

In Vitro Antibacterial Activity of four Saudi Medicinal Plants

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Abstract

In the current study, we tested, evaluated and compared the antibacterial activity of twenty four extracts prepared from four traditional Saudi medicinal plants (*Jatropha pelargonifolia*, *Euphorbia inaequilatera*, *Cadaba glandulosa* and *Cadaba rotundifolia*). The plant materials were extracted in six organic solvents with different polarity by two different extraction methods. The activity of these plant extracts were tested against three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus megaterium* and *Bacillus cereus*) and four Gram-Negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella enteritidis*). Minimum inhibitory concentrations (MICs) of the extracts were determined using the agar dilution method. Our results indicated that most of the tested extracts exhibited a significant broad-spectrum antibacterial activity against both Gram-Positive and Gram-Negative bacteria. The *Euphorbia inaequilatera* extracts were active against all tested bacterial strains whereas the other plant extracts showed selective activity at the level of both organic solvent and bacterial strain. The use of different extraction solvents resulted in a substantial differences in the potency of the antibacterial activity of the extracts with acetone being the most effective solvent in the extraction of bioactive compounds from the tested plant species. In addition, a number of the tested plant extracts successfully recovered more than 70% of the Ciprofloxacin antibacterial activity. *Cadaba glandulosa* extracts showed the most potent antimicrobial activity against *B. megaterium* (MIC of 16 and 32 mg/l). Different organic solvents as well as different extraction methods can be effectively employed to mine plants' phytochemical profiles and consequently illustrate their antibacterial activity against antibiotic resistant bacterial strains.

Keywords: Antimicrobial activity; Medicinal plants; Agar diffusion assay; Minimum inhibitory concentration

Introduction

Medicinal plants play a vital roles in the healthcare provision of numerous countries and they have been used as a drug treatment of major human diseases [1,2]. These plants have been classified by World Health Organization (WHO) as the best source of drugs [3]. In fact, important drugs like morphine, cocaine, codeine, digitoxin, and quinine are all of plant origin [4]. Interestingly, different drugs are obtained from different plant organs such as root, stem, flowers, and fruits [5,6]. Most of the bioactive medicinal metabolites are synthesized via plant secondary metabolic pathways during the vegetative stage of plant's life cycle and these compounds are responsible for the therapeutic properties of the medicinal plants [7]. Chemical or synthetic drugs and antibiotics have been used for treatment of human diseases and infections. In general, these compounds effectively inhibit and/or stop microbial growth via disruption of the synthesis of microbial nucleic acids, proteins and cell walls [8,9]. However, they are currently facing many health concerns. Recently, constant increase in microbial resistance to different trade drugs or antibiotic forms has been reported [10,11]. In addition, numerous studies have reported critical side effects of synthetic antibiotics on the human immune system [11,12]. Therefore, there is an emerging need for new innovative strategies to develop new and safe antibiotics. In this regard, traditional medicinal plants provide unique and promising alternative for achieving this goal because of the limited side effects and accessibility of plant derived drugs. In addition, these plant-derived drugs have the potential for restoring the activity lost because of bacterial resistant mechanisms. Unlike traditional antibiotics, the wide diversity of phytochemicals in plant extract put severe challenges on microorganisms to develop resistant mechanisms against them. As a result, investigation and screening of these plants for biologically active compounds with considerable antimicrobial activity

has been a continuous process [13,14] Medicinal plants are represented in almost all taxonomic plant families worldwide. Examples of families and specific examples include compositae (Chamomile, *Matricaria chamomilla*, Artichoke, *Cynara cardunculus*), Labiatae (Lavander, *Lavandula officinalis*, rosemary, *Rosmarinus officinalis*), Umbelliferae (carrot, *Daucus carota*, sea holly, *Eryngium maritime*, anise, *Pimpinell aanisum*), Leguminosae (false acacia, *Robinia pseudoacacia*, alfalfa, *Medicago sativa*, and fenugreek, *Trigonella foenumgraecum*), Rosaceae (Almond, *Prunusdulcis*), Rutaceae (orange, *Citrus aurantium*, lemon, *Citrus limon*) and Solanaceae (white henbane, *Hyoscyamus albus*, the Mediterranean withania, *Withania somnifera*). These plants have been used as a source of active compounds with differential abilities to inhibit the growth of microorganisms and drug resistant microbes [15,16]. The existence of a large number of medicinal plants in the flora of Saudi Arabia has also been reported [17]. These plants are distributed over about 36 various plant families. The two families, Euphorbiaceae and Capparidaceae are among these families which have a considerable number of traditional medicinal plant species. In Saudi Arabia, Euphorbiaceae family is represented by about 15 genera and 66 species that vary from herbs to shrubs and trees and from

