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A Qualitative Phytochemical Analysis and a Comparative Study of the Antibactrial Activity of *Retama stalks* (*raetam*)

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ABSTRACT

The present work is aimed mainly to investigate and compare the antibacterial activities of methanolic, diethyl ether and ethyl acetate extracts of *retama* on *Escherichia coli, Salmonella, Proteus mirabilis* and *Staphylococcus aureu* using well difusion method. The results of study showed a significant effect on all bacterial species except *Proteus mirabilis*. The preliminary test of *retama* constituents revealed the presence of active material : Resins, Volatil oils, Coumarins, Terpenes, Phenols, Tannins, Alkaloids, Saponins, Cardiac glycosides, and Flavons. The highest Inhibition rate of *Salmonella* is 16 mm at the concentration 100 mg/ml, while the lowest inhibition rate was 8 mm for *Escherichia coli* at concentration 1 mg/ ml in methanolic extract. The results obtained in the present study suggest that the *retama* stalks (broom broom) can be used in treating deseases 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.

Key words : phytochemical analysis, sretama talks, *salmonella*, antibactrial activity.

INTRODUCTION

The urinary tract infection 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 *Bacillus* colon *Escherichia coli*, which occupies a leading position among the races of this family.[5] As well as other pathogens include *Staphylococcus aureus* and *Streptococci* and sometimes as types fungus Candida fungal.[6]

The virulence of bacteria attacking and vulnerability to the host of fundamental importance

in the occurrence and development of the infection, which relies on a series of interactions between pathogen and host. The infection of the urinary tract occurs as a result of excessive growth of the bacteria with high virulence in the urinary tract and then the displacement of these bacteria to the bladder, and may include the urethra injury, ureters and bladder and kidney.[7,8] Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramitically without resorting to drugs and synthetic materials.

Therefore, we have chosen the study of medicinal *retama* plant (broom plant), a desert plant, it reaches a hight of more than 2 meters. It has small leaves, rapid falling (precipitation), to reduce the transpiration process, flowers butterfly shaped white Color and cup pink color purple, oval-shaped fruits contain one seed. Moreover we have chosen to study the stalks and flowers of this medicinal plant because of the availibility of year-round and represents the most of the plant size. This plant used in the treatment of allergies, stopping the bleeding and to treat the wounds. 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.[8-10] An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available.[11-15]

The aim of of this study was to evaluate the activity of aquous and alcoholic, diethyl ether and ethyl acetate extracts against several Gram-positive and Gram-negative bacterial strains in vitro.

MATERIALS AND METHODS

Fresh plant/plant parts : *retama* plant was collected randomly from the Lamniaa desert-Ghardaia Algeria in November 2013. The medicinal plant was deposited at Laboratoire de Dynamique Interaction et Réactivité des Systèmes, Department of Process engeneering, Faculty of Applied Sciences, University of Kasdi Merbah-Ouargla. Fresh stalks *plant* material was washed under running tap water, air dried under dark and then homoggenized to fine powder and stored in closed container away from light and moisture.

Preliminary Phytochemical Analysis

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

Resins (200 mg plant material in 10 ml distilled water, filtered) ; a 10 ml filtrate + 4% HCl, the apearance of turbidity indicated the presence of *Resins*. *Volatile oils* (200 mg plant material in 10 ml distilled water, filtered), then a filter paper saturated by the filtrate and exposed to the UV rays, bright rose color indicated the presence of Volatile oils.[15] *Coumarins* : In a test tube was placed 1g in 10 ml of distilled water, then covered with filter paper after being soaked in a diluted solutin of NaOH. The test tube was placed in boil water bath for a few minutes and then exposed to UV rays, yellow-green indicated the presence of Coumarins.[17] *Terpenes* (Liebermann-Burchard reaction : 200 mg plant material in 10 ml chloroform, filtered) ; a 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of Terpenes.[18] *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.[18] *Tannins* (200 mg plant material in 10 ml distilled water, filtered) ; a 2 ml filtrate + 2 ml $FeCl_3$, 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 Mayor's reagents/Wagner's reagent/Dragendroff reagent, creamish prcipitate/ brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids.[20] *Saponins* (frothing test : 0.5 ml filtrate + 5 ml distilled water) ; frothing persistence indicated the presence of saponins. *Cardiac glycosides* (Keller-Kilani test : a 2 ml filtrate + 1 ml glacial acetic acid + Fe Cl_3 + conc. H_2SO_4) ; green-blue color indicated the presence of cardiac glycosides. *Steroids* (Liebermann-Burchard reaction : 200 mg plant material in 10 ml chloroform, filtered) ; a 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring 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.[21] *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.[22]

Extraction of plant material

Each extrat was prepared by soaking 200 g of the plant powder in a mixture of EtOH/H_2O (70/30) evaporated under reduced pressure. The second extract was prepared by soaking 200 g in diethyl ether, and the third extract was prepared by soaking 200g in ethyl acetate. Each of the resulting extracts was diluted with distilled water and left overnight. The ethanolic filtrates were subjected to extraction by various solvents with increasing polarity (petroleum ether, dichloromethane, ethyl acetate, and butanol). All organic phases were separated and evaporated. The rsulting residue was stored at 4°C.

