

COMPARATIVE STUDY OF IN VITRO ANTIMICROBIAL ACTIVITIES OF *FOENICULUM VULGARE* MILL. (UMBELLIFERAE) EXTRACT

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ABSTRACT

The importance to push scientifically the investigations on the organic extracts of the plants aromatic as potential source of new antimicrobial compounds comes from the traditional use of the plants. However, the consumption of these natural products requires a thorough research in this field. The antimicrobial effect of organic and aqueous leaves extracts of *Foeniculum vulgare* Mill., However, which makes difficult this antimicrobial activity, is the insolubility of organic extracts in water. The standard M27-T technique is basically used to cure this problem. The microorganisms under examination were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae*, *Escherichia coli* and *Candida albicans*. The M27-T technique allowed us to determine the Minimum Inhibitory Concentrations (MICs) of different extracts. Therefore, the test's results showed that the all samples were clearly different in terms of antimicrobial activities. All extracts of *Foeniculum vulgare* showed the most activity on all the microorganisms tested. The most significant and active extract under study were methanol and ethyl acetate on all the bacteria tested in comparison to the hexane and aqueous extracts. On the other hand, the results of antimicrobial activity of aqueous extract were more compelling than the hexane and dichloromethane extracts when used on *Candida albicans* (ATCC and CBS) (MIC = 0,78 mg mL⁻¹). It then appear that *C. albicans* ATCC is the least susceptible microorganisms to the ethyl acetate extract. The chloramphenicol, amoxicillin and amphotericin B were used as standard antibiotics to carry this study.

Keywords: Leaves Extracts, *Foeniculum Vulgare*, Antimicrobial Activity

1. INTRODUCTION

Foeniculum vulgare Mill., which is commonly known as fennel, belongs to the Apiaceae (Umbelliferae) family. It is an annual, biennial or perennial herbs and typical aromatic plant that grows in several regions all over the world. It is growing to a height ranging from 70 to 200 cm. it grows wild in most regions, especially the west and south regions of turkey.

Fennel, which is largely planted in temperate and tropical regions of the world, is extensively used for

medicine purposes and as a culinary spice (Tanira *et al.*, 1996; Beaux *et al.*, 1997; Patra *et al.*, 2002; Barros *et al.*, 2010).

They are also known for a wide range of activities, like diuretic, anti-inflammatory, analgesic, antipyretic, antispasmodic, antidiabetic and antioxidant actions (Beaux *et al.*, 1997; El Bardai *et al.*, 2001; Oktay *et al.*, 2003; Choi and Hwang, 2004; Heinrich, 2005; Conforti *et al.*, 2006; Abed, 2007; Surveswaran *et al.*, 2007; Marino *et al.*, 2007). Fennel's leaves and mature fruits are basically used as flavoring agents in food

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product such as liqueurs, pickles, bread, pastries and cheese. They are also used in cosmetic and pharmaceuticals (Telci *et al.*, 2009).

The essential oil, one of the wide uses of fennel, was the most frequently investigated, in which antioxidant, antimicrobial, antidiabetic and hepatoprotective were actively showed (Ruberto *et al.*, 2000; Ozbek *et al.*, 2003; Faudale *et al.*, 2008; El-Soud *et al.*, 2011). They entail several monoterpenes and phenylpropanoids, where trans-anethole, estragole, fenchone, α -phellandrene and limonene exist as main components. The relative concentration of these compounds varies considerably depending on the origine and phonological state of the fennel (Guillen and Manzanos, 1996; Choi and Hwang, 2004; Diaz-Maroto *et al.*, 2006; Gross *et al.*, 2009).

The essential oil is largely used as favoring agent in culinary preparations, confectionary, cordials, liqueurs and occasionally added in scenting soaps (Rupam *et al.*, 2003). Other studies showed that some species extract of this family (*Anethum graveolens*, *Foeniculum vulgare* and *ferula halophila*) have antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (Baldemir *et al.*, 2006; Gurinder and Daljit, 2008; Pai *et al.*, 2010).

The current study was meant to assess the antimicrobial properties of the extracts of *Foeniculum* by using the standard M27-T broth microdilution technique of the National Committee for Clinical Laboratory Standards (NCCLS) (Barchiesi *et al.*, 1994; Espinel-Ingroff *et al.*, 1995; Bouamama *et al.*, 2006).

We are more interested here on the results of antimicrobial testing of *Foeniculum vulgare* extracts against *Escherichia coli*, *Enterococcus hirea*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

2. MATERIALS AND METHODS

2.1. Plant Material

Leaves of *Foeniculum vulgare* Mill. were collected in march 2009 from Ouaouizerth, the area of Azilal (Morocco). Specimens were botanically identified at the laboratory of vegetable ecology in the department of Biology, University Cadi Ayyad, Faculty of Sciences Semlalia, of Marrakesh, Morocco.