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succulent to non-succulent plants. It has more than twenty species with diverse medicinal properties with about two endemic species [17]. The diverse medicinal properties of Euphorbiaceae members may be associated with their wide distribution in different habitats which seems to occur as a result of their high adaptive mechanisms such as Crassulacean Acid Metabolic (CAM) pathway and succulence [18]. Interestingly, a patent describing the extraction of bioactive flavonoid metabolites (4'-methoxy-luteolin-7-O-rhamnoglucoside and aromadendrin) from *Euphorbia cuneata* in Saudi Arabia has been issued by the United States Patent and trademark office (<http://patft.uspto.gov>, Patent # 20140288286,) [19]. Capparidaceae family is also widely spread in Saudi Arabia. It's members grow in the Red Sea coastal region, particularly in, Nagran, Abha, Bisha, south Jeddah to Medina area, and the area between Dahna and Arabian Gulf [20] It has about 28 genera and about 700 species of annual or perennial herbs, sub-shrubs, shrubs or trees [21] In addition, more than 12 medicinal species have been investigated and about four species being endemic to Arabian Peninsula. Interestingly, various members belonging to the family Euphorbiaceae have been widely used as traditional medicine in many parts of Eastern Africa and south Asia [22]. Also, different members of the family Capparidaceae are commonly used for treating various human diseases in many parts of India, Africa and Asia [23,24]. Successful identification of the right medicinal plant is crucial for the success of the entire process. Therefore, screening the planta kingdom for identification of wild plants with potential medicinal significance and selection of the appropriate extraction methods are the first critical steps in the discovery of plant-derived drug. As a result, the objectives of the current study were: (1) to test and evaluate the potential antimicrobial activity of extracts prepared from four Saudi's medicinal plants belonging to Euphorbiaceae and Capparidaceae and (2) compare the efficiency of various organic solvents and different extraction methods on the antibacterial activity of the selected plant extracts.

Methods

Plant materials

Four plant species were used in the current investigation. The selected plants were *Jatropha pelargonifolia*, *Euphorbia inaequilatera*, *Cadaba glandulosa* and *Cadaba rotundifolia* (Table 1). The first two plants belong to the family Euphorbiaceae whereas the other two belong to the family Capparidaceae. These plants were collected on November 2014 either from Jeddah, AlQuonfodha, or from Makkah-AlQuonfodha road as indicated in (Table 1). The collected plants were identified and classified by Prof. Dr. Kadry Abdel Khalik, a plant taxonomist at Biology Department - Umm Al-Qura University, Makkah, Saudi Arabia according to [25].

Bacterial stocks

Seven bacterial strains belong to both Gram-Positive and Gram-Negative bacteria were used in testing and evaluation of possible antibacterial activity of plant extracts. Gram-Positive bacteria were *Staphylococcus aureus* (NCTC 12989), *Bacillus megaterium* (DSM319), and *Bacillus cereus* (ATCC 15816), whereas Gram-Negative bacteria included *klebsiella pneumoniae* (ATCC-BAA 1705), *Pseudomonas*

aeruginosa (ATCC 10145), *Escherichia coli* (MTCC 40), and *Salmonella enteritidis* (ATCC13076). The bacterial strains were obtained from the Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia. Bacterial isolates were maintained on Mueller-Hinton Agar medium. Twenty four hour old pure cultures of bacteria were prepared for use each time according to [26-28].

