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A Comparative study of the antibacterial activity of *Juncus maritumus Asch* & *Buschen*; its synergic effect with some standard antibiotics and some other medicinal plants.

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ABSTRACT

The antibacterial effect of some selected algerian plants like *Juncus maritumus Asch* & *Buschen* were evaluated on several bacterial strains : Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATTC 27853, Staphylococcus Coagulasse (ATTC 5118), Staphylococcus aureus ATCC 25923, Klebcsiella pneumonie and Enterococcus faecalis. The *in vitro* antibacterial activity was performed by agar disc diffusion method. The combination of *Juncus maritumus Asch* & *Buschen* with each of the standard antimicrobs E (Erythromycine), C (Chloramphenicol), CTX (Cefotaxime), AMX (Amoxicillin), CZN: (Cefazoline), CXN (Cefalexine) were most active and showed significant synergic effects. Moreover, *Juncus maritumus Asch* & *Buschen* / other extracts of screened medicinal plants showed also high synergic effects. The results obtained in the present study suggest that *Juncus maritumus 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.

Keywords: *Juncus maritumus Asch*, bacterial strains, synergic effect, ethanolic extract.

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INTRODUCTION

Since the 1940's, chemists have developed all sorts of highly effective antibiotics (Sulfa drugs, penicillins, tetracyclines, and others that are effective) against bacterial and viral infections. In recent years there has been a flood of papers describing the synthesis of new antibacterial compounds and isolation of some natural products and study of their biological antimicrobial activities [1-6]. 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 [7-9].

Today there is an imperative necessity to find out new antibacterial compounds with various chemical structures and new mechanisms of action for new and re-emerging contagious syndoms [10]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs that are effective against bacterial infections and viral infections from infuenza and the common cold and even the more serious herbs infections and AIDS (Aquired Immune Defectious Syndrome). The viral infectious account for about 60% ilnesses, contrasted with about 15% for bacterial infections.

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 [11-14]. 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 [15].

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 [16-17]. An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available [18-23].

The literature concerning the *Juncus maritumus Asch* & *Buschen* plant contains little or no information on its antibactrial activity except that its use in local folk medicine to cure some diseases such as urinary tract infection. In the field of industry, it is used as raw material for the manufacture of paper [24]. Moreover it is used in the field of agriculture to dry the figs on teir stalks. In the past, it was used in the roofs of our houses.

We wish to report the study and evaluation of the Antibacterial activity of EtOH/H_2O extract of *Juncus maritumus Asch* & *Buschen*and ; its synergic effect with some standard antibiotics and extracts of some medicinal plants against several *Gram-positive* and *Gram-negative* bacterial strains *in vitro*.

Juncus maritumus Asch & *Buschen* known locally as "addees and also called Ithals". It grows in all Algerian mountains such as Djourdjoura mountains W.Tizi Ouzou, Bejaia, Jijel mountains and Arris Mountains, W. Batna.

MATERIALS AND METHODS

Fresh *Juncus maritumus Asch* **&** *Buschen* plant and other plants were collected from the mountains of Arris-Batna- East of Algeria. The plants were deposited at Laboratory of "Dynamique Interaction et Réactivité des Systems", Department of Process engineering, Faculty of Applied Sciences, University of Kasdi Merbah-Ouargla, Algeria. Fresh 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 apearance of turbidity indicated the presence of *Resins* [25].

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* [26].

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 solution 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*] [16].

Terpenes and ***steroids*** (Liebermann-Burchard reaction) : 1mg plant material in 10 ml chloroform, filtered) ; a drop of acetic anhydride + a drop conc. H_2SO_4. The brown color indicated the presence of *Terpenes*. If the mixture left for few minutes; the apearance of blue color indicated the presence of *steroids* [27].

Phenols **:** (200 mg plant material in 10 ml distilled water, filtered) ; a 2 ml filtrate + 2 ml $FeCl_3$, blue-green precipitate indicated the presence of *Phenols* [27].

Tannins: (10 g plant material in 50 ml distilled water, filtered); a 2 ml filtrate + 2 ml of 1% $FeCl_3$, 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/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of alkaloids [28].

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 apearance 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 [29].

Flavons: 10 ml of solution of plant powder in ethanol (50%) was added to 10 ml of KOH solution (50%), and then equal amounts of this solution and extracted plant were mixed, yellow color, indicated the presence of *Flavons* [29].

