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ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OIL AND METHANOLIC EXTRACT OF MOROCCAN *CORIANDRUM SATIVUM*

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Abstract

Coriandrum sativum (*C. sativum*) is amongst the most useful spices in Morocco. To the best of our knowledge, no previous research has evaluated the antioxidant capacities of Moroccan coriander. In the present study, we evaluated for the first time the antimicrobial and the antioxidant potential of the methanolic extracts and essential oil of Moroccan coriander seeds. On the one hand, the antimicrobial activity was tested by disc diffusion method. This test was done on bacteria and yeast isolates from clinical environments. On the other hand, the antioxidant activity was evaluated by two methods DPPH and β-Carotene bleaching test. Coriander oil expressed highest antibacterial action against *Listeria monocytogenes* which was significantly ($P < 0.05$) better than Ampicilin. The least activity was expressed against Candida albicans (18 ± 0.25). Noteworthy, antioxidants of Coriander's essential oils and methanolic extracts were put to the test using DPPH radical scavenging and b-carotene-linoleic. Methanolic extracts of coriander have shown better antioxidant activity in all tests than essential oils. Worth noting, adding coriander to food will increase the antioxidant content and inhibit harmful oxidation.

Keywords: *Coriandrum sativum; antimicrobial activity; antioxidant activity.*

Introduction

Medicinal plants, aromatic herbs, spices and their extract have many uses in the food flavouring and preservation, fragrance and pharmaceutical industries [1-2]. Noteworthy, there has been a growing demand for antimicrobials to keep clear of microbial food spoilage and bacterial infections. To that end, interest in the curative properties of herbs is increasing as a substitute for synthetic preservatives and antibiotics [3-4]. Many EOs are already used in the food industry as flavoring agents and some are known to have antimicrobial activity [5-6].

Coriandrum sativum is a plant from the family of Apiacease. The dry fruits, named coriander seeds or coriandi seeds, are used as seasoning. Seeds from coriander are used to treat indigestion, cough, bronchitis, vomiting, diarrhea, dysentery. The antibacterial and antioxidant activities of *Coriandrum sativum* varies both qualitatively and quantitatively in relation to the method of extraction, type of the cultivar and the area of harvest [7-10]. The spice is also recognized for its effects on the metabolism of carbohydrates and lipids. It has been shown that the essential oil and various coriander extracts exhibit antibacterial, anticancer, antimutagenic, antioxidant, and free radical scavenging activities.

On the basis of the above, the present study aims at evaluating antibacterial activity of methanolic extract and oil essentiel of *coriandrum sativum* against Gram-positive and Gram-negative bacteria.

Materials and methods

Preparation of methanolic extracts

The plants had been ground with a grinder to a fine powder. Thereafter, the powdered plant material (10 g) was extracted using a Soxhlet type extractor with 100 mL methanol (MeOH) at 60°C for 6 h. Then, the extract was filtered and evaporated (Rotavator, Buchi, Switzerland) to dryness under vacuum at 40°C with a rotary evaporator. After determining the yield, the extract was stored at 4°C until further analyses [11].

Preparation of EOs

The plant hydrosols were generated by hydrodistillation method Clevenger. Plant materials (10 g), cut into small pieces, were placed in a flask with 100 mL of double distilled water and hydrodistilled for 1 h. After hydrodistillation, the oil was collected in cooling vapor to separate the essential oil of the plant. The blend without essential oil in the flask was identified as hydrosol. The hydrosol was filtered and kept in 4 ° C sterile dark bottles until further analysis [12].

Determination of antagonistic activities of Coriander

Disc diffusion method was used. The disks were made ready using Whattman paper with a diameter of about 6 mm. Next, the paper has been pasteurized for 2 hours using a 1600 C hot air oven. The prepared paper had been immersed aseptically into the extract. Then, sterile nutrient agar was aseptically poured into sterile Petri dishes and allowed to harden. Test organisms were taken from overnight culture to inoculate the dried agar Splates by streaking. Sterile forceps was used and discs were aseptically placed on the surface of Agar plates. Ampicilin was used as a control. The plates were incubated at 37°C for 24 hours. The plates were examined for zones of inhibitions [13].