2.2. Extracts Preparations

The extract preparation involves taking dried and finely powdered leaves (100 g) of the fennel and then

extracting it with methanol, using Soxhlet apparatus for 48h. This methanol extract is filtered and then evaporated. The process in question is carried out through an rotary evaporator adjusted under a temperature of 45°C. Yielding 24 g (24%). Both extracts were redissolved in distilled water and successively extracted with hexane, dichloromethane and ethyl acetate. Each organic extract was then evaporated to dryness and labeled as indicated in **Table 1**.

Afterwards, we prepare approximately 100 mg mL⁻¹ of each extract solution in distilled water. The pH was adjusted between 5 and 7. Extracts were sterilized over a membrane filter unit of 0.2 μ m of pore size and preserved at +4°C until used.

2.3. Fungal and Bacterial Strains

Tests were performed on two fungi and four bacteria reference strains obtained from department of biology, Faculty of sciences and technology, Cadi Ayyad University of Marrakesh, Morocco: *Candida albicans* (ATCC 2091) and *Candida albicans* (CBS 562), *Escherichia coli* (CIP 54125), *Staphylococcus aureus* (CIP 53154), *Enterococcus hirea* (CIP 5855) and *Pseudomonas aeruginosa* (CIP A22).

2.4. Standard Microdilution Method (SMM)

2.4.1. Antifungal Activity

The same M27-T method, which was described by Bouamama *et al.* (2006), was used again with some modification in terms of culture medium and the concentrations of the extract and of the Amphotericin B. used the same method M27-T described by Bouamama with some modification on the culture medium and the concentrations of the extract and of the Amphotericin B. The culture medium is the Sabouraud dextrose broth (BIOKAR) buffered to pH 7. Amphotericin B was used as standard antibiotic with concentrations ranging from 25 to 0,024 μ g mL⁻¹. The antifungal concentrations ranged from 100 to 0.09 mg mL⁻¹. After inoculation, the plates were incubated for 24, 48 and 72 h. at 35 °C.

2.4.2. Antibacterial Activity

We used the same method M27-T for the antibacterial test. The culture medium was the Mueller-Hinton broth. Amoxicillin and chloramphenicol (Sigma) were used as standards. Concentrations ranged from 320 to 0.31 μ g mL⁻¹ for amoxicillin and for chloramphenicol. After inoculation, the plates were incubated at 37°C for 24 and 48 h.

2.4.3. Inoculum Preparation

Yeast colonies, which obtained from 24h cultures, were taken to another environment called Sabouraud dextrose broth at 35°C under agitation. The first suspensions were used for the inocula preparation in the same culture medium. Thomas hematimeter was utilised to determine the concentrations of yeast cells. The concentrations of yeast cells were determined using a Thomas's hematimeter. Budding organisms were counted as two. The original concentrations were adjusted to a concentration of 10^4 - 10^5 colony forming units (CFU)/ml with broth used in the susceptibility test.

Stock bacterial inocula suspensions were obtained from 18 h culture on Mueller-Hinton broth at 37°C. The suspensions finally yielded serve as the inocula preparation. The cell density of each suspension was determined using a Thomas hematimeter and then adjusted to a concentration of 10^5 - 10^6 CFU mL⁻¹ by dilution with Mueller-Hinton broth.

2.4.4. Readings and Control

The experiments were repeated three times and the results were determined as an average value. The result readings were made visually. The MIC was considered as the lowest drug concentration of antifungal or antibacterial agent inhibiting the total growth of microorganisms. MIC was detected by lack of visual turbidity (matching the negative growth control). Subcultures were made from the clear wells which did not show any growth after incubation during the MIC assays on nutritive agar with 2% for bacteria and Potato Dextrose Agar (PDA) (BIOKAR) for fungi.

3. RESULTS

The **Table 1** shows that the extracts tests had an antimicrobial activity. This activity depends on the nature of the extract, there concentration and bacterial or fungal strain. These results are indeed in line with the previous analysis of the leaves extracts of *Foeniculum vulgare* Mill., which possessed antimicrobial properties with MIC values ranging from 0.78 to 6.25 mg mL⁻¹ for bacteria and 0.39 to 25 mg mL⁻¹ for fungi.

Extract such as the methanol, hexane, dichloromethane, ethyl acetate and the aqueous extracts of *F.vulgare* show the lowest MIC and inhibit the development of *Escherichia coli*, *Enterococcus hirea* and *Staphylococcus aureus*. P3 and P4 are the most active extracts (MIC = 0, 78 mg mL⁻¹ for *E. coli* and *E. hirea*). P2 and P5 are the less active extracts (MIC = 3,125 for *E. coli*, *E. hirea* and *P. aeruginosa*). *S. aureus* is the most sensitive species to *F. vulgare* with MIC values between 0, 78 mg mL⁻¹. Among them, hexane extract show the least active extracts inhibiting *S. aureus*, *E. hirea* and *P. aeruginosa* with MIC values of 0,78 mg mL⁻¹.

