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Anti-microbial Activity of the Sterols and Steroids Extracted from the Algerian *Oudneya africana* R. Br.

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Abstract: The aim of this study was to investigate the chemical composition and the anti-microbial activity of *Oudneya africana* R.Br. The anti-microbial assays showed that the extracts of sterols and steroids of the leaves and the fruits inhibited the growth of *Pseudomonas* and showed a broad spectrum antifungal.

Key words: *Oudneya africana* R.Br., medicinal plant, sterols, steroids, bacteria, antibacterial, antifungal, fungi

INTRODUCTION

Natural products contribute in a great extent to the fight against pathogenic micro-organisms. Several plants or parts of them are used in food as spices and are thought to display some therapeutic activity or to provide a natural conservation by inhibiting the microbial growth. The anti-microbial activity is another widely studied feature of essential oils[1]. Recently, many studies on plants revealed antibacterial and anti-inflammatory activity and therefore, have been made in order to understand their anti-microbial properties[2-4].

Flavonoid compounds exhibit inhibitory effects against multiple viruses[5-7]. Since flavones, flavonoids and flavonols are known to be synthesized by plants in response to microbial infections[8], it should not be surprising that they have been found in vitro, to be effective anti-microbial substances against a wide array of micro-organisms. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumour activity and a wide range of anti-infective actions have been assigned to tannins[9]. The anti-microbial properties of tannins and coumarins have been carried out in several papers[10,11].

Pseudomonas aeruginosa is an ubiquitous micro-organism, inhabitant of fresh waters, soil and plants. Their strains are capable of producing enterotoxins. These bacteria have been recognized as an enteric pathogen and causative agent of diarrhea with infants and children[12-14]. In man, *Pseudomonas aeruginosa* is an opportunist pathogen well known in the hospital environment. It seems likely to be the cause of 10 to 20% of nosocomial infections.

Worldwide spending on finding new anti-infective agents (including vaccines) is expected to increase 60% from the spending levels in 1993[15]. The human is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. The dangerous bacterial strains from hospital sources demonstrated an increased resistance to the most common antibiotics. Thus, the search for new substances with biological activities is necessary. Hospital bacteria isolated from the medical devices (catheters, vesicle probes) at the Centre Hospitalo-Universitaire (CHU) of Tlemcen, Algeria have been revealed to be resistant to antibiotic (ATB) and antifungic (ATF).

This study reports an evaluation of the anti-microbial effect against human pathogens Gram (-) and Gram (+) bacteria, yeasts and moulds tested against some sterols and steroids extracted from *Oudneya africana* R.Br. This later was collected from the Algerian Sahara and is commonly used as medicinal plant. The genus *Oudneya* belongs to the Brassicaceae family (*cruciferae*) and the brassicoideae subfamily; it comprises about four thousand species[16]. They occur mainly in temperate and cold regions of the Northern Hemisphere[17]. Locally, known as Hanet L'Ibel, *Oudneya africana* R.Br. is an endemic plant of Sahara and is used in folk medicine by local people of Ouargla (Algeria) to treat wound cicatrisation and against the scorpion's bites. However, to the best of our knowledge, there is no previous study on this particular plant. Therefore, the lack of the phytochemical information in the literature prompted this

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investigation in order to evaluate the chemical composition of the *Oudneya africana* R.Br. extracts and its anti-microbial activity.

MATERIALS AND METHODS

Plant material: The botanical identification is made according to the classification given by Ozenda[18] and has been confirmed by (Département de Botanique, Institut National Agronomique, INA, El Harrach, Algiers-Algeria), and in Laboratoire de Biologie (LB), Ouargla-Algeria. A plant sample is kept at the herbarium of the LB. The material made up of fruits and leaves was collected on May in Oued N'ssa (Ouargla). After drying and crushing, the material has been subjected to phytochemical tests and various extractions.

Phytochemical tests: Arial parts including fruits and leaves were subjected to classical phytochemical tests.

Extractions: Two solvents namely, the ethyl acetate and methanol, were used successively for the fruits and the leaves.

Microbial strains, inoculation and media

Bacteria: The strains were isolated from the medical devices (catheters and vesicle probes) of the service of surgery at the CHU of Tlemcen. It concerns four Gram (-): *Pseudomonas aeruginosa* (Pa 1 and 2), *Pseudomonas putida* (Pp.) and *Proteus* (Pr.) and two Gram (+): *Staphylococcus aureus* (St.) and *Listeria monocytogens* (Lm.).