Preparation of plant extracts

Plants were collected from different regions as indicated in (Table 1). The collected plants were then thoroughly washed with running water then blotted using filter paper. Plant organs of interest were collected and air dried at room temperature. Dried tissues were ground into fine powder using electric grinder and stored in dark colored bottles until use. Powdered plant tissues were extracted separately in water as well as in five individual organic solvents. Two methods of extraction were employed, namely, method A and method B. In method A, amounts of fifty grams of powdered plant tissues were extracted separately in 100 ml of water and 85 % four different organic solvents including methanol, ethanol, acetone, and petroleum ether [29,30]. These solvents were used for the extraction because of their different polarity and their ability to dissolve many phytochemical compounds like tannins, polyphenols, flavones, terpenoids and alkaloids [31]. The solvents- powder mixtures were kept at room temperature for 48 hr with rapid stirring every 8 hr. The extracts were then filtered through Whatmann No.1 filter papers to exclude undissolved cellular particles. Filtrates were concentrated down to a known volume by evaporation using water bath at 40°C. Crude plant extracts were then transferred to screw cap bottles and stored at 4°C until use [32]. In method B, fifty grams of the powdered plant tissues were suspended in enough distilled water to form a homogeneous suspension. The mixture was adjusted to pH 2.0 with HCl (1N) and then incubated at 37 °C for 30 min in a shaking water-bath. The mixture was then neutralized with NaOH (1N) to pH 7.0, filtered, extracted with diethyl ether and concentrated to a known volume by evaporation using water bath at 40°C. Crude extract obtained for each plant was transferred to screw cap bottles and stored at 4°C until use [33]. All solvents were purchased from Merck (Milan, Italy).

Culture media

Aliquots of 100 ml of nutrient broth were inoculated with individual tested organisms and then incubated at 37°C for overnight. Using a sterile pipette, 200 µl of the broth culture of each test organism was added to 20 ml of molten agar, which were then cooled at 45°C, mixed well and poured into a sterile Petri plate. Agar test plates of each test organism were prepared according to [34,35].

Antibacterial bioassay

To test the antibacterial activity of plant extracts, concentrations of 1024 mg/l of each extract were used. This was done by dissolving 0.1024 g of the extracts in 100ml of 2.5% Dimethyl sulfoxide (DMSO). This concentration of DMSO was used because it doesn't affect bacterial growth [36]. Wells of five mm diameters were prepared in the solidified medium and filled with 100 µl of different plant extracts. Ciprofloxacin at 20 µg/disc was used as positive control [37]. Whereas 2.5% DMSO

| Scientific name | Taxonomic family | Local name | Part of plant | Collection site |
|---------------------------------|------------------|------------|------------------|------------------------|
| <i>Cadaba glandulosa</i> | Capparidaceae | Qurmot | Leaves | Jeddah province |
| <i>Cadaba rotundifolia</i> | Capparidaceae | Qadab | Leaves | Makkah-Al-Qunfudahroad |
| <i>Euphorbiaina equilatera</i> | Euphorbiaceae | Abu alaban | Entire plant | Jeddah province |
| <i>Jatrophapel argoniifolia</i> | Euphorbiaceae | Obob | Leaves and stems | Al-Qunfudah province |

Table 1: Plant species used in the current study.

was used as a negative control. The inoculated plates were incubated at 37°C for 24 h and the diameters of inhibition zones were measured.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration which inhibits any visible bacterial growth [38]. To measure the MIC values of the tested extracts, serial dilutions from the stock extracts (1024 mg/l) were made to give extracts of 512, 256, 128, 64, 32, 16, 8, 4 mg/l [39]. The antibacterial activities of these diluted extracts were assayed against the tested bacteria using agar diffusion method as described earlier.

Results

The antibacterial activity

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Antibacterial activity of *Cadaba glandulosa*

Table 2 shows the antibacterial activities of plant extracts prepared from *Cadaba glandulosa*. In general, the negative control (2.5% DMSO) showed no inhibition effect on the growth of the tested organisms. On the other hand, the positive control (antibiotic Ciprofloxacin) induced inhibition zones of bacterial growth with different diameters that varied over a range from 20 mm to 30 mm. These results indicate differential effects of the antibiotic Ciprofloxacin against all tested bacterial strains which justify its choice as a positive control in the current investigation. In addition, the Ciprofloxacin exhibited its maximum inhibitory effect against the growth of *K. pneumonia*, *E. coli*, and *B. megaterum*, whereas the least Ciprofloxacin-induced inhibitory effect was recorded against *S. aureus*. Our results also indicated that *C. glandulosa* extract exhibited inhibitory effects on the growth of all tested bacterial strains except *S. enteritidis*. The diameters of the inhibition zones varied over a range from 8 mm to 19 mm which translates to a reduction in bacterial growth from 34% to 68% among the tested organisms compared to the Ciprofloxacin antibiotic induced bacterial growth inhibition. Regardless the organic solvent and the method of extraction, the *C. glandulosa* extracts showed their maximum inhibitory effects against the growth

of *B. megaterum*, *K. pneumonia* and *P. aeruginosa*. The diameters of the plant extract-induced inhibition zones against these organisms ranged from 16 mm to 19 mm. On the other hand, the least *C. glandulosa* extracts-induced bacterial growth inhibition was recorded against *S. aureus*, *B. cereus* and *E. coli* where the inhibition zone diameters varied from 8 mm to 13 mm.

