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Full Length Research Paper

Antimicrobial and antioxidant activities of essential oil and methanol extract of *Matricaria chamomilla* L. from Djibouti

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The essential oil and methanol of *Matricaria Chamomilla* L. were subjected to screening for their possible antioxidant activity by two complementary test systems, namely 2,2-Diphenykpicrylhydrazyl (DPPH) free radical scavenging and β-carotene-linoleic acid assays. BHT was used as positive control in both test systems. In the DPPH test system, the IC50 value of essential oil and methanol extract were respectively 4.18 and 1.83 µg/ml. In the β-carotene-linoleic acid system, oxidation was effectively inhibited by *M. Chamomilla*, the RAA value of essential oil and methanol extract were respectively 12.69 and 11.37%. When compared to BHT, the oil and methanol extract were nearly the same value. The essential oil and methanol extract were tested against bacterial and fungal strains using a broth microdilution method. The results suggest that *M. Chamomilla*, oil and methanol extract have significant antimicrobial activity.

Key words: *Matricaria chamomilla* L., essential oil, methanol extract, antioxidant activity, antimicrobial activity.

INTRODUCTION

In the last years, there is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceutics (Azuma et al., 1995). The antioxidant action, is supposed to protect living organism from oxidative damages resulting in the prevention of various diseases. Plants are the primary sources of naturally occurring antioxidants for humans. It has been well-know that essential oils and plant extracts have antimicrobial and antioxidant effects (Özer, 2006).

Matricaria chamomilla L. is an annual plant of approximately 50 cm, with drawn up stem, oarswoman. The sheets are very divided, in thin straps. The flowers are grouped in solitary flower heads at the top of the branches. The fruit very small, white yellowish is slightly arched. *M. chamomilla* is a hardy perennial with rather strong, fibrous and hairy roots. The stems, from 10 to 30 cm, are hails, green, hairy, lying, spread out, or right, they carry sheets alternate, sessile, divided into very short, lobed and pointed leaflets. *M. chamomilla* forms part, like the lime, mint or vervain, of the most consumed plants, because most known for their multiple benefits. *M. chamomilla* is used as tonic of the appetite before the meals, to facilitate digestion after the meals, to fight against the aerophagia, flatulence. It is also useful to calm the headaches, the various pains, the aches, the tooth aches, to facilitate the menstruation and to relieve

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the pains of the rules. Anti-inflammatory drug, it softens the eyes and the eyelids (Shivananda, 2007; Owlia, 2007). The chemical composition of *M. chamomilla* L. essential oils was determined by Gas chromatography-mass spectrometry (GCMS). Three main fractions with high degree of purity in E-β-farnesene were isolated from the oil of *M. chamomilla* (Heuskin, 2008). Antistreptococcal and antioxidant activity of essential oil from *M. chamomilla* have been reported (Owlia, 2007). As part of our work on the characterization medicinal plants, we report here the *in vitro* antibacterial and antioxidant activities of the essential oils and methanol extracts of *M. chamomilla* collected in Djibouti.

MATERIALS AND METHODS

Isolation of essential oil

Essential oil of *M. chamomilla* was obtained by hydrodistillation of the leaf from the garden of Ambouli in Djibouti town (Djibouti Republic) in June 2008. Voucher specimens have been deposited in Centre d'Etudes et de Recherches en Biotechnologie, Djibouti. The obtained essential oil was dried over anhydrous sodium sulfate and, after filtration, stored at - 4°C until tested and analyzed.

Preparation of the methanol extract

The dried and powdered leaf (500 g) were extracted with 1 L of methanol using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent (Lin, 1999). The extract was filtrated using Whatman filter paper (no 1) and then concentrated in vacuum at 40°C using a rotary evaporator. The residues obtained were stored in freezer at -4°C until further tests.

Microbial strains

The microorganisms used were: *Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 103907, *Escherichia coli* CIP 11609, *Listeria innocua* LMG 1135668, *Salmonella enteric* CIP 105150, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Proteus mirabolis* 104588 CIP, *Staphylococcus camorum* LMG 13567 and *Candida albicans* ATCC 10231. *Pseudomonas aeruginosa, Streptococcus pyogenes, Aspergillus niger, Aspergillus sp.* and *Candida albicans* were vaginal isolated at the Laboratoire de Biologie Médicale Saint Camille de Ouagadougou.

