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Biological Activities and Importance of the Medicinal Plant, *Commiphora gileadensis* Collected from Makka Region, Saudi Arabia

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Abstract

Objective: This study aimed to discover safe antibacterial and antibiofilm agents from natural sources to control resistant bacteria.

Methods: *C. gileadensis* leaves and stems were collected, identified, and *extracted using different organic solvents, methanol, ethyl acetate, chloroform, and hot water. The tested bacteria were multidrug-resistant Escherichia coli, Acinetobacter baumannii, Listeria monocytogenes, Serratia marcescens, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa*. Crystal violet method was used to detect biofilm formation while the phenol-sulphonic acid method to measure exopolysaccharide contents, which are an effective factor in biofilm formation.

Results: No significant differences were noted in the biological activities between leave and stem. Ethyl acetate and chloroform extracts showed lower activities. Still, hot water extract showed the weakest activities.There is a synergistic effect between the *Commiphora* extract and some antibiotics, especially Amoxicillin, Polymixin B, and Tetracycline. The highest inhibition of biofilm was recorded against *K. pneumonia* which had the highest EPS content (0.29 ± 0.04 µg/mg of cells), decreased after treatment with plant extract by 39%. Cytotoxicity studies using *Artimia salina* as a test organism were conducted for the methanolic extracts, which showed antitumor activity against two cell lines, MCF-7 (breast cancer) and HepG2 (hepatocellular carcinoma). *Commiphora* extract showed antioxidant activity using two different protocols.

Conclusion: The methanolic extract of *Commiphora* singly or in combination with antibiotics inhibit many pathogenic bacteria that form biofilm and recorded antitumor and antioxidant activities.

Keywords: *Commiphora gileadensis*, biofilm, antimicrobial agent, plant extracts, multidrug-resistant, bacteria

Introduction

Many thousands of plant species have been recognized, but lower numbers are considered to have medical significance. It's interesting to note that Saudi Arabia's flora is rich in medicinal plants that offer numerous secondary products and drugs, as documented by Kaur and Arora, 2009, Joshi et al., 2011, Balasankar et al., 2013).[1-3] The effectiveness of the current antibiotics is limited or not effective and pharmacological industries need new products from plants to control dangerous microbes and contribute to the improvement of human health. The search for new biologically active materials led to the discovery and identification of many antimicrobial agents that act as antibacterial, antifungal, antitumor, or antioxidant drugs to stimulate the immune system and destroy different pathogens.[4,5] In recent years, there has been a surge in the use of plant materials or their extracts in alternative and complementary medicine, and their counts were enhanced every year because they are easily used, common, wild available, have low prices, and are suitable to the poor people.[6] Moreover, the data obtained by many authors reported that plants from Saudi Arabia demonstrated biological activities and many plants need to be studied. *Commiphora* is a flowering plant genus that belongs to the Burseraceae family and is found in tropical and subtropical areas. It is one of the most important genera that form fragrant resins used to make incense, perfume, and medicines.[7,8] The genus *Commiphora* is characterized by small trees with thorny, short branches, distributed along the Red Sea shore and in western and southwest Saudi Arabia. Out of six species from the genus *Commiphora* present in Saudi Arabia, *C. gileadensis* is a well-known traditional medicinal plant, commonly found in different regions of Saudi Arabia like Jeddah, Jizan, and Riyadh[6,9] and used by the popular to treat many dangerous diseases in the Arabian region due to its bioactive constituents such as phenols, flavonoids, and alkaloids.[10,11] After phytochemical analysis, *Commiphora* extract was rich in phenolic compounds, flavonoids, alkaloids, saponins, and tannins.[9] In recent years, studies have investigated the antimicrobial properties of *Commiphora* extracts and their potential as alternative treatments for bacterial infections caused by various bacterial species. For example, Al-Snafi and Al-Baghdadi (2017) study showed that *C. gileadensis* essential oil exhibited potent antibacterial activity against *S. aureus, E. coli,* and *P. aeruginosa*.[12] Similarly, Al-Bayati and Al-Mola (2008)[13] found that *C. gileadensis* resin extract displayed significant antibacterial activity against methicillin-resistant *S. aureus* (MRSA). In addition to its antimicrobial properties, *Commiphora* extracts have also been shown to inhibit bacterial biofilms, complex structures of three-dimensional communities of bacteria on various surfaces, and often lead to chronic infections that are difficult to treat with conventional antibiotics. A study conducted by Al-Musayeib et al. (2014)[14] found that *C. gileadensis* resin extract inhibited the biofilm formed by MRSA and *P. aeruginosa*, suggesting its potential as an effective treatment for biofilm-related infections. The antimicrobial and anti-biofilm properties of *Commiphora* extracts are believed to be due to their high content of bioactive compounds, such as terpenoids and flavonoids. These compounds have been shown to disrupt bacterial cell membranes, interfere with bacterial quorum sensing, and inhibit the production of virulence factors, among other mechanisms of action. *Commiphora* extracts, particularly *C. gileadensis*, have shown promising antimicrobial and anti-biofilm properties against various bacterial species that had different degrees of antibiotic resistant. However, these findings suggest that *Commiphora* extracts could be potential sources of novel antimicrobial

