Chemical Indices and Antibacterial Properties of Some Essential Oils of Apiaceae and Lauraceae Spices in Southwest of Algeria

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ABSTRACT
This study is a part of the valorization of extract from three most commonly used Algerian spices, namely; caraway and cumin seeds and cinnamon bark. On the one hand, it aims at characterizing the chemical indices of extracted essential oils and evaluating the antibacterial activity of each essential oil by titration and disc diffusion method respectively. On the other hand, it attempts at evaluating the combined action of essential oils against four reference pathogenic bacterial strains, namely Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis by well and Chabbert-type diffusion method. The essential oils obtained by the hydrodistillation method have a relatively average extraction about 1.43, 2.3 and 2.5%, respectively for caraway, cumin, and cinnamon. The acid index indicates the behavior and amount of free acids present in the essential oil, in which the acid and saponification indices of cinnamon essential oil indicate a value of 4.48 and 168.56 respectively. It can also inform us about the susceptibility of the oil to undergo alterations. The antibacterial activity results showed that cinnamon essential oil (EO) proved to be the most active against the tested bacterial strains; caraway EO was active against Enterococcus faecalis, and the antibacterial action of cumin EO was the lowest. However, the association of the extracted essential oils has a higher synergistic effect than the independent effect of each essential oil, in which the MIC value found was estimated at 10 to 20 (V/V), 40 to 50 (V/V) and 50 to 70 (V/V) respectively for cinnamon, cumin and caraway. The obtained results show that the response to the antibacterial activity varies according to the plant species used and the extract tested alone or in combination.

Keywords: Antibacterial effects, Bechar (Algeria), Chemical indices, Essential oils, Interaction, Spices.

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INTRODUCTION
The antimicrobial effects of different species of herbs and spices have long been known and used to increase the shelf life of foods. Thus, essential oils and their components, currently used as food flavorings are also known to have antimicrobial activities and could, therefore, serve as food preservatives, especially since they are mostly classified as generally recognized as safe (GRAS), or approved as food additives by the Food and Drug Administration. Therefore, they do not need food authorization (Baser et al. 2002; Burt, 2004). Based on the current functionality of the GRAS list, it is advisable not to use it as a basis for counseling patients on the safe use of aromatherapy or essential oils, so preliminary studies are needed to better understand their antimicrobial activity (Manion and Widder, 2017).

Caraway (Carum carvi L.), cinnamon (Cinnamomum verum) and cumin (Cuminum cyminum L.) occupy a prominent place in the food, used not only as an additive (Saiedirad et al. 2008), but also it has medicinal properties known since the antiquity as an antioxidant, diuretic, astringent, hypoglycemic, chologogue, stomachic and antimicrobial effect (Dhandapani et al. 2002). For this reason, scientists strongly return to nature and use new phytosynthetic substances which prove the effectiveness of plants and their extracts (Vaubourdolle, 2007).

Indeed, the evaluation of phytotherapeutic properties as an antimicrobial agent of aromatic and medicinal plants led us in this study to characterize the chemical indices and evaluate the antibacterial activity of spices essential oils which belong to the family of Apiaceae and Lauraceae.

MATERIALS AND METHODS
Plant Raw Material
This study aims at analyzing the fresh vegetative parts that were collected on the local market of Bechar city (Algeria) as follows: cumin and caraway seeds and cinnamon bark during the year 2018.

The extraction of essential oils was carried out by hydro-distillation method in a Clevenger-type apparatus (Clevenger, 1928; Kalemba and Kunicka, 2003).
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Extraction of the Essential Oil
This method consists of immersing a quantity sample (100 g) of the plant raw material part in a ball ‘monocolumn flask’ containing distilled water. The mixture is brought to the boil using a ball heater for 5 to 6 hours. The vapor charged of essential oil passes through the refrigerant to be condensed and recovered in an Erlenmeyer flask (Bourrel, 1993).

