



Original Research Article

Preliminary Phytochemical Analysis and Comparative Study of the Antibacterial Activity of *Juncus maritimus* Asch & Buschen Leaves

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ABSTRACT

The present work is aimed mainly to investigate and compare the antibacterial activities of some extracts of the *Juncus maritimus* Asch & Buschen leaves against six bacteria strains : *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Staphylococcus Coagulasse* (ATTC 5118), *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae*, and *Enterococcus faecalis* using Disc diffusion method. The results revealed that all extracts exhibited a certain bioactivity against all tested gram positive and gram negative bacteria at 1000 and 5000 µg/ml. Moreover the Ethanol/H₂O extracts showed higher activity compared to ethyl acetate, dichloromethane and n-butanol extracts, where the maximum activity was recorded against *Staphylococcus aureus* and a maximum inhibition diameter of 13 mm with the EtOH/water and n-butanol extracts at the concentrations of 1000 and 5000 µg/ml ; whereas, the ethyl acetate and dichloromethane and n-butanol extracts showed no effect against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis* at 500 and 700 µg/ml. The results obtained in the present study suggest that the *Juncus maritimus* Asch & Buschen can be used in treating diseases caused by the tested organisms. Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in the selected plants responsible for the antimicrobial activity.

Keyword: *Juncus maritimus* Asch; phytochemical; *Staphylococcus aureus*; ethanolic extract

INTRODUCTION

Extracts of medicinal plants are useful in the treatment of several health problems; bacterial

infections such as the urinary tract infection, that is the most common bacterial diseases in

children, as it ranks second in terms of spreading infection after respiratory tract [1-4]. The urinary tract infection comes usually from attacking microorganisms urinary system that are mostly negative gram bacteria, from digestive system, as most of the infections at urinary system caused by bacteria intestinal Enterobacteriaceae including *Escherichia coli*, which occupies a leading position among the races of this family [5].

Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramatically and sometimes without resorting to drugs and synthetic materials. However, due to the appearance of new strains of the bacteria and the weakness of chemotherapeutics and antibiotic resistance exhibited by pathogens has led to the screening of several medicinal plants for their potential antimicrobial activity [6-8]. An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available [9-14].

Since the literature concerning the *Juncus maritimus Asch & Buschen* plant contains little or no information on its antibacterial activity, we wish to report the study and evaluation of the activity of EtOH/H₂O, n-butanic alcohol, dichloromethane, and ethyl acetate extracts against several *Gram-positive* and *Gram-negative* bacterial strains *in vitro*.

Juncus maritimus Asch & Buschen known locally as "addees" is used in local folk medicine to cure some diseases such as urinary tract infection.

EXPERIMENTAL

Materials and methods

Fresh *Juncus maritimus Asch & Buschen* plant was collected from the mountains of Arris-Batna-Algeria. The plants were deposited at Laboratory de Dynamique Interaction et Réactivité des Systèmes, Department of Process engineering, Faculty of Applied Sciences,

University of Kasdi Merbah-Ouargla, Algeria. Fresh roots material was washed under running tap water, air dried under dark and then homogenized to fine powder and stored in closed container away from light and moisture.

Preliminary Phytochemical Analysis

Qualitative Phytochemical analysis of the plant powder was determined as follows:

Resins: 10 ml plant material in 20 ml distilled water, filtered ; a 10 ml filtrate + 4% HCl, the appearance of turbidity indicated the presence of *Resins* [15].

Volatile oils: 10 ml plant material in 10 ml distilled water, filtered, the filter paper was then impregnated with the filtrate and exposed to the UV rays, bright rose color indicated the presence of *Volatile oils* [16].

Coumarins: In a test tube was placed 1g of plant material in 10 ml of distilled water, and then covered with filter paper after being soaked in a diluted solutin of NaOH. The test tube was placed in boil water bath for few minutes and then exposed to a source of UV rays, yellow-green indicated the presence of *Coumarins* [6].

Terpenes and **steroids** Liebermann-Burchard reaction: 1mg plant material in 10 ml chloroform, filtered); a drop of acetic anhydride + a drop conc. H₂SO₄. The brown color indicated the presence of *Terpenes*. If the mixture left for few minutes; the appearance of blue color indicated the presence of *steroids* [17].

Phenols: 200 mg plant material in 10 ml distilled water, filtered; a 2 ml filtrate + 2 ml FeCl₃, blue-green precipitate indicated the presence of *Phenols* [17].

Tannins: 10 g plant material in 50 ml distilled water, filtered; a 2 ml filtrate + 2 ml of 1% FeCl₃, blue-black precipitate indicated the presence of *Tannins*.