agents to treat bacterial infections, further studies are needed to investigate their efficacy and safety in clinical settings. This study aimed to detect the antimicrobial and antibiofilm activities of the extracts of *C. gileadensis* and detect its effects on the EPS content, toxicity, MIC, and antitumor activities in addition to the synergism with some of antibiotics.

Materials and Methods

Pathogenic Bacterial Strains

Standard local biofilm forming pure culture of the Gram-negative, *Escherichia coli, Acinetobacter baumannii, Serratia marcescens, Klebsiella pneumonia, Pseudomonas aeruginosa,* and Gram-positive *Listeria monocytogenes, Staphylococcus aureus,* were collected in agar plates from Clinical and Molecular Microbiology Laboratory, King Abdulaziz University Hospital, Jeddah, Saudi Arabia. All cultures were checked up again for purity.[15] For preservation, The selected isolated strains were maintained on tryptic soy agar slants at 4°C and regenerated every six months by subculturing at 37°C for 18–24 hrs. A stock of 15% sterile glycerol was inoculated with a single colony of each bacterial isolate and the vial was sealed and immediately preserved at –80°C until used.

Sensitivity of the Tested Bacterial Pathogens for Some Antibiotics

The isolates were examined for sensitivity to several common commercial antibiotics (ampicillin, amoxicillin\clavulanic acid, piperacillin, piperacillin\tazobactam, cefuroxime, cefotaxime, cefepime, meropenem, amikacin, gentamicin, ciprofloxacin, nitrofurantoin, and trimethoprim\sulfamethoxazole) by disc-diffusion method according to Kirby-Bauer technique described by Bauer et al. (1966)[16] using Clinical Laboratory Standards Institute Guidelines to interpret diameter of growth inhibition zone (CLSI, 2012).[16,17] Lawn culture of the bacterial isolates was made on Muller-Hinton agar plates, the specific antimicrobial agent discs were tested and diameters of inhibition zones were measured in mm after incubation at 37°C for 24 hrs.

Plant Material and Preparation of Plant Extracts

Healthy plants of *Commiphora gileadensis,* free from disease were collected from the Makka region, Saudi Arabia during the winter of 2021. All plant materials were identified at the Biology Department, Faculty of Science, KAU, Jeddah (Migahid, 1996)[18] and a voucher specimen was deposited in the herbarium. The collected *C. gileadensis* were washed individually with distilled water and oven-dried for 24 hrs at 60°C, cut into small pieces, and transformed into a fine powder using an electrical blender. About 10 g of each dried leaves or stems of the plant were extracted using 250 ml of hot water or organic solvents 99%, (methanol, ethyl acetate, chloroform) for 24 hrs. The slurry was filtered using sterile filter paper. The obtained extract was concentrated using a rotary evaporator (HAHNVAPOR HS-2005S) at 40°C for solvent elimination and the residue was dissolved in DMSO. The water extract was lyophilized until dryness. Finally, each extract was kept in a sterile bottle under refrigeration until use. Moreover, each 100 g of the dry shoot material was extracted with methanol as described before, dried, weighted, and used to detect toxicity, antitumor, and antioxidant activities in addition to MIC and MBC.