The essential oil yield has been determined in relation to the dry matters which are stored in tight opaque vials at +4°C in the presence of sodium sulfate anhydrous until used (AFNOR, 2000).

Chemical Indices

Acid Index
The acid index is used to check the quality of essential oil, especially concerning its degradation over time during storage (Dipage, 2010).

The acid index has been measured by volumic titration, which in an erlenmeyer flask, a solution of m in (mg) of essential oil is mixed with 5 mL of 95% ethanol and 5 drops of phenolphthalein 0.2%. This solution is neutralized with an ethanolic solution 0.1 M of potassium hydroxide (KOH).

The titration is over when the pink color begins to appear and persists for at least 15 sec, we note the volume V in (mL) of the potassium hydroxide solution ‘KOH’ that causes this color change, and it was calculated by the following formula (NF ISO 1242, 1999):

\[ I_a = \frac{5.61 \times V}{m} \]

Where:
I_a: Acid index
V: Volume in (mL) of the used KOH solution
m: Mass of the test sample in (g)

Saponification Index
The saponification index corresponding to the mass of potassium hydroxide (KOH) (in milligrams) that is necessary for the neutralization of the combined fatty acids in one gram of fat body.

In a 250 mL monocolumn flask equipped with a reflux condenser, the test sample m (mg) is introduced with 25 mL of 0.5 M alcoholic potassium hydroxide and a few glass beads. The refrigerant is adapted and refluxed for 30 minutes. 1ml of phenolphthalein as color acid-bases indicators is added to the solution. The latter is titrated immediately (while the solution is still hot) with 0.5 M hydrochloric acid (sample test).

A blank test is carried out under the same conditions (Blank test).

The saponification index is calculated by the following formula (NF EN ISO 3657, 2013):

\[ I_S = \frac{(V_t - V_e) \times C_{HCl} \times M_{KOH}}{m} \]

Where,
I_s: Saponification index
V_t: Volume in (mL) of HCl (0.5M) poured, control or blank test.
V_e: Volume in (mL) of HCl (0.5M) poured, test sample.
C_{HCl}: Concentration of HCl in (mol /L)
M_{KOH}: Molar mass of KOH (56.10g/mol)
m: Mass of the test sample in (g)

Ester’s Index
This is the needed amount (in milligrams) of potassium hydroxide (KOH) to saponify one (1) gram of oil-free of fatty acid, which is calculated from the saponification index (I_s) and the acid index (I_a) according to the following formula (NF ISO 709, 2002):

\[ I_e = I_s - I_a \]

Where:
I_e: Ester’s index.
I_s: Saponification index.
I_a: Acid index.

Microorganisms
The evaluation of the antibacterial activity of spices’ essential oils selected for this study was conducted in accordance with official methods.

However, the bacterial strains tested were chosen based on their pathogenicity. These are the reference strains that come from the Pasteur Institute of Algeria, under ATCC collection (American Type Culture Collection), cited as follows: Escherichia coli ATCC 25922; Pseudomonas aeruginosa ATCC 27856; Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212.

Antibacterial Tests
The bacterial colonies that have been isolated after incubation at 37 °C for 18 to 24 hours on nutrient agar medium (Fluka, India) were transplanted into tubes containing sterile physiological water to prepare bacterial suspensions having a turbidity equivalent to 0.5 McFarland. After that, the previously prepared bacterial suspension was seeded over the entire Mueller Hinton (MH) agar surface (Himedia, India) by tight streaks.

The study of the antibiotic susceptibility was performed by disc diffusion method on MH agar medium using antibiotic discs as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2017).

Antibiotic resistance results were interpreted according to the criteria of the Antibiogram Committee of the French Society for Microbiology (SMF) (Soussy et al. 2000).

