



IN-VITRO ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *CALLIGONUM COMOSUM* PLANT AGAINST FOUR HUMAN PATHOGENS IN SAUDI ARABIA

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ABSTRACT: The present investigation evaluated the *in-vitro* antibacterial activity of the crude ethanolic extracts of the leaves, stem and roots of *Calligonum comosum* on two Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The antibacterial activity of the extracts (90% ethanol) was evaluated using a gar well diffusion technique. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined in this investigation. Generally, all plant extracts showed a remarkable antibacterial action against the tested microbes at significant levels. Of the plant parts tested, leaves exhibited the highest inhibitory effect on Gram negative bacteria while stem part showed the highest inhibitory effect on Gram positive bacteria. Based on these present findings it might be concluded that *Calligonum comosum* possessed great potential against these human pathogens. It might also be speculated that these plant parts can be subjected to further biochemical analysis to characterize and explore their chemical constituents. Knowledge of the specified chemical components responsible for the activity in these plant tissues would definitely uncover the mystery of the antimicrobial capacity of *Calligonum comosum* against some human pathogens. Further experimental investigations are no doubt required to validate and enhance our current findings which might eventually assist in the development of new pharmaceuticals to meet the ever increasing therapeutic demand.

Key words: *Calligonum comosum*, antimicrobial activity, minimum inhibitory concentration, minimum bactericidal concentration, human pathogens.

INTRODUCTION

Bacterial diseases are considered one of the most important causes of mortality worldwide. Moreover, a real problem is the microbial resistance which is common in antibiotic resistant bacteria [1] constituting a horrible threat to the well-being of human-kind. To address such situations, many recent research studies have been focused on exploring the potential of plant extracts on bacteria in different parts of the world [2, 3, 4, 5].

Plants have been recognized long ago as rich sources of natural products for the treatment of a wide spectrum of diseases. It has been reported that plant extracts are commonly used in traditional medicine and its contribution with respect to health coverage was estimated for over 80% of the world's population, especially in the developing world [6]. Many potent and powerful drugs are of plant origin [7]. Raw drugs are prepared from different plant parts such as leaves, stem, roots, fruits, flower and twigs via various extraction methods. Some of these raw drugs are collected in meager quantities by the local communities to meet their local needs, while commercial raw drugs are usually collected in greater quantities as raw materials for herbal industries [8]. Nowadays, medicines derived from plants have gained great acceptance worldwide and considered as alternatives to the synthetic drugs. The preference of these plant-derived medicines could be due to the low cost particularly in the poor countries, affect a wide range of antibiotic resistant microorganisms and also because of the relatively fewer adverse effects of these natural medicines relative to modern conventional pharmaceuticals [9]. However, the potential of higher plants as a source of new pharmaceuticals and drugs is still not thoroughly known. In this study *Calligonum comosum* L. Her., "Arta", a member of the family *Polygonaceae* was evaluated for its antimicrobial activity. It is a plant of tropical and subtropical regions with a wide spread in United Arab Emirates.

Former studies showed that ethanolic extract of the aerial parts of *Calligonum comosum* significantly reduced the increase in hind paw oedema induced by carrageenan in rats. Furthermore, a pre-treatment with the extract produced a significant and dose-dependent inhibition to the acute gastric ulcers induced by phenylbutazone, indomethacin [10]. Chemical analysis showed that anthraquinones and flavonoids are the common chemical constituents in Arta [11,12]. Furthermore, late investigations on *Morinda angustifolia* resulted in the isolation of a new anthraquinone, which demonstrated significant antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* [13]. Some of the phytochemical compounds, e.g., glycoside, saponin, tannin, flavonoids, terpenoids, alkaloids, have been reported to have antimicrobial activity [14,15]. It has been documented that flavone, quercetin and naringenin were effective in inhibiting the growth of different organisms such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [16]. On the other hand, due to the complex nature of the phytochemicals present in a plant extracts, the extraction solvent system needs to be adequately considered. A recent study provided data on the importance of selection of an appropriate solvent type and concentration and indicated that ethanol extracts of plants can offer significant potential for the development of novel antibacterial therapies [17].

To date, limited information is available on *Calligonum comosum* with respect to its potential in the treatment of human pathogens. Therefore, the present study was conducted and the principal aim was to analyze and evaluate the antibacterial activity in the leaves, stem and roots extracts of *Calligonum comosum* against some human pathogenic bacteria.

