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Antioxidant Activities of *Cuminum cyminum* Extracts and Their Antibacterial Effects against Some Dental Caries Pathogens

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ABSTRACT

Background: The seeds of cumin (*Cuminum cyminum* L.) are widely used as the spice for their distinctive aroma, they are also commonly used in traditional medicine to treat a variety of diseases.

Methods: The present study aimed to evaluate the antioxidant activities of cumin extracts and their antimicrobial effects against six oral pathogens isolated from dental caries and supragingival plaque. The extracts were obtained by maceration with acetone, ethanol and methanol at 70% (v/v).

Result: The greatest content of polyphenols, flavonoids and tannins, was obtained with acetone (42±3.46 mg GAE/g DW), (24.49±1.24 mg EQ/g DW) and (34.95±8.71 mg EC/g DW) respectively. HPLC analysis performed to determine acetonic extract components revealed the presence of p-coumaric acid, diosmin as the most abundant compounds and several apigenins. Antioxidant activity of cumin extracts showed the greatest activity with the acetonic extract (940±42 µg/mL) followed very closely by the ethanolic extract (960±50 µg/mL). The agar well diffusion method has shown that all the extracts have an antimicrobial power against all the Gram+strains. The minimum inhibitory concentration values recorded by the studied extracts vary from 12.5 to 200 mg/mL. These findings suggest that cumin seed extracts might be effective as antibacterial agent against oral pathogens.

Key words: Antioxidant activity, Antibacterial activities, *Cuminum cyminum,* Dental caries, Phenolic extracts.

INTRODUCTION

In terms of the richness of bacterial species in the human body's microbiome, the oral cavity ranks in the top (Gao *et al*., 2018). Dental caries and periodontitis are biofilm-dependent diseases manifested within oral environment (Bowen *et al*., 2018). The majority of the components of a dental biofilm are microorganisms, which are encased in an intercellular matrix made up of organic and inorganic substances produced from saliva, gingival crevicular fluid and bacterial byproducts (Flemming *et al*., 2016). The capacity of the bacteria and microcolonies contained within the biofilm to communicate with one another is necessary for some of the special activities of biofilms (Jakubovics *et al*., 2021). Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria and Proteobacteria were shown to be the five main phyla of bacteria in dental plaque according to research utilizing molecular biology techniques (Peterson *et al*., 2013). They constitute 80-95% of the oral cavity microflora (Mosaddad *et al*., 2019). One of the main etiological factors of oral infectious diseases is *Streptococcus mutans* (Khan *et al*., 2011).

Emergence of multidrug resistance bacteria is threatening world population (Flemming and Wuertz, 2019). Among the therapeutic approaches for managing bioûlms, medicinal plants are considered as potential sources of new chemotherapeutic drugs because of their diverse phytochemicals and little or no toxic effect (Beg and Ahmad, 2000). Cumin (*Cuminum cyminum* L.) also known as cumin is a medicinal herb native to Mediterranean region belonging to the Apiaceae family. Its fruit, known as cumin seed, is yellow to brownish-gray in color and is elongated in shape with nine protuberances (Lacobellis *et al*., 2005). It is an aromatic herb and has been used in the treatment of mild digestive disorders as a carminative and eupeptic, as an astringent in broncopulmonary disorders and as a cough remedy, as well as an analgesic (De *et al*., 2003). Numerous studies have suggested that Cumin possesses various therapeutic properties, including antioxidant, antibacterial, antifungal and anti-inflammatory properties. Cumin aldehyde, Cuminum cyminum's main chemical component, contributes to its antibacterial activity (Ebada, 2017; Srinivasan, 2018).

Therefore, the purpose of the current study was to examine how different solvents affected the extraction of polyphenol from cumin extract. This study is a contribution to the prevention and treatment of dental caries pathogens and research of compounds with therapeutic potential.

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MATERIALS AND METHODS

Preparation of plant extracts

The experiments were carried out in the Laboratory of Microbiology and Vegetal biology (University of Mostaganem. Algeria) during 2021.

The seeds of cumin have been purchased at the regional market of Mostaganem province (Algeria). The solvent extraction process was completed using the Soares *et al*. (2009) method's instructions. The extracts were made by combining 5 g of grounded seeds (powder) with 100 ml of acetone 70%, ethanol 70% and methanol 70% (v/v), three different solvents with increasing polarity. Before filtering, the mixture was agitated for 24 hours at room temperature. The filtrates were concentrated using a rotary evaporator (Heidolph Laborota 4000) at 40°C under reduced pressure. For further investigation, the resultant crude extracts were kept at 4°C.