Yeasts: Two strains of *Candida albicans* were used throughout this study. One (Ca 1) has been isolated at the service of dermatology (CHU, Tlemcen) and the other one (Ca 2) 444, is obtained from IPP (Institut de Pasteur de Paris).

Fungi: The four tested strains were obtained from the collection of the Musée National d'Histoire et Nature de Paris (MNHNP): *Aspergillus flavus* (Av. 9942), *Cladosporium herbarum* (Ch. 3369), *Botrytis cinerea* (Bc.76.3127) and *Fusarium oxysporium* (Fo. 963917).

Preparation of the *inoculum*

Bacteria: The strains preserved in the nutrient agar at 4°C, were revivified in nutrient solution and incubated at 37±1°C during 18 to 24 h. 0.1 mL of each culture was added to 10 mL BHIB (Brain Heart Infusion Broth, Pronadisa Hispanalab, S.A.).

Yeasts: The strains preserved at 4°C in the Sabouraud agar supplemented with *chloramphenicol* were revivified in nutrient solution and incubated at 30±1°C during 24 to 48 h. 0.1 mL of each culture was added to 10 mL sterile physiological water.

Fungi: The *inoculum* is presented in the form of spores' suspension in sterile physiological water at 0.1% of Tween 80[19]. The *inoculum* used for all the assays reached the microbial density of the order of 10^6 to 10^7 UFC mL^{-1} for the bacteria and yeasts and 10^8 to 10^9 spores mL^{-1} for the moulds.

Culture media: Muller Hinton for the bacteria. Sabouraud Dextrose Agar + *chloramphenicol* for the yeasts. Sabouraud Dextrose Agar for the moulds (Pronodisa Hispanalab, S.A).

Anti-microbial assay: Two different techniques were used to test the microbial activity: the paper disc diffusion and the dilution broth method.

Paper disc diffusion: The agar plate containing the appropriate medium was spread with the *inoculum* previously adjusted to the microbial densities cited above. Several discs (6 mm diameter) have been impregnated with different extracts at a rate of 4 μL for the methanol extract and 8 μL for the ethyl acetate extract. After incubation, the diameters of inhibition zones and the sensitivity were measured with a caliper.

Dilution broth method: Bacteria and yeasts. One milliliter of the extracts solubilised in methanol (for the methanol extract) and acetone (for that of the ethyl acetate) was added to the test tube and adjusted to 10 mL with sterile distilled water. The resulting mixture must have a final concentration less than 5%. The mixture was strongly agitated during few minutes to disperse the extract in distilled water. The obtained Solution Mother (SM) was carried out in successive dilutions going from 10^{-2} up to 10^{-3}. Three controls were included in this test. Each tube contains respectively, sterile distilled water, the culture medium and the solvent. 1.5 mL of each dilution and 0.5 mL of a fresh bacterial culture were added to tubes containing 8 mL of the sterile nutrient solution. After incubation at 37±1°C during 24 h for the bacteria and at 30±1°C during 48 h for the yeasts, the culture strains were spread in scratches on a solid medium. After a second incubation under the same conditions, the development or the inhibition of each culture stock was recorded. The appreciation of the anti-microbial activity is based on the measure of the inhibition diameter from the concentrations 1.3 mg mL^{-1} for the bacteria and 0.8 mg mL^{-1} for the moulds.

RESULTS AND DISCUSSION

Phytochemical tests of *Oudneya africana* R. Br.: The phytochemical tests of the aerial parts of *Oudneya africana* R.Br. showed the presence of saponosids, flavonoids, sterols, steroids and tannins in different quantities (Table 1). These results reveal that leaves and fruits contain high amounts of sterols, steroids and saponosids, medium quantities of tannins and low quantities of flavonoids.

Yield in extracts of *Oudneya africana* R. Br.: Occasionally tannins and terpenoids as well as flavonoids can be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents. Both ethyl acetate and methanol were proved to be good solvents in extracting substances from the plants. Presumably, ethyl acetate did extract sterols and steroids from the leaves and fruits of our plant material. The obtained extracts are denoted, respectively LA and FA. Other compounds remaining in leaves and fruits as flavonoids, saponosids and tannins have been extracted with methanol and have been denoted, respectively LM and FM. The yields of the methanol extracts were found to be more relevant than those of the ethyl acetate (Table 2). Moreover, the yields of the leaves extracts were found to be more or less important than the ones of the fruits. These results suggest that the leaves of *Oudneya africana* R.Br. contain more quantities of the compounds than the fruits.