Microorganisms

All bacterial standard strains : *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Proteus mirabilis* and *Salmonella* were obtained from Colonel Chaabani Hospital, Lamniaa, W. Ghardaia. ALGERIA.

Preparation of the bacterial culture media

3.7 of muller Hilton agar was mixed with hot distilled water and autoclaved at 121°C and 2 atm for 15 minutes. After autoclavingit 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.[20]

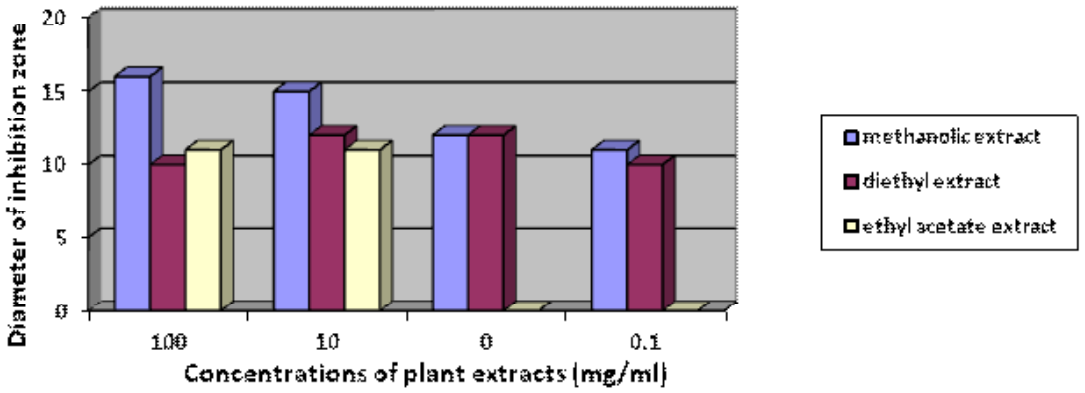

Fig.1 : The influence of three extract concentration of *Retama* plant *vs* the inhibition diameter on Salmonella

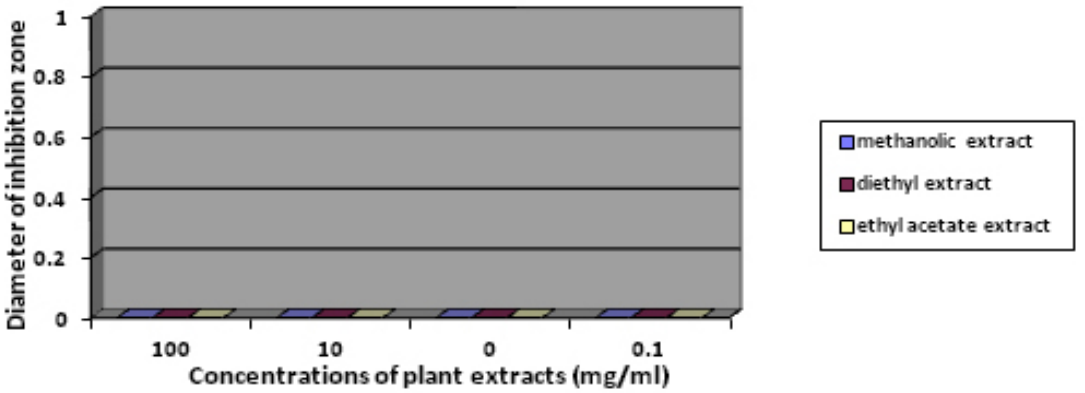

Fig.2 : The influence of three extract concentration of *Retama* plant *vs* the inhibition diameter on Proteus mirabilis

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.[23]

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.[22] A bacterial suspention 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 disces 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 rsulting residue of all extracts stored at 4°C were tested at concentrations of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} g/ml and were prepared in DMSO.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the crude powder of *Retama* plant collected showed that the stalks of *Retama* plant contains many active ingrediants : *Coumarins, tannins, volatile oils, terpenes and alkaloids*, one of the

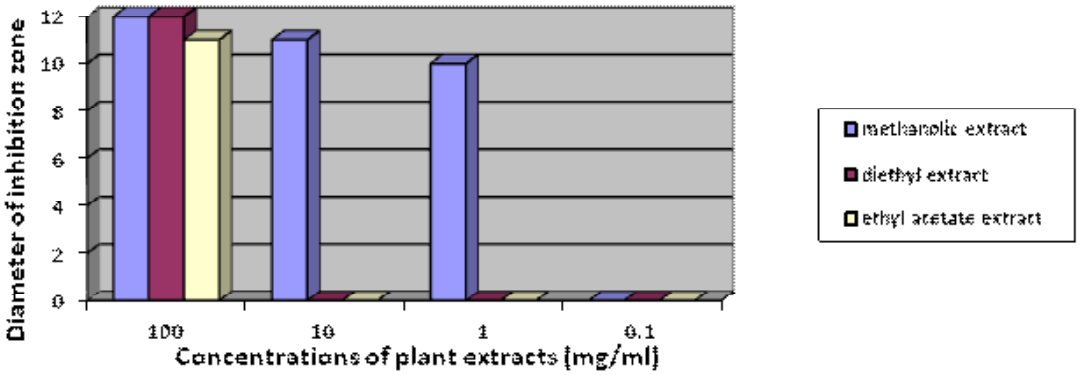

Fig.4 : The influence of three extract concentration of *Retama* plant *vs* the inhibition diameter on Staphylococcos aureus