Alkaloids: 200 mg plant material in 10 ml methanol, filtered ; a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayer's reagents/Wagner's reagent / Dragendorff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the

presence of alkaloids [Oguyemi et al., 1979] [18].

Saponins: method 1: 1g of plant material in 10 ml distilled water was placed in the test tube and shaked strongly; frothing persistence indicated the presence of saponins.

Method 2: 1 to 3 ml of sol. 1% $HgCl_2$ was added to 5g of plant material; the appearance of white precipitate indicated the presence of saponins.

Glycosides Keller-Kilani test: a 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4 ; green-blue color indicated the presence of Glycosides.

Steroids Liebermann-Burchard reaction: 200 mg plant material in 10 ml $CHCl_3$, filtered; a 2 ml

acetic anhydride + conc. H_2SO_4 . Blue-green color indicated the presence of steroids.

Flavonoids: 200 mg plant material in 10 ml ethanol, filtered; a 2 ml filtrate + conc. HCl + magnesium ribbon, pink-tomato red color indicated the presence of flavonoids [19].

Flavons: 10 ml of solution of plant powder in ethanol (50%) was added to 10 ml of KOH soltion (50%), and then equal amounts of this solution and extracted plant were mixed, yellow color, indicated the presence of Flavons [19].

The results of preliminary phytochemical analysis of *Juncus maritimus Asch & Buschen* leaves are summarized in table 1.

Table 1: Preliminary phytochemical analysis of *Juncus maritimus Asch & Buschen* leaves.

Phyto-constituents	Tests
Glycoside	+
Alkaloides	+
Phenol	+
Terpenes	+
Steroids	+
Resins	+
Saponines	+
Tannins	+
Flavones	+
Coumarines	+
Volatile Oils	-

(+): indicates the presence of Phyto-constituent; (-): indicates the absence of Phyto-constituent.

Extraction of plant material

The extracts were prepared by soaking 200 g of the leaves powder in petroleum ether for 24 hours in order to get rid of the fat and chlorophyll. The mixture was then filtered and the residue soaked again in a mixture of EtOH/water (70/30) for 24 hours with shaking from time to time and then filtered. The procedure was repeated three times and the filtrates were combined before being evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left overnight. The filtrates were subjected to extraction by various solvents with

increasing polarity (petroleum ether, dichloromethane, ethyl acetate, and butanol). The organic phases were separated and evaporated. The resulting residue was stored at 4°C.

Microorganisms

All bacterial standard strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Staphylococcus Coagulasse* (ATTC 5118), *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae*, and *Enterococcus faecalis* were obtained and diagnosed in Microbiology Laboratory, Arris-Batna Hospital, Algeria.

Preparation of the bacterial culture media

3.7 g of muller Hilton agar were mixed with hot distilled water and autoclaved at 121°C and 2 atm for 15 min. After autoclaving, it was allowed to cool to 45°C in a water bath. Then the medium was poured into sterilized petri dishes with a uniform depth of approximately 5 mm [20].

Preparation of plant extract impregnated discs

Whatman N°1 filter paper was used to prepare discs of 6 mm in diameter. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants [21].

Disc diffusion method

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of antibacterial activities of plant extracts. A bacterial suspension adjusted to 0.5 Mc Farland standard (1.5×10^8 CFU/ml) was used to inoculate Mueller Hinton agar plates evenly using a sterile swab. The discs impregnated with the plant extracts were placed individually on the Mueller Hinton agar surface. The discs were spaced far enough to avoid both reflection waves from the edges of the petri discs and overlapping rings of inhibition. The plate was then incubated at 37°C for 18 hours in inverted position to look for zones of inhibition. Zones of inhibitions produced by the sensitive organisms were demarcated by a circular area of clearing around the plant extract impregnated discs. The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter. The resulting residue of all extracts stored at 4°C were tested at concentrations of 500, 700, 1000 and 5000 µg/ml and were prepared in DMSO.

RESULTS

The preliminary phytochemical analysis of the crude powder of *Juncus maritimus* Asch & Buschen plant collected showed that this plant contains many active ingredients: Coumarins, tannins, volatile oils, terpenes and alkaloids, one of the antioxidants of the bacteria responsible for the effect of microorganisms, also contains flavonoids including glycosides antioxidant and phenols and saponins.