DPPH assay

The evaluation of the DPPH radical scavenging activity was determined in consonance with Maadabe et al. (2015) [14]. DPPH solution was prepared in methanol at 0.2 mmol. Then, two mL of methanolic extracts and essential oils at different concentrations were blended with 0.5 mL DPPH methanolic solution. After that, the blend was incubated in the dark for 30 min. The absorbance was evaluated at 517 nm. The samples were tested in triplicate. BHT has been used for positive control. The antiradical activity was typified as IC50 (µg / mL), the concentration required to induce a DPPH inhibition of 50%. The potential to scavenge the DPPH radical was calculated using the equation below:

DPPH scavenging effect (%) = $[(A_0 \times A_1) / A_0] \times 100$

A_0: is the absorbance of the control at 30 min,.

A_1: is the absorbance of the sample at 30 min.

β-Carotene bleaching test

The method reported by Loucif et al. (2020) [15] has been applied with a slight modification. To prepare the stock solution of B-carotene and linoleic, 0.5mg of b-carotene was liquified in 1mL of chloroform. Then, 40 mg of linoleic acid and 400 mg of Tween 40 were added to the solution of b-carotene. The chloroform was evaporated. One-hundred mL of aerated water was added to the remainder. Reference compounds (BHT and BHA) and sample extracts were prepared in methanol. 3 mL of emulsion was added to a tube that contains 0.2 mL of different extract and essential oils concentrations (500, 700, and 1000 µg / mL). The absorbance was immediately measured at 470 nm and the test emulsion was incubated during 120 min in a water bath at 50°C, when the absorbance was evaluated again. In the negative control, the extract was replaced with an equal volume of methanol and BHT was used as positive control. The antioxidant activity (%) of extracts was evaluated in terms of the bleaching of the b-carotene using the following formula:

% Inhibition = $[(A_t - C_t) / (C_0 - C_t)] \times 100$

Where

A_t is the absorbance value obtained for the test sample

C_t is the control after 120 min incubation

C_0 is the absorbance value for the control obtained at zero time during incubation.

The values are expressed as IC50 values (µg / mL), the concentration required to induce a 50 per cent inhibition of b-carotene bleaching.

Statistical analyses

All experiments were done in triplicate. Statistical analysis was performed using SPSS software. The results showing $p < 0.05$ were considered as significant.

Results and discussion

Though spices and herbs have been added to foods to impart, intensify or change their flavours, their antioxidant and antimicrobial effects have applied to their applications. Coriander is one of the most important spices used in Morocco. Accordingly, it was important to study its antimicrobial and antioxidant activities.

The antibacterial activity was measured in mm and expressed as Mean±SD. The findings of bacterial growth inhibition of coriander oil, methanolic extract and ampicillin are listed in Table 1.

The research was conducted on isolates from clinical pathogens. The findings of the antibacterial effect of EO and methanolic extract were compared with standard ampicillin antibiotic, which were tested under similar conditions against the same above mentioned bacteria.

Coriander oil has had an inhibitory effect on all bacteria tested ($p<0.05$). Coriander oil has shown full antibacterial activity against *Listeria monocytogenes* (30.5 ±1.02) superior to ampicilin (19.5±0.5; $p<0.05$). The least activity against *Candida albicans* was demonstrated (18 ± 0.25), and it was also higher to the behavior of Ampicillin (16.5 ±0.3; $p<0.05$). Additionally, antimicrobial effect of Ethanol has a similar effect against *Listeria monocytogenes, Salmonella enteritidis, Enterococcus faecium and Candida albicans*. In general, *Coriandrum sativum* essential oil had a greater and wider pectrum of antimicrobial activity than ethanol extract ($p<0.05$).

EOs of aromatic and medicinal plant usually have antimicrobial and antioxidant properties. They can be used as food flavoring agents or preservatives and for medicinal purposes as well. Moreover, the most important microorganisms in safety food were tested (*Listeria monocytogenes, Salmonella enteritidis* and *Staphylococcus aureus*). These bacteria were influenced by EO of *C. sativum*. However it would be useful to use EO of C. sativum in the food preservation industry because it allows for the best reduction of microorganisms and it is also known for its excellent sensory influence.

Microbial resistance to antimicrobials is a matter of considerable importance if resistant strains supplant susceptible strains. Simply put, resistance can increase in micro-organisms by the spread of

resistance gene between different microbial species Which is necessary for the development of new antimicrobial drugs.

Interestingly, the findings of the present study are consistent with those of Yildiz (2016) [16] as strong antibacterial activity of coriander oil against Listeria monocytogene and important antibacterial activity against all bacteria tested in this study were also observed by Serban et al. (2011) [17] and Suganya et al. (2012) [18].

