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Antimicrobial activity of aqueous and methanolic extracts of two medicinal plants from the algerian Sahara

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Abstract

The antimicrobial activity of several extracts of two medicinal plants from the algerian Sahara, which known by their various therapeutic properties, *Randonia africana* Coss. and *Oudneya africana* R.Br. was investigated using the disc diffusion method. The aqueous and methanolic extracts of the two plants were obtained by decoction of the aerial parts (leaves and stems) and maceration in 80 % (v/v) methanolic solution respectively. The four extracts were tested against seven reference microorganisms; two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), two molds (*Aspergillus flavus* NRRL 391, *Aspergillus Niger* 2CA 936) and one yeast (*Candida albicans* ATCC 1024). Neither the aqueous extracts of the two species studied nor the methanolic ones showed any antibacterial activity against the Gram-negative bacteria and *A.flavus*. However, the methanolic extract of *O.africana* and the aqueous extract of *R.africana* were the most active among all the four extracts tested, mainly against Gram-positive bacteria and *C.albicans*.

Keywords: *Randonia africana*, *Oudneya africana*, antimicrobial activity, aqueous extract, methanol extract

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1. Introduction

Treating microbial infections with chemiotherapeutic agents began in the 1930s; it was one of great medical breakthroughs of the twentieth century. Most antimicrobial drugs in use today were discovered by empirical screening for inhibitors of microbial growth during the so-called “golden period” of antimicrobial drug discovery from the 1940s to the 1970s (Chopra et al., 2002; Odds et al., 2003). Unfortunately, the last two decades have seen a marked decline in the discovery and development of new antibiotics and a remarkable increase in resistance to those currently (Galani et al., 2008). Nowadays there is a need to find naturally occurring substances with antimicrobial activity as an alternative to available antibiotics or chemiotherapeutics. Plants have been shown to be a potential source for multiple antimicrobial agents, as they produce a wide variety of secondary compounds as natural protection against microbial attack (Goyal et al., 2008; Maksimović et al., 2008).

Randonia africana Coss. is a spinescent perennial deciduous woody shrub of 5-10 dm, with small linear or spatulate leaves and long clusters of small yellow flowers, it belongs to Resedaceae family. This plant inhabits the sandy plains and rocky soils; it has a fairly continuous range of distribution in the African continent, extending from Senegal, Mauritania eastwards to North Africa, Ethiopia and Somalia. The local people of the Sahara use this plant to treat scorpion bites (Abdelghani et Marei, 2006; Martín-Bravo et al., 2007; Berrehal et al., 2010). The second plant, *Oudneya africana* R.Br. is a Saharan plant of the Brassicaceae family. It is a shrub with long leaves (2 to 3 cm) and flowers in short racemes, pink or purple. This plant, endemic to Algeria, Tunisia and Morocco live in gypsum soil and desert rock, and widespread throughout the northern Sahara. Local people use this plant to treat skin diseases and scorpion stings (dermatological effect), the leaves and seeds of this plant are also prepared for the treatment of digestive problems, arthritis, colds, flu and fever (Quézel et Santa, 1963; Ozenda, 1991; Chehma, 2007).

2. Materials and methods

2.1. Plant material

Aerial parts of *R.africana* and *O.africana* were collected from Tarfaya (Oaurgla, Algeria) on october and december 2012 respectively. They were identified and authenticated at the Departement of Biology and Vegetal Ecology, Faculty of nature and life sciences, Ferhat Abbas Setif-1 University, (Setif, Algeria) and voucher specimens were prepared and deposited at the herbarium of the Biochemistry Departement. The plants material was air dried at room temperature, and stored in the shade until used for analysis.

2.2. Preparation of the extracts

The aqueous extracts were prepared with decoction of 20 g crude powder in 200 ml of water, for 10 min. While the methanolic extracts were obtained by maceration of the plant material with methanol for 1 day at room temperature, and this procedure was repeated twice, then the extracts were filtered and dried under reduced pressure at a temperature below 45 °C (Belhattab et al., 2004).

2.3. Preparation of samples

In this study, the different extracts were diluted in dimethylsulfoxide (DMSO). The corresponding concentrations are expressed in terms of mg of extract per ml of solvent. It was found that DMSO at the final concentration used had no influence on the growth of the tested microorganisms. Six different concentrations were prepared for each extract (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25mg/ml).

2.4. Antimicrobial activity

The crude methanol and aqueous extracts obtained from the aerial parts of *R. africana* and *O. africana* were screened for *in vitro* antimicrobial activity using the disc-diffusion method (Choi et al., 2006), against a panel of reference strains of microorganisms, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and fungi (*Aspergillus flavus* NRRL 391, *Aspergillus Niger* 2CA 936, *Candida albicans* ATCC 1024). All these microorganisms came from American Type Culture Collection (ATCC), routinely used for the evaluation of antimicrobials. Bacterial cultures were first grown on nutrient agar plates at 37 °C for 24 h, while fungi were grown on PDA (potato dextrose agar). Microbial suspensions were prepared in sterile saline solution (0.9 % NaCl) with a density of 0.5 McFarland for bacteria and 10^6 UFC for fungi. Blank discs of 6 mm diameter were impregnated with 20 µl of each extract dilution. Blank discs impregnated with DMSO were used as negative controls, and different discs of antibiotics (OX, FA, RD, TEL, P, K, LVX, FT, NA, AM, CFP, ATM, TIC, PIP, GEN) and antifungals (Nystatin, Clotrimazol, Amphotericin B) as positive controls. The plates were incubated for 24 h at 37 °C (bacteria), 48 h at 37 °C (yeast) and 72 h at 28 °C (molds) and the diameter of any resulting zones of inhibition (mm) measured. Each experiment was repeated at least three times and the mean of the diameter of the inhibition zones was calculated.

