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A Comparative study of the antibacterial Activity of Retama stalks (raetam) ; its synergic effect with some of standard antimicrobes.

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ABSTRACT

The present work is aimed mainly to investigate and compare the antibacterial activities of methanol, diethyl ether and ethyl acetate extracts of ***retama*** stalks, and their synergic effect with some standard antibiotics on *Escherichia coli, Salmonella, Proteus mirabilis* and *Staphylococcus aureu* using well diffusion method. Results for antibacterial activity as obtained with *Retama plant* revealed that the three different extracts tested 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 methanol extract at concentration 10^{-1} g/ml. As far as the synergic effect is concerned the combination of methanol extract with each of the standard antimicrobics, CZ, VA, and CN; ether extract with each of the standard antimicrobics, CZ, VA, and CN ; ethyl acetate with E2 & CZ; E2 & VA. were most active and showed high synergic effect. The results obtained in the present study suggest that the *retama* stalks 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: phytochemical analysis, retama plant, *salmonella*, antibacterial activity, synergic effect.

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INTRODUCTION

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 [1-7]. The urinary tract infection is the most common bacterial diseases in children, as it ranks second in terms of spreading infection after respiratory tract [8-13]. 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 [12]. As well as other pathogens include *Staphylococcus aureus* and *Streptococci* and sometimes as types fungus Candida fungal [14].

Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramatically 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 [15-17]. An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available [18-23].

Therefore, we have decided to study the medicinal plant *retama,* since the literature contains little information in its use as antimicrobial activity. Moreover we have chosen to study the stalks and flowers of this medicinal plant because of the availability of year-round and represents the most of the plant size. Retama is a desert plant; it reaches a high 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 [24]. This plant used in the treatment of allergies, stopping the bleeding and to treat the wounds [1,18].

The aim of this study was to evaluate the activity of aqueous and alcoholic, diethyl ether and ethyl acetate extracts; its synergic effect with some of standard antimicrobs against several Gram-positive and Gram-negative bacterial strains in vitro.

EXPERIMENTAL

Materials and methods

Fresh plant/plant parts: *retama* plant was collected randomly from the El-mineaa desert W. Ghardaia, south of Algeria in April 2015. 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 homogenized to fine powder using an electrical mixer "Panasonic Type" for 20 minutes, and stored in closed container away from light and moisture.

Preliminary Phytochemical Analysis

We recently reported that the preliminary phytochemical analysis of the crude powder of *Retama* plant collected showed that the stalks of *Retama* plant contains many active ingredients : *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, *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 [1].

Extraction of plant material

Each extrat was prepared by soaking 200 g of the plant powder in a mixture of MeOH/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. All organic phases were separated and evaporated. The resulting 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, El-mineaa, W. Ghardaia. 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 minutes. 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 [25].

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 [26].

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 [27]. 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 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 10^{-1} g/ml and were prepared in DMSO.

Standard ant microbes

All standard antimicrobs: Cefazolin (CZ), Vancomycin (VA) and Gentamicin (CN) were obtained from Italian company "*Liofilchem.*"

RESULTS

Two standard antimicrobs: Cefazolin (CZ), and Gentamicin (CN) exhibited a positive effect against all tested bacterial strains: Escherichia coli, Samonella, Staphylococus aureus and Proteus mirabilis. On the other hand Vancomycin (VA) was ineffective against Escherichia coli, Samonella, and Proteus mirabilis. Moreover the solvent DMSO showed no effect against all tested bacterial strains. Table 1. and Table-2 summarized the microbial growth inhibition of these standard antibiotics.

Table 1: Conc. of some standard antibiotics with their antibacterial activity

Antibiotic	**Conc.**	Antibacterial activity
Cefazolin	30 mcg	Gram positive and negative bacteria
Vancomycin	30 mcg	Gram positive bacteria
Gentamicin	10 mcg	Gram positive and negative bacteria

Cefazolin: CZ; Vancomycin: VA; Gentamicin: CN

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Table 2: Antibacterial activity of some and DMSO

Bacteria / Antibiotic	E.coli	Salmonella	Staphylococcus aureus	Proteus mirabilis
Cefazolin	17	26	23	24
Vancomycin	-	-	15	-
Gentamicin	24	24	19	12
DMSO	-	-	-	-

Results for antibacterial activity as obtained with *Retama plant* revealed that the three different extracts tested (Methanolic, diethylether and ethyl acetate extracts) in vitro by agar disc diffusion against 4 bacterial species: Escherichia coli, Samonella, Staphylococus aureus and Proteus mirabilis. Table 3 : 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*.

Table 3: Antibacterial activity of methanolic, diethl ether and ethyl acetate extracts of screened plant Retama.