Results for antibacterial activity as obtained with *Juncus maritimus* Asch & Buschen plant revealed that the four different tested extracts in vitro by agar disc diffusion against six bacterial species. Table 2 summarizes the microbial growth inhibition of tested extracts of this plant that showed significant bacterial activity against all the tested bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus Coagulasse*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*), where the maximum activity was recorded against *Staphylococcus aureus* and a maximum inhibition diameter of 13 mm with the EtOH/water and n-butanol extracts at the concentrations of 1000 and 5000 µg/ml; whereas, the ethyl acetate and dichloromethane and n-butanol extracts showed no effect against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis* at 500 µg/ml. Moreover the dichloromethane extract showed no effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis*). Moderate inhibition was recorded with ethyl acetate extract at concentrations 1000 and 5000 µg/ml against all the bacteria tested. As far as the Ethanol/water extract is concerned a significant bacterial activity against all the bacteria was recorded at the concentrations 1000 and 5000 µg/ml.

Table 2: Antibacterial activity of extracts of *Juncus maritimus* Asch & Buschen leaves.

Plant extracts ($\mu\text{g/ml}$)	Diameter of inhibition zone (mm)					
	<i>Escherichia coli</i> (TTC25922)	<i>Pseudomonas aeruginosa</i> (TTC 27853)	<i>Staphylococcus aureus</i> (TTC 25293)	<i>Staphylococcus coagulasse</i> (TTC 5118)	<i>Klebsiella pneumoniae</i>	<i>Enterococcus faecale</i>
Dichloromethane extract	500	-	-	07	06	-
	700	-	-	07	07	-
	1000	08	09	10	10	06
	5000	08	09	10	10	06
Ethyl acetate extract	500	-	-	07	-	-
	700	08	-	08	06	-
	1000	08	06	11	09	09
	5000	09	07	11	10	09
n-Butanol extract	500	06	-	-	-	-
	700	08	09	09	-	-
	1000	10	11	13	08	09
	5000	10	12	13	08	11
EtOH/H ₂ O extract	500	-	-	-	-	-
	700	-	-	-	-	-
	1000	11	11	13	10	09
	5000	11	12	13	10	07

On the other hand Ethanol/water extracts were ineffective against *all bacteria* at concentration 500 and 700 $\mu\text{g/ml}$. Figures-1, 2, 3, and 4

showed the influence of the extract concentration four the different extracts on the growth of the the tested bacteria.

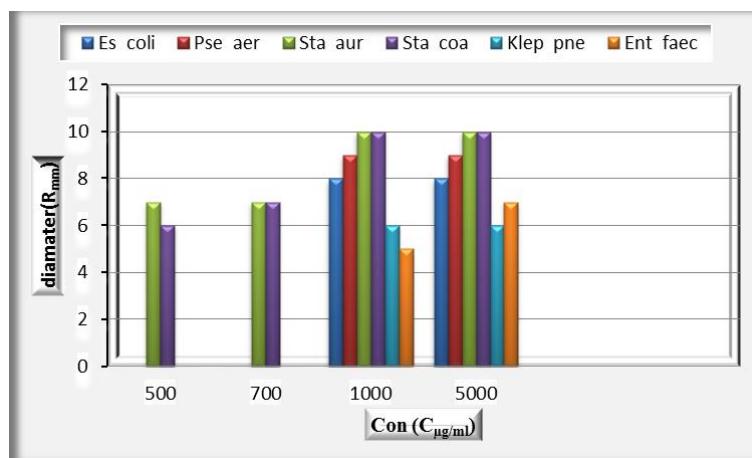


Fig. 1: The influence of three different extracts concentrations ($\mu\text{g/ml}$) of *Juncus maritimus* Asch & Buschen leaves vs the inhibition diameter (mm) on the tested bacteria

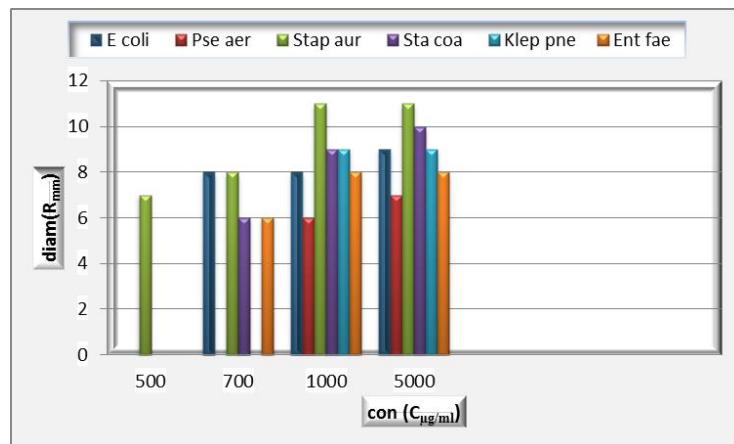


Fig. 2: The influence of three different extract concentrations of ethyl acetate ($\mu\text{g}/\text{ml}$) of *Juncus maritimus* Asch & Buschen leaves vs the inhibition diameter (mm) on the tested bacteria.