Antimicrobial Activities

Ampicillin (Sigma®, A9518), polymyxin B (Bio-Chemical Technology Co., Ltd), tetracycline Nutricare Bio Science Private Limited) were used as control antibiotics. The antimicrobial activities of the obtained extracts were studied using Muller Hinton agar plates and the Agar well diffusion method.[19] Further, the susceptibility of the tested bacteria to the tested plant extract was determined on Muller Hinton agar.[16] The plates were incubated at 37°C for 24 hrs and the diameter of the inhibition zones was measured in mm. Minimal inhibitory concentration was determined using the Broth microdilution method and ELISA reader as described by Bonnavero et al (1998).[20] Muller Hinton broth medium was used to grow the bacteria overnight and the growth was diluted to approximately 10^4 cell/ml and phenol red was used as a colorimetric indicator. MIC was determined by changing the color from yellow to pink. To determine the minimum bactericidal concentration (MBC) of the extract, 100 µL of the culture from each well of the micro-broth assay was subcultured on Muller Hinton agar plates for 24 hours at 37°C and the lowest concentration of extracts that showed no bacterial growth was considered MBC.

The experiments were performed in triplicate for each bacterial isolate. Similarly, the effect of a mixture of *Commiphora* extract with different antibiotics like Amoxicillin, Polymixin B, or Tetracycline was detected. to evaluate the synergistic antimicrobial activity. The bacterial suspension was spread on MHA plates (turbidity, 0.5 McFarland), and the discs were separately impregnated with a mixture of 100 µL of the tested plant extracts and antibiotic at the MIC value (V/V), the inoculated agar plates were kept for 24 hrs at 37°C and the zones of inhibition were estimated.[21] Synergism means the zones of combination > zone of plant extract + zone of the used antibiotic while antagonism was recorded when the inhibition zone of combination < zone of plant extract + zone of the corresponding antibiotic.

Toxicity Assay of Plant Extracts

A bioassay test using Brine shrimp lethality assay was carried out to investigate the toxicity of the methanolic plant extracts and *Artemia salina* was used as the test organism. The percentage of mortality was determined and LC_{50} was calculated.[22]

Antitumor Activity

The antitumor activity of the three plant extracts against MCF-7 (breast cancer) and Hep G2 (hepatocellular carcinoma), tumor cell lines was studied using *In vitro* MTT and Neutral Red assays. The MTT Test on two cell lines was evaluated.[23] Under sterile conditions and in 96-well plates, seeded cells (6×10^3 cells/well) in 100 µl of the culture medium were grown for 24 hrs and treated with different concentrations of the tested plant extracts for 72 hrs. Cells were collected and treated with MTT solution (10 µl) and after the formazan crystals were dissolved, cell viability was calculated (the absorption at A_{550} nm). Culture medium plus MTT was used as control. The plant extract concentration reduced the cell viability by 50% recorded as IC_{50} for the tested extract. Three replicates

for each experiment were applied, and the mean values and standard deviations were calculated.

Measurement of Biofilm Formation for the Bacterial Isolates for Selection of the Most Biofilm Forming Isolates

All the collected isolates were screened for biofilm formation using the quantitative biofilm assay method described by Christensen et al. (1995).[24] Biofilm formation by the bacterial isolates was tested in a microtiter plate using a Crystal violet stain. About 20 µl of broth culture (1.5×10^8 CFU/ml) was added to 180 µl broth medium in the first well of a sterile flat-bottomed 96-well microtiter plate and the other wells in the same row were filled with 100 µl of the fresh broth medium. Then, 100 µl from the first well was transferred to the second well and 100 µl from it was transfered to the third well and so on to make serial dilutions. The plate was incubated at 37°C for 24 hr. After incubation, the contents of each well were removed by gentle tapping. The wells were washed with phosphate buffer saline (pH 7.2). The formed biofilm by bacteria, adherent to the wells, was fixed by 225 µl of 2% sodium acetate and stained by crystal violet (0.1%). The microtiter plate was left at room temperature for 10 min. Then, 225 µl of 30% acetic acid in water was added to each well in the microtiter plate to solubilize the crystal violet. Finally, 225 µl of the solubilized crystal violet was transferred to a new flat-bottomed microtiter dish and absorbance in a plate reader was quantified at 570 nm using a Microplate reader (model DNM-9602, Wincom, Hunan) and 30% acetic acid in water was used as the blank.

