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Anti-microbial Activity of Extracts from Algerian *Aristida pungens* L.

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Abstract: The antimicrobial activity of the *Aristida pungens* L. extracts was reported in this study. It was found that, the ethyl acetate extracts of the leaves of *Aristida pungens* L. show a significant antibacterial activity on *Pseudomonas* and on a large spectre of fungi.

Key words: *Aristida pungens* L., medicinal plant, bacteria, antibacterial, antifungal, fungi

INTRODUCTION

A wide variety of bacterial and fungal agents are implicated in nosocomial infections. For instance, *Candida albicans*[1, 2] is an important pathogen to human and causes serious systemic complications for immune-compromised patients. *Pseudomonas aeruginosa* strains are on the other hand, capable of producing enterotoxins. These bacteria have been recognized as an enteric pathogen and causative agent of diarrhea with infants and children[3-6].

The complications from the hospital sources caused by this category of microorganisms are in increase. Indeed, the strains are more and more antibioresistant. It is urgent to find new substances with biological activities. 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). It has been proved that those bacteria especially *Pseudomonas* produce β-lactamases.

This study reports an evaluation of the antimicrobial effect against Gram (-) and Gram (+) human pathogens, yeasts and moulds tested against some extracts of *Aristida pungens* L., collected from the Algerian Sahara and commonly used as medicinal plants.

The genus *Aristida* belongs to the graminaceae family (*poacées*) and comprises about three hundred species. They occur in the dry areas of tropical Africa, in Western Sahara, Eastern Sahara, Central Asia, United States and in Australia[7-11]. In Algeria, we record about twenty species[12] of *Aristida pungens* L. that represents a wide distribution regarding its psamo-xerophyte characters[13]. Locally, known as *'Drinn'*, *Aristida pungens* L. is a spontaneous plant used in popular medicine, to treat constipation, stomachache, indigestion and wound cicatrisation. Aerial parts of this plant have been used for the investigation of the antimicrobial activity. It is worthwhile to note that *Aristida pungens* L. is a fodder species[12] and its seeds are used to make wafers called Lûl. Futhermore, the plant has a great interest for the paper industry in particular. Harche *et al.*[14,15] studied the chemical composition of polyosides, lignins and phenolic acids of *Aristida pungens* L. Except this previous work, to our knowledge, there is a lack of phytochemical information in the literature about this plant and the identification of other metabolites and their biological and pharmacological activities as well[16, 17].

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-Algerie. A plant sample is kept at the herbarium of the LB. The material made up of stems and leaves was collected on May in Oued N'ssa (Ouargla). After drying and crushing, the material has been subjected to various extractions.

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

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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, 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 flatus* (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 CFU 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.).

Antimicrobial 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 antimicrobial 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

Yield in extracts of *Aristida pungens* L.: According to the results obtained, the yields of the methanol extracts were more important than those of the ethyl acetate (Table 1). This result can be explained by the fact that methanol extracts the majority of the chemical compounds present in our tested plant. Moreover, the yields of the leaves extracts were found to be more or less relevant than the ones of the stems. This suggests that the leaves of *Aristida pungens* L. contain more chemical families of compounds than the stems.

Table 1: Yields (%) of the extracts of *Aristida pungens* with respect to the dry matter. TM, FM, TA and FA denote, respectively methanol extract of the stems, methanol extract of the leaves, ethyl acetate extract of the stems and ethyl acetate of the leaves

Extract	Yield (%) with respect to the dry matter
TM	4.41
TA	1.03
FM	6.86
FA	1.65

Table 2a: 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*

	TM	TA	FM	FA	AMX	S	E	SXT	RA	CZ	TE	NA	CB
LM	6	3	6	7	8	6	8	12	9	6	6	6	6
St	6	8	14	6	12	7	11	13	12	6	6	6	6
Pa1	21	6	13	22	6	6	6	6	7	6	6	6	6
Pa2	21	6	13	22	6	6	6	6	7	6	6	6	6
Pp	6	7	6	6	6	6	6	6	6	6	6	6	6
Pr	6	6	6	6	6	6	6	6	6	6	6	6	6

Table 2b: 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. Ca. 1, Ca. 2, Af., Ch., Bc. and F_0 represent, respectively *Candida albicans*, *Candida albicans* PIP 444, *Aspergillus flavus*, *Cladosporium herbarum*, *Botrytic cinerea* and *Fusarium oxysporium*