Total phenolic and flavonoid content

The total phenolic content (TPC) of the obtained extracts was determined using the Folin-Ciocalteu (Singleton and Rossi, 1965). Gallic acid concentrations were prepared as standard in methanol the results were represented as mg of gallic acid equivalents per gram of dry weight (mg GAE/g of DW). To determine the flavonoid content (TFC), the aluminum chloride colorimetric assay was used as described by Kumazawa *et al*. (2004). Quercetin was used for the calibration curve.The results were given as mg QE/g DW, or mg of quercetin equivalent per gram of dry weight.

Proanthocyanidin content

The vanillin test was used to determine the proanthocyanidin content. 1 mL of the appropriate dilution of the extract in distilled water (1 mg/mL) was combined with 2.5 mL of each of the methanolic solutions of a 1:3 (v/v) sulfuric acid and 1% (w/v) vanillin solution. A spectrophotometer (6715 UV/VIS, Jenway) was used to measure the absorbance after the tubes were incubated at 30°C for 15 minutes at a wavelength of 500 nm. The calibration curve (range from 50 to 400 g/mL) is used to quantify the tannin content in mg equivalent of catechin per gram of dry weight (CE/g).

Free radical scavenging assay (DPPH)

Using the 1, 1-dipheny l-2-picrylhydrazyl (DPPH), the antioxidant activity of the cumin extracts was evaluated in terms of their capacity to scavenge free radicals. The absorbance was measured using a spectrophotometer at 515 nm. Methanol was employed as a blank and no extract was used in the preparation of the control.

$$\text{Scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where,

A_c = Absorbance of the control reaction.

A_s = Absorbance of the test compound.

The nonlinear graph of scavenging activity (%) versus concentration of samples was used to determine the EC50 value, which is the concentration of the sample required to reduce the initial concentration of DPPH by 50%. While a greater IC 50 value implies a lesser ability to scavenge DPPH radicals, a lower absorbance of the sample suggested a stronger free radical scavenging activity.

Bacterial isolates and culture media

The current investigation includes clinical isolates that had previously been isolated by our research team (Benslimane *et al*., 2020). All samples were cultivated on various media and traditional procedures for isolation and identification were applied. Six strains (*Streptococcus mutans*, *Enterococcus faecalis*, *Gemellamorbillorum*, *Staphylococcus epidermis*, *Enterococcus bugandensis* and *Klebsiellaoxytoca*) with notable biofilm development were chosen and their molecular identities were determined (data not shown).

Antibacterial activity of cumin extracts

The agar-well diffusion assay was performed as descriebd by Valgas *et al*. (2007). Briefly, after spreading a standardized bacterial inoculum on a Mueller-Hinton agar, 50 µL of each cumin extract dissolved in DMSO (200 mg/mL) was added into the wells of 6 mm in diameter, the dishes were incubated at 30°C for 24 h and the measure of the diameter of inhibition zones was done. All tests were done in triplicate.

The microdilution method was used to test the MIC of cumin's crude ethanolic, methanolic and acetonic extracts. Each extract was prepared as a 200 mg/mL stock solution in 10% dimethylsulfoxide (DMSO) and from this solution, two serial dilutions were made, ranging in concentration from 200 mg/mL to 0.0125 mg/mL. The first line of the microplate's wells receive 100 µL of stock solution and the subsequent lines receive 100 µL of each of the different dilutions in turn. In each well, 90 µL of nutritional broth and 10 µL of inoculums (adjusted at 0.5 Mc Farland) were added. The microplates were incubated at 37°C for 24 hours. The MIC was established as the lowest extracts concentration that allowed for no discernible microbial growth (no turbidity). By subculturing 10 µL of the culture from each negative well on Mueller Hinton Agar and incubating at 37°C for 24 hours, the minimum bactericidal concentrations (MBC), or the lowest concentrations of the extract that prevented observable growth of the microorganism, were identified. These tests were all run in triplicate.

Statistical analysis

The results of each analysis were performed in triplicate and were then provided as average values with their usual derivations. With SPSS version 25, one-way analysis of variance (ANOVA) and Tukey's test were used to statistically examine the data. If $p<0.05$, the results were deemed significant.