The antibacterial activity of extracts prepared from *C. rotundifolia* is presented in (Table 3). Our results revealed antibacterial activity similar to that of *C. glandulosa*, extracts of *C. rotundifolia* inhibited the growth of all tested bacterial strains except that of *S. enteritidis*. The diameter of the bacterial growth inhibition zones varied from 7 to 19 mm. The highest inhibitory effects were generally obtained against *B. cereus*, *E. coli* and *P. aeruginosa* regardless the kind of organic solvent. Compared to the rest of organic solvents, petroleum ether extract exhibited the highest inhibitory effect on the growth of all tested bacteria. The highest value of the inhibition zones diameter exhibited by petroleum ether extract was against *B. cereus* and *P. aeruginosa*. Interestingly, the petroleum ether extract recovered 76% and 77% of the Ciprofloxacin-induced bacterial growth inhibition against these 2 organisms respectively. On the other hand, the lowest recovery percentage of the Ciprofloxacin antibacterial activity (33%) was recorded by the aqueous extract against *K. pneumoniae*. The rest of the tested extracts exhibited intermediate recovery percentages against bacterial strains.

Antibacterial activity of *Euphorbia inaequilater*

The antibacterial activity of both *E. inaequilater* and *J. Pleargoniifolia* (members of the Euphorbiaceae family) against the same set of bacterial strains were tested and evaluated. Table 4 shows the antibacterial activity of *E. inaequilater* extracts prepared by both extraction method A and B against the tested bacterial strains. Our results indicated that *E. inaequilater* extracts exhibited inhibitory effect on the growth of all tested bacterial strains including *S. enteritidis* regardless the organic solvent and the method of extraction. Different plant extracts induced significant difference in the diameters of the bacterial growth inhibition zones among bacterial strains. The diameters of the plant extract-induced inhibition zones ranged from 3 mm to 16 mm. interestingly, the acetone extract tended to have the highest inhibition effect against the growth of the tested bacterial strains compared to the rest of organic solvents. The acetone extract-induced inhibition zones were generally

| Organism | M | E | W | A | PE | DE |
|-----------------------|------|------|------|------|------|------|
| <i>S. aureus</i> | 0.0 | 0.0 | 10.0 | 0.0 | 0.0 | 11.0 |
| <i>B. megaterum</i> | 0.0 | 0.0 | 12.0 | 7.0 | 12.0 | 15.0 |
| <i>B. cereus</i> | 10.0 | 16.0 | 17.0 | 16.0 | 20.0 | 14.0 |
| <i>E. coli</i> | 16.0 | 15.0 | 12.0 | 16.0 | 15.0 | 12.0 |
| <i>S. enteritidis</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>K. pneumonia</i> | 12.0 | 11.0 | 10.0 | 14.0 | 16.0 | 12.0 |
| <i>P. aeruginosa</i> | 14.0 | 14.0 | 14.0 | 14.0 | 19.0 | 14.0 |

Table 2: Inhibition zone (mm) of *Cadaba glandulosa* extracts prepared in different organic solvents by two methods of extractions against selected bacterial strains. Organic solvents are: M: Methanol; E: Ethanol; W: Water; A: Acetone; PE: Petroleum Ether; DE: Diethyl Ether.

| Organism | M | E | W | A | PE | DE |
|-----------------------|------|------|------|------|------|------|
| <i>S. aureus</i> | 0.0 | 8.0 | 8.0 | 13.0 | 12.0 | 11.0 |
| <i>B. megaterum</i> | 19.0 | 16.0 | 18.0 | 19.0 | 17.0 | 18.0 |
| <i>B. cereus</i> | 0.0 | 0.0 | 10.0 | 0.0 | 0.0 | 12.0 |
| <i>E. coli</i> | 10.0 | 0.0 | 11.0 | 10.0 | 00.0 | 10.0 |
| <i>S. enteritidis</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>K. pneumonia</i> | 16.0 | 17.0 | 16.0 | 15.0 | 16.0 | 15.0 |
| <i>P. aeruginosa</i> | 14.0 | 18.0 | 16.0 | 0.0 | 15.0 | 16.0 |