Regarding the fungi studied, we showed that all tested extracts are inhibitory effects on *Candida* with CMI which vary from 0.39 to 6,25 mg mL⁻¹. *F. vulgare* extracts have the most compelling inhibition activities against *Candida*. The species *Candida* CBS is strongly inhibited by P4 and P5 (MIC = 0,78 mg mL⁻¹) and P1 (MIC = 0, 39 mg mL⁻¹). *C. albicans* CBS and ATCC are the least sensitive strain to the *F. vulgare* extracts P2 and P3 (MIC = 6, 25 mg mL⁻¹).

Table 1. Antibacterial and antifungal activities of *F. vulgare* extracts from Morocco

Microorganisms	MIC (mg/ml)					MIC (mg/ml)		
	Extracts					Antibiotics		Antifongic APH B
	P1	P2	P3	P4	P5	AMX	CHL	
Gram-positive bacteria								
<i>Staphylococcus aureus</i>	0,78	3,125	1,56	0,78	0,78	0,00025	0,00125	
<i>Enterococcus hirea</i>	1,56	3,125	0,78	0,78	3,125	0,00052	0,00052	
Gram-negative bacteria								
<i>Escherichia coli</i>	1,56	3,125	0,78	0,78	3,125	0,0035	0,00125	
<i>Pseudomonas aeruginosa</i>	1,56	6,25	3,125	1,56	3,125	0,00025	0,00125	
Yeast								
<i>Candida albicans</i> ATCC	3,125	6,25	6,25	25	0,78			0,00156
<i>Candida albicans</i> CBS	0,39	6,25	6,25	0,78	0,78			0,00156

P1: Methanol extract; P2: Hexane extract; P3: Dichloromethane extract; P4: Ethyl acetate extract; P5: Remaining aqueous layer. AMX: Amoxicilline; CHL: Chloramphenicol; APH B: Amphotericin B

4. DISCUSSION

For many age, medicinal plants have been used to cure diseases. Herbal medicines have increasingly been used to treat effectively infections that are difficult to manage. We are fully aware that plants perfectly produce certain natural chemicals that are toxic to bacteria. A large body of literature has validated the antimicrobial activity of plant extracts (Basile *et al.*, 1999; Pesewu *et al.*, 2008; Babri *et al.*, 2012).

4.1. Antibacterial Activity

The results indicated that all extracts have antibacterial activity against examined Gram negative and Gram positive bacteria. Compared with other studies, our data show better antimicrobial activities for *Foeniculum* species. While observing the therapeutic activity against most of the pathogenic bacteria, gram-positive bacteria were more sensitive to extract than gram-negative bacteria. Gnan and Demello (1999) reported an antimicrobial activity of *Goiaba* leaves extract at a concentration of 6, 5 mg mL⁻¹ against *Staphylococcus aureus*. Chandrasekaran and Venkatesalu (2004) reported that MIC values of *Syzygium jambolanum* seed extracts ranged between 0,0031 and 0,5 mg m mL⁻¹ against *E. coli*, *S.aureus*, *P. aeruginosa* and *C. albicans*. Tanis *et al.* (2009) reported that chloroform and methanol extract of *N. arvensis* and *N. unguicularis* were the least effective against the microorganisms. These data coincide with those of Okoli and Iroegbu (2004), who reported that water and methanol extracts of some plants displayed a significant antimicrobial activities. Similarly, Basri and Fan (2005) had reported that the aqueous and acetone extracts of galls of *Quercus infectoria* (Oak) displayed similarities in antimicrobial activity on the bacterial species and as such, it is the potentially source of antimicrobials.

4.2. Antifungal Activity

Compared to Amphotericin B, organic extracts of fennel leaves (as shown on the table above) have an effective antifungal activity. these results are perfectly in accordance with those of Zahid *et al.* (2012) who had reported that the aqueous extract of fennel (*Foeniculum vulgare* Mill.) had potential antifungal activity against three soil borne: fungi namely: *Macrophomina phaseoli*, *Rhizocotina Solani* and *Fusarium moniliforme*.

5. CONCLUSION

To sum up, we basically use the standard M27-T technique to survey the organic and aqueous leaves extracts of *Foeniculum vulgare* Mill. (Apiaceae) used in traditional medicine, for their antimicrobial properties. Our study has emphasized the fact that the antimicrobial activities are much more significant at *Foeniculum* species. The leaves extracts of *F. vulgare* species displayed the inhibitory activities against some of the microorganisms involved in many infections and skin diseases.

Although fungi have been the main source of antibiotics up to now, the discovery of new plant products with potential antimicrobial application is of considerable interest in view of the increasing antibiotic resistance to many microorganisms.

This study enables us for further attention of a phytochemical analysis of different extracted and research to identify the active compounds responsible for the biological activity of this plant. More studies t conducted to explicate the exact mechanism of action by which extracts exert their antimicrobial effect.

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