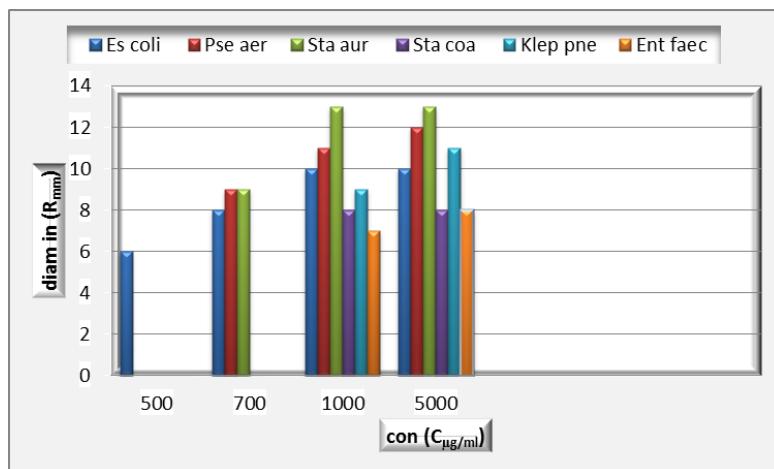


Fig. 3: The influence of three different extract concentrations of butanol ($\mu\text{g}/\text{ml}$) of *Juncus maritimus* Asch & Buschen leaves vs the inhibition diameter (mm) on the tested bacteria

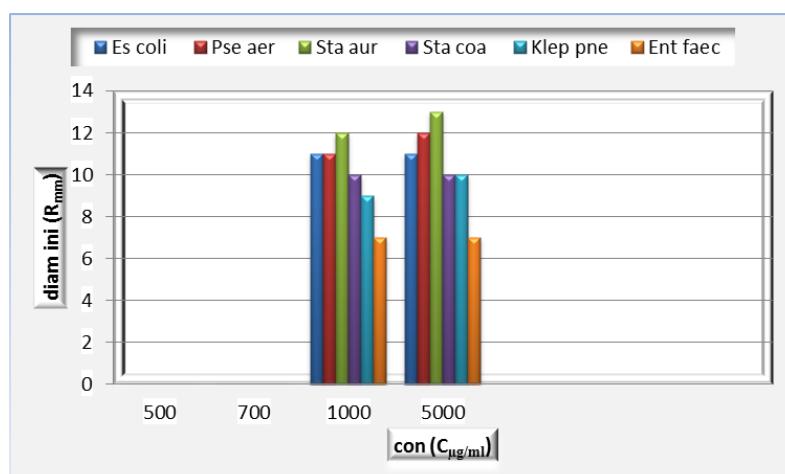


Fig. 4: The influence of three different extract concentrations of EtOH/H₂O ($\mu\text{g}/\text{ml}$) of *Juncus maritimus* Asch & Buschen leaves vs the inhibition diameter (mm) on the tested bacteria

DISCUSSION

The increase in the effect of the alcoholic extracts of some plants may be due to the extract effect on the permeability of the cell membrane and the function of the bacterial cell Al-Abed, Kaouther Fouad [22]. Since the alcoholic extracts (EtOH and n-BuOH) are more polar than dichloromethane and ethyl acetate extracts, so it has the ability to extract the largest quantities of the active substances such as phenols flavonoids [14] ; [10]. Therefore the high activity of the alcoholic extracts of *Juncus maritimus* Asch & Buschen leaves compared with the dichloromethane and ethyl acetate as shown in the results can be attributed to the presence of phenolic compounds and flavonoids that have inhibitory effect on the positive and negative gram bacteria.

Generally, the four different extracts of this plant are more or less effective towards the tested bacteria and ethanolic/H₂O extracts are more potent compared to ethyl acetate and dichloromethane ether extracts.

CONCLUSION

This study underscored the antimicrobial activity of one chenopodiaceae species namely: *Juncus maritimus* Asch & Buschen using four different solvents: Dichloromethane, Ethyl acetate, n-butanol and ethanol/H₂O with increasing polarity against six bacteria strains. The results partially justify the claimed uses of the selected plant in the traditional system of medicine to treat various infectious diseases caused by the microbes. Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in the selected plant responsible for the antimicrobial activity.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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