Studying the Growth of the Selected Bacterial Isolates

Each selected bacterial isolate was subcultured and standardized to get the 1.5×10^8 CFU/ml preculture to start the experiment. After inoculating each bacterial isolate broth culture in microtiter plate wells in triplicate, growth was estimated by measuring turbidity at the starting point using a microplate reader (model DNM-9602, Wincom, Hunan) at wavelength 650 nm after 24 hrs.[4]

Exopolysaccharide Extraction and Estimation for the Selected Bacterial Isolates

After biofilm formation, Exopolysaccharide (EPS) quantity was estimated after the extraction according to Smitinont et al. (1999).[25] The overnight cultures of the selected bacterial isolates were taken separately into vials and centrifuged at 10,000 rpm for 20 min at 4°C to remove bacterial cells. The obtained supernatant was collected into a fresh new vial, precipitated with two volumes of absolute chilled ethanol, and incubated the mixture at 4°C overnight. The precipitated EPS was collected by centrifugation at 10,000 rpm at 4°C for 20 min and the pellet containing EPS was dried at room temperature and the total exopolysaccharides were estimated by phenol-sulphuric acid method and spectrophotometer.[26,27] Ten µl of the EPS was added to a glass vial followed by the addition of 200 µl of 5% phenol, and one ml of concentrated sulfuric acid. After the vortex, the absorbance was detected at 490 nm after 60 minutes at room temperature. The quantity of EPS (µg/mg of cells) was detected from a standard curve of glucose and the comparison between the tested bacterial strains was made.

In vitro Antioxidant Activities (Free Radical Scavenging Determination)

In vitro, the antioxidant activity of *Commiphora* extract was proved by determining the total antioxidant capacity and free radical scavenging activity. The total antioxidant potential was determined using two different methods.

1. ***DPPH Free radical scavenging activity***

The method of Ishakani et al., (2016) was used to estimate the free radical scavenging activity of the plant extracts.[28] A final concentration of 10 mg/ml was dissolved in DMSO and in a 96-well plate, 100 µL, 0.1 mM DPPH (Sigma- Aldrich, USA) in methanol solution was added to 100 µl of the plant extract. The plate was incubated in the dark at room temperature for 30 minutes. Ascorbic acid and butylated hydroxytoluene (BHT) (Sigma-Aldrich, USA) are positive controls while 200 µl of DPPH solution was used as a negative control. The decrease in the absorbance of DPPH is the antioxidant activity of the plant extract, which was measured at 515 nm using a Bio-Tek microplate reader (Synergy HT, USA). Three triplicates were performed and the ability to scavenge the DPPH radical was calculated as a percent of DPPH scavenging using the following Equation:

DPPH radical scavenging activity (%) = $[A_0 - A_1]/A_0 \times 100$, where A_0 is the absorbance of the DPPH control and A_1 is the absorbance of the plant extract

The extract concentration that caused a 50% reduction of DPPH (EC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

2. ***Ferric Reducing Antioxidant Power***

Ferric reducing antioxidant power (FRAP) was measured according to the manufactured kit OxiSelect™ (Cell Biolabs, USA and the reaction reagent was prepared just before use (immediate applications). Serial dilutions of 1 mM of Fe^{+2} standards solution were prepared (500, 250, 125, 62.5, 31.3, 15.6,7.8, 3.9 and 0.0 µg/ml) in 500 µl of deionized water. Equal amounts of Colorimetric Probe and ferric Chloride Solution (500 µl) were added to 4 ml of 1X Assay Buffer and was completed to 5 ml total. In a 96-well plate, 100 µl of reaction reagent (ferric chloride solution and Colorimetric Probe) was added to 100 µl of plant extract that was prepared freshly in methanol (1 mg/ml) and to 100 µl of standard serial dilution.[29] The experiment was performed in triplicate and the color changing was read at 540 nm by a microplate reader (Synergy HT, USA). Results are expressed as mM ferrous equivalent/mg of extract.

Statistical Analysis

All the experiments were performed in triplicates. For each result, data were summarized as mean ± standard deviation (SD). Statistical analysis was performed using SPSS (version 11, Chicago). One tail Student's *t*-test was used to calculate the significant difference between the mean values, calculated from the given experimental sample and the control sample, and the *P*-value < 0.05 was considered significant results.