Microbiological methods

Antibacterial screening

The agar disc diffusion method was employed for the screening of antimicrobial activities of essential oil and methanol extract (NCCLS, 1999). The dried plant extracts were dissolved in methanol to final concentration of 30 mg/ml and sterilized by filtration through 0.45 µm Millipore filters (Schleicher and Schuell, Microscience, Dassel, Germany), Antimicrobial tests were then carried out by disk diffusion (Murray, 1995) using 100 µl of suspension containing 108 colony forming units (CFU)/ml of bacteria, 104spore/ml of fungi spread on nutrient agar (NA) and potato dextrose agar (PDA) medium, respectively. The disk impregnated with 10 µl of essential oil or 10 µl of the methanol solution of the dried plant extracts (300 µg/disk) were placed on the inoculed agar. Negative controls were prepared with the same solvent used to dissolve the plant extracts. Tetracyclin (30 UI) and ticarcillin (75 µg) were used as standard antibiotics (BIO-RAD Marnes–la coquette-France). The positive controls were used to determine the sensitivity of one strain/isolated in each microbial species tested. The inoculated plates were incubated aerobically at 30°C (Gram-negative) or 37°C (Gram-positive) according to strain for 24 and 72 h for fungi isolated. Plants-associated microorganisms were incubed at 27°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated twice (Kordali et al., 2005a; Agnaniet et al., 2009).

Antimicrobial activity

The minimum inhibitory concentration (MIC) values were determined for the bacteria strains that were sensitive to the essential oil in the disk diffusion assay. The inocula of the bacterial strains were prepared from 10 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils and extracts of *M. chamomilla,* dissolved in 10% dimethyl sulfoxide (DMSO), were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial –fold dilutions were made to obtain a concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing nutrient broth . The MIC values of *M. chamomilla* extracts against bacterial strains and fungal isolates were determined on the basis of a microwell dilution method (Obame et al., 2007) with some modification. The 96-weel plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculums into each well. One hundred microliters from the stock solutions of *M. chamomilla* essential oil prepared at the 500 µg/ml concentration was added into the first wells. Then, 100 µl from the serial dilutions was transferred into the six consecutive wells. The last well containing 195 µl of nutrient broth without compound and 5 µl of the inoculums on each strip was used as a negative control. The final volume in each well was 200 µl. Maritime at a concentration range of 500 to 7.8 µg/ml was prepared in nutrient broth and used as a standard drug for positive control. The plate was covered with a sterile plate shaker at 300 rpm for 20 s and then incubated at appropriated temperatures for 24 h. Microbial growth in each well determined by reading the respective absorbance (Abs) at 600 nm using the ELx 800 universal microplate reader (Biotek Intrument Inc., Highland Park, VT) and confirmed by plating 5 µl samples from clear wells on nutrient agar medium. The oil tested in this study was screened twice against each organism.

MIC Agar dilution assay. The agar dilution method, was used to determine the MIC values of the fungal isolates. The essential oil of *M. chamomilla* were added aseptically to sterile molten PDA medium, containing tween 20 (Sigma 0.5%, v/v), at appropriate volume to produce the concentration range of 7.8-500 µg/mL. The resulting PDA solutions were immediately poured into Petri plates after vortexing. The plates were spot incubated with 5 µl (10^4spore/ml) of each fungal isolate. In addition, PDA plates treated with benomyl (12.0 mg/Petri dishes) and griseofulvin (100 µg) were used as positive controls (BIO-RAD Marnes–la coquette-France). The inoculated plates were incubated at 27 and 37°C for 72 h for plant and clinical fungus isolates, respectively. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oil at which the absence of growth was recorded. Each assay was repeated at least twice (Obame et al., 2008).

Antioxidant activity

2,2-Diphenykpicrylhydrazyl (DPPH) assay. The hydrogen atoms or

Table 1. Antimicrobial activities of *M. Chamomilla*: screening by inhibition zone diameters (mm) measuring.

