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Oliveria decumbens and *Pistacia atlantica* Gum's Essential Oils: Assessment of Antimicrobial and Chemical Properties During Thermal Process

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Abstract

The essential oils usually have a good effect against undesirable microorganisms; therefore, they can be utilized as natural antimicrobial agents in food or their packaging. In this research, the antimicrobial attributes of two essential oils (*Oliveria decumbens and Pistacia atlantica* gum), have been investigated before and after thermal process (200°C - 10 minutes) against bacterial and mold spoilage in bread. Also, the compounds of essential oils were detected by gas chromatography-mass spectrometry. The main compounds of the essential oil of *O. decumbens* were carvacrol, thymol, and elemicin before and after thermal treatment. In the case of *P. atlantica* gum, only one prominent peak was observed in the chromatogram, which was related to the α-pinene. For both essential oils, the MIC and MFC against *Aspergillus niger* were 4000 and 8000 µL/ml, respectively. In comparison, the antimicrobial effect of both essential oils against *Bacillus subtilis* was higher than the mold. The amount of MIC and MBC were 125 and 250 µL/ml for *Oliveria decumbens* and 62.5 and 125 µL/ml for *Pistacia atlantica* gum, respectively. The results showed that these two essential oils have a promising effect against the main microorganisms of bread spoilage. The thermal process did not significantly affect the antimicrobial activity of *Pistacia atlantica* gum essential oil against *A. niger* but significantly decreased the antimicrobial activity against *B. subtilis*, while in the case of antimicrobial activity *of Oliveria decumbens* essential oil, the results were the opposite. Considering the fact that the most spoilage agents of the bread are molds so the use of *Pistacia atlantica* gum essential oil is recommended as natural preservatives in products that tolerate high heat treatment, such as bread and bakery products.

Keywords: GC-Mass, MBC/MFC, *Oliveria decumbens*, *Pistacia atlantica* gum, Thermal stability

Introduction

The most common molds detected in bakery products are genera of *Penicillium*, *Aspergillus*, *Monilia*, *Mucor*, *Endomyces*, *Cladosporium*, *Fusarium*, *Alternaria*, and *Rhizopus* (Alhendi and Choudhary, 2013; Cioban *et al.*, 2010;

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Gerez *et al.*, 2009; Rodriguez *et al.*, 2008). According to recent scientists' findings, about 60% of the dough spoilage is due to the presence of *Penicillium* genus and *Aspergillus niger* (Alhendi and Choudhary, 2013). Faparusi and Adewole, 2019 reported that *A. niger* is the most common bread mold spoilage agent. On the other hand, ropiness is the utmost bacterial spoilage of bread, usually caused by *Bacillus* spp., especially *Bacillus subtilis* (Faparusi and Adewole, 2019). This spoilage often occurs when the bacterial population reaches 10^5 CFU per gram of sourdough (Sadeghi *et al.*, 2011).

The use of a limited number of synthetic preservative compounds, in trace amounts, is allowed in baking products., However, due to consumers' awareness of the dangers of chemical preservatives, in recent years researchers have focused on finding a substitution natural ingredients such as plant essential oils for preventing spoilage in food products (Borghei *et al.*, 2010; Jideani and Vogt, 2016). The antimicrobial, antioxidant and anti-carcinogenic properties of essential oils, which is mainly due to the presence of phenolic compounds, have been proven (Bagamboula *et al.*, 2004; Neffati *et al.*, 2017).

Pistacia atlantica (wild pistachio) gum (Fig. 1), called "Saqqez" or "Banneh" in Iran, is secreted from the outer layer of the plant's inner skin. The predominant composition of this gum's essential oil is α- pinene (Rahimi *et al.*, 2016). Antimicrobial activity of the essential oils and extract of this plant has been proven by many researchers (Azeez and Gaphor, 2019; Ellahi *et al.*, 2019; Mahjoub *et al.*, 2018). Ahmed *et al.* (2020) reviewed ethnobotany, phytochemistry, and pharmacology properties of some subspecies of *Pistacia*, including *atlantica*, *cabulica*, *kurdica*, and *mutica*. They reported gum antimicrobial properties against various Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Acenitobacter boumanii*) and emphasized its antifungal activities against *Aspergillus* species (*A. niger*, *A. flavus*, *A. fumigates*) (Ahmed *et al.*, 2021).