The antibiotic discs used by disc diffusion assays were ranked firstly for E. coli as follows: fosfomycin, chloramphenicol, ceftazolin and amoxicillin + clavulanic acid; secondly for S. aureus: sulfamethoxazole, erythromycin, oxacillin, vancomycin and fosfomycin, and thirdly for P. aeruginosa: ticarcillin, fosfomycin, tobramycin, ceftazidim and...
ciprofloxacin, and finally for *E. faecalis*: ampicillin, clindamycin, tetracycline and gentamicin.

The antibacterial activity of the essential oils was determined by disc diffusion method mentioned by Sacchetti *et al.* (2005); Yesil-Celiktas *et al.* (2007). This method consists of replacing the antibiotic discs by other confined discs from Whatman paper impregnated with the essential oil (Hayes and Markovic, 2002).

The results of antibacterial activity were interpreted according to the diameter of the inhibitory zone around the discs and that is as follows (Benyagoub *et al.* 2015; Benyagoub, 2015):

- < 8 mm : resistant strains (–)
- 9 mm ≤ diameter ≤14 mm: sensitive strains (+)
- 15 mm ≤ diameter ≤19 mm: very sensitive strains (++)
- > 20 mm: extremely sensitive strains (+++)

For studying the combined antibacterial effect of two essential oils, we used *Chabbert*-type diffusion method, where the two essential oils tested are diffused from two confined strips of filter paper arranged at right angles (Daguet and Chabbert, 1985). Also, the conjugated action of three essential oils was evaluated by well diffusion method. There were mixed in 1:1:1 ratio. The agar plate surface (Mueller Hinton agar) is inoculated by spreading a volume of the bacterial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm has punched aseptically with a sterile cork borer or a tip, and a volume 10 µL of the extract is introduced into the well. Then, agar plates are incubated at 37°C for 24 hours (Balouiri *et al.* 2016).

**Determinations of Minimum Inhibitory Concentrations (MICs)**

The aim of agar dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine the susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents. Agar dilution involves the incorporation of different concentrations of the antimicrobial substance (Essential oils diluted in dimethyl sulfoxide 'DMSO' as diluent) into a nutrient agar medium (Mueller Hinton agar) followed by the application of a standardized number of cells (10^6 CFU/mL) to the surface of the agar plate (Wiegand *et al.* 2008).

**Results**

**Essential Oil Yield**

The average yield of the essential oils has varied according to the used plant. The extracted oils have organoleptic properties characterized by a yellow color, limpid and strong odor. Through these results, we note that cinnamon is the richest in essential oil (2.50 %) compared to cumin (2.3 %). However, the caraway seeds have a low yield estimated at (1.43%).

**Chemical Indices**

The chemical analyses results of the essential oils are presented in Table 1.

| Essential Oil | Extraction Yield (%) | Acid Index | Saponification Index | Ester’s Index |
|---------------|-----------------------|------------|----------------------|--------------|
| Caraway       | 1.43                  | 2.75       | 148.56               | 145.81       |
| Cumin         | 2.3                   | 2.244      | 153.11               | 150.866      |
| Cinnamon      | 2.5                   | 4.488      | 168.56               | 164.072      |

The values of the acid and saponification indices of the cinnamon essential oil were higher than other examined essential oils.

**Antibacterial Tests**

The results of the multidrug-resistant profiles of the bacterial strains are represented in Table 2.

These results demonstrate that the resistance rate was increased in most tested strains with several antibiotics namely oxacillin, fosfomycin and clindamycin for *Staphylococcus aureus*. However, *Escherichia coli* was resistant to amoxicillin + clavulanic acid. The strain *E. faecalis* was resistant to ampicillin, clindamycin, erythromycin, and sensitive to tetracycline. While, *Pseudomonas aeruginosa* was sensitive to all tested antibiotics namely fosfomycin, gentamicin, ticarcillin, tobramycin and ceftazidime (Fig. 1).

**Agar Diffusion Method**

For the evaluation of the antimicrobial potential of these extracts, it has been preferred to test them against several targets, since each of them has cell structures and a particular metabolism.