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, *Klebcsiella pneumonie*, and *Enterococcus faecalis* were obtained and diagnosed in Microbiology Laboratory, Arris-Batna Hospital, Algeria.

Preparation of the bacterial culture media

3.7 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 sterillized petri dishes with a uniform depth of approximately 5 mm [30].

Preparation of plant extract impregnated discs

Whatman N°1 filter paper was used to prepare discs of 6 mm in diameter. They were sterillized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants [31].

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 antibecterial activities of plant extracts. A bacterial suspension adjusted to 0.5 McFarland standard ($1.5x10^8$ 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 dishes 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 a concentration of 10^{-3} g/ml and were prepared in DMSO.

RESULTS

The preliminary phytochemical analysis of the crude powder of *Juncus maritumus Asch* **&** *Buschen* plant and other tested plants showed that they contain many active ingrediants: *Coumarins, tannins, volatile oils, terpenes and alkaloids*, one of the antioxidants of the bacteria responsible for the effect of microbs, also contains flavonoids including glycosides antioxidant and phenols and saponins.

The antibacterial activity of nine species extract tested in vitro by agar disc diffusion against 6 bacterial species. **Table 1** summarizes the microbial growth inhibition of these extracts of the screened plant species. These extracts of nine plants showed significant bacterial activity against all the bacteria tested (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus Coagulasse, Staphylococcus aureus, Klebcsiella pneumonie, Enterococcus faecalis). The maximum antibacterial activity was recorded against Staphylococus aureus and a maximum inhibition diameter of 16 mm with Cyndon dactylon (L) Pers, Nigella sativa and Camilia sinensis. As far as Rosmarinus officinalis is concerned, the maximum antibacterial activity was recorded against Staphylococus aureus and a maximum inhibition diameter of 14 mm. Similar results were obtained with *Juncus maritumus Asch* & *Buschen* with a maximum inhibition diameter of 13 mm against Staphylococus aureus. A weak antibacterial activity was recorded with Malva parviflora, Mentha viridis Hort and Mentha,pulegiumL with inhibition diameters of 05-06 mm against Escherichia coli and Staphylococus aureus.

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Table 1: Antibacterial activity of extracts of screened medicinal plants.

Bacteria strains / Plant extracts	Diameter of inhibition zone (mm)					
	Escherichia coli (ATTC 25922)	Pseudomonas aeruginosa (ATTC 27853)	Staphylococcus aureus (ATTC 25923)	Staphylococcus coagulasse (ATTC 5118)	Klebcsiella pneumonie	Enterococcus faecale
Juncus.maritimus ,Asch	11	12	13	10	10	07
Cynodon dactylon (L) Pers	11	07	16	12	10	08
Nigella sativa	09	7	16	10	11	07
camellia,sinensis	08	15	16	12	14	12
Malva parviflora	10	09	05	10	10	08
Mentha viridis Hort	05	09	11	11	08	08
Mentha,pulegiumL	06	07	09	09	08	07
Artemisia	08	11	11	12	10	08
Rosmarinus officinalis	09	12	14	09	08	07

All the standard antimicrobs (C, CTX, CZN, CXN and AMX) exhibited a positive effect against Escherichia coli, Staphylococus and Klepsiela pneumonie. On the other hand E, CZN, CXN and AMX were ineffective against Pseudomonas and Staphylococus Coagulasse G. Table-2 summarized the microbial growth inhibition of these standard antimicrobics.

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Table 2: Antibacterial activity of some antibiotics (standard antimicrobs)

Bacteria strains / Anti-biotics	Diameter of inhibition zone (mm)					
	Escherichia coli (ATTC 25922)	Pseudomonas aeruginosa (ATTC 27853)	Staphylococcus aureus (ATTC 25923)	Staphylococcus coagulasse (ATTC 5118)	Klebcsiella pneumonie	Enterococcus faecale
E (15µg)	_	_	26	_	_	_
C (30µg)	29	-	23	**28**	**23**	**11**
CTX (30µg)	30	20	25	**24**	**27**	_
CZN (30µg)	29	_	26	_	**22**	**15**
CXN (30µg)	24	-	27	-	**26**	-
AMX (25µg)	27	-	30	-	**15**	**28**

E : Erythromycine ; C : Chloramphenicol ; CTX : Cefotaxime ; AMX : Amoxicillin ; CZN : Cefazoline ; CXN : Cefalexine.