Reference strains	Origin	*M. chamomilla*		Te	Ti
		Oil	MeOH		
B. cereus LMG 13569	LMG	17	17	18	50
E. faecalis CIP 103907	CIP	14	13	19	30
E. coli CIP 105182	CIP	14	17	22	8
L. innocua LMG 1135668	LMG	20	12	14	50
S. enterica CIP 105150	CIP	20	17	16	50
S. dysenteria CIP 5451	CIP	25	22	21	31
S. aureus ATCC 9244	ATCC	21	16	17	48
S.camorum LMG 13567	LMG	22	19	20	16
P. mirabilis 104588 CIP	CIP	17	15	15	16
Hospital strains					
P. aeruginosa	Vaginal liquid	30	20	21	19
S. pyogenes	Vaginal liquid	24	18	20	24
Fungal strains				Be	Gr
C.albicans ATCC 10231	ATCC	20	15	5	15
C. albicans	Clinical	19	15	5.5	15
A.niger	Clinical	17	14	6.4	6
Aspergillus sp	Clinical	14	13	5.2	7

Te: tetracycline, Ti: ticarcilline. Be: benomyl and Gr: griseofulvin, ND: not determined, MeOH: methanol extract, Oil: Essential oil.

electron-donating ability of the corresponding extracts and butylated hydroxytoluene (BHT) was determined from the bleaching of purple-colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits, 2000; Tepe et al., 2005). Briefly, 0.5 mM DPPH radical solution in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of the sample solution in ethanol. Final concentrations of essential oils or methanol extract were 20, 40, 60 and 100 µg/mL (Kordali et al., 2005b). BHT was used as a positive control at 100 µg/ml concentration. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as percent DPPH radical scavenging, which is calculated with the equation:

%DPPH radical scavenging = [(control absorbance- sample absorbance)]/control absorbance] X 100

Control contained 1 ml of DPPH solution and 3 ml of ethanol. The measurements of DPPH radical scavenging activity were carried out for three sample replications, and values are an average of three replicates.

β-carotene-Linoleic Acid Assay. In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide formation from linoleic acid oxidation (Dapkevicius, 1998). A stock solution of β-carotene linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 ml of Chloroform (HPLC grade); 25 µl of linoleic acid and 200 mg of tween 40 were added as emulsifier because β-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen was added with vigorous shaking at a rate of 100 ml/min for 30 min; 2500 µl of this reaction mixture was dispersed to test tubes, and different concentrations of essential oil or methanol extract, were added. The emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with a positive control BHT and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Antioxidant capacities of the extracts were compared with those at the BHT and the blank. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

The yields of the essential oil and methanol extract of *M. chamomilla* were respectively 0.25% (w/v) and 2.35% (v/v).

Antibacterial screening

Table 1 shows the average inhibition zones. Diameter of the essential oil varied from 14 to 30 for bacteria. The best zone of inhibition for bacteria was obtained for *P. aeruginosa* (30 mm), *S. dysenteria* CIP 5451 (25 mm), *S. pyogenes* (24 mm) and *S. camorum* LMG 13567 BHI (22 mm). The other strains had sensitivities between 14 and 21 mm. Diameter of the methanol extract varied from 12 to 22 mm for bacteria. The best zone of inhibition for bacteria was obtained for *S. dysenteria* CIP 5451 (22 mm) and *P. aeruginosa* (20 mm). The other strains had sensitivities between 12 and 19 mm. Essential oils

Table 2. Antibacterial (MIC, MBC) and antifungal parameters (MIC, MFC) of *M. chamomilla* (µg/ml).

Reference strains	Origin	*M. Chamomilla*			
		Oil		MeOH	
		MIC	MBC	MIC	MBC
B. cereus LMG 13569	LMG	4	4	100	100
E. faecalis CIP 103907	CIP	4	8	100	100
E. coli CIP 105182	CIP	4	8	25	25
L. innocua LMG 1135668	LMG	2	2	100	>100
S. enterica CIP 105150	CIP	2	2	100	100
S. dysenteria CIP 5451	CIP	1	1	25	25
S. aureus ATCC 9244	ATCC	2	2	100	100
S.camorum LMG 13567	LMG	2	2	100	100
P. mirabilis 104588 CIP	CIP	4	4	50	50
Hospital strains					
P. aeruginosa	Vaginal liquid	1	1	25	25
S.pyogenes	Vaginal liquid	2	2	25	50
Fungal strains		MIC	MFC	MIC	MFC
C. albicans ATCC 10231	ATCC	1	1	100	100
C. albicans	Clinical	2	2	100	100
A. niger	Clinical	2	2	100	>100
Aspergillus sp	Clinical	16	>16	200	>200

MeOH: methanol extract, Oil: Essential oil.