MATERIALS AND METHODS

Plant material

Calligonum comosum seedlings (35 – 40 cm height) were provided from Dirab near Riyadh city, Saudi Arabia. In this study, leaves, stem and roots of *Calligonum comosum* were used for testing their antimicrobial activity against four types of human pathogenic bacteria.

Preparation of *Calligonum comosum* plant extracts

Leaves, stem and roots of *Calligonum comosum* were cut, rinsed in water, spread on trays and air-dried under the sun light. After drying, the plant material were subjected to mechanical grinding and powdered by electrical blender. For ethanol extract, ten grams of the powder was soaked in 100 ml of 90% ethanol for 24 hours under room temperature, 22°C. Thereafter, the resultant solutions were filtered through Whatman filter paper No.1 grade. The filtrate was concentrated through evaporation process using water bath at 100°C. The extracts were stored in sterile glass bottles at 4°C until further use.

Source of pathogens and cultures medium

Four pathogenic bacteria: *Escherichia coli*, *Pseudomonas*, *Bacillus subtilis*, *Staphylococcus aureus* were provided from the department of pathology – King Saud University, Riyadh, Saudi Arabia. Nutrient agar medium was used as a growth medium for investigated microorganisms in this study.

Antimicrobial activity

The antibacterial activity was evaluated by noting the zone of inhibition against the test organisms [18]. Two colonies of 24 hours cultured plates were transferred to 10 ml distilled water in test tubes and mixed thoroughly to maintain uniform distribution. A sterile cotton swab was then used to spread the resulting suspension on the nutrient agar and allowed to dry for 10 minutes. Subsequently, two adequately spaced wells (holes) of 4 mm diameter each were made per plate at the culture agar surface using sterile metal cup borer. In each hole, 0.2 ml of each extract and control were put under aseptic conditions, kept at room temperature for one hour to allow the agent to diffuse into agar medium and incubated accordingly. Distilled water was used as negative control. The plates were then incubated for 24 hours at 37°C. At the end of the incubation period the zones of inhibitions were measured to the nearest millimeter [19]. The inhibition zone is the area surrounding the hole with no growth of inoculated microorganisms. For confirmation of the results each test was performed in duplicate.

Determination of minimal inhibitory concentration (MIC) of the extract

The lowest concentration of the extract that inhibits the growth of the tested microorganisms is conventionally known as the minimal inhibitory concentration. The initial concentration of the plant extract (100mg/ml) was diluted using double fold serial dilution by transferring 5 ml of the sterile plant extract into 5 ml sterile distilled water to obtain the concentration of 50mg/ml. [20].

Different concentrations were prepared from the crude extracts by doubling dilution in distilled water to get the following concentrations (50, 25, 12.5, 6.25 and 3.13 mg/ml). Each individual dilution was introduced in to nutrient agar plates already seeded with the microorganisms and all plates were incubated for 24 hours at 37°C. Agar plates with the lowest concentration of the extract (with no microbial growth) are considered as the minimal inhibitory concentration [18].

Minimal bactericidal concentration (MBC)

The Minimum bactericidal concentration (MBC) of the plant extract was determined using the method described by [21]. Samples were taken from the plates with no visible growth in the MIC assay and sub-cultured on freshly prepared nutrient agar and incubated for 48 hours at 37°C. The MBC was taken as the concentration of the extract that did not show any growth on the new set of agar plates.

Statistical analysis

Data obtained in this investigation was statistically treated with the statistical programme JMP 5.1 Start Statistics, third edition (SAS Institute, Inc., Cary, North Carolina, USA). The variations among the different treatments were tested using analysis of variance, ANOVA. The results presented are means (4 replicates \pm SD). Separation of means was performed by Tukey – test. A probability level of $P < 0.05$ was considered to indicate significant differences.

RESULTS AND DISCUSSION

Taking into consideration medicines from plant origin as an alternative form of health care is increasing because they are serving as promising sources of novel antibiotic prototypes [22]. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial properties as anticancer agents, anti-diarrheal as well as antifungal activities [16, 23]. In our current investigations we have found that the activity of ethanol extract of *Calligonum comosum* against the two Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) was different from plant part to another as shown below (Table 1). The leaf extract showed the highest zone of inhibition for Gram negative bacteria (*E. Coli* 17.5 \pm 0.75 mm and for *P. aeruginosa* is 20.5 \pm 1.38 mm) and lowest zone was observed for *S. aureus* (13.0 \pm 1.05 mm). On the other hand, stem part showed the highest inhibitory effect on Gram positive bacteria (*B. subtilis* 15.7 \pm 0.75 mm, *S. aureus* 16.8 \pm 1.17 mm) compared to the other plant parts. Interestingly, the root extracts exhibited the lowest inhibition zone for all the tested organisms compared to leaves and stem extracts (Table 1).

