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Full Length Research Paper

Antibacterial activities of essential oil and crude extracts *from Matricaria pubescens* (Desf.) growing wild in Bechar, South west of Algeria.

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Crude extracts (Aqueous, ethanolic) and hydrodistillated-essential oil from aerial parts of *Matricaria pubescens* (Desf.) *were* investigated for their antibacterial activities against seven strains of bacteria. We wanted to proof an antibacterial activity of *Matricaria pubescens* (Desf.) with two most commonly used methods: disc diffusion method and broth dilution method. With the disc diffusion method we have obtained the inhibition zone. Minimal inhibitory concentration (MIC) corresponding at the lowest concentrations, where no visible bacterial growth was recorded, were assumed as values (MIC). Overall, extracts from *M. pubescens* (Desf.) showed stronger antibacterial activities than their essential oil obtained from hydrodistillation. The diameters of growth inhibition zone ranged from 12 to 33 mm (including the diameter of the disc-6 mm) with the highest inhibition zone values observed against *Escherichia coli* (31 mm) and *Klebsiella pneumonia* (33 mm). We determined MIC values in the ranges from 0.5 to 2.33 mg/ml for extracts and essential oil in the medium. Aqueous extracts exhibited MIC values of 9 mg/ml against *Bacillus cereus*. The MIC values of the ethanolic extract against *Listeria monocytogenes and Staphylococcus aureus* were 0.5 and 0.833 mg/ml, respectively. In the other hand, the best inhibitory activity *of M. pubescens* (Desf) essential oil (EO) was observed on *E. coli* and *K. pneumonia* 1.66 mg/ml.

Key words: *Matricaria pubescens* (Desf.), essential oil, crude extracts, antibacterial activity.

INTRODUCTION

Disease causing bacteria have always been considered a major cause of morbidity and mortality in humans. The appearance of resistant microorganisms paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents. The emergence of resistant Gram negative bacteria presents a major challenge for the antimicrobial therapy of infectious diseases and increases the incidence of mortality and morbidity. Bacterial resistance to antimicrobial agents is a medical problem with public health, socioeconomic, and even political implications (Abdel massih et al., 2010).

Matricaria pubescens (Desf.) is a small annual plant, 10 to 20 cm high, rarely reaching 40 cm., with numerous prostrate stems, that become erect. The thin dark green stems are only very slightly ramified.

The deeply dissected leaves, with each lobe ending in a white tip, are slightly fleshy and are between 10 and 20 mm long. The tubular yellow flowers are grouped in hemispherical discoid heads. The flower heads are about 5 to 8 mm in diameter and are set at the ends of the stems. The fruits are achenes with a small membranous pappus to help dispersal.The entire plant have a very agreeable scent. Flowering takes place in spring in the northern Algerian Sahara, and any time after rain in the central Algerian Sahara (Bounaga and Brac, 1989; Ozenda, 2004). The whole plant is collected fresh in spring, and sold in the market in several oases in the south (Béchar, Djanet, El Golea). It is prepared as an infusion or powder and used internally. It has antiseptic properties. It is not reported as toxic by nomads. It is

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Figure 1. *M. pubescens* (Desf.)(Bechar Zone, Algeria).

used for gastro-intestinal troubles and calculus, and is a much appreciated medicinal herb. The crushed stems and leaves are used as a filter for goat's butter, giving a nice aroma to the butter and helping to conserve it. It is also added to the traditional soup and gives the food a very nice smell.

MATERIALS AND METHODS

M. pubescens (Desf.) specimens (Figure 1) were collected from Bechar region, at four different time intervals, namely, October and December 2010, January and February, 2011. These biomasses were dried for fifteen days in the dark at ambient laboratory temperature (20 to 28°C).

Distillation of essential oil

The dried aerial parts were grounded before the operation, and then, 100 g of grounded *M. pubescens* (Desf.) were submitted to hydrodistillation for 3 h using a Clevenger apparatus (Amarti et al., 2008). The distilled essential oils were dried over anhydrous sodium sulfate, filtered and stored at +4°C until it was used (Ayoughi et al., 2011; Chanthaphon et al., 2008).

Preparation of ethanolic extract

Fresh plants were dried in the shade at room temperature and ground in a coffee bean grinder. 15 g of dried plant material was soaked in 100 ml of ethanol for 24 h with continuous shaking in a shaker at room temperature. The plant material was filtered and the filtrate collected (Abbassi et al., 2005). This was repeated and the filtrates were combined and concentrated in a rotary evaporator at 30°C to obtain the crude extract and stored at 4°C until further (Kassi et al., 2008; Iqbal and Arina, 2001).