3. Results and discussion

3.1. Antibiotic and antifungal effects

All the sixteen antibiotics used in the tests showed a very good antibacterial activity (Table 1), with inhibition diameters which, exceeded 17 mm. All fungi were sensitive to the action of various antifungal used (nystatin, clotrimazole, amphotericin B) (Table 2). Nystatin was more active against the yeast than the two molds, producing an inhibition diameter of 21 mm.

Table 1: Inhibition Diameters (mm), produced by the different antibiotics.

Bacteria	Antibiotics	Inhibition Diameters
Staphlococcus aureus	TEL	29
	OX	31
	RD	31
	P	37
	FA	32
	K	23
	GEN	24
	LVX	26
Bacillus subtilis	K	23
	GEN	21
	OX	24
	TIC	26
	CFP	29
	RD	18
	NOR	20
Escherichia coli	NA	30
	AM	21
	FT	28
	CFP	33
	ATM	34
	LVX	36
Pseudomonas aeruginosa	TIC	29
	PIP	34
	CFP	34
	ATM	33
	GEN	29
	LVX	23

Table 2: Inhibition Diameters (mm), produced by the different antifungals.

Fungi	Antifungal Inhibition Diameters		
	Nystatine	Clotrimazol	Amphotericine B
Aspergillus flavus	10	31	14
Aspergillus niger	12	15	13
Candida albicans	21	33	19

3.2. Plant extracts effects

The results of the antimicrobial activity by the disc diffusion method, of *O.africana* and *R.africana* aqueous and methanol extracts are presented in (Table 3). The four extracts did not exhibit any activity against *E. coli*, *P. aeruginosa* and *A. flavus* (data not shown). The methanolic extract (MeOH. E) of *O. africana* and the aqueous extract (Aq. E) of *R. africana* were the most active among all the four extracts tested. We noted that inhibition diameters were concentration-dependent. The methanolic extract of *O. africana* showed an interesting effect against *S. aureus*, *B. subtilis* and *C. albicans* with inhibition diameters of 11.17 mm, 11.17 mm and 10.67 mm respectively (at

200 mg/ml). While, the aqueous extract of *R. africana* showed a good activity against *B. subtilis* with an inhibition diameter of 13 mm at 200 mg/ml. Moreover, from the results obtained it seems that the antibacterial action of the extracts was more pronounced on Gram-posit

ive than on Gram-negative bacteria and these findings correlate with the observations of previous screenings of medicinal plants for antimicrobial activity, where most of the active plants showed activity against Gram-positive strains only (few are active against Gram-negative bacteria) (Rabanal et al., 2002). The Resistance of Gram negative bacteria is related to the nature of their cell wall , which, unlike that found in Gram positive, has a multilayer structure composed with an outer, membrane rich of phospholipids, proteins and lipopolysaccharides making the cell wall impermeable for the most of biocides (Faucher et Avril, 2002).

Table 3: Inhibition Diameters (mm), produced by different extracts of *Oudneya africana* and *Randonia africana*. The data are expressed as mean ± SD (*n* = 3).

Plants	Extracts	Dilutions (mg/ml)	*S.aureus*	*B.subtilis*	*C.albicans*	*A.niger*
O.africana	Aq. E	200	9 ± 0.5	7 ± 0		
		100	7 ± 0	6.66 ±0,3		
		50	-	-		
		25	-	-	-	-
		12.5	-	-		
		6.25	-	-		
	MeOH. E	200	11,17±0,8	11,17±0,3	10,67±0,6	8 ± 0
		100	10,17±0,3	10 ± 0	8 ± 0	7 ± 0
		50	9,17±0,3	9 ± 0,5	7 ± 0	-
		25	7 ± 0	8,5 ± 0	-	-
		12.5	-	7 ± 0	-	-
		6.25	-	-	-	-
R.africana	Aq. E	200	8 ± 0.5	13± 0.5		
		100	6.5±0.5	12,17±0,3		
		50	-	11,17±0,3		
		25	-	9 ± 0.5	-	-
		12.5	-	6.5 ± 0		
		6.25	-	-		
	MeOH. E	200				
		100	-	-	-	-
		50				
		25				
		12.5				
		6.25				

Aq. E: *(Aqueous extract),* ***MeOH. E:*** *(Methanolic extract),* ***(-):*** *no effect.*

Conclusion

The data summarised above indicate that the two plants under study showed good antimicrobial activity, specially against Gram-positive bacteria. Further phytochemical studies are required to determine the types of compounds responsible for the antibacterial effects of these medicinal plants.

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