RESULTS AND DISCUSSION

Cumin extracts analysis

Total phenolic, flavonoid and proanthocyanidin content of the 70% (acetonic, ethanolic and methanolic) cumin extracts

are summarized in Table 1. The total polyphenol contents (TPC) are in the range of 30±4.01 to 42±3.46 mg gallic acid equivalents (GAE)/g dry weight (DW), the highest TPC was obtained with 70% acetone. The total flavonoids contents in cumin extract ranged from 14.09±0.8 to 24.49±1.24 mg quercetin equivalent (QE) /g DW. The amount of flavonoids in acetonic extract was highest, whereas methanolic extract had the lowest amount. Proanthocyanidin are ranged from 25.15±0.73 to 34.95±8.71 mg CE/g, the highest level of these condensed tannins was observed with each of the acetonic and ethanolic extracts, followed by methanolic extract.This result is in line with those obtained with Bettaieb *et al*. (2012), acetonic extract at 80% gave a better performance in polyphenols in comparison with ethanolic and methanolic extracts. Another study found a higher content of polyphenols with the methanolic extract (35.5 mg EAG/g MS) (El-Ghorab *et al*., 2007), which is slightly higher than our found result (30±4.01 EAG/g MS). The difference in the yield of total phenols obtained from different plant extracts was mainly due to the difference in the nature of phenolic compounds obtained by each solvent used (Ignat *et al*., 2011).

The HPLC anlysis of acetonic extract (data not shoun) resulted in the identification of several compounds, among which the most abundant was the p-coumaric acid as the major phenolic acid and diosmin as flavonoid, the apigenines was also detected in cumin extract.These results concur with earlier research, but to a different amounts (Akroum *et al*., 2010; Bettaieb *et al*., 2012). Cuminaldehyde is present in high concentrations in the cumin extract (61.65%), Cumene, p-cymene, -pinene, acetic acid, p-cymen-7-ol and terpinene were among other constituents of the extract (Amalia *et al*., 2019). Tashtoush *et al*. (2016) revealed the presence of gallic, vanillic and chlorogenic acides in cumin seeds.

Antioxidant activity

The proportion of DPPH-scavenging activity in cumin extracts was calculated and it was contrasted with a reference ascorbic acid. The mean ± standard deviation of twice replications are shown for the data. As seen in Fig 1, the antioxidant activity of phenolic compounds varied significantly depending on the investigated solvent. At the lowest concentration (25 µg/mL), DPPH radical scavenging activity of ethanolic (2, 6%) and acetonic (2, 25%) extracts was not significantly different, whereas no antiradical activity was observed for methanolic extracts. At the highest concentration (1000 µg/mL), scavenging activity were around 16.44%, 13.87% and 17.75% for acetonic, ethanolic and methanolic extracts respectively.

The plant extracts under investigation had EC 50 values that ranged from 940±42 µg/mL to 1180±123 µg/mL. Comparing the different extracts, the acetonic cumin extract's EC50 had the lowest value (Table 2). The total phenolic content, flavonoid content and antioxidant activity of the cumin extract are positively correlated in the current study. This is due to the fact that 70% acetonic extract, which also displayed the largest quantities of phenolics and flavonoids, demonstrated the best antioxidant capacity. Likewise, the levels of phenolics, flavonoids and antioxidant activity were lowest in the methanolic cumin extract.Other authors found EC50 (6.24 µg/mL to 236 µg/mL) with acetonic extract

Table 1: The total phenolic, flavonoid and tannin contents of cumin extracts.

Cumin extract	Total phenolic mg GAE/g	Total flavonoid mg QE/g	Proanthocyanidin mg CE/g
Acetonic	42±3.46	24.49±1.24	34.95±8.71
Ethanolic	32.74±1.08	22.49±0.34	34.1±0.74
Methanolic	30±4.01	14.09±0.8	25.15±0.73

The data are displayed with mean standard deviation of twice replications.

Table 2: DPPH scavenging activity at EC50 levels in bioactive extracts of *Cuminum cyminum*.

Bioactive compounds	EC50 (µg/mL)
Acetonic extract	940±42data
Ethanolic extract	960±50data
Methanolic extract	1180±123
Ascorbic acid	200±26

EC50: The amount of sample needed to scavenge 50% of free radicals is known as the. A positive control was ascorbic acid. Values are expressed as mean of three replicates.

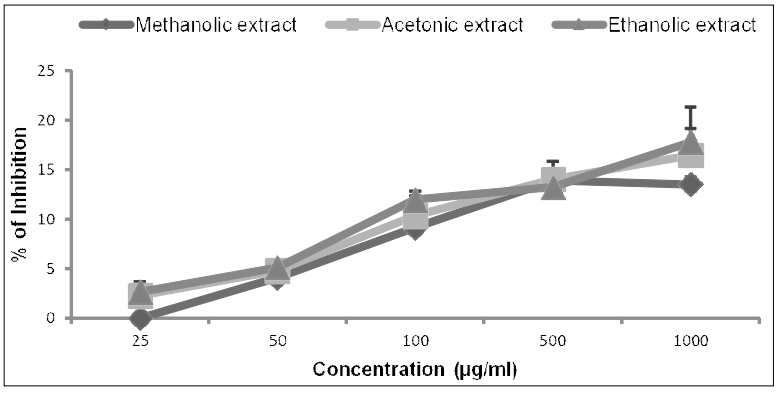


Fig 1: DPPH radical scavenging activities (%) of 70% acetonic, 70% ethanolic and 70% methanolic cumin extract.