The disc diffusion method is often used to evaluate the antibacterial activity of natural substances and plants’ extracts. The diameters means of the inhibition zone around the impregnated discs of essential oil are presented in the figure below (Fig. 2).

We concluded that the essential oil of cinnamon has a very strong activity on all tested bacterial strains. This effect is represented by a maximum inhibition value of 27.77% against *E. faecalis*. Then, the cumin is placed in second place. However, the antimicrobial activity of caraway appears lower (Table 3).
Well Diffusion Method

The results of the antibacterial activity carried out by good diffusion show that radially oils diffusion giving an inhibitory effect with an estimated inhibition percentage of 27.77; 32.22; 33.33 and 35.55% against E. coli, P. aeruginosa, S. aureus and E. faecalis strains, respectively (Table 4) (Fig. 3).

Determinations of MICs

After evaluating the antibacterial effect of the extracted essential oils, those showed a variable activity on the tested strains where it was necessary to determine the MIC value of each EO. These results are presented in the table below (Table 5).

Based on the results shown in the table above, cinnamon essential oil had a MIC of 10 to 20 (v/v), 40 to 50 (v/v) for cumin EO and 50 to 70 (v/v) for caraway EO.

Chabbert-type Diffusion Method

The obtained results from the effect of the associations of two essential oils revealed switching between indifference and synergetic effect (Table 6).

The results of the antibacterial activity of the combined action of two essential oils show that the majority of the

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**Table 2: Multidrug-resistant profiles of the tested bacterial strains.**

| Multidrug       | Bacterial strains           |
|-----------------|-----------------------------|
| AMC             | Escherichia coli            |
| FOS-CM-OX       | Staphylococcus aureus       |
| AM-CM-E         | Enterococcus faecalis       |

AMC, amoxicillin+clavulanic acid; FOS, fosfomycin; CM, clindamycin; OX, oxacillin; AM, ampicillin; E, erythromycin

**Fig. 1**: Antibiogram assay of the bacterial strains tested on Mueller Hinton agar.

(a) E. coli, (b) P. aeruginosa, (c) S. aureus, (d) E. faecalis

**Fig. 2**: Antibacterial tests of essential oils against the bacterial strains tested on Mueller Hinton agar.

(a) E. coli, (b) P. aeruginosa, (c) S. aureus, (d) E. faecalis

A: C. carvi L EO; B: C. cyminum L EO; C: C. verum EO; T: Diluent for the essential oils (DMSO) used as a control (a) E. coli, (b) P. aeruginosa, (c) S. aureus, (d) E. faecalis.

**Table 3**: Diameters zone values (mm) and inhibitions percentage (%) of the tested essential oils

| Bact. S.    | C. carvi L EO | C. cyminum L EO | C. verum EO |
|-------------|---------------|-----------------|-------------|
|             | D (mm) | I (%)         | D (mm) | I (%)         | D (mm) | I (%)         |
| E. coli     | 6      | 6.66          | 9      | 10            | 23     | 25.55         |
| P. aeruginosa | 9      | 10            | 6      | 6.66          | 22     | 24.44         |
| S. aureus   | 6      | 6.66          | 10     | 11.11         | 23     | 25.55         |
| E. faecalis | 14     | 15.55         | 8      | 8.88          | 25     | 27.77         |

Bact. S., bacterial strains; D, diameter of inhibition zones (mm); I (%), inhibition percentage; EO, essential oil; C. carvi, carum carvi; C. cyminum, cuminum cyminum; C. verum, cinnamomum verum
Several researches confirmed the technical impact of extraction method. It should be mentioned that the studies on the extraction of essential oils from cumin and caraway have shown that their essential oil yield is important according to the method of steam distillation by water compared to the hydrodistillation method (Aouf, 2002).

Among the factors influencing the essential oil yield is the duration extraction, according to Careaga et al. (2003), where the extraction yield of the essential oils of some Apiaceae conducted by Naenia et al. (2011), shows a variation between the distillation for 6 and 12 hours.