present an antimicrobial activity stronger than tetracycline and ticarcycline for *P. mirabilis* CIP 104588, *S. camorum* LMG13567, *S.pyogenes* and *P. aeruginosa.* Essential oils present an antimicrobial activity stronger than the tetracycline for *E. faecalis* CIP 103907, *S. dysenteria* CIP 5451, *B. cereus* LMG 13569, *L. innocua* LMG 1135668, *Salmonella enterica* CIP105150 and *S. aureus* ATCC 9244. Essential oils present an antimicrobial activity stronger than the ticarcycline for *E. coli* CIP 105518. The methanol extracts of *M. Chamomilla* have an antimicrobial activity weaker than the tetracycline and ticarcycline except for *E. coli* CIP 105182, *S. dysenteria* CIP 5451, *P. aeruginosa* and *S. enterica* CIP105150. The essential oil and methanol extract of *M. Chamomilla* were tested against *C. albicans* and *Aspergillus* as pathogenic fungal species in human body and compared with benomyl and griseofulvin. The result showed that the growth of fungal species was significantly inhibited by the essential oil and methanol extract (Table 1).

The methanol extract was more active on clinical origin and reference *C. albicans*. *A. niger* and *Aspergillus sp* were sensitive to the extract with the diameters were 14 to 13 mm. The essential oil was more active on clinical origin and reference *C. albicans. A. niger* and *Aspergillus sp* were sensitive to the extract with the diameters were 14 to 13 mm. Oil and methanol extract were also interesting to find that the inhibition effect against fungal strains were higher than that of benomyl and griseofulvin. *C. albicans* was more sensitive to the methanol extract and oil essential than *Aspergillus* (Heuskin et al., 2008; Agnaniet et al., 2009).

Although the MICs and MBCs result varied between tests organisms (Table 2), in the most cases the MIC was equivalent to the MBC, indicating a bactericidal action of the oil. The more bactericidal effect of methanol extract was for *Escherichia coli* CIP 105182, *S.dysenteria* CIP 5451 and *P. aeruginosa* (MIC and MBC=25 µg/ml). Methanol extract showed antimicrobial activity for *P. mirabolis* 104588 CIP (MIC and MBC=50 µg/mL), *E. faecalis* CIP 103907, *B. cereus* LMG 13569, *S. enterica* CIP105150, *S. aureus* ATCC 9244 and *S. camorum* LMG13567 (MIC and MBC=100 µg/mL). Methanol extract showed bacteriostatic activity for *S. pyogenes*. The essential oil of *M. Chamomilla* showed the strongest bactericidal activities on *C. albicans* ATCC 10231 (1 µg/ml). It was fungicidal activity for *C. albicans* and *A. niger*. Methanol extract was microbicidal action for *C. albicans* and *C. albicans* ATCC 10231(Agnaniet et al., 2009).

Antioxidant activity

The essential oil was subjected to screening for their possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging (Figure 2)