Fig. 1- *Pistacia atlantica* gum

Oliveria decumbens (Fig. 1) is a medicinal plant locally called "Dan" or "Danak" (Zali and Tahmasb, 2016). Thymol and carvacrol are the major constituents of *O. decumbens* essential oil (Amin *et al.*, 2005). Some researchers, such as Behrooz (2016), demonstrated that the antifungal activity of the *O. decumbens* essential oil was higher than *Thymus vulgaris*, *Zataria multiflora*, rosemary, and cinnamon. The essential oil was able to prevent the growth of *A. niger* and *A. fumigatus* as well as two species of *Penicillium* (Behrooz, 2016).

Fig. 2- *O. decumbens* plant

There are two main challenges when adding essential oils in bread. Firstly, they might change bread`s taste and secondly, the high temperature during baking can alter the composition of the essential oils. Using natural antimicrobial compounds in bread and baking products packaging can reduce the problems mentioned above to some extent. However, high temperature is also used in the production of packaging film; therefore, in addition to investigating the antimicrobial properties of essential oils against the main bread spoilage microorganisms, their thermal resistance should also be carefully studied.

This study aimed to investigate the antimicrobial properties of *P. atlantica* gum and *O. decumbens* essential oils (before and after thermal treatment) against the most common mold and bacterial spoilage agents of bread (*A. niger* and *B. subtilis*). Moreover, the effect of heat treatment on the type and amount of constituents of essential oils was evaluated.

Materials and Methods

A. niger isolated from raisins and identified by the molecular method was prepared from the microbial bank of the Research Institute of Food Science and Technology, Iran. Lyophilized powder of *B. subtilis* (UBBS-14) was bought from Unique Biotech Company (India). Microbial culture mediums, PDB (Potato Dextrose Broth), PDA (Potato Dextrose Agar), MHB (Mueller Hinton Broth), and MHA (Mueller Hinton Agar) as well as ringer tablets and N–hexane (HPLC Grade) were purchased from the Merck Company (German). Antibiotic discs such as gentamicin, ampicillin, and erythromycin were purchased from a pharmacy and Tajhiz Avaran-e Shargh Company in Iran.

Essential oils extraction

Clevenger was used for extracting essential oils. Fresh *P. atlantica* gum and the flower and stem of *O. decumbens* were purchased from local markets in Mashhad, Iran. The samples were air-dried to a constant mass at room temperature (in the shade) and then were grounded with a miller (Model 8300- Toos Shekan-e Khorasan Company, Iran) for about 1 min and sieved with the size of 35 mesh. After that 40–50 g of milled powder were poured into a volumetric balloon (1000 cc), and 70% of the balloon was filled with distilled water. After heating (100˚C) for 6 hours, the essential oil was collected and stored in sealed vials in a dark place at 4°C, until the subsequent experiments (Elyemni *et al.*, 2019). It should be noted that the sterilization of extracted essential oils for antimicrobial tests was done using 0.45 µm filters.

The yield of essential oils extraction

The extraction yield was expressed in g in 100 g of dry plant powder and was calculated according to Equation (1) (Elyemni *et al.*, 2019):

$$\text{Extraction yield (\%)} = \frac{\text{Amount of extracted essential oil (g)}}{\text{Amount of dry plant powder (g)}} \times 100 \quad (1)$$

Thermal processing

In order to investigate the effects of thermal processing on the essential oils and their active compounds, they were kept in oven (Memmert-Germany) at 200°C for about 10 min (Krepker *et al*., 2018).

Determination of the chemical composition of Essential oils by GC-MS

The chemical composition of Essential oils before and after thermal processing of essential oils was resolved using gas chromatography. Five microliters of each essential oil was dissolved in n–hexane solvent (HPLC Grade), and one microliter was injected into a gas chromatography with a mass spectrometry detector (GC-MS 5977 A- Agilent Technologies, USA) equipped with an HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 µm o.d– Agilent). The injection temperature was 279 °C. The oven temperature was programmed from 60 °C to 200 °C with an increase of 5°C/min. The helium carrier gas was injected at a volume of 1 ml, and the flow rate of helium gas was maintained at 1 ml/min. The mass spectrometer was also fixed to EI mode at 70 eV. The interface temperature and the mass spectrum were set at 280°C and 35 to 700 m/z, respectively (Paventi *et al*., 2020).