Bacteria / Extract	E.coli	Salmonella	Staphylococcus aureus	Proteus mirabilis
Methanol	11	16	12	-
Diethyl ether	09	10	12	-
Ethyl acetate	-	11	11	-

The resulting residue of all extracts stored at 4°C were tested at concentrations of 100 mg/ml were prepared in DMSO.

As far as the synergic effect is concerned the combination of Ethanolic extract with each of the standard antimicrobics, CZ, VA, and CN were most active and showed high synergic effect. The maximum antibacterial activity was recorded with E1 & CZ against Esherichia coli, Salmonella, and Staphylococcus aureus. Moreover, the maximum antibacterial activity was recorded with E1 & CN against Proteus mirabilis, whereas E1 & VA showed no synergic effect against Esherichia coli, and Salmonella. The combinations of diethyl ether extract with each of the standard antimicrobics, CZ, VA, and CN were also most active and showed significant synergic effect. The maximum antibacterial activity was recorded with E2 & CZ against Esherichia coli, Salmonella, and Staphylococcus aureus, whereas E2 & VA showed no synergic effect Salmonella. Similar results were recorded with E2 & CZ; E2 & VA. Table-4 Summarizes the microbial growth inhibition of *Retama* & standard antimicrobics.

Table 4: Microbial growth inhibition of *Retama* & standard antimicrobics.

Bacteria / Extract antibiotic &	E.coli	Salmonella	Staphylococcus aureus	Proteus mirabilis
E1 &(CZ 30 mcg)	18	25	27	17
E1 &(VA 30 mcg)	-	-	20	09
E1 &(CN 10 mcg)	10	24	22	26
	----------	----------	----------	----------
E2 &(CZ 30 mcg)	16	25	26	17
E2 &(VA 30 mcg)	08	-	20	08
E2 &(CN 10 mcg)	09	24	19	17
	----------	----------	----------	----------
E3 &(CZ 30 mcg)	18	25	28	19
E3 &(VA 30 mcg)	08	-	19	07
E3 &(CN 10 mcg)	08	22	24	24

E1: methanolic extract , **E2:** Diethyl ether extract, **E3:** Ethyl acetate extract

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The diameters of inhibition zone (mm) of *Retama* extracts and tested antibiotics on the bacterial species: Escherichia coli, Samonella, Staphylococus aureus and Proteus mirabilis are summarized in Fig.1, Fig. 2, Fig. 3, and Fig. 4.

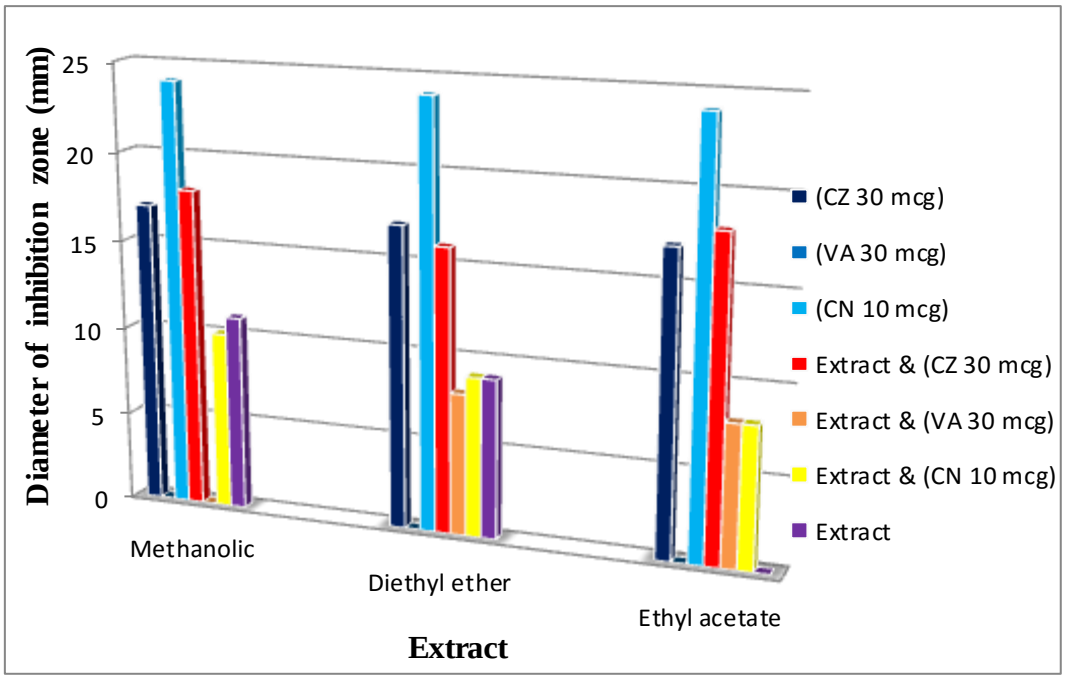

Figure 1: The diameters of inhibition zone (mm) of *Retama* extract and tested antibiotics