Preparation of aqueous extract

Aqueous extract of *M. pubescens* (Desf.) was prepared by boiling 25 g in 500 ml sterile distilled water for 15 to 20 min. The flasks were then plugged and removed from heat and allowed to cool. After cooling, the contents of flasks were filtered (Sqalli et al., 2007; Loubaki et al., 1999). This was repeated and the filtrates were combined and concentrated in a rotary evaporator to obtain the crude extract (Kassi et al., 2008).

Antimicrobial activity

Microbial strains

The antibacterial activity was evaluated by paper disc diffusion and dilution methods against seven selected Gram-positive and Gram-negative species: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* CIP 106818, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC27853, *Bacillus cereus* ATCC 11778, *Enterococcus feacalis* ATCC 29212 and *Listeria monocytogenes* ATCC19115.

Disc diffusion method

The qualitative antibacterial was carried out by the disc diffusion

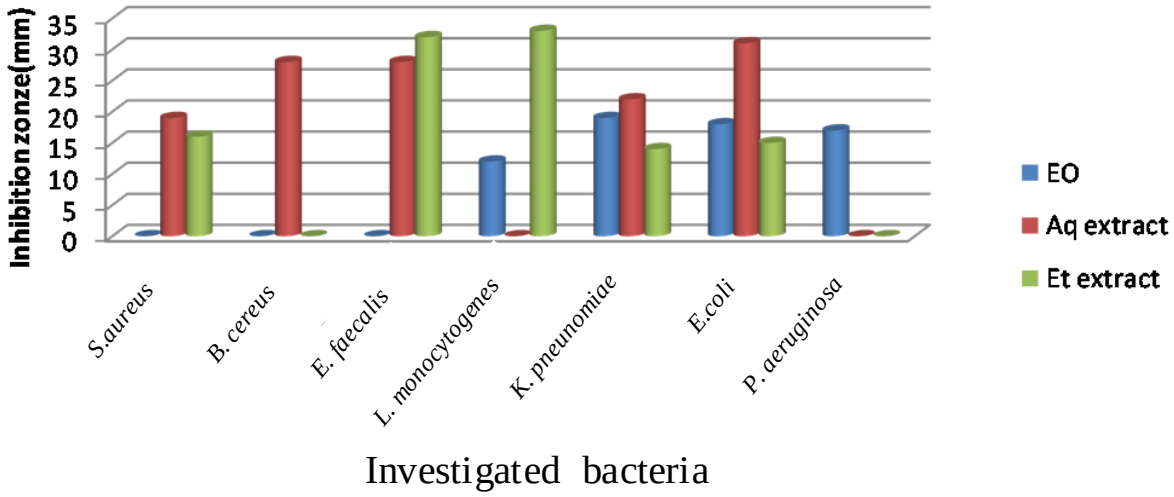

Figure 2. Antibacterial activity of essential oil (EO),Aq and EtOH extract by disc diffusion assay.

method (Boussaada et al., 2008). It was performed using culture growth at 37°C for 18 h and adjusted to approximately 10^8 colony forming unit per milliliter (CFU/ml). The culture medium used for the bacteria was Mueller Hinton Agar (MHA) (Gachkar et al., 2007; Joffin and Leyral, 2001). Five hundred microliters of the inoculums were spread over plates containing MHA and a Whatman paper disc (6 mm) impregnated with 5 µl of the essential oil or crude extracts was placed on the surface of the media. The plates were left for 30 min at room temperature to allow the diffusion of the oil. They were incubated 24 h at 37°C (Bekhechi et al., 2008; Bourkhiss et al., 2007 and Shunying et al., 2005). After incubation period, the inhibition zone obtained around the disc was measured. Two controls were also included in the test, the first was involving the presence of microorganisms without test material and the second was two standard antibiotics: Ampicillin used to control the sensitivity of the tested bacteria. The experiments were run in triplicate, and the developing inhibition zones were compared with those of reference discs (Yesil et al., 2007).

Dilution method minimum inhibitory concentration (MIC)

Essential oil

Antimicrobial tests were performed according to the method reported by Remmal et al. (1993) and Farah et al. (2001). The essential oil is emulsified with an agar solution of 0.2% in order to disperse the compounds and improve their contact with the tested germs, and then diluted to one tenth in the agar solution. Quantities of this dilution are added to test tubes containing Mueller Hinton agar for bacteria. The final concentrations of essential oil are from 1 / 100, to 1 / 1000 (v / v). In parallel, Control assay containing only the culture medium and agar solution at 0.2% were also used. The minimum inhibitory concentration (MIC) is the lowest concentration of essential oil giving no visible growth in the naked eye (Kaloustian et al., 2008).