Disk diffusion method for determination of inhibition zone

Antimicrobial activity of the stock essential oil (32000 µL/ml) against *A. niger* and *B. subtilis* was investigated by the disk diffusion technique. The lyophilized *B. subtilis* were grown in Mueller-Hinton Broth at 37 °C for 18-24 hours. Then, the turbidity equivalent to 0.5 McFarland standard was prepared and diluted to 10^6 CFU/ml. For *A. niger*, the hemocytometer chamber was used to reach 10^6 spores per ml of fungal suspension. After that, 100 µl of microbial suspensions was spread on the surface of the plate containing MHA medium. Paper disks (6 mm) were impregnated with 30 µl of essential oils and alcohol (70 %) was used as negative control. Moreover, the antibiotic standard disks, including Erythromycin, Ampicillin, and Gentamicin were applied as positive controls (10 µl per disc). The plates were incubated at 37 °C for 18 h and 25 °C for 3-5 days for bacteria and fungi, respectively. Finally, inhibition zone diameters (mm) were measured (Aljeldah, 2022).

Determination of the MIC, MBC and MFC of essential oils by Microdilution Method

The microdilution method was used to determine the MIC of essential oils. Aliquots (100 µl) of different concentrations (32000, 16000, 8000, 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7. 8125, and 3.9062 µl /ml) of two essential oils were added to each well of 96 well-plate, which contained 95 µl Mueller-Hinton Broth and Potato Dextrose Broth for bacteria and mold, respectively. Then, five microliters of 10^5 CFU/ml suspensions of microorganisms were added to each well. The negative controls were prepared with MHB and PDB media containing the essential oils without the microorganisms. The positive control was prepared with the MHB and PDB media containing the examined microorganisms without adding essential oils. The 96 well-plates were incubated at 37 °C for 24 h for bacteria and 25 °C for 48 h for molds. The turbidity of the microorganism's growth was detected by an ELISA reader (ELX 808, Biotech USA). After the incubation period, the lower concentration of the essential oils with turbidity similar to negative wells was defined as MIC. To detect MBC/MFC, 100 µl of each well with turbidity similar to the negative controls was spread on the surface of MHA and BDA media and incubated at a suitable temperature and time for the microorganisms. The minimum bactericidal / fungicidal concentration was the lowest concentration of essential oils that caused the death of microorganisms in a way that no colony of microorganisms are observed (Behrooz, 2016).

Results and Discussion

Essential oils extraction yield

The extraction yield was 1/5 % for *O. decumbens* and 3% for *P. atlantica*.

GC-MS results

GC-MS measured the most important compounds of essential oils before and after thermal treatment. The results are as follows

GC-MS chromatogram of essential oils (before and after heat treatment)

GC-MS chromatogram of *O. decumbens* essential oil, before and after thermal treatment are presented in Fig. 3. Also the main compounds of this plant is available in Table 1.