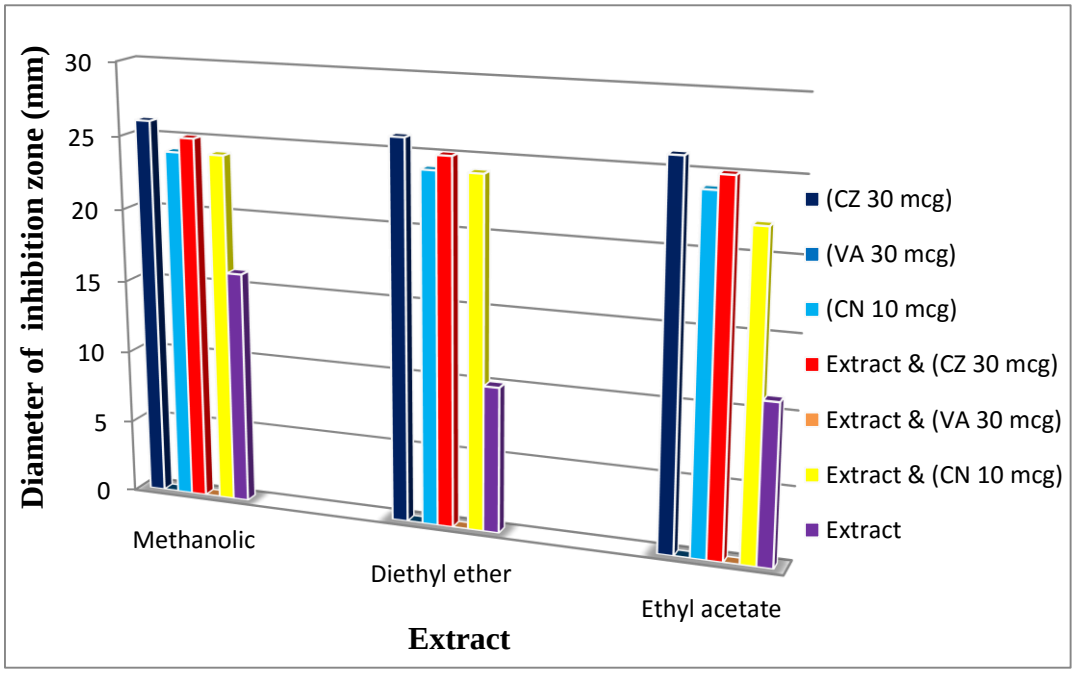

Figure 2: The diameters of inhibition zone (mm) of *Retama* extract and tested antibiotics on *Salmonella.*

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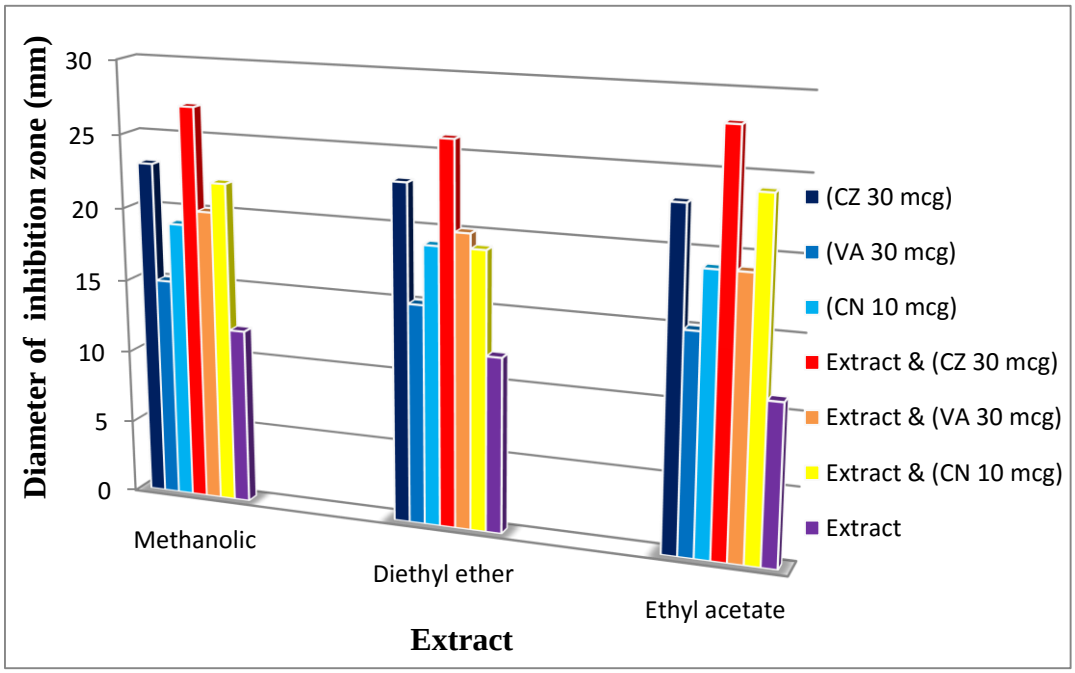