Anti-microbial activity of the extracts

Paper disc diffusion: Table 3 summarises the anti-microbial activities on various germs. The results are presented in the form of histograms in Fig. 1 and 2. As shown in Table 3a, all tested ATB were inactive against both Gram (+) and it was reported that

Table 1a: Phytochemical tests of leaves of *Oudneya africana* R. Br.

Chemical family	Result
Alcaloïdes des sels	-
Stérrols et steroide	+++
Flavonoïdes	+
Saponosides	+++
Anthocyanosides	-
Anthracénosides	-

Table 1b: Phytochemical tests of fruits of *Oudneya africana* R. Br.

Chemical family	Results
Alcaloïdes des sels	-
Stérols et steroids	+++
Flavonoïdes	+
Saponosides	+++
Anthocyanosides	-
Anthracénosides	-
Tanins	++

Table 2: Yields (%) of the *Oudneya africana* R.Br. with respect to the dry matter. FM, LM, FA, and LA denote, respectively methanol extract of fruits, methanol extract of leaves, ethyl acetate extract of fruits and ethyl acetate of leaves

Extract	Yield (%) with respect to the dry matter
LM	5.93
LA	1.83
FM	3.73
FA	1.09

Table 3a: Diameter (mm) of the inhibition zones of the various strains. AMX, S, E, SXT, RA, CZ, TE, NA and CB denote, respectively Amoxicilline, Streptomycine, Erytromycine, Trimethoprine-sulphamides, Rifampicine, Cephazoline, Tetracycline, Acid nalidixic and Carbenicillin. Lm, St, Pa, Pp and Pr represent, respectively. *Listeria monocytogens, Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas putida* and *Proteus*

	LM	LA	FM	FA	AMX	S	E	SXT	RA	CZ	TE	NA	CB
LM	8	6	6	7	8	6	8	12	9	6	6	6	6
St	6	8	6	10	12	7	11	13	12	6	6	6	6
Pa1	8	25	11	24	6	6	6	6	7	6	6	6	6
Pa2	8	25	11	24	6	6	6	6	7	6	6	6	6
Pp	6	8	8	8	6	6	6	6	6	6	6	6	6
Pr	6	8	6	8	6	6	6	6	6	6	6	6	6

Table 3b: Diameter (mm) of the inhibition zones of the various strains. EC, MTR, AB, FC, MCZ and CTR denote, respectively Econazole, Mitraconazole, Amphotericine, Flucytosine, Miconazole and Clotrimazole. Ca1, Ca2, Af, Ch, Bc and F_0 represent, respectively *Candida albicans*, *Candida albicans* PIP 444, *Aspergillus flavus*, *Cladosporium herbarum*, *Botrytic cinerea* and *Fusarium oxysporium*

	LM	LA	FM	FA	EC	MTR	AB	FC	MCZ	CTR
Ca1	6	14	6	10	15	6	20	6	12	12
Ca2	6	20	8	20	14	6	15	6	18	22
Af	6	23	11	20	20	6	6	6	10	12
Ch	6	11	6	12	11	6	12	6	6	6
Bc	6	15	12	20	12	6	15	6	17	8
F_0	6	25	6	30	13	6	20	6	19	9

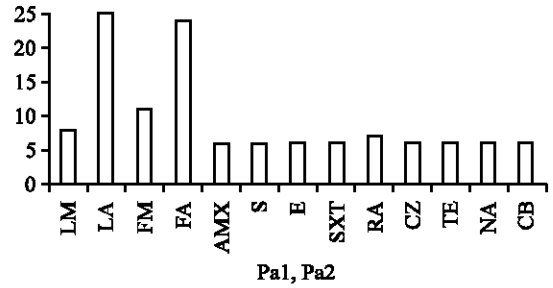

Fig. 1: The mean diameter of the inhibition zones of *Pseudomonas aeruginosa* versus the four extracts and the ATB

inhibited by sterols and steroids extracted from the leaves and the fruits of *Oudneya africana* R.Br.