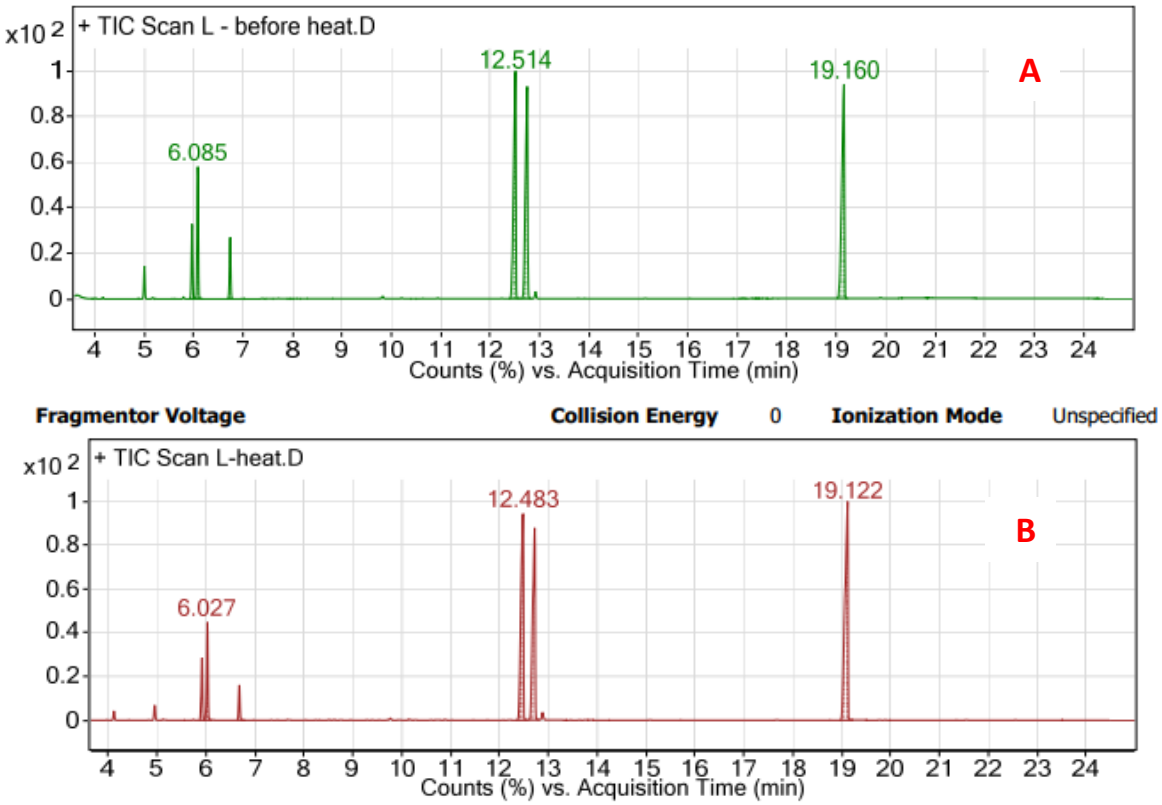

Fig. 3- GC-MS chromatogram of Oliveria decumbens (A= Before heating, B= After heating treatment)

As shown in Table 1, the main compounds of *O. decumbens* before heat treatment were elemicin (28.71 %), carvacrol (27.91 %), and thymol (24.76 %), that totally account for about 80% of *O. decumbens* composition. By comparing the peak of main compounds of *O. decumbens* essential oil before and after heat treatment, it is clear that after heating the retention time of all compounds was slightly decreased although the main compounds remained unchanged. In addition, the amount of minor compounds decreased 6% in total, and the same amount was added to the elemicin (34.58 %); possibly due to the chemical interactions and material decomposition/ composition.

Table 1- The main compounds and retention time of *Oliveria decumbens* (before and after heating treatment)

Number	Treatment	Name of compound	Retention time	Area
1	Before heating	o-Cymene	5.97	5.03
	After heating	o-Cymene	5.918	4.44
2	Before heating	D-Limonene	6.085	9.26
	After heating	D-Limonene	6.027	6.82
3	Before heating	γ-Terpinene	6.744	4.33
	After heating	γ-Terpinene	6.679	2.56
4	Before heating	Carvacrol	12.514	27.91
	After heating	Carvacrol	12.483	27.27
5	Before heating	Thymol	12.752	24.76
	After heating	Thymol	12.720	24.33
6	Before heating	Elemicin	19.16	28.71
	After heating	Elemicin	19.122	34.58

Sereshti *et al*. (2011) declared that the main components of the essential oil of *O. decumbens* vent are: thymol (47.06%), carvacrol (23.31%), gamma-terpinene (18.94%), p-cymene (8.71%), (Sereshti *et al*., 2011). Esmaeili *et al*. (2018) studied the aerial parts of *O. decumbens* and reported that, according to the GC and GC-MS results, γ-terpinene (33.6%), carvacrol (16.9%), and thymol (16%) in the vegetative stage were the main components; whereas during the flowering stage thymol (37.8%) and carvacrol (29.38%) were dominated components (Esmaeili *et al*., 2018). Karami *et al*. (2020) studied the variation in the content of essential oil and composition of 12 types of *O. decumbens* populations in several habitats of Iran. They found that, the highest essential oil content was obtained from Behbahan (8.52%) and it was significantly higher than those previously reported for other areas in Iran and elsewhere. They announced that carvacrol (18.8–51.8%), thymol (20.3–38.7%), γ-terpinene (0.9–28.8%) and p-cymene (1.6–21.3%) were the major volatile compounds of *O. decumbens* based on GC-FID and GC-MS analysis (Karami *et al*., 2020). The differences observed in this regard are associated with the plant origin, the parts of the plants selected or gathered for extraction (Karami *et al*., 2020; Sereshti *et al*., 2011) different phenological stages and also the extraction methods (Esmaeili *et al*., 2018).

