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Full Length Research Paper

Antimicrobial and antioxidant activities of essential oil and methanol extract of *Jasminum sambac* from Djibouti

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The essential oil was subjected to screening for their possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging and - carotene-linoleic acid assays. Butylated hydroxytoluene (BHT) was used as positive control in both test systems. In the DPPH test system, the IC_{50} value of essential oil and methanol extract were respectively 7.43 and 2.30 µg/ml. In the - carotene-linoleic acid system, oxidation was effectively inhibited by *Jasminum sambac*, the RAA value of essential oil and methanol extract were respectively 96.6 and 93.9%. When compared to BHT, the oil and methanol extract were nearly the same value. Furthermore, the essential oil and methanol extract were evaluated for its antimicrobial activity using disc diffusion and microdilution methods. The essential oil and methanol extract showed better activity against bacterial species than against yeast.

Key words: *Jasminum sambac*, antimicrobial activity, antioxidant activity, essential oil, methanol extract.

INTRODUCTION

In the recent years, the antimicrobial and antioxidant actions have received much attention. This is so because of the increasing interest in human health and have been studied *in vitro* and *in vivo* by many researchers. The antioxidant may be useful in retarding oxidative deterioration of food materials especially those with high lipid content. The natural antimicrobial agents protect living organisms from damages resulting in the prevention of various diseases.

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, 1995). Although, much work has been done on the antimicrobial and antioxidant effects of different plants species. Plants are the primary sources of naturally occurring antioxidants for humans. It has been well known that essential oils and plant extracts have antimicrobial and antioxidant effects (Özer, 2007). This kind of *Jasminum* gathers more than 200 species originating from all the continents. The spe-cies *Jasminum sambac*, probably originating in tropical India and Burma, is cultivated in Ambouli (Djibouti city) for its perfume. *Jasminum sambac* is a persistent shrub, which often reaches 5 feet height in pots. The sheets are persistent, green-beds, brilliant and opposite. The flowers are white. The flowers are used in the manufacture of the perfumes and aromatizing. The plant is used in the form of liana to decorate the hair and the neck in the form of collar (Isa, 2005).

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The traditional use of this plant suggests analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant and tonic (uterine) effects. Essential oil of *J. sambac* is used as fragrance for skin care products. Jasmine oil and absolute reduce skin inflammation, tones the skin and lifts up your mood.The present work reports results of a detailed analysis of antioxidant and antibacterial activities of the essential oils and methanol extracts of *J. sambac* contributing to the search for beneficial uses of this plant.

MATERIALS AND METHODS

Isolation of essential oil

Essential oil of *Jasminum sambac* 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 7 h at a temperature (64°C) not exceeding the boiling point of the solvent (Lin, 1999). The extract was filtrated using Whatman filter paper (n° 1) and then concentred in vacuo at 40°C using a rotary evaporator. The residues obtained were stored in freezer at -80°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 enterica* CIP 105150, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Staphylococcus camorum* LMG 13567 BHI and *Candida albicans* ATCC 10231. Clinical strains of *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Aspergillus niger, Aspergillus sp* and *Candida albicans* were isolated at the Laboratoire de Biologie Médicale Saint Camille de Ouagadougou.

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 10^8 colony forming units (CFU)/ml of bacteria, 10^4spore/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 h and 72 h for fungi isolated. Plants-associated micro-organisms were incubated at 27 °C.

Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated twice (Edris et al., 2003; Kordali et al., 2005b).

Antimicrobial activity

The MIC values were determined for the bacterial 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 *J. sambac*, 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 10ml sterile test tubes containing nutrient broth . The MIC values of *J. sambac* extracts against bacterial strains and fungal isolates were determined on the basis of a microwell dilution method with some modification (Obame et al., 2007a and b).

The 96-weel plate transparent microwells 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 *J. sambac* 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. Maxipime at a concentration range of 500 - 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 Instrument 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 fungus isolates. The essential oil of *J. sambac* 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°C 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.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The hydrogen atoms or electron-donating ability of the corresponding extracts and butylated hydroxytoluene (BHT) was determined from the bleaching of purple-colored methanol solution of

DPPH (Hatano et al., 1988) . 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., 2005a) . 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 (µQuant Bio-teck Instrument Inc. USA). 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] × 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.

-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 (Dapkevicus, 1998). A stock solution of -carotene-linoleic acid mixture was prepared as follows: 0.5 mg of -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 -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, 2,6-ditert -butyl-4-methylphenol (BHT) and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm (µQuant Bio-teck Instrument Inc. USA). 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 essential oil and methanol extract of flowers of *J. sambac* were respectively 0.19% (w/w) and 3.56% (v/v).

Antibacterial screening

The test of sensitivity of the extracts of *J. sambac* to the various bacterial strains shows that the methanol extracts and essential oils have an inhibiting potential. The methanol extracts and essential oils are active on bacteria gram + and -. They gave a great antimicrobial activity and an inhibition with the diameters from 8 to 41 mm for oil essential and from 7 to 17 mm for the methanol extract. Essential oils are more active than the methanol extract.