Table 2 presents the antioxidant activity as evaluated by two different methods, DPPH and β-carotene-linoleic acid. The DPPH method with stable organic 1,1-diphenyl-2-picrylhydrazyl was used to measure the efficiency of free radical scavenging, usually expressed as IC50, The Antioxidant content required to lower the initial concentration of DPPH by 50%. The greater IC50 value has shown weak antioxidant activity. The methanolic extract of coriander has demonstrated higher scavenging ability on DPPH radicals (IC50 values is 32.00±3.57 lg/mL). When compared to this reported for essential oil (IC50 = 60000 µg/mL: $p<0.05$). The antioxidant activity results are similar to those reported by Sriti et al. [19] for Tunisian coriander. Besides, Moroccan *Coriandum* was higher antioxidant activity in comparison with Canadien *Coriandum* [19]. A study conducted by Yildiz (2016) [16] revealed that the essential oil did not show any activity with DPPH.

The observations reported here are in accordance with several previously published studies which have shown more antioxidant activity in polar solvent extracts than in lower polarity solvents. Further, the antioxidant activity of methanol extract and essential oil was also analyzed using the β carotenelinoleate bleaching method. β-carotene has strong biological activity and is a compound with important physiological importance. Methanolic extract demonstrated height ability to prevent the bleaching of b-carotene (IC50= 240.25 ± 16.35 ug/mL) in comparison with essential oil (IC50= 54000 ±33.34 ug/mL). Basically, methanolic extracts and essential oil had lower antioxidant activities than BHT with IC50 of 76.65 ± 7.68 ug/mL. These results were similar to those reported by Msaada et al. (2017) [20] for Egyptian and Syrian coriander.

Worth noting, oxidation profoundly affects the quality of food and shortens its shelf life by adversely affecting its appearance, texture, sensory properties, and nutritive value. That is to say, all major food components are susceptible to oxidation, resulting in changes in flavor and aroma (lipids), texture and functionality (proteins), and loss of nutritive value (vitamins). Moreover, eating a wide variety of chemical additives is linked to health risks. That is why; the use of natural oxidation inhibitors in food processing is extremely important.

Conclusion

As has been noted above, significant antibacterial activity was shown by coriander (*Coriandrum sativum*) essential oils and significant antioxidant activity was observed. More extensive studies are called for to isolate and formulate the active components responsible for antibacterial and antioxidant action. This will pave the way for formulating powerful biological additives.

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Table 1. Antagonistic effect of the methanolic extracts and essential oil (mm) of Cordiandrum Sativum bacterial isolates.

Bacteria species	Oil essential	Methanolic extract	Ampicilin
E. coli	20.63 ± 0.4^{a1}	13.7 ± 0.3^{b1}	$15.67\pm0.4c^{1}$
Enterococcus faecalis	18.81 ± 0.2^{a2}	11.2 ± 0.25^{b2}	$16.11\pm0.5^{c1,2}$
Staphylococcus aureus	$21\pm0.35^{a1,4}$	14.5 ± 0.35^{b1}	17.1 ± 0.2^{c2}
Pseudomonas aeruginosa	28.25 ± 0.8^{a3}	10 ± 0.2^{b2}	$19.93\pm0.25c^{3}$
Enterococcus faecium	$22.9\pm0.2^{a4,5}$	16 ± 0.3^{b3}	17.5 ± 0.3^{b3}
Bacillus subtilis	24.5 ± 0.4^{a5}	14.5 ± 0.25^{b1}	$16\pm1.2^{b1,2}$
Listeria monocytogenes	30.5 ± 1.1^{a3}	16 ± 0.35^{b3}	19.5 ± 0.5^{c3}
Salmonella enteritidis	18.5 ± 0.4^{a2}	16 ± 0.4^{b3}	$18.5\pm0.5^{a2,3}$
Candida albicans	18 ± 0.25^{a2}	16.5 ± 0.45^{b3}	17.5 ± 0.35^{a2}

Diameter of inhibition zone [mm] around the disks (6 mm) impregnated with 12 µL of essential oil.
Values are means of triplicates±SD.
Values in the same row with different superscripts (a–c) are significantly different at P< 0.05.
Values in the same colomn with different superscripts (1–5) are significantly different at P< 0.05.

Table 2. Antioxidant activities of methanolic extracts and essential oils from coriander.

Antioxidant activities	Methanol extract	Essential oil	BHT	P-value
IC50 of DPPH (µg/mL)	32±3.57	60000.00±26.67	20.10±1.61	**
IC50 of β-Carotene bleaching (µg/mL)	240.25 ±16.35	54000.00 ±33.34	76.65± 7.68	**

** P <0.01.