GC-MS chromatogram of *P. atlantica* gum's essential oil (post and pre-heating) is shown in Fig. 4. As seen in Fig. 4, before the heat treatment, only one sharp peak appears in the chromatogram (4.12 min), related to a-pinene (3-carene), the main composition of *P. atlantica* gum (67 %). It should be noted that there were other minor compounds, but they could be ignored. After heat treatment, it was observed that the amount of α- pinene decreased from 67% to 60.5% in comparison to pre-heat treatment; however the amount of some trace compounds such as trans-verbenol rose increasedafter heat treatment. Hasheminya and Dehghannya (2020) also declared that α-pinene is the main compound found in essential oil of hull of *P. kurdica* subsp. (Hasheminya and Dehghannya, 2020). Elahi *et al*. (2019) reported that α-pinene (92.08%) is the main ingredient of *P. atlantica* gum essential oil (Ellahi *et al*., 2019). In another study carried out by Azeez and Gaphor (2019), it was reported that α-pinene made up 79.76% of the gum's essential oil of *P. atlantica Kurdica* (Kurdistan, Iraq) (Azeez and Gaphor, 2019). According to the research of Rahimi *et al*. (2013), it is worth mentioning that in both males and females of *P. atlantica* subsp. *Kurdica*, α-pinene (92.42%-84.10%), and Limonene (5.23%-1.26%) were the major compounds of the gum, (Rahimi *et al*., 2013). Jaradat *et al*. (2022) also found that Limonene and α-pinene were characterized as the major *Pistacia lentiscus* essential oil components (Jaradat *et al*., 2022). Sharifi *et al*. (2011) determined the gum's composition of *P. atlantica* subsp *kurdica* and showed that α-pinene contents (97.18) giving a unique

characteristic to this species. Overall, in the present research in line with other relevant studies working on all other parts of this plant, such as fruit (71.9%), (Fathollahi *et al.*, 2019) leaves (5.54-66.1% from 34 samples), (Gourine *et al.*, 2010) hull (20.80%)(Rezaie *et al.*, 2015), industrial essential oil (Saqez Company of Kurdistan– 91.47%) (Hesami *et al.*, 2014); the main component of the essential oils of *P. atlantica* was α-pinene (Memariani *et al.*, 2017; Rahimi *et al.*, 2013; Sharifi and Hazell, 2011).