	TM	TA	FM	FA	EC	MTR	AB	FC	MCZ	CTR
Ca1	6	8	6	10	15	6	20	6	12	12
Ca2	6	10	8	18	14	6	15	6	18	22
Af	6	10	14	18	20	6	6	6	10	12
Ch	6	9	6	12	11	6	12	6	6	6
Bc	6	6	13	20	12	6	15	6	17	8
F_0	6	6	6	31	13	6	20	6	19	9

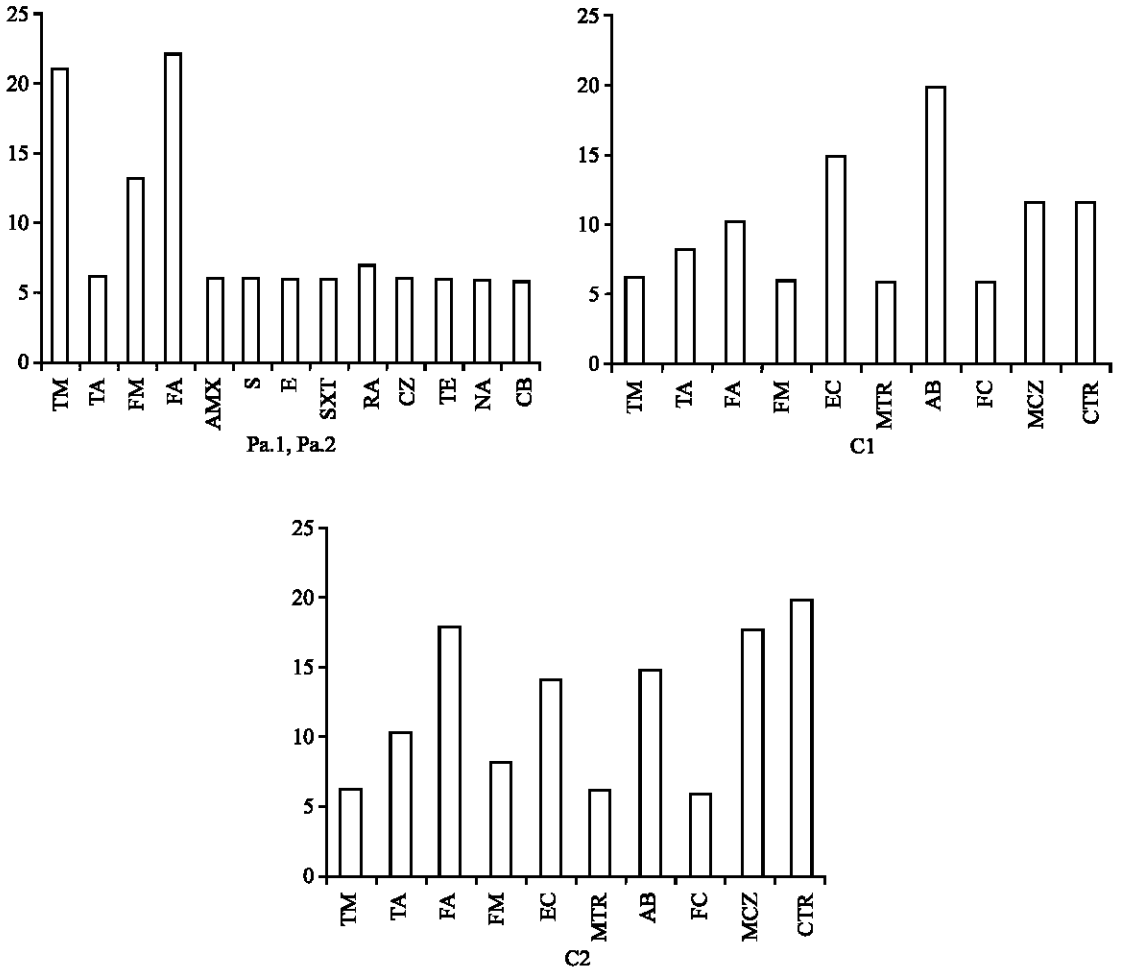

Fig. 1: The mean diameter of the inhibition zones of *Pseudomonas aeruginosa* versus the four extracts and the ATB