Table 3: Inhibition zone (mm) of *Cadaba rotunifolia* extracts prepared in different organic solvents by two methods of extractions against selected bacterial strains. Organic solvents are: M: Methanol; E: Ethanol; W: Water; A: Acetone; PE: Petroleum Ether; DE: Diethyl Ether.

| Organism | M | E | W | A | PE | DE |
|-----------------------|------|------|------|------|------|------|
| <i>S. aureus</i> | 12.0 | 12.0 | 5.0 | 15.0 | 3.0 | 4.0 |
| <i>B. megaterum</i> | 12.0 | 12.0 | 10.0 | 11.0 | 11.0 | 14.0 |
| <i>B. cereus</i> | 12.0 | 12.0 | 4.0 | 16.0 | 3.0 | 4.0 |
| <i>E. coli</i> | 12.0 | 12.0 | 3.0 | 16.0 | 3.0 | 3.0 |
| <i>S. enteritidis</i> | 13.0 | 12.0 | 4.0 | 16.0 | 4.0 | 3.0 |
| <i>K. pneumonia</i> | 12.0 | 12.0 | 3.0 | 16.0 | 4.0 | 5.0 |
| <i>P. aeruginosa</i> | 16.0 | 16.0 | 3.0 | 16.0 | 10.0 | 10.0 |

Table 4: Inhibition zone (mm) of *Euphorbia inaequilatera* extracts prepared in different organic solvents by two methods of extractions against selected bacterial strains. Organic solvents are: M: Methanol; E: Ethanol; W: Water; A: Acetone; PE: Petroleum Ether; DE: Diethyl Ether.

around 16 mm. In addition, it is important to point out that extracts prepared by the extraction method B seemed to have inhibitory effects against bacterial strains similar to that induced by the aqueous and petroleum ether extracts prepared by method A. The three extracts exhibited significantly lower inhibition effects on the growth of the tested bacteria than the rest of organic solvents prepared by method A. Compared to the synthetic antibiotic Ciprofloxacin, our results revealed that some *E. inaequilatera* extracts can recover a large portion of the Ciprofloxacin antibacterial activity against certain bacterial strains. For example, the alcoholic extracts (methanolic and ethanolic) can recover about 60% of its antibacterial activity against both *S. aureus* and *P. aeruginosa*. Whereas the acetone extract can recover about 53%-75% of Ciprofloxacin antibacterial activity against all tested organisms.

Antibacterial activity of *Jatropha pleargoniifolia*

The antibacterial activity of plant extracts prepared from *J. pleargoniifolia* against the tested bacterial strains indicated that extracts prepared according to method A did not induce any visible inhibition against any of the tested bacterial strains. On the other hand, extracts prepared by method B showed inhibition zones of growth of all bacterial strains except *S. enteritidis*. The diameters of the extract-induced inhibition zones fluctuated over a range of 12 mm to 14 mm. The highest inhibitory effect of this extract was recorded against *S. aureus* where the diethyl ether extract recovered 70% of the Ciprofloxacin antibacterial activity against this bacterial strain. The lowest antibacterial activity of the diethyl ether extract was recorded against *E. coli* where the extract recovered only 41% of the antibacterial activity of Ciprofloxacin against the same organism. On the other hand, the diethyl ether extracts attained antibacterial activity of about 46% of that of Ciprofloxacin against the rest of bacterial strains (Table 5).

Minimum inhibitory concentration (MIC)

The promising and differential antibacterial activities of the tested plant extracts encouraged us to determine the minimum inhibitory concentration of each extract. Therefore, serial dilutions were made from stock extracts (1024 mg/l) prepared from each plant using methods A and B. The antibacterial activities of the diluted extracts were each tested against the same set of bacterial strains.

Minimum inhibitory concentration (MIC) of *Cadaba glandulosa*

The MIC data of plant extracts prepared from *C. glandulosa* against the tested bacterial strains are shown in (Table 6). Our results revealed that these extracts exhibited MIC values from 16 to 1024 mg plant tissue/l. The MIC values differed significantly among different organic solvents and tested organisms. The lowest MIC values (16 mg/l or 32 mg/l) were obtained against *B. megaterum* regardless the organic solvents and method of extraction been used. On the other hand, the highest MIC value (1024 mg/l) was obtained by the aqueous extract

against *B. cereus*. Interestingly, all *C. glandulosa* extracts showed MIC value of 64 mg/l against *K. pneumonia* except the petroleum ether extract which exhibited MIC value of 128 mg/l. Similarly, the active *C. glandulosa* extracts attained MIC value of 512 mg/l against both *E. coli* and *S. aureus* except the acetone extract against *S. aureus* which had a MIC value of 256 mg/l.