Figure 3: The diameters of inhibition zone (mm) of *Retama* extract and tested antibiotics on *Staphyloccus aureus*.

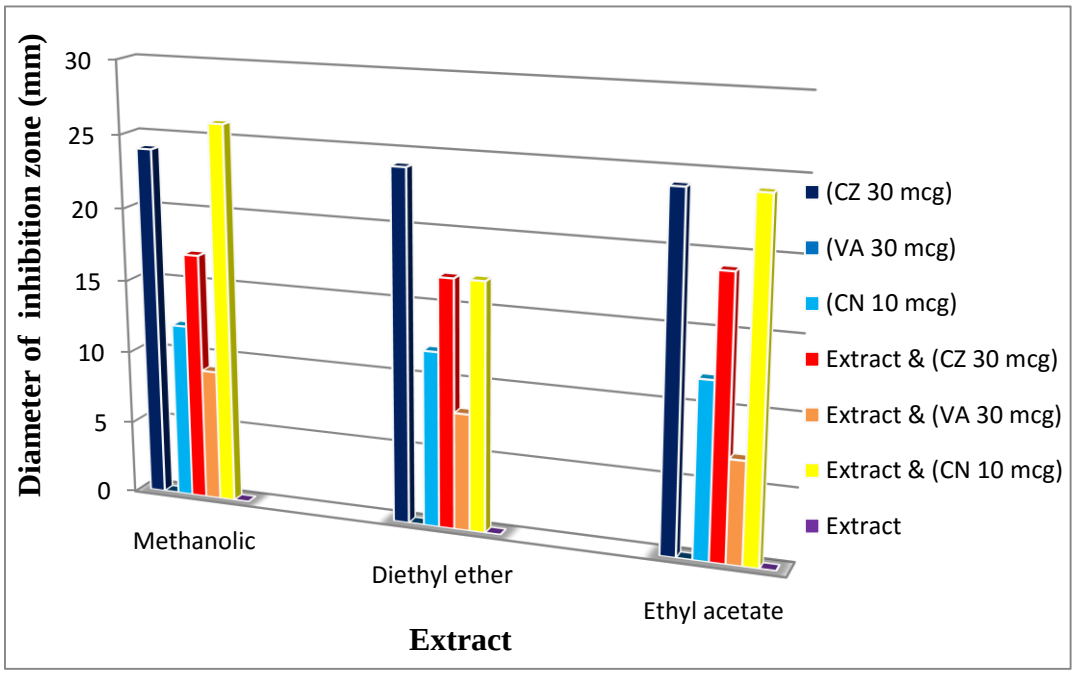

Figure 4: The diameters of inhibition zone (mm) of *Retama* extract and tested antibiotics on *Proteus mirabilis*.

DISCUSSION

As far as the synergic effect is concerned the combination of methanol extract with each of the standard antimicrobics, CZ, VA, and CN were most active and showed high synergic effect. The maximum antibacterial activity was recorded with E1 & CZ against Esherichia coli, Salmonella, and Staphylococcus aureus. Moreover, the maximum antibacterial activity was recorded with E1 & CN against Proteus mirabilis, whereas E1 & VA showed no synergic effect against Esherichia coli, and Salmonella. The high polarity of

MeOH/H_2O extracts, increase the ability of extracting the largest quantities of the active substances such as phenols flavonoids [19, 28]. 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.

The combinations of diethyl ether extract with each of the standard antimicrobics, CZ, VA, and CN were also most active and showed significant synergic effect. The maximum antibacterial activity was recorded with E2 & CZ against Esherichia coli, Salmonella, and Staphylococcus aureus, whereas E2 & VA showed no synergic effect Salmonella. Similar results were recorded with E2 & CZ; E2 & VA. These significant effects may be due to the extract effect on the permeability of the cell membrane and the function of the bacterial cell [29].

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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