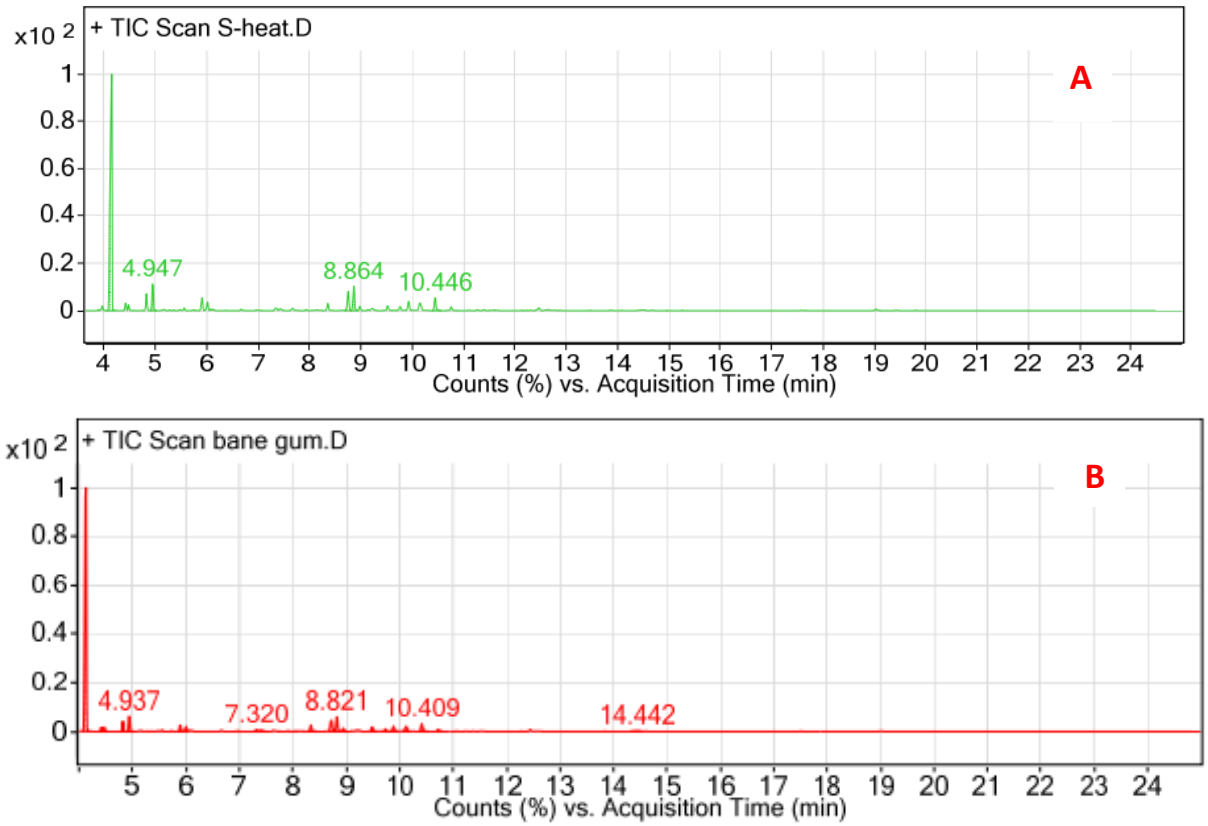

Fig. 4- GC-MS chromatogram of *Pistacia atlantica* (A=before heating, B=after heating)

Microbial tests

Molds

According to the results of the disk diffusion test, the inhibition zone of *O. decumbens* essential oil against *A. niger* was 25.50±0.08 mm before heat treatment and 20.87±0.43 mm after heat treatment, which showed the antimicrobial activity after heat treatment decreased significantly, although due to the high antimicrobial activity of the essential oil based on inhibition zone, after the heat treatment, it can be used as an antifungal compound. On the other hand, no significant difference was observed before (11.00±0.16) and after (10.85±0.04) the heat treatment in inhibition zone of *P. atlantica* essential oil showing entire resistant to the heat treatment (200°C- 10 minutes). Although Erythromycin and Gentamicin showed the biggest inhibition zone (40.61±0.27 mm and 40.50±0.41 respectively), Ampicillin inhibition zone was lower (20.15±0.28 mm).

Bacteria

The inhibition zone of *P. atlantica and O. decumbens* essential oil with a concentration of 32000 µL/ml against *B. subtilis* was 28.28±0.39 and 42.75±0.20 mm before heat treatment and 13.86±1.29 and 42.77±0.22 mm after heat treatment respectively; whereas inhibition zone of Erythromycin, Ampicillin and Gentamicin were 28.40±0.33, 25.94±0.11 and 24.88±0.68

mm respectively. Based on the results, heat treatment reduced the antimicrobial properties of *P. atlantica* essential oil against *B. subtilis* significantly, while the inhibition zone of the heated *O. decumbens* essential oil on the mentioned bacteria was not different from the unheated essential oil, which indicates the stability of *O. decumbens* essential oil to heat treatment.

Ghalem and Mohamed (2009) evaluated the antimicrobial activity of *P. atlantica Desf* essential oil (PEO) against *S. aureus*, *Streptococcus pyogenes*, and *E. coli*. The PEO concentration was 10^{-1} dilution of the extracted essential oil. They declared that the largest inhibition zone was 9 mm obtained against *E. coli* (10^3 CFU/ml), and the lowest was against *S. pyogenes* (10^2 CFU/ml) with no inhibition zone. Moreover, the PEO at 10^{-2} and 10^{-3} µg/ml showed moderate antimicrobial activity. (Ghalem and Mohamed, 2009). Hama Amin *et al*. (2022) investigated antifungal activity of *Pistacia atlantica subsp. kurdica* oil gum extract (100, 50, 25 µl/ml) against *Aspergillus brasiliensis*. According to their results, oil gum extract showed strong antifungal activity because no growth was observed at different concentrations of the extract added to the inoculated PDA medium (HamaAmin *et al*., 2022).