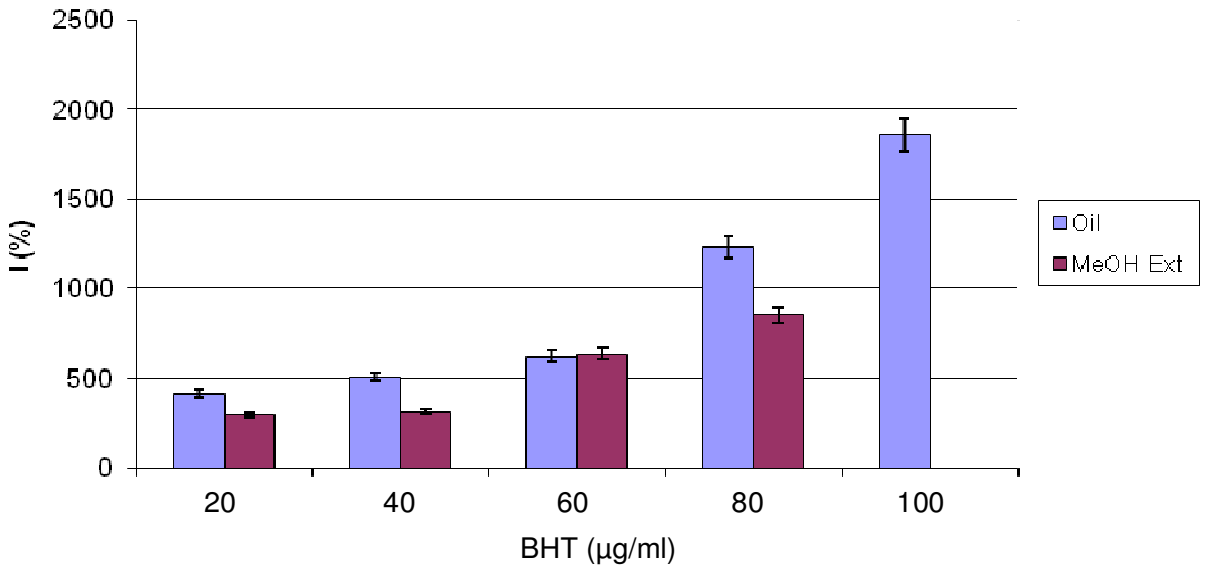

Figure 2. DPPH radical scavenging activity of essential oil and methanol extract of *M. Chamomilla*
IC_{50} of essential oil and methanol extract respectively 4.18 µg/mL and 1.83 µg/ml, I=%DPPH radical scavenging=[(control absorbance- sample absorbance)]/control absorbance] X 100.

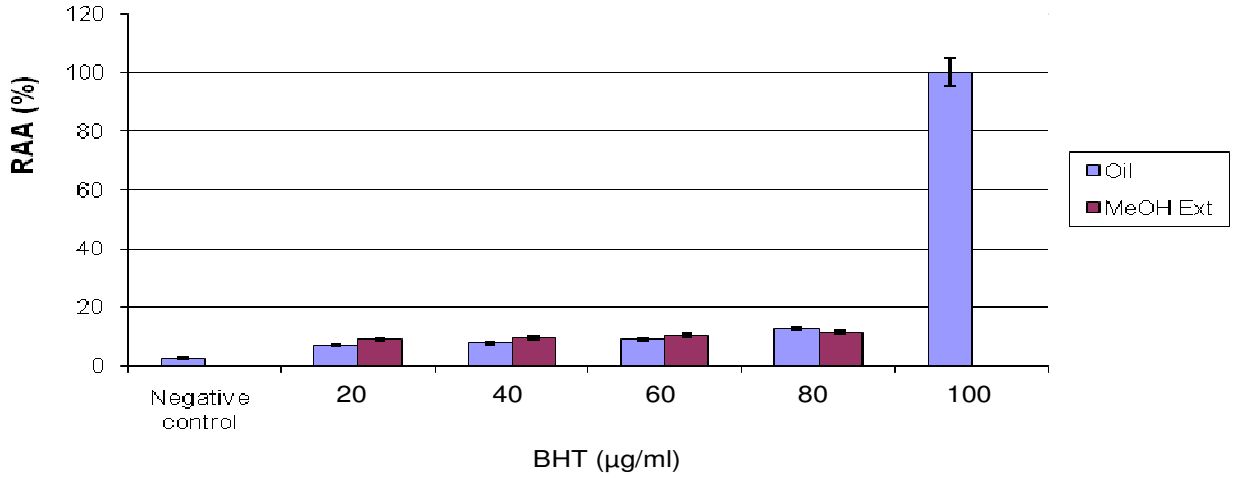

Figure 1. β-carotene bleaching test of *M. Chamomilla,* essential oil and methanol extract. RAA: Relative Antioxidant Activity= (sample absorbance/BHTabsorbance) X 100**,** BHT: 2, 6-di-tert-butyl-4-methylphenol or 2, 6-Bis (1, 1-dimethylethyl)-4-methylphenol.

and β-carotene-linoleic acid assays (Figure 1). BHT was used as positive control in both test systems. In the DPPH test system, the IC50 value of essential oil and methanol extract were respectively 4.18 and 1.83 µg/ml. In the β-carotene-linoleic acid system, oxidation was effectively inhibited by *M. Chamomilla*, the RAA value of essential oil and methanol extract were respectively 12.69 and 11.37%. When compared to BHT, the oil and methanol extract were nearly the same value.

In conclusion, this study shows *in vitro* high antimicrobial activities and antioxidant activity of the *M. chamomilla* essential oil and methanol extract. It was bactericidal and low fungicidal for most of the reference strains and some clinic strains tested. Its effect is most effective against reference bacteria than clinical bacteria. The essential oil exhibits also antioxidant activity. These results indicate that the *M. chamomilla* could be used as a natural antimicrobial agent for human and infectious diseases and in food preservation. Furthermore, the development of natural antimicrobial agents will help to

decrease negative effects (pollution of environment, resistance) of synthetic chemicals and drugs. The interesting antimicrobial effects observed in some cases support the traditional use of this plant, particularly by the local population, which needs cheap medicine.

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