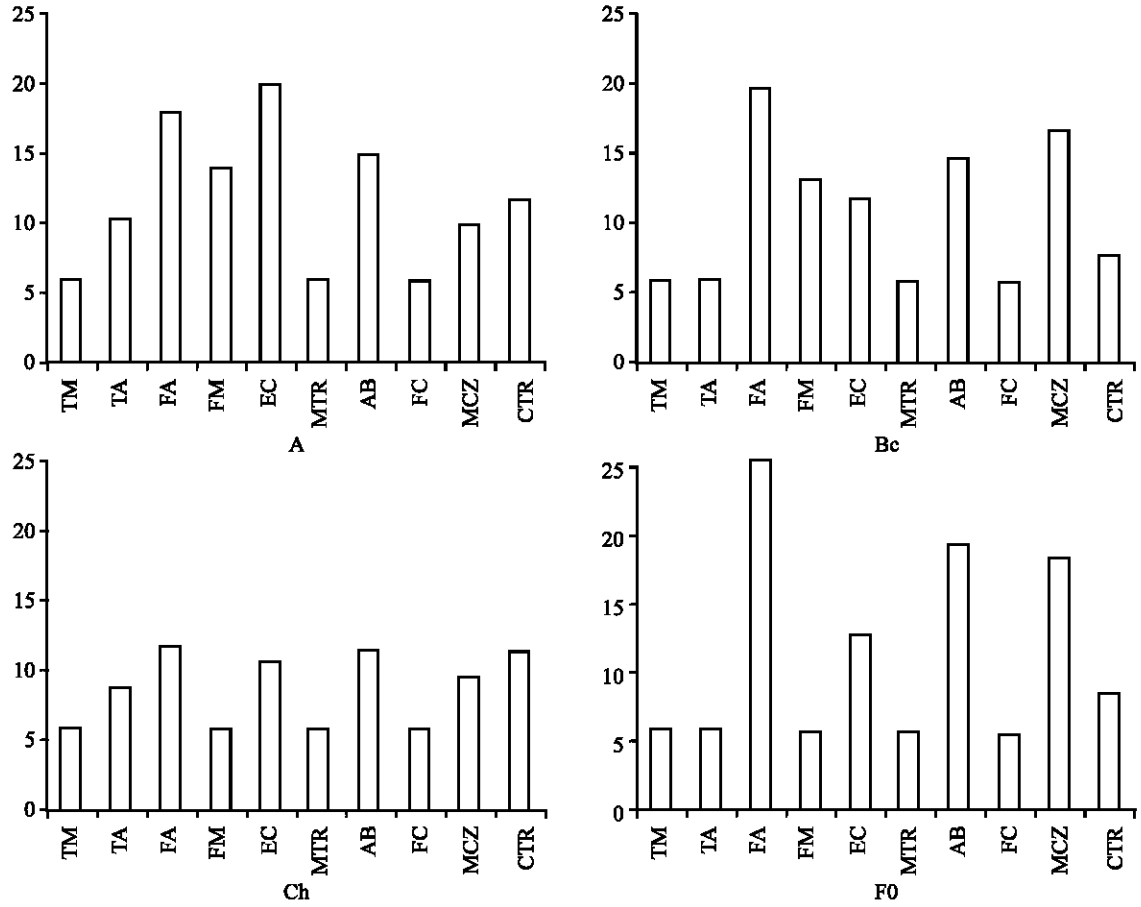

Fig. 2: The mean diameter of the inhibition zones of some strains versus the four extracts and the ATF

Antimicrobial activity of the extracts

Paper disc diffusion: The results obtained for the various germs are summarized in Table 2 and presented in the form of histograms in Fig. 1 and 2 where it was found that the *Pseudomonas aeruginosa* and the strains are sensitive, respectively to the extracts and the ATB and to the extracts and the ATF.

The extracts of *Aristida pungens L.* have antifungal and antibiotic activities since they were active against yeast, fungus and Gram (-) bacteria in several antimicrobial assay systems tested. The extracts showed antimicrobial activities on the germs better than those of the ATB and the ATF. Indeed, the most ATB did not exhibit any inhibition on *Pseudomonas*, whereas the extract FA and FM showed good antibiotic activity against *Pseudomonas* (Gram (-) bacteria are less susceptible to the antibiotic due to their outer membrane and *Pseudomonas aeruginosa* is known to be resistant against the antibiotic)[20].

Moreover, the ATF as, *Mitraconazole* and *Flucytosine* did not have any inhibition activity on all strains. FA had a large fungi spectrum, since inhibited the growth of the yeast Ca.2 and many the fungus tested including *Aspergillus flavus* Af., *Botrytis cinerea* Bc. and *Fusarium oxysporium* Fo. Thus, the analysis of the variance of FA with respect to the tested strains is very significant as compared with the other extracts.

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