The MIC and MBC of both essential oils (*P. atlantica* and *O. decumbens*) against *A.niger* and *B. subtilis* were measured using microdilution test. The results are shown in Table 2.

Table 2- The MIC and MBC of both tested essential oils (*P. atlantica* and *O. decumbens*) against the tested microorganism (*A. niger* and *B. subtilis*) before heat treatment

Type of Microorganism	Test	*Pistacia atlantica*	*Oliveria decumbens*
niger	MIC	4000 µL/ml	4000 µL/ml
	MBC	8000 µL/ml	8000 µL/ml
subtilis	MIC	62.5 µL/ml	125 µL/ml
	MBC	125 µL/ml	250 µL/ml

Azeez and Gaphor (2019) reported that both MIC and MBC of the essential oils of *P. atlantica* gum against *Porphyromonas gingivalis* ($5*10^5$ CFU/ml) were 12.5 µL/ml (Azeez and Gaphor, 2019). In another study, Sharifi *et al*. (2011) evaluated the antimicrobial activity of essential oil of *P. atlantica kurdica* crude gum against nine strains of *Helicobacter pylori* ($1.5*10^8$ CFU/ml) and some other Gram-positive and negative bacteria. They reported that the MIC values ranged from 500-1000 mg/mL (Sharifi and Hazell, 2011). Ghalem and Mohamed (2009) determined the MIC of the resin oil of *P. atlantica* in different concentrations (0.1, 0.01, and 0.001 µg/ml). They proved that its values ranged from 3- 11 µg/ml against *E. coli*, 1- 10 µg/ml against *S. aureus*, and 0– 8 µg/ml against *S. pyogenes* (Ghalem and Mohamed, 2009). In addition, Doosti (2019) investigated the antimicrobial effects of *P. atlantica*'s gum against *S. aureus*, *P. aeruginosa*, *E. coli*, *Candida albicans* and *Candida glaberata* by disc diffusion, and then MIC, MBC, MFC was determined. The results revealed that *S. aureus* had the greatest inhibition zone diameter at a concentration of 5 mg/ml while *P. aeruginosa* showed the least diameter of the inhibition zone at a concentration of 0.156 mg/ml. The lowest MIC and MBC for *S. aureus* were 5.312 and 625 µg/ml, respectively. Among the fungi, the biggest diameter of the inhibition zone at a concentration of 5 mg/ml was related to *C. albicans*, and the smallest diameter at a concentration of 0.156 mg/ml was related to *C. glaberata*. *C. albicans* had the lowest MIC and MFC, 625 and 1250 µg/ml, respectively (Doosti, 2019). In another study, Mokhtari *et al*. (2021) compared the effects of the leaf extract and gum of *p. atlantica* (0/2 g/ml) with 0.2 % chlorhexidine (CHX) against the growth of *Streptococcus mutans*. The results demonstrated a 24-mm inhibition zone around CHX, but no inhibition zones were observed

neither of the extract nor the gum of *P. atlantica* against *S. mutans*. Furthermore, they declared that the MIC values for CHX, the leaf and gum extract of *P. atlantica* were 1.256, 1.8, and 1 mm, respectively (Mokhtari *et al*., 2021).

To the best of our knowledge, few articles have been published about the heat stability of essential oils. Pina-Pérez *et al.* (2018) investigated the antimicrobial potential of Açaí (*Euterpe oleracea*), Ginseng (*Panax quinquefolius* L.), and Stevia (*Stevia rebaudiana* Bertoni) extracts treated with microwave, pulsed electric field, and conventional thermal treatment (90 °C, 60 s) against sporeforming/ non-sporeforming foodborne pathogens. The results pointed out that both microwave and pulsed electric field increased the antimicrobial potential of the extracts against vegetative and sporulated microorganisms; while the bioactive compounds of Açaí extract were sensitive to the conventional heating method and caused its antimicrobial activity to decrease. They declared that new lines of research should be opened regarding validation of the antimicrobial potential of these ingredients when integrated into real food matrices (Pina-Pérez *et al*., 2018).

