics, anti-inflammatories, antivirals, and antimicrobials. Many previous papers have shown that these two compounds have excellent antibacterial and antifungal properties (Roy et al. 2010 ; Esfandyari-Manesh et al., 2013). Notably, a degree of synergy between the majority and minority constituents of the caraway seed essential oil is possible.

Conclusion

The chemical composition analysis of caraway seeds essential oil, obtained with hydrodistillation, reveals that 99.74% of the constituents were identified. The main compound in this oil was carvone (75.64%), followed by limonene (20.70%). Due to its chemical composition, which is rich in monoterpene compounds, *Carum carvi* L. essential oil is effective against all strains of bacteria and molds isolated from infected potato (pink-pigmented), notably against *Citrobacter freundii* and *Aspergillus clavatus*. Thus, Caraway seeds essential oil can be used as a preservative to prevent contamination of potatoes due to the presence of enterobacteria and mold.

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