The bacteria most sensitive to the essential oil of *J. sambac* were *S. pyogenes* (41 mm), *S. enterica* CIP 105150, *E. coli* CIP 105182 (31 mm), *S. dysenteria* CIP 5451 (29 mm), *L. innocua* LMG 1135668 (28 mm). The other bacterial strains were sensitive with diameters of inhibit-tion going from 17 to 25 mm. The bacterial strain *S. camorum* LMG 13567 is resistant to the essential oil of *J. sambac.* The methanol extract of *J. sambac* was more active on *E. faecalis* CIP 103907 (17 mm), *Salmonella enterica* CIP 105150, *S. pyogenes* (16 mm). The other bacterial strains were sensitive with diameters of inhibit-tion going from 11 to 15 mm. *S. camorum* LMG 13567, *E. faecalis*, *P. aeruginosa*, *S. aureus* are resistant to the methanol extract of *J. sambac*. The antimicrobial activity of essential oil is stronger than that of the methanol extracts. The essential oil of *J. sambac* presents an antimicrobial activity stronger than the tetracycline. However, methanol extract of *J. sambac* has an antimicrobial activity weaker than the tetracycline. Essential oil of *J. sambac* presents an antimicrobial activity stronger than the ticarcycline for *E. coli* CIP 105182, *P. aeruginosa* and *S. pyogenes*. The methanol extract of *J. sambac* have an antimicrobial activity weaker than the ticarcycline except for *E. coli* CIP 105182.

The essential oil and methanol extract of *J. sambac* 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). Clinical origin *C. albicans* was less sensitive to the essential oil (13 mm) than reference *C. albicans* strain (15 mm). Oil and methanol extract were also interesting to find that the inhibition effect against *C. albicans* were higher than that of benomyl (*C. albicans*, 13 mm) and griseofulvin (*C. albicans*, 15 mm). *Aspergillus niger* was resistant to the essential oil (9 mm) . *C. albicans* was more sensitive to the methanol extract (15 mm) than *Aspergillus sp.* and *A. niger* (14 and 13 mm). The values of the MIC and MBC of oil essential and the extract of *J. sambac* with the bacterial strains are consigned (Table 2). The methanol extract of *J. sambac* contains bactericidal properties with MIC and MBC equal to 80 µg/ml on *E. faecalis* CIP 103907, *E. coli* CIP 105182, *S. enterica* CIP 105150 and *S. pyogenes*. The extract is bactericidal on *B. cereus* LMG 13569 with MIC and MBC equal to 240 µg/ml. It is bacteriostatic on *L. innocua* LMG 1135668.

The essential oil of *J. sambac* showed the strongest bactericidal activities on *E. faecalis* CIP 103907 (4 µg/ml). It is bacteriostatic effect on *B. cereus* LMG13569, *E. coli* CIP 105182, *L. innocua* LMG 1135668, *Salmonella enterica* CIP 105150, *S. aureus* ATCC 9244 and *S. pyogenes*. Essen-tial oil was fungicidal activity for *C. albicans* and *C. albicans* ATCC 10231. Methanol extract was microbicidal action for C*. albicans, C. albicans ATCC* 10231 and *Aspergillus spp.*

Table 1. Inhibition (mm) of bacterial growth.

	Origin	*Jasminum sambac*			
		Oil	MeOH	Te[b]	Ti[b]
Reference strains					
Bacillus cereus LMG 13569	LMG	17	14	18	50
Enterococcus faecalis CIP 103907	CIP	22	17	19	30
Escherichia coli CIP 105182	CIP	31	15	22	8
Listeria innocua LMG 1135668	LMG	28	13	14	50
Salmonella enterica CIP 105150	CIP	31	16	16	50
Shigella dysenteria CIP 5451	CIP	29	13	21	31
Staphylococcus aureus ATCC 9244	ATCC	23	11	17	48
Staphylococcus camorum LMG 13567	LMG	8	10	20	16
Hospital strains					
Enterococcus faecalis	Foecal	25	9	20	28
Pseudomonas aeruginosa	Vaginal liquid	23	7	21	19
Staphylococcus aureus	Vaginal liquid	23	8	21	27
Streptococcus pyogenes	Vaginal liquid	41	16	20	24
Fungal strains				**Benomyl**	**Griseo**
Candida albicans ATCC 10231	ATCC	15	15	5	15
Candida albicans	Clinical	13	15	5.5	15
Aspergillus niger	Clinical	9	13	6.4	6
Aspergillus sp	Clinical	10	14	5.2	7

[a]Tested at a concentration of 5 µl/disc [b]Te: tetracycline, Ti : ticarcilline. Values given as mm, ND: not determined, MeOH: methanol extract**,** Griseo: Griseofluvin.