However, some articles investigate the thermal stability of encapsulated essential oils. For example, Garcia-Sotelo *et al.* (2019) encapsulated rosemary essential oil within β-cyclodextrin and proved that thermal stability and antimicrobial capacity of the encapsulated essential oil (Garcia-Sotelo *et al*., 2019). Fonseca *et al*. (2020) encapsulated the thyme essential oil with starch by electrospinning method and found that nanofibers with 5% Thyme essential oil retained up to 50% of the phenolic compounds after thermal treatment (Fonseca *et al*., 2020). Based on the mentioned articles, it seems that the encapsulation process is an effective solution in preserving the bioactive compounds of essential oils during thermal processing.

Conclusion

The results showed that both essential oils of *O. decumbens* and *P. atlantica*'s gum have antimicrobial activity against examined microorganisms that are the leading cause of bread spoilage according to the MIC, MBC, MFC, and also disk diffusion test. Overall, the essential oil's content does not change significantly during the thermal process, so the authors declared that both oils were resistant to thermal processing (200°C- 10 minutes). These essential oils have the potential to be employed at active film to preserve food products. The authors suggested that the essential oils of *O. decumbens* and *P. atlantica* gum could be used in food packaging, such as bread, and bakery products, and also in the formulation of bakery products to increase food safety and reduce food pathogens risks.

Conflict of interest

The authors declare that there is not any conflict of interest between them.

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اسانس لعل کوهستان و صمغ بنه: ارزیابی خواص ضد میکروبی و شیمیایی در طی فرآیند حرارتی

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چکیده

اسانس‌های ضروری معمولاً در برابر میکروارگانیسم‌های نامطلوب تاثیر خوب و بسزایی دارند. بنابراین می‌توان از آن‌ها به عنوان عوامل ضد میکروبی طبیعی در مواد غذایی یا بسته‌بندی آن‌ها استفاده کرد. در این تحقیق خصوصیات ضد میکروبی دو اسانس (صمغ بنه و لعل کوهستان) قبل و بعد از فرآیند حرارتی (۱۰ دقیقه– ۲۰۰ درجه سانتی‌گراد) در برابر فساد باکتری و کپک در نان بررسی شده است. همچنین ترکیبات اسانس‌ها با کروماتوگرافی گازی- طیف‌سنجی جرمی شناسایی شدند. ترکیبات اصلی اسانس لعل کوهستان کارواکرول، تیمول و المیسین قبل و بعد از عملیات حرارتی بودند. در مورد صمغ بنه، تنها یک پیک شاخص در کروماتوگرام مشاهده شد که مربوط به آلفا-پینن بود. برای هر دو اسانس، کمترین غلظت ممانعت کنندگی (MIC) و کمترین غلظت قارچ‌کشی (MFC) در برابر *آسپرژیلوس نایجر* به ترتیب ۴۰۰۰ و ۸۰۰۰ میکرولیتر بر میلی‌لیتر بود. اثر ضد میکروبی هر دو اسانس در برابر *باسیلوس سوبتیلیس* بیشتر از کپک بود. مقدار MIC و MBC برای لعل کوهستان به‌ترتیب ۱۲۵ و ۲۵۰ میکرولیتر در میلی‌لیتر و برای صمغ بنه ۶۲/۵ و ۱۲۵ میکرولیتر بر میلی‌لیتر بود. نتایج نشان داد که این دو اسانس اثر امیدوارکننده‌ای بر میکروارگانیسم‌های اصلی فساد نان دارند. فرآیند حرارتی تأثیر معنی‌داری بر فعالیت ضدمیکروبی اسانس صمغ بنه در برابر آسپرژیلوس نایجر نداشت، اما به طور قابل‌توجهی فعالیت ضدمیکروبی علیه باسیلوس سوبتیلیس را کاهش داد، در حالی که در مورد فعالیت ضد میکروبی اسانس لعل کوهستان، نتایج برعکس بود. با توجه به اینکه کپک‌ها بیشترین عامل فساد نان هستند، استفاده از اسانس صمغ بنه به عنوان نگهدارنده طبیعی در محصولاتی که عملیات حرارتی بالایی را تحمل می‌کنند مانند نان و فرآورده‌های نانوایی توصیه می‌شود.

واژه‌های کلیدی: پایداری حرارتی، صمغ بنه، کروماتوگرافی‌گازی، لعل کوهستان، MBC/MFC

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