Results

The tested bacteria, *A. baumannii, E. coli, K. pneumoniae, L. monocytogenes, P. aeruginosa, S. aureus,* and *S. marcescens* were obtained from KAUH as multidrug-resistant bacteria. The antibiotic sensitivity of these bacteria to some antibiotics was detected and compared. They resist at least one agent in the different tested antimicrobial categories. The results of the resistance of the these bacteria are summarized in Table 1. The tested isolates, *A. baumannii* and *K. pneumoniae* were resistant to all tested antibiotic except Cotrimoxazole for *A. baumannii* and Cotrimoxazole and Ampicillin for *K. pneumoniae. Commiphora gileadensis* is commonly found in the Makka region, Saudi Arabia, and is used popularly to treat many diseases. *Commiphora* leaves and stems were collected and extracted using organic solvents and hot water. The antimicrobial activities of the tested extracts were recorded by measuring the diameter of the inhibition zones (mm) on Muller Hinton agar using the Agar well diffusion method. Methanolic extract of both leaves or stems was active against all the tested pathogens except *A. baumannii* which recorded the lowest activities compared to standard commercial antibiotics which showed excellent activities. For methanolic extract of both leaves and stems, the inhibition zone diameters ranged from 15-23 mm, from 12 to 21 mm for ethyl acetate extract, and from 12 to 18 mm for chloroform extracts. The lowest activities were recorded by hot water extract whereas the inhibition zone diameters ranged from 10-14 mm (Table 2). Thus, the methanolic extract of the shoot system was used in the following experiments. Using the microdilution method and phenol red as an indicator, minimal inhibitory concentrations (MIC) and minimal bacteriocidal concentrations (MBC) of the methanolic *Commiphora* extract, were recorded and compared (Table 3). The MBC values were determined by viable counts on MHA. The MIC ranged from 0.5 to 8.5 mg/mL while MBC ranged from 5 to 40 mg/ml. In the present study, combining *Commiphora* extract with amoxicillin revealed synergistic activity with a wider zone of inhibition with maximum synergism reported against *E. coli*, *S. marcescens,* and *K. pneumoniae* (26–32 mm). *Commiphora* extract combined with tetracycline showed a high synergism and widest zone of inhibition against *E. coli* and *S. marcescens* compared to *A. baumannii*, which recorded the lowest results (Table 4). The results showed that *A. baumannii* and *K. pneumoniae* produce high biofilm, A_{595} nm = 0.59 and 0.60, respectively while *E. coli* and *S. marcescens* produce moderate biofilm, A_{595} nm = 0.45 and 0.27, respectively. The quantity of EPS for the selected bacterial isolates was detected and the maximum quantity of EPS was recorded for both *A. baumannii* and *K. pneumoniae.* Treatment of the bacterial cells with MIC of the *Commiphora* extract recorded high inhibition in biofilm formation and EPS content (Table 5). Moreover, the highest inhibition

Table 1. **Resistant of the selected bacteria to different antibiotics measured by inhibition zone diameter (mm)**

Pathogenic isolates	Tested antibiotics					
	Nalidixic acid (30 µg)	Nitrofurantion (30 µg)	Cephalothin (30 µg)	Ampicillin (25 µg)	Cotrimoxazole (25 µg)	Norfloxacin (10 µg)
A. baumannii	ND	ND	ND	ND	17 ± 0.5	ND
E. coli	25 ± 2.0	17 ± 0.9	ND	15.9 ± 0.4	20 ± 0.5	25 ± 0.1
K. pneumoniae	ND	ND	ND	15.8 ± 0.8	15 ± 0.4	ND
L. monocytogenes	19 ± 0.5	17 ± 0.6	19 ± 0.0	ND	15 ± 0.0	19 ± 1.5
P. aeruginosa	25 ± 0.4	15 ± 0.4	ND	ND	25 ± 0.0	30 ± 0.3
S. marcescens	35 ± 0.4	25 ± 0.5	ND	25.0 ± 0.9	25 ± 0.0	34 ± 0.2
S. aureus	20 ± 0.3	20 ± 0.5	ND	ND	20 ± 0.0	30 ± 0.1

ND: Inhibition zone not detected or less than 15 mm.