Table 7 shows the MIC values of plant extracts prepared from *C. rotundifolia* against the tested bacterial strains. Our results revealed significant differences in MIC values among plant extracts and tested bacterial strains with MIC values changing over a range from 32 mg/l to 512 mg/l. The lowest MIC values (32 mg/l) were obtained by petroleum ether plant extract against *B. cereus* and *P. aeruginosa* using extraction method A. In addition, these two bacterial strains tended to be more responsive to the ethanolic, methanolic and the diethyl ether plant extracts than the rest of organic solvents with MIC value of 128 mg/l. The highest MIC values (512 mg/l) were obtained by the aqueous extracts against *S. aureus*, and *K. pneumonia*, methanol extract against *B. cereus*, ethanol extract against *K. pneumonia*, acetone extract against *B. megaterum*, and diethyl ether extract against *S. aureus*. The rest of plant extracts-bacterial strains interactions showed MIC value of 256 mg/l.

Minimum inhibitory concentration (MIC) of *Euphorbia inaequilatera*

The MIC data of plant extracts prepared from *E. inaequilatera* against the tested bacterial strains are presented in (Table 8). Our results indicated significant differences in MIC values among plant extracts and tested bacterial strains. The MIC values varied over a range from 64 mg/l to 1024 mg/l. The lowest MIC value (64 mg/l) was obtained by the acetone plant extract against *B. megaterum*. Interestingly, this plant extract also showed small MIC values (128 mg/l) against the rest of the tested bacterial strains. Similar MIC values (128 mg/l) were obtained by both the methanolic and ethanolic extracts against *E. coli*. In addition, these alcoholic extracts also showed relatively small MIC value (256 mg/l) against the rest of the tested bacterial strains. The aqueous, petroleum ether, and diethyl ether plant extracts showed the highest MIC values (1024 mg/l) against almost all tested organisms except for *B. megaterum* and *P. aeruginosa* where each of which attained MIC value of 512 mg/l. Among the plant extracts prepared by method A, the aqueous and the petroleum ether extracts were the closest to the diethyl ether extracts (method B) in their MIC values.

Minimum inhibitory concentration (MIC) of *Jatropha pelargoniifolia*

The MIC data of plant extracts prepared from *J. pelargoniifolia* against the tested bacterial strains are presented in (Table 9). Our results indicated that only plant extracts prepared in diethylether according to method B showed MIC values against most of the tested organism. Interestingly, the active diethylether extracts maintained consistent

| Organism | M | E | W | A | PE | DE |
|-----------------------|-----|-----|-----|-----|-----|------|
| <i>S. aureus</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 70.0 |
| <i>B. megaterum</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 46.0 |
| <i>B. cereus</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 46.0 |
| <i>E. coli</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 41.0 |
| <i>S. enteritidis</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>K. pneumonia</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>P. aeruginosa</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12.0 |

Table 5: Percentages of inhibition zones (mm) of the tested plants extracts compared to the Ciprofloxacin antibacterial activity. Organic solvents are: M: Methanol; E: Ethanol; W: Water; A: Acetone; PE: Petroleum Ether; DE: Diethyl Ether. Wheats, A: *Cadaba glandulosa*, B: *Cadaba rotundifolia*, C: *Euphorbia inaequilater*, D: *Jatropha pelargonifolia*.

| Organism | Method A | | | | | Method B |
|-----------------------|-----------|-----------|-----------|-----------|-----------------|-----------|
| | Methanol | Ethanol | Water | Acetone | Petroleum ether | |
| <i>S. aureus</i> | 0.0 | 512 | 512 | 256 | 512 | 512 |
| <i>B. megaterum</i> | <u>32</u> | <u>32</u> | <u>16</u> | <u>16</u> | <u>32</u> | <u>16</u> |
| <i>B. cereus</i> | 0.0 | 0.0 | 1024 | 0.0 | 0.0 | 512 |
| <i>E. coli</i> | 512 | 0.0 | 512 | 512 | 0.0 | 512 |
| <i>S. enteritidis</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>K. pneumonia</i> | 64 | 64 | 64 | <u>64</u> | 128 | <u>64</u> |
| <i>P. aeruginosa</i> | 256 | 256 | 256 | 0.0 | 256 | 256 |