Table 2. Minimum inhibitory concentration, minimum bactericidal concentration data (%v/v) obtained by microdilution method.

	Origin	*Jasminum sambac*			
		Oil		MeOH	
		MIC	MBC	MIC	MBC
Reference strains					
Bacillus cereus LMG 13569	LMG	0.5	1	240	240
Enterococcus faecalis CIP 103907	CIP	1	1	80	80
Escherichia coli CIP 105182	CIP	0.5	1	80	80
Listeria innocua LMG 1135668	LMG	0.5	1	80	240
Salmonella enterica CIP 105150	CIP	1	2	80	80
Shigella dysenteria CIP 5451	CIP	1	>8	240	>400
Staphylococcus aureus ATCC 9244	ATCC	1	4	400	>400
Staphylococcus camorum LMG 13567	LMG	8	>16	>400	>400
Hospital strains :					
Enterococcus faecalis	Foecal	4	>8	>400	>400
Pseudomonas aeruginosa	Vaginal liquid	1	>8	>400	>400
Staphylococcus aureus	Vaginal liquid	8	>8	400	>400
Streptococcus pyogenes	Vaginal liquid	0.5	1	80	80
Fungal strains					
Candida albicans ATCC 10231	ATCC	0.5	0.5	50	50
Candida albicans	Clinical	0.5	0.5	50	50
Aspergillus niger	Clinical	>8	>8	>200	>200
Aspergillus sp	Clinical	>8	>8	50	50

ND: not determined, MeOH: methanolic extract, Oil: essential oil.

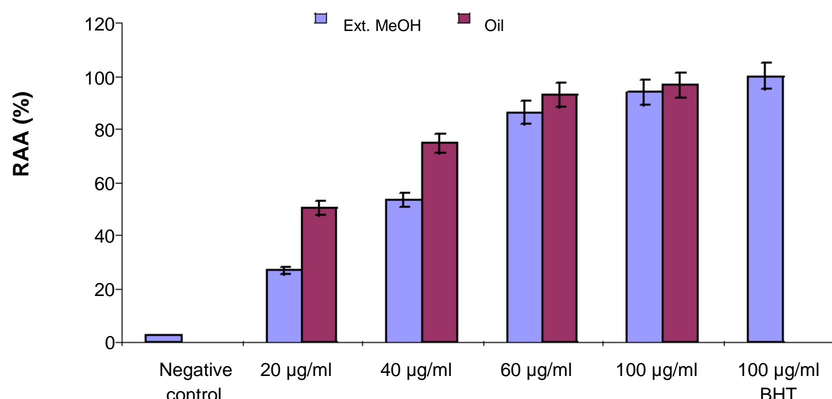

Figure 1. -carotene bleaching test of *J. sambac*, essential oil and methanol extract. RAA: relative antioxidant activity= (sample absorbance/BHT absorbance) × 100, BHT: 2, 6-di-tert-butyl-4-methylphenol or 2, 6-Bis (1, 1-dimethylethyl)-4-methylphenol.

Antioxidant activity

The essential oil was subjected to screening for their possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging and -carotene-linoleic acid assays. BHT was used as positive control in both test systems. In the DPPH test system (Figure 2), the IC_{50} value of essential oil and methanol extract were respectively 7.43 and 2.30 µg/ml. In the -carotene-linoleic acid system (Figure 1), oxidation was effectively inhibited by *J. sambac*, the RAA value of essential oil and methanol extract were respectively 96.6% and 93.9%. When compared to BHT, the oil and methanol extract were nearly the same value.

In conclusion, the study has shown that essential oil and methanol extract from *J. sambac* have in vitro antimicrobial and antioxidant activities which could support the use of the plant by traditional healers to treat various infective diseases. Further studies could lead to the most active essential oil and could lead to a new antimicrobial agent.

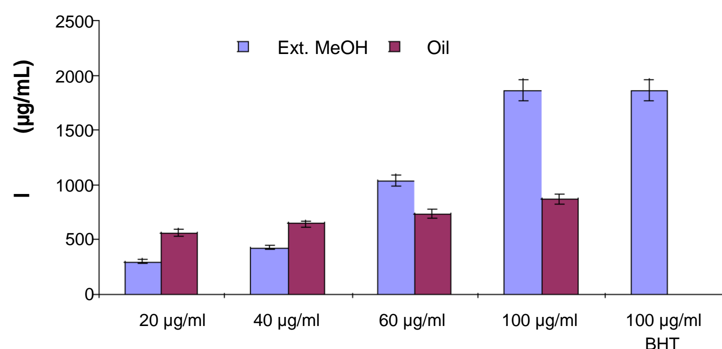

Figure 2. DPPH radical scavenging activity of essential oil and methanol extract of *J. sambac.* IC_{50} of essential oil and methanol extract respectively 7.43 and 2.30 µg/ml, I=%DPPH radical scavenging= [(control absorbance- sample absorbance)]/control absorbance] × 100.

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