Table 2. **Antimicrobial activity (measured by inhibition zone diameter, mm) of different *Commiphora* extracts of leave and stem using agar well diffusion assay and different bacterial pathogens as test organisms, Amoxicillin as control antibiotics**

Tested pathogens	Leave extract				Stem extract				Amoxicillin (30 µg) (control)
	Methanol* extract	Ethyl acetate extract	Chloroform extract	Water extract	Methanol extract	Ethyl acetate extract	Chloroform extract	Water extract	
A. baumannii	13.0 ± 0.41[a]	10.0 ± 1.11	10.2 ± 1.00	10.2 ± 1.22	13.0 ± 1.01[a]	10. ± 2.11	10.0 ± 0.71	14.0 ± 1.34	10.0 ± 1.34
E. coli	18.0 ± 2.11[b]	16.2 ± 1.14	11.2 ± 2.11	10.0 ± 101	19.9 ± 2.19[b]	16.0 ± 2.11	14.0 ± 1.34	10.0 ± 2.11	19.0 ± 2.11
K. neumoniae	20.1 ± 2.22[c]	20.0 ± 3.09	13.4 ± 2.22	13.1 ± 1.22	22.0 ± 2.09[c]	21.4 ± 1.02	14.1 ± 0.29	13.1 ± 2.22	13.1 ± 200
L. monocytogenes	15.8 ± 2.80[d]	12.5 ± 2.21	11.1 ± 2.80	10.8 ± 1.00	15.5 ± 2.12[d]	12.9 ± 1.09	16.8 ± 2.06	10.8 ± 2.80	28.8 ± 2.00
P. aeruginosa	22.2 ± 1.99[e]	20.1 ± 1.03	17.4 ± 1.99	12.4 ± 1.01	23.1 ± 1.54[e]	20.6 ± 1.22	17.2 ± 1.35	14.2 ± 1.99	19.0 ± 1.22
S. marcescens	16.2 ± 2.44[f]	16.1 ± 2.00	14.8 ± 2.44	13.0 ± 1.00	19.4 ± 1.14[f]	16.0 ± 2.45	18.2 ± 0.78	14.2 ± 2.44	19.0 ± 2.34
S. aureus	21.2 ± 2.88[g]	21.0 ± 2.18	15.0 ± 2.88	14.0 ± 1.12	21.4 ± 2.00[g]	20.0 ± 2.19	14.2 ± 0.80	13.2 ± 2.88	18.8 ± 2.11

*The number with the same letters mean no significant results at $P \leq 0.05$ compared to control.

was recorded for *A. baumannii* and *K. pneumoniae*. Figure 1 showed the growth, biofilm formation, exopolysaccharide (EPS), and protein inhibition in the four tested bacteria, treated with *Commiphora* methanolic extract at MIC compared to control (without treatment or extract addition). The toxicity of any plant extracts or essential oils must be carried out because active agents may be toxic in high concentrations. No cell toxicity for *Commiphora* extract was determined against *Artemia salina* as a test organism, detected by measuring surviving percentages (LD_{50}) of larvae after 8 hr. which were more than 0.5 mg/ml as summarized in Table 6. Exopolysaccharide contents were estimated for each isolate before and after treatment with the plant extract. MCF-7 (breast cancer) and Hep G2 (hepatocellular carcinoma) are cell lines inhibited with the tested *Commiphora* extract, LD_{50}, 0.75 + 0.11 and 1.00 + 0.28 µg/ml, respectively. Bleomycin was used as a control antitumor, LD_{50} = 0.40 + 0.12 µg/ml (Table 6). The DPPH scavenging activity was detected in the *Commiphora* extract. The percentage of reduced DPPH radical at a concentration of 1 mg/ml is shown in Table 6. The effectiveness of the antioxidant quality was evaluated using the parameter EC_{50}. The moderate scavenging percentage was more than 50% compared to the control BHT (synthetic antioxidant, 97%). Ferric-reducing antioxidant power (FRAP) is used to measure the *Commiphora* extract-reducing ability of (Fe^{+3}) to (Fe^{+2}) at a concentration of 1 mg/ml as shown in Table 6. The FRAP value was 1.95 mM FE/mg of extract. This result is close to the activity of the control antioxidant BHT (2.5 mM FE/mg of extract) followed by the natural control, Ascorbic acid (2.1 mM FE/mg of extract). Figure 2 summarizes the different steps used for *Commiphora* plant extraction and detection of its antibacterial and antibiofilm activities against different resistant bacteria.