Table 6: Minimum Inhibitory Concentration (mg/l) of *Cadaba glandulosa* extracts prepared in different organic solvents by two extraction methods against Gram-Positive and Gram-negative bacterial strains

| Organism | Method A | | | | | Method B |
|-----------------------|----------|---------|-------|---------|-----------------|----------|
| | Methanol | Ethanol | Water | Acetone | Petroleum ether | |
| <i>S. aureus</i> | 0.0 | 0.0 | 512 | 0.0 | 0.0 | 512 |
| <i>B. megaterum</i> | 0.0 | 0.0 | 256 | 512 | 256 | 256 |
| <i>B. cereus</i> | 512 | 128 | 256 | 128 | <u>32</u> | 128 |
| <i>E. coli</i> | 128 | 256 | 256 | 256 | 256 | 256 |
| <i>S. enteritidis</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>K. pneumonia</i> | 256 | 512 | 512 | 256 | 256 | 256 |
| <i>P. aeruginosa</i> | 256 | 128 | 256 | 128 | <u>32</u> | 128 |

Table 7: Minimum Inhibitory Concentration (mg/l) of *Cadaba rotundifolia* extracts prepared in different organic solvents by two methods of extractions against Gram-positive and Gram-negative bacterial strains.

| Organism | Method A | | | | | Method B |
|-----------------------|----------|---------|-------|-----------|-----------------|----------|
| | Methanol | Ethanol | Water | Acetone | Petroleum ether | |
| <i>S. aureus</i> | 256 | 256 | 1024 | 128 | 1024 | 1024 |
| <i>B. megaterum</i> | 256 | 256 | 512 | <u>64</u> | 1024 | 512 |
| <i>B. cereus</i> | 256 | 256 | 1024 | 128 | 1024 | 1024 |
| <i>E. coli</i> | 128 | 128 | 1024 | 128 | 1024 | 1024 |
| <i>S. enteritidis</i> | 256 | 256 | 1024 | 128 | 1024 | 1024 |
| <i>K. pneumonia</i> | 256 | 256 | 1024 | 128 | 1024 | 1024 |
| <i>P. aeruginosa</i> | 256 | 256 | 1024 | 128 | 512 | 512 |

Table 8: Minimum Inhibitory Concentration (mg/l) of *Euphorbia inaequilater* extracts prepared in different organic solvents by two methods of extractions against Gram-positive and Gram-negative bacterial strains.

MIC value of 512 mg/l against *S. aureus*, *B. megaterum*, *B. cereus*, *E. coli*, and *P. aeruginosa*.

Discussion

In the current study, The antibacterial activity as well as the minimum inhibition concentrations of twenty four plant extracts against seven bacterial strains belonging to Gram-Positive (*S. aureus*, *B. megaterium*, and *B. cereus*) and Gram-Negative bacteria (*K. pneumonia*, *P. aeruginosa*, *E. coli*, and *S. enteritidis*) were investigated. The plant extracts were prepared from four Saudi's medicinal plants belonging two different taxonomic families: Cappariaceae (*Cadaba glandulosa* and *Cadaba rotundifolia*) and Euphorbiaceae (*Jatropha pelargonifolia* and *Euphorbia inaequilatera*) [40-42]. Because of different chemical

nature of plant phytochemicals, we employed two different extraction methods with aqueous and six different organic solvents of different polarities for preparation of plant extracts. Our results revealed that all plant extracts, except that of *J. pleargonifolia* prepared by method A, had antibacterial activity against the tested Gram-Positive and Gram-Negative bacteria. Interestingly, significant differences in the diameters of the inhibition zones as well as in the MICs values were observed in different experiments among the tested organisms and different organic solvents. Similar results were obtained by [43]. These results suggest that the tested plant extracts have differential inhibitory efficiencies against bacterial growth. In fact, it has been reported that *Cadaba* species synthesize a wide variety of bioactive secondary metabolites such as flavonoids, alkaloids terpens [44]. Similarly, members of the family Euphorbiaceae synthesize a wide range of secondary metabolites such

| Organism | Method A | | | | | Method B |
|-----------------------|----------|---------|-------|---------|-----------------|----------|
| | Methanol | Ethanol | Water | Acetone | Petroleum ether | |
| <i>S. aureus</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 512 |
| <i>B. megaterum</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 512 |
| <i>B. cereus</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 512 |
| <i>E. coli</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 512 |
| <i>S. enteritidis</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>K. pneumonia</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>P. aeruginosa</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 512 |