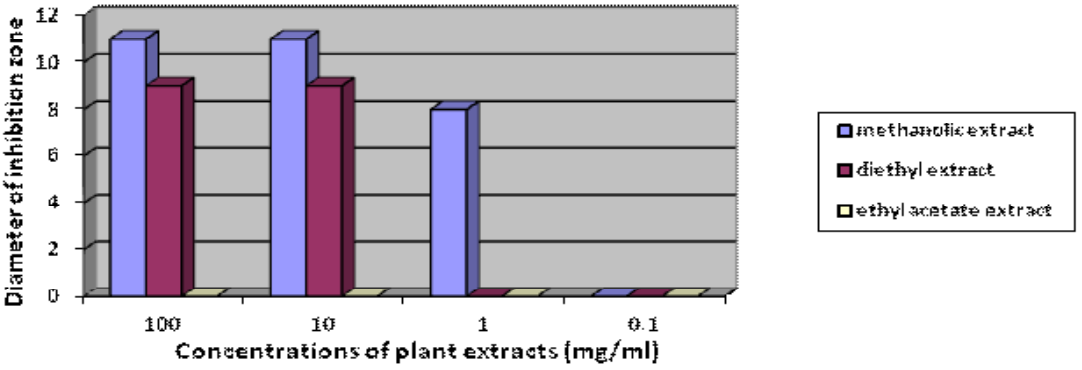

Fig. 3 : The influence of three extract concentration of *Retama* plant *vs* the inhibition diameter on Proteus Escherichia coli

Table 1 : Antibacterial activity of methanolic, diethl ether and ethyl acetate extracts of screened plant Retama

Concentration of ethyl acetate extract (g/ml)			Concentration of Diethl ether extract (g/ml)			Concentration of methanolic extract (g/ml)			Type of gram	Bacteria strains
10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{-4}		
Diameter of inhibition zone (mm)			Diameter of inhibition zone (mm)			Diameter of inhibition zone (mm)				
0	0	11	11	10	12	12	10	11	12	15	16	-	*Salmonella*
0	0	0	0	0	0	0	0	0	0	0	0	-	*Proteus mirabilis*
0	0	0	0	0	0	9	9	0	8	11	11	-	*Escherichia coli*
0	0	0	11	0	0	0	12	0	10	11	12	+	*Staphylococcos aureus*

antioxidants of the bacteria responsible for the effect of microbs, also contains flavonoids including glycosides antioxidant and phenols and saponins. As for the nature of the extracts were characterized by strength viscous dark green color and aromatic smell, due to the emergence of green chlorophyll pigment and material xanthine. The aromatic smell of *Retama* plant can be attributed to the volatile oils, also contains vegetarian jelly and glues.[24]

Results for antibacterial activity as obtained with *Retama plant* revealed that the three different extracts tested in vitro by agar disc difusion against 4 bacterial species. **Table 1** : summarizes the microbial growth inhibition of tested extracts of this plant that showed significant bacterial activity against all the bacteria tested (*Escherichia coli, Salmonella, and Staphylococcus aureu*) except *Proteus mirabilis* where the maximum activity was recorded against *Salmonella* and a maximum inhibition diameter of 16 mm with the methanolic extract at concentration 10^{-1} g/ml. On the other hand the three extracts were ineffective against *Proteus mirabilis*.

Moreover the ethyl acetate extract showed no effect against *Escherichia coli, Proteus mirabilis* and *Staphylococcus aureu* at different concentrations. Moderate inhibition was recorded with the methanol extracts at 10^{-3} against *Escherichia coli*. As far as the concentration of 10^{-4} is concerned the three extracts exhibits no actions against *Escherichia coli, Proteus mirabilis* and *Staphylococcus aureu*. However moderate effects against *Salmonella* are recorded at this concentration. Figures-1, 2, 3, and 4 showed the influence of the extract concentration on the growth of the the bacteria tested.

The increase in the effect of the alcoholic extract of the ***retama*** plant may be due to the extract effect on the permeability of the cell membrane and the function of the bacterial cell. The activity of the extracts of this plant can be attributed to the presence of phenolic compounds that have inhibitory efficacity on the positive and negative gram bacteria.

Generally, the three extracts of this plant are more or less effective towards the tested bacteria and methanolic extracts are more potent compared to ethyl acetate and Diethyl ether extracts.

CONCLUSION

This study underscored the antimicrobial activity of one chenopodiaceae species namely : *Retama* using three different solvents : Diethyl ether, Ethyl acetate, and Methanol with increasing polarity against four bacteria strains. This medicinal plant averred to be effective against three types of gram negative bacteria : *Escherichia coli, Salmonella, and Proteus mirabilis* and one type of gram positive *Staphylococcus aureus*. 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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