Table 3: Mean zone of inhibition (mm) of all extracts of Cuminum cyminum on oral pathogens.

Cumin extracts	*E. faecalis*	*S. mutans*	*G. morbillorum*	*S. epidermidis*	*K. oxytoca*	*E. bugandensis*
Acetone	17.30±0.49 (++)	15.00±0.10 (++)	13.50±0.35 (+)	14.30±0.06 (+)	0 (-)	0 (-)
Ethanol	0 (-)	14.00±0.2 (+)	0 (-)	12.00±0.28 (+)	0 (-)	0 (-)
Methanol	0 (-)	15.00±0.15 (++)	13.50±0.25 (+)	14.30±1.00 (+)	0 (-)	0 (-)

Values + and - represent degree of sensitivity of strains: Non sensitive: Ø <8 mm; sensitive (+): 9 <Ø <14 mm; very sensitive (++): 15 <Ø <19 mm and extremely sensitive (+++):Ø > 20 mm [40] (Ponce *et al.*, 2003).

Table 4: MIC and MBC of acetonic, ethanolic and methanolic cumin extract.

Species	MIC (mg/mL)			MBC (mg/mL)		
	Acetone	Ethanol	Methanol	Acetone	Ethanol	Methanol
S. mutans	100	100	12.5	100	100	200
G. morbillorum	100	-	50	100	-	>200
E. faecalis	100	100	100	100	100	>200
S. epidermis	100	100	100	100	-	-
K. oxytoca	-	-	-	-	-	-
E. bugandensis	-	-	-	-	-	-

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.

(Bettaieb *et al.*, 2012) and (419.86±18.88 µg/mL) with ethanolic extract (Tuekaew *et al.*, 2014). Demir and Korukluoglu (2020) reported the EC50 values of 1480 µg/mL for methanolic extract and 3250 µg/mL for ethanolic extract. The differences in results led to the conclusion that the antioxidant activity does not depend only on the high content of total phenolics but also on the phenolic composition, as well as solvent concentration and method of extraction (Milica *et al.*, 2016).

Antibacterial effect

In the present work, the mean zone of inhibition by cumin extracts against dental caries pathogens ranged from 12 mm to 17, 30 mm (Table 3). The three extracts of *C. cyminum* showed antibacterial activity against Gram+ bacteria except *E. faecalis* that has shown resistance to methanolic and ethanolic extracts ($P<0.05$). The Gram-bacteria were insensitive to cumin extracts ($P<0.05$). Acetonic extract caused the largest zone of inhibition, among the extracts tested and was the most effective against the four Gram+ bacteria.The results of the MIC assay suggest moderate antibacterial activity of the extracts that varied depending on the species of bacteria. Results show the highest values of CMBs with methanolic extract (≥200 mg/mL) (Table 4).

These results indicate that the Gram-positive microorganisms were more sensitive to the plant extracts studied than the Gram-negative microorganisms. However, controversial data have reported that cumin extract is effective against Gram-positive and Gram-negative bacteria (*E. coli*, S. *aeruginosa, S. aureus, S. flexneri, B. cereus, E. faecalis* and *S. typhimurium*). The higher susceptibility of the Gram-positive organisms to the plant extracts may be due to their cell wall structure, which is of a single layer while the gram-negative cell wall is a multi-layered structure and quite complex (Nazzaro *et al.*, 2013). Bactericid activity of *C. cyminum* extracts was observed in all the strains, except *S. mutans* for wich a bacteriostatic activity has been noted with the methanolic extract (data not shown). Recent results showed an inhibitory effect of cumin against *S. mutans* and *S. pyogenes*with bactericide effect (Ghazi *et al.*, 2019), MIC and MBC values found were inferior to those obtained in this study (Pillay *et al.*, 2019). The cumin extract contains numerous active chemical compounds with antibacterial potency (Patil *et al.*, 2016).

CONCLUSION

All extracts of cumin have inhibitory effect against Gram-positive bacteria. Acetonic extract of this plant revealed the greatest antioxidant activity. The results of this study suggest the potential use of cumin as antibacterial agent against the cariogenic oral bacteria tested, the effects of this extract may be more beneficial if it is incorporated in gum, tooth paste, mouthwash and dental products to reduce plaque and dental caries.

Conflict of interest

All authors declare that they have no conflicts of interest.

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