Table 3. **The minimum inhibitory concentration MIC (mg/ml) and minimum bactericidal concentration MBC (mg/ml) of *Commiphora* methanolic extract detected using the microdilution method**

Tested pathogens	*Commiphora* extract		MIC of Amoxicillin (µg/ml)
	MIC (mg/mL)	MBC (mg/mL)	
A. baumannii	8.50*	40.0*	≥10
E. coli (control)	1.50	10.0	10.0 ± 1.25
K. pneumoniae	0.50*	5.0*	10.0 ± 2.50
S. marcescens	0.75*	10.0*	5.0 ± 1.25

*Significant result at $P \leq 0.05$ compared to control.

Discussion

However, the increase of antibiotic resistance of bacteria to already used antibiotics become a pressing global health concern, necessitating the exploration of alternative antimicrobial agents. Plant extracts offer a promising avenue due to their rich chemical diversity and historical use in traditional medicine.[30-32] For a long period, medicinal plants have been consumed as a source of natural therapy products and folk medicine. Plants are the best selection of food and drugs for maintaining good human health. Tea extract and oil were used to treat bacterial infection with *E. coli*.[33] According to the World Health Organization, herbal plants are safe as traditional treatments for thousands of years compared to synthetic materials which have unsafe effects on human health. Almost all medical plants have therapeutic importance and human health care due to many active alkaloids and secondary metabolites in different plants.[34,35]

Pathogenic bacteria recorded increasing antibiotic resistance and new antibiotics must be developed.[36] *Escherichia coli*, *A. baumannii, P. aeruginosa,* and *S. marcescens* are Gram-negative bacteria known for developing a wide range of resistance

Table 4. **Synergistic and additive antimicrobial effects of the plant extract in combination of some antibiotics**

	Methanol extract (control)	Amoxicillin	Extract + Amoxicillin	Action	Polymixin B	Extact + Polymixin B	Action	Tetracycline	Tetracycline + Extract	Action
A. baumannii	13.0 ± 1.01	00..0 ± 0.00	15 ± 1.00	A	00..0 ± 0.00	19. ± 2.11	A	ND.	17.3 ± 1.34	A
E. coli	19.9 ± 2.19	20.2 ± 1.14	31.2 ± 2.11	A	29.9 ± 2.19	36.0 ± 2.11	A	13.0 ± 2.11	29.9 ± 2.11	A
K. pneumoniae	22.0 ± 2.09	17.0 ± 3.09	26.4 ± 2.22	A	15.0 ± 2.09	11.4 ± 1.02	N	13.1 ± 200	25.1 ± 2.0	A
S. marcescens	19.4 ± 1.14	22.5 ± 2.21	32.1 ± 2.80	A	29.5 ± 2.12	37.9 ± 1.09	A	17.8 ± 2.90	29.8 ± 2.9	A

Extract: *C. gileadensis* methanolic extract of stem, A: Additive effect, N: Antagonistic effect.

Table 5. **Bacterial growth, biofilm formation, and exopolysaccharide (ESP) quantity detected in normal and treated cells with methanolic extract of *C. gileadensis***

	Normal cells				Treated cells with MIC		
Tested isolate	Growth A (595 nm)	Biofilm (A_{570} nm ± SD)	Biofilm strength	EPS concentrtion (A_{490} nm)	EPS quantity (µg/mg of cells)	Biofilm inhibition (%)	EPS inhibition (%)
A. baumannii	0.46	0.592 ± 0.01	High	1.234	0.169 ± 0.04*	33	37
E. coli (control)	0.59	0.452 ± 0.001	Moderate	0.597	0.084 ± 0.05	29	18
K. pneumoniae	0.42	0.606 ± 0.007	High	1.456	0.29 ± 0.04*	40	39
S. marcescens	0.50	0.276 ± 0.006	Moderate	0.555	0.073 ± 0.01	25	14

*Significant results compared to control *(E. coli)*.