Crude extracts

Dry extracts were weighed and dissolved in sterile distilled water. The solutions were filtered through 0.22 µm sterile filter membranes and stored at 4°C for further use. The concentration of the original solution of the plant extract/fraction corresponds to the concentration obtained after resuspension of the dried plant extracts. This was used as the stock solution and the most concentrated one from which the MIC series were prepared (Abdel et al., 2010).

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors (Akroum et al., 2009). The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

RESULTS AND DISCUSSION

Antimicrobial activity

Disc diffusion assay

The increase of antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases, the result of different studies provide evidence that some medicinal plants might indeed be potential source of new antibacterial agents (Syed et al., 2011).

M. pubescens (Desf.) has provided a yield of 0.8% essential oil. According to these results, this essential oil has an antibacterial activity against the investigated strains except *S. aureus*, *E. feacalis* and *B. cereus*. The growth inhibition zones measured by disc diffusion method are presented in Figure 2. The diameters of growth inhibition zone ranged from 12 to 19 mm (including the diameter of the disc 6 mm) with the highest inhibition zone values observed against *K. pneumoniae*

Table 1. MIC of essential oil (EO) and crude extracts (mg/ml).

Bacteria	essential oil (EO) MIC mg/ml	EtOH extract MIC mg/ml	Aqu essential oil (EO)us extract MIC mg/ml
S. aureus	-	8	11
B. cereus	-	-	9
E. feacalis	-	13	18
L. monocytogenes	20	5	-
K. pneumoniae	15	12	6
E. coli	15	13	10.5
P. aeruginosa	16	-	-

Table 2. Phytochemical properties of extracts of *M. pubescens* (Desf.)

Variable	Chloroform extract	Diethylic ether extract	EtOH extract	Aqu essential oil (EO)us extract
phenols	+	+	+	+
tannins	+	+	+	+
glycosides	+	+	+	+
saponins	+	+	+	+
flavonoids	+	+	+	+
steroids	+	+	+	+
Alkaloids	-	-	-	-
Quinone	-	-	-	-

(+) presence,(-) absence.

(19 mm) and *E. coli* (18 mm). These values are lower than those found with Ampicillin (46 mm).

On the other hand, the results indicated that the *M. pubescens* (Desf.) extracts showed antibacterial activity (Figure 2), mainly against the Gram positive bacteria (*E. feacalis 32 mm* and *L. monocytogenes* 33 mm). The extracts also exhibited an effect against the Gram-negative bacteria (*E. coli* 31 mm and *K. pneumonia* 22 mm). However, this effect was less efficient than that presented against the Gram positive bacteria, since a higher MIC value was obtained with the Gram negative bacteria.

Dilution method (MIC)

M. pubescens (Desf.) essential oil (EO) exerted an inhibitory effect on *E. coli*, *P. aeruginosa, L. monocytogenes* and *K. pneumoniae*. The best MIC was observed against *E. coli* and *K. pneumonia* 1.666 mg/ml (Table 1).

These effects were observed with the crude extracts (aqueous, EtOH), the best inhibitory activity represented by the lowest MIC was observed with EtOH extract at 0.5 and 0.833 mg/ml for *L. monocytogenes* and *S. aureus*, respectively (Table 1).

The lowest MIC (1.666 mg/ml) was recorded for the aqueous extract with *B. cereus* and *E. coli* (Table 1). The crude extracts exhibited as well as bactericidal activity on the majority of the strains at concentrations between 1.333 and 1.833 mg/ml. The EO inhibited most of the strains at a concentration of 2 mg/ml.

The solvents' controls that were systematically run for all solvents did not exert any antibacterial activity. Bacterial growth was observed for the positive controls while no growth was observed for the negative controls. Phytochemical analysis of extracts demonstrated the presence of common phytoconstituents like phenols, terpenes, tannins, glycosides, saponins, flavonoids, steroids and the absence of alkaloids (Table 2).

The antibacterial activity of essential oil (EO) and crude extracts is due to the presence of a mixture of compounds and not to a single one. The effect of some terpenes on microorganisms has already been studied. Terpenes, have shown increasing promise *in vivo*, inhibiting multiple species of bacteria (Zwenger and Basu, 2008). Tannins, flavonoids and other glycosides were present in the ethanolic extracts of *M. pubescens* (Desf.) which could be responsible for its antibacterial properties. Pamploma- Roger (1999) earlier reported that

plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections (Egwaikhide et al., 2009).

Conclusion

Hydro distillated-essential oils from *M. pubescens* (Desf.) had less inhibitory activity, compared to the crude extracts (Aqueous and EtOH). The strong antibacterial activity of *M. pubescens* against array of bacteria strains is an indication of the broad spectrum antibacterial potential of the oil and extracts. This could make the oil and the extracts a promising group of natural compounds for development of safer antibacterial agents.

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