Table 1: Zone of inhibition (mm) of ethanol extract of various parts of *Calligonum comosum* plant on tested microorganisms. Values are mean \pm std of four replicates. Values (means) within a row with different alphabets are significantly different from each other at a probability level of $P \leq 0.05$ Means with no inhibition zone detected are indicated as dash (-).

Treatment	Leaves	Stem	Root	Distilled water
<i>E. coli</i>	17.5 \pm 0.75 a	16.0 \pm 1.17 ab	12.5 \pm 0.55 b	-
<i>P. aeruginosa</i>	20.5 \pm 1.38 a	16.2 \pm 1.72 b	13.3 \pm 0.75 c	-
<i>B. subtilis</i>	14.3 \pm 1.21 a	15.7 \pm 0.75 a	12.3 \pm 1.37 b	-
<i>S. aureus</i>	13.0 \pm 1.05 b	16.8 \pm 1.17 a	13.7 \pm 1.37 b	-

These findings might be in well agreement with Riadh [24] who investigated *C. comosum* and observed that the significant level of antibacterial activity was in a similar range of inhibition zone for the growth of the bacterium, *Listeria ivanovii* (9 – 18 mm) when laboratory tested by the agar well diffusion method. The authors also demonstrated that the most active plant part causing inhibition is the extract from the leaves, which is the very case the Gram positive in our current investigation. With regard to the minimal inhibitory concentration (MIC) of plant extracts against the bacterial strains it was also varied from plant part extract to another. Moreover, we have observed that the MIC value of the same plant part extract has changed according to the test organism (Table 2). The MIC of leaves extract was between 3.13 and 6.50 mgml⁻¹ while that of stem extract was 3.13 mgml⁻¹ and that for the root extract was in the range between 6.5 and 12.5 mgml⁻¹. On the other hand, the minimal bactericidal concentration of the ethanol extract of the leaves ranged between 3.13 and 6.50 mgml⁻¹ while that of the stem extract was 6.5 mgml⁻¹ and for the root extract it was 12.5 mgml⁻¹ (Table 2).

Furthermore, results of present investigation clearly indicated that the antibacterial activity vary with plant part used. Thus, the study ascertains the value of plant parts used, which could be of considerable interest towards the development of new drugs. The observed differences in sensitivity the *Calligonum comosum* extract between Gram-positive and Gram-negative bacteria can probably be attributed to the structural and compositional variations in the nature of the cell wall between the two groups [25].

Table 2: Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in mg/ml of the ethanol extract of *Calligonum comosum* plant against tested organisms.

Plant parts	Tested organisms							
	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>B. subtilis</i>		<i>S. aureus</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Leaves	3.13	3.13	3.13	3.13	3.13	6.50	6.50	6.50
Stem	3.13	6.50	3.13	6.50	3.13	6.50	3.13	6.50
Root	6.50	12.50	6.50	12.50	12.50	12.50	12.50	12.50

In conclusion, the present findings strongly highlighted the capability of the different parts of *Calligonum comosum* to suppress the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. This inhibitory effect of *Calligonum comosum* extract might be due to the action of special organic compounds such as anthroquinones and flavonoids as reported in previous studies for the chemical composition of *Calligonum comosum* [11,12]. Further supporting evidence was provided by Song [26] that anthroquinones, terpenoids and flavonoids have positively controlled the growth of dental caries caused by *Streptococci* when treated with separated fraction from *Polygonum cuspidatum*.

In the light of our present findings we can conclude that the ethanol leaf, root/bark extracts of *Calligonum comosum* Showed antibacterial effect in experimental models against *B. subtilis*, *E. coli*, *S. aureus*, and *P. aeruginosa* which therefore offer a scientific basis for using this plant as a good source of traditional microbiological references, further studies are required to validate our findings and improve our knowledge on the potential of the *Calligonum comosum* extract as antimicrobial in relation to its chemical composition.

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