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As far as the synergic effect is concerned the combination of *Juncus.maritimus,Asch* with each of the standard antimicrobics, E, C, CTX, CZN and AMX were most active and showed significant synergic effect (05-08 mm) with inhibition diameters of 05-31 mm compard with the other tested plants, without antibiotics, (inhibition diameters: 05-16). The maximum synergic effect of **08** mm was recorded with Juncus.maritimusAsch/CTX against Klebcsiella pneumonie, whereas *Juncus.maritimus,Asch*/CXN Showed no synergic effect against Pseudomonas aeruginosa, and Staphylococus coagulasse. **Table-3** summarizes the microbial growth inhibition of Cyndon dactylon (L)/standard antimicrobics.

Table 3: Antibacterial activity of *Juncus.maritimus,Asch* with some antibiotics

	Diameter of inhibition zone (mm)					
Bacteria strains / Plant extract/ Antibiotic	Escherichia coli (TTC25922)	Pseudomonas aeruginosa (TTC 27853)	Staphylococcus aureus (ATTC 25923)	Staphylococcus coagulasse (ATTC 5118)	Klebcsiella pneumonie	Enterococcus faecale
Juncus.maritimus ,Asch / E (15µg)	07	07	26	07	08	05
Juncus.maritimus ,Asch / C (30µg)	30	08	23	22	22	13
Juncus.maritimus ,Asch / CTX (30µg)	31	22	25	24	27	09
Juncus.maritimus ,Asch / CZN (30µg)	27	07	26	05	12	12
Juncus.maritimus ,Asch / CXN (30µg)	25	06	27	07	08	06
Juncus.maritimus ,Asch / AMX (25µg)	31	05	30	06	06	30

A high antibacterial activity was recorded with Juncus.maritimus,Asch / *Artemisia*, Juncus.maritimus,Asch / Mentha,pulegiumL, Juncus.maritimus,Asch / Mentha viridis with inhibition diameters of 20-25 mm against Staphylococus aureus and Enterococcus faecale. Table-4 summarizes the microbial growth inhibition of Juncus.maritimus,Asch / other extracts of screened medicinal plants.

Table 4: Antibacterial activity of a mixture of *Juncus.maritimus,Asch* and some extracts of screened medicinal plants.

	Diameter of inhibition zone (mm)					
Bacteria strains / Plant extracts	Escherichia coli (TTC25922)	Pseudomonas aeruginosa (TTC 27853)	Staphylococcus aureus (ATTC 25923)	Staphylococcus coagulasse (ATTC 5118)	Klebcsiella pneumonie	Enterococcus faecale
Juncus.maritimus,Asch /Cynodon dactylon (L) Pers	1 3	23	11	12	07	15
Juncus.maritimus,Asch / *Nigella sativa*	12	12	15	14	18	14
Juncus.maritimus,Asch / camellia,sinensis	11	12	11	13	20	23
Juncus.maritimus,Asch / Malva parviflora	13	12	11	12	21	05

Juncus.maritimus,Asch / Mentha viridis Hort	**12**	**08**	**25**	**15**	**15**	**23**
Juncus.maritimus,Asch / Mentha,pulegiumL	**12**	**10**	**24**	**14**	**20**	**25**
Juncus.maritimus,Asch / *Artemisia*	**11**	**07**	**20**	**16**	**16**	**23**
Juncus.maritimus,Asch / Rosmarinus officinalis	**15**	**12**	**19**	**12**	**18**	**11**

In conclusion, Juncus.maritimus,Asch) pers with each of the standard antimicrobics, E, C, CTX, CZN and AMX were most active and showed high synergic effect against Esherichia coli, Pseudomonas aeruginosa, Staphylococcus coagulasse and Enterococcos faecale. Antibacterial activity of mixture of Juncus.maritimus , Asch and other extracts of screened medicinal plants possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissssory extracts open the possibility of finding new clinically effective antibacterial compounds. 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.

DISCUSSION

The increase in the effect of the alcoholic/H_2O extracts of Cyndon dactylon (L) Pers, Nigella sativa, Camilia sinensis and *maritumus Asch* & *Buschen* (maximum inhibition diameter of 13-16 mm) may be due to the extract effect on the permeability of the cell membrane and the function of the bacterial cell [32]. The high polarity of EtOH/H_2O extracts, increase the ability of extracting the largest quantities of the active substances such as phenols flavonoids [19, 23]. Therefore this high activity of these plants 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/H_2O extracts are more potent compared to ethyl acetate and dichoromethane ether extracts.

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