Table 9: Minimum Inhibitory Concentration (mg/l) of *Jatropha pleargoniifolia* extracts prepared in different organic solvents by two methods of extractions against Gram-positive and Gram-negative bacterial strains.

asphenols, flavonoids, tannins, alkaloids and triterpens, saponins and essential oils [45]. These bioactive secondary metabolites can effectively interact with critical structural and functional microbial cellular components and thus induce disruption of biological membranes, proton motive force, interruption of electron flow, and coagulation of cellular components. Such interactions usually inhibit or completely stop the microbial growth depending on the quality and quantity of the bioactive compounds in the plant extract as well as the sensitivity of microbial cells to plant phytochemicals. In this regard, it is worth mentioning that the phytochemical content of plants is influenced by many plant and environment-related factors such as the genetic constitution of the plant, developmental stages, plant organs, and mechanisms of plant physiological adaptation to surrounding environmental conditions such as biotic and abiotic stresses. These factors most likely contribute to the possible differences in the phytochemical profiles of the tested plants. Compared to the conventional antibiotic Ciprofloxacin, our results revealed that the tested crude plant extracts prepared from *C. glandulosa*, *C. rotundifolia*, and *E. inaequilatera* by method A can recover a significant portion of Ciprofloxacin antibacterial activity against the tested bacterial strains. In addition, significant difference in the recovery efficiency was also observed among the organic solvents. In *C. glandulosa*, a number of extracts were successfully recovered up to 70% of Ciprofloxacin's antibacterial activity against the tested bacterial strains. Examples include acetone (65%) and petroleum ether (60 %) against *S. aureus*, all extracts against *B. megaterum*, ethanolic (72%) as well as the aqueous extract (64%) against *P. aeruginosa*. In *C. rotundifolia*, all extracts (62-77%), except methanol against *B. cereus* and petroleum ether (76%) against *P. aeruginosa*. In *E. inaequilatera*, methanolic, ethanolic, and acetone extracts recovered about 60 %, 64%, 75% of the Ciprofloxacin antibacterial activity against *S. aureus*, respectively, whereas the recovery efficiency of these extracts were about 64% against *P. aeruginosa*. On the other hand, in *J. pleargoniifolia* only plant extracts prepared by method B recovered 70 % against *S. aureus* [46]. Unlike extracts of *C. glandulosa* and *C. rotundifolia* which showed selective antibacterial activity at the level of both organic solvent and bacteria strains, the *E. Inaequilatera* extract exhibited antibacterial activity against all tested organisms regardless the solvent used. Interestingly, the *E. Inaequilatera* extract showed strong antibacterial activity against *B. enteritidis* which attained consistent resistance against all other tested plant extracts. Among the tested solvents on this plant species, the acetone extract consistently maintained the highest antibacterial activity against all tested organisms. Further, this extract also showed the lowest MIC values (64 or 128 g/l) against all tested organisms. Interestingly, acetone has an intermediate polarity among the tested solvents. It has been reported that solvents with intermediate polarity are more efficient in extraction of bioactive compounds than polar and non-polar solvents [47]. These result suggest a number of important points: (1) the *E. Inaequilatera* synthesizes a wide diversity of bioactive secondary metabolites of different chemical nature and

solubility in aqueous and organic solvent, (2) these compounds are synthesized in amounts high enough to inhibit the growth of bacterial strains, (3) acetone is the most efficient organic solvent in extraction of bioactive compounds in *E. Inaequilatera*, (4) the low MIC values exhibited by acetone extract as well as by some of the other tested extracts suggest that these extracts either have strong antibacterial potency and/or contain high concentrations of bioactive ingredients, and (5) the *E. Inaequilatera* synthesis unique bioactive compound(s) that can effectively inhibit the growth of bacteria and that compound(s) can be extracted in any of the tested solvents with acetone.

Conclusion

The results of the current investigation indicated that most of the tested plant extracts had strong antibacterial activity against the tested Gram-Positive and Gram-Negative bacteria. The antibacterial activities of the tested extract can significantly recover high percentage of that of conventional antibiotics like Ciprofloxacin. The tested extracts also exhibited differential MIC values against the tested bacterial strains. Different organic solvents as well as different extraction methods can be effectively employed to mine plants' phytochemical profiles and consequently illustrate their antibacterial activity against recalcitrant bacterial strains.

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