The quality of essential oil and its commercial value are defined by accepted standards relating to physicochemical indices (Taleb-Toudert, 2015). According to the obtained results, the values of the acid index of the extracting essential oils are in agreement with the bibliographic data which is (1 to 3.33) and (1 to 2.55) for the caraway and cumin seeds respectively (Aouf, 2002). The chemical indices results were slightly lower compared to the French standard (AFNOR, 2000).

It should be noted that the saponification index classifies oils according to the length of the fatty acid chains that compose them, a criterion related to the molecular weight (MW) of fatty acids. So when the molecular weight (MW) of the fatty acids is high, the saponification index is low. Indeed, when the MW is high, so more carbon chains of fatty acids are long and less labile (hydrolyzable) (Baaziz, 2018).

The study results conducted by Gachkar et al. (2007) on the antibacterial activity of cumin seeds’ essential oil showed an antibacterial effect against Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes.

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**Table 4:** Diameters values of the inhibitory zones (mm) and inhibition percentage (%) of combined three essential oils

| Bacterial strains | Mixture of the three essential oils |
|-------------------|------------------------------------|
|                   | D (mm) | I (%) |
| E. coli           | 30     | 33.33 |
| P. aeruginosa     | 32     | 35.55 |
| S. aureus         | 29     | 32.22 |
| E. faecalis       | 25     | 27.77 |

Essential oils have a more synergetic effect between them against the tested bacterial strains compared to the antibacterial effect of each essential oil. The combined essential oils of caraway/cumin had an indifference effect for all tested bacterial strains. While, the combined essential oils of (caraway/cumin), (cumin/cinnamon) and (caraway/cinnamon) had an indifference effect for E. faecalis strain (Fig. 4).

**Discussion**

The essential oil yield of the aromatic plants that we have studied was variable. This variability is probably due to the variation of the following factors: extraction conditions, a period of harvest and the variations in the culture condition (Galambosi et al. 1996), stage of growth, pedo-climatic and seasonal conditions (Gonny et al. 2004).

Thus, other factors contribute to the variation of the essential oil yield such as the treatments which can be carried out before the hydro-distillation (grinding, dilacerations, chemical degradation, pressure, and agitation).

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**Table 5:** MICs of the extracted essential oils against the tested bacterial strains

| Bacterial strains | MICs (V/V) | C. carvi | C. cuminum | C. verum |
|-------------------|-----------|---------|---------|--------|
| E. coli           | 50        | 40      | 10      |
| P. aeruginosa     | 60        | 50      | 20      |
| S. aureus         | 70        | 50      | 20      |
| E. faecalis       | 60        | 50      | 20      |

C. carvi, carum carvi; C. cuminum, cuminum cuminum; C. verum, cinnamomum verum

**Table 6:** Combined antibacterial activity of two essential oils against the tested bacterial strains

| Bacterial strains | Combined essential oils |
|-------------------|-------------------------|
|                   | A          | B          | C          |
| E. coli           | Ind        | Ind        | Syn        |
| P. aeruginosa     | Ind        | Syn        | Syn        |
| S. aureus         | Ind        | Ind        | Syn        |
| E. faecalis       | Ind        | Ind        | Ind        |

A, caraway/cumin, B, cumin/cinnamon; C, caraway/cinnamon; Ind, indifference effect; Syn, synergetic effect
Another study conducted by Fabry et al. (1998) showed that cinnamon essential oil was active against *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*. According to Naik et al. (2017). The biological activity of *Cinnamomum* essential oils is probably due to its prominent concentration of cinnamaldehyde. Thus, caraway essential oil is able to inhibit the growth of *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholera* and *Mycobacterium tuberculosis* (Bocianowski, 2012), as well as the membrane of Gram-negative bacteria is richer in lipopolysaccharides and in protein compared to gram-positive bacteria which makes these bacteria more hydrophilic and prevents terpenes from adhering to the membrane (Marzouk et al. 2006).