The fungus *Aspergilus flavus* Af, *Cladosporium herbarum* Ch, *Botrytis cinerea* Bc and *Fusarium oxysporium* F_0 and the *Candida albicans* were insensitive to the both *Mitraconazole* MTR and *Flucytosine* FC.

In accordance with previous antifungal properties of sterols and steroids and as shown in Table 3b, LA and FA

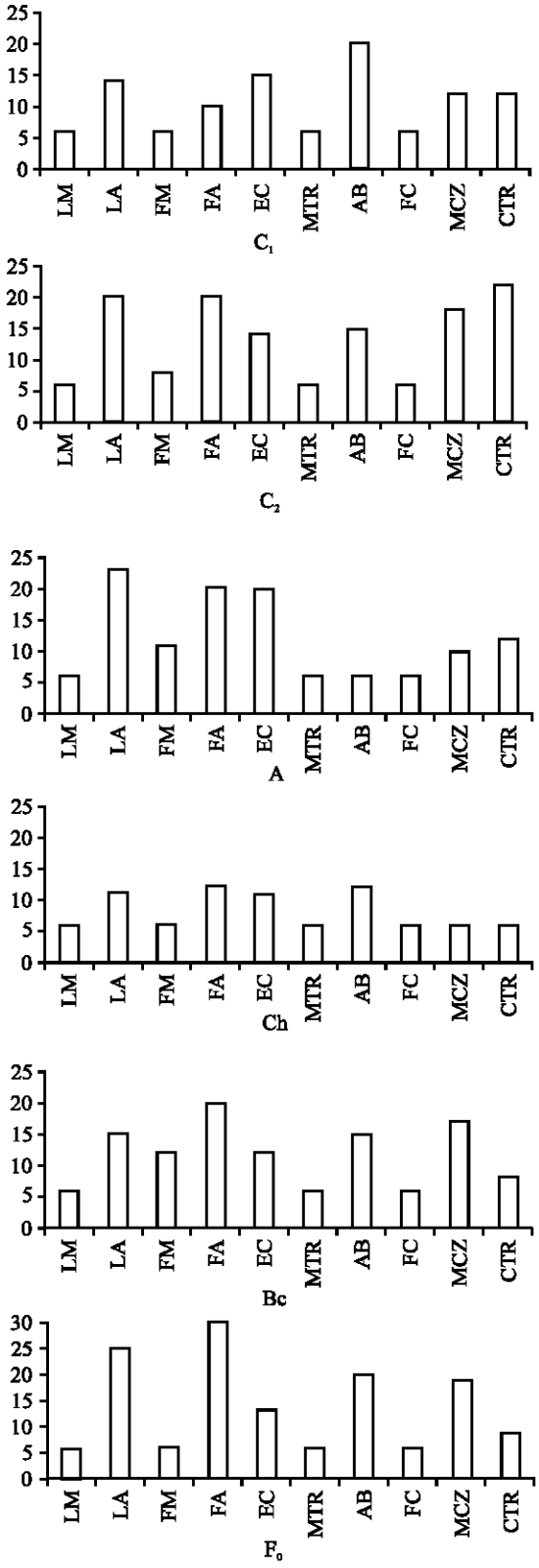

Fig. 2: The mean diameter of the inhibition zones of some strains versus the four extracts and the ATF

demonstrated significantly antifungal activities. FA were more active against both F_0 and Bc than LA with the inhibition zones of 30 and 20, 25 and 15 mm, respectively. In contrast LA were more active against Af than FA with inhibition zones of 23 and 20 mm. On the other hand, while LA and FA inhibited the growth of yeast *Candida albicans* Ca2 with inhibition zones of 20 mm, the yeast *Candida albicans* Ca1 showed a lower inhibition with the inhibition zones of 14 and 10 mm, respectively. The analysis of the variance of FA and LA with respect to the tested strains is then very significant as compared with the other extracts.

Note that, the results of this investigation are similar to the ones obtained from a recent study on *Aristida pungens* L.[20]. The similarity on the anti-microbial results of the *Aristida pungens* L. and *Oudneya africana* R.Br. is basically due to the fact that both plants are from Sahara with the psamo-xerophyte characters[21]. From our findings, it should be noted also that *Oudneya africana* R.Br. and *Aristida pungens* L. could play a major role in searching a new substance with potential anti-microbial activity.

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