The essential oils have a broad spectrum of action since they inhibit the growth of bacteria as well as those of molds and yeasts. Their antimicrobial activity is mainly a function of their chemical composition, and in particular of the nature of their major volatile compounds. They act by preventing the multiplication of bacteria; their sporulation and the synthesis of their toxins (Oussalah et al. 2006).

The activity of the association of essential oils is related to its chemical compositions, the functional groups of the major compounds (alcohols, phenols, terpene compounds, and ketones) of each essential oil and the possible synergistic effects between these components.

Noting that the major volatile compounds that exhibit the most important antimicrobial properties, and in particular phenols, alcohols and aldehydes.

In this regard, many researchers argued that there are many mechanisms of antimicrobial interaction that produce synergism. Probably the main reasons for this are sequential inhibition of a common biochemical pathway, inhibition of enzymes, protein synthesis, nucleic acid synthesis, disintegrated the outer membrane. Other authors proposed that the synergistic effect could be due to the similarity of their mechanism; or maybe due to act on the different targets (Gadisa et al. 2019).

Thus, the natures of the chemical structures that constitute it and their proportions play a determining role. In this way, the combined effect of essential oils is determined in a significant way (Lahlou, 2004).

The study carried out by Hermal (1993), showed a synergistic effect of the association of essential oil of cinnamon and thyme against *S. aureus*. However, the combined action of essential oils on gram-negative bacteria
i.e., P. aeruginosa and E. coli was not effective compared to the independent action of each essential oil.

It is reported in the literature that essential oils have antioxidant and antiradical properties that improve the shelf life of the food and also interest the consumer in their nutraceutical values and health benefits.

Thus, the incorporation of essential oils directly into the food or the application by spraying the surface of the food help to control the microbial flora and preserve the food oxidation phenomena.

The addition of essential oils in food could give it a nutraceutical value. Other biological properties of oils, such as antiparasitic, insecticide, antifungal, and antiviral properties could be considered to meet the requirements of organic farming by developing biopesticides or animal feed supplements, enriched with natural substances effective against infections.

Although these properties could be an answer, in the future, to the problem of antibiotics and their resistance, and have an application in human and animal health (Burt, 2004).

CONCLUSION

The study aims at characterizing the chemical indices and at evaluating the antibacterial power of the essential oils of three spices selected based on them wide consumption in Algeria namely caraway, caraway and cinnamon.

The extraction yield carried out by hydrodistillation revealed that the best yield is given by cinnamon, followed by caraway and then caraway by a rate of 2.5, 2.3 and 1.43%, respectively.

The antibacterial activity results showed that cinnamon essential oil (EO) proved to be the most active one against the tested bacterial strains; caraway EO was active against Enterococcus faecalis, whereas the antibacterial action of cumin EO was the lowest.

Based on the present study combined the three essential oils were found to have a more antibacterial effect than single EO or that conjugated of two essential oils which had most often a synergistic or indifference effect.

More in-depth studies on the composition of extracted essential oils make it indispensable to relate the synergistic effect between the functional groups and the majority compounds (alcohols, phynols, terpenic and ketonic compounds). However, it’s likely that compounds of each essential oil act in a different way (synergistic, indifference or antagonism).

Based on the results found, it can be predicted that the extracted essential oils from Apiaceae and Lauraceae family possess an important antibacterial activity, and can be helpful in the pharmaceutical field for combating multidrug-resistant strains or in the food field, which is particularly relevant for its potential use as a preservative.

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AUTHORS' CONTRIBUTION

• Spices’ collection/essential oils’ extraction: Dr L Rouisset and Ms D Razni
• Antibacterial and chemical tests: Dr E Benyagoub, Ms D Razni
• Writing of the original manuscript: Dr E Benyagoub
• Writing-examination and editing: Dr L Rouisset.

All authors have read and approved the final draft of the manuscript.

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