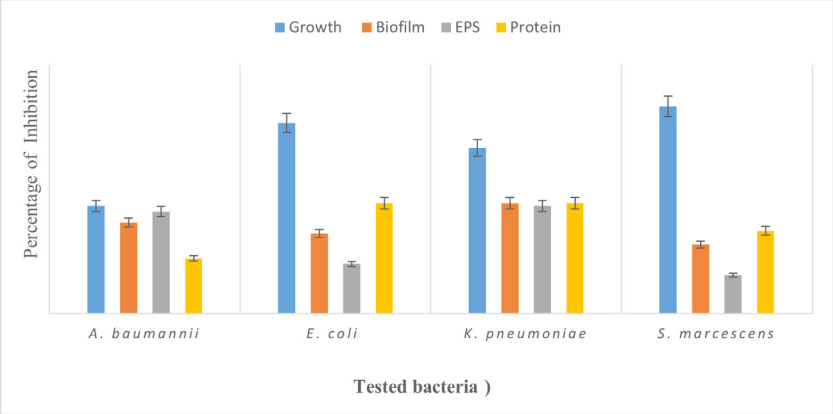

Fig. 1 **Percentage of growth, biofilm formation, exopolysaccharide, (EPS) and protein inhibition in the four tested bacteria treated with *Commiphora* methanolic extract at MIC compared to control (without treatment).**

Table 6. **Toxicity, antitumor, and antioxidant activities of the plant extract compared to the antitumor (Bleomycin) and the antioxidant materials (Ascorbic acid and BHT)**

Tested compound	Toxicity against *Artemia salina* (LD_{50}, mg/ml)	Antitumor activity (LD_{50}, µg/ml)		Antioxidant activity compared to control	
		Hep G2	MCF-7	DPPH reducing %	FRAP value (mM FE/mg of extract)
Metanolic extract	≥ 0.5	0.75 + 0.11*	1.00 + 0.28*	50 ± 7.9*	1.95 ± 0.019*
Bleomycin (control antitumor)	≥ 0.5	0.40 + 0.12	0.40 + 0.04	ND	ND
Ascorbic acid (control)	ND	ND	ND	100	2.1 ± 0.07
BHT (control)	ND	ND	ND	97.08 ± 0.67	2.5 ± 0.95

*Significant result at $P \leq 0.05$ compared to control, ND = not detected.

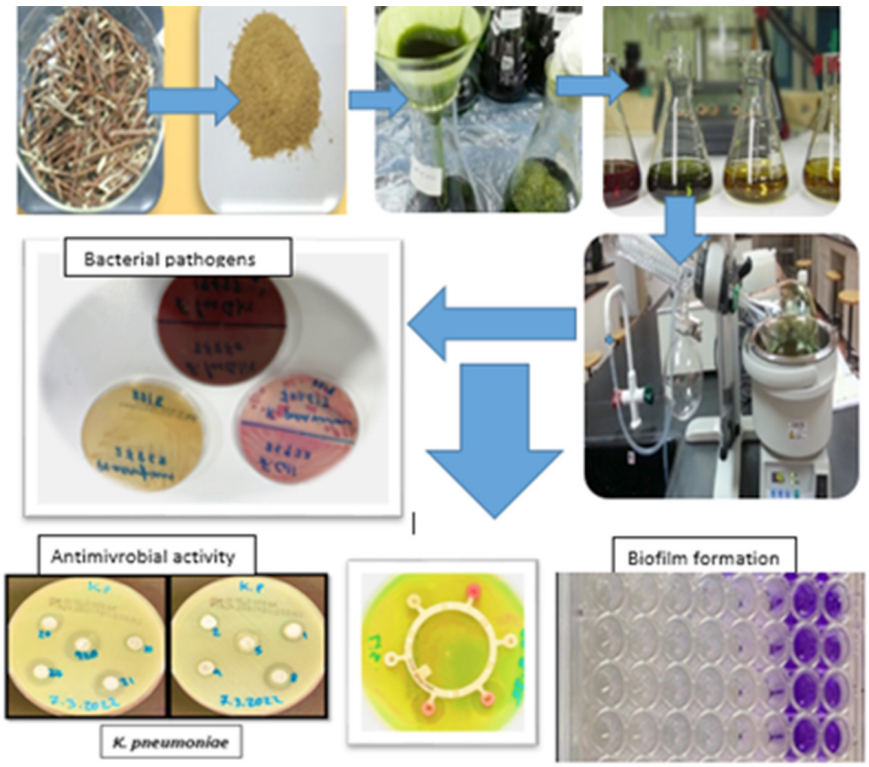

Fig. 2 **Diagram showing the extraction process of the plant, the bacterial pathogens, their sensitivity to antibiotics, antimicrobial activity of the extract, and inhibition of biofilm formation.**

against certain antibiotics, one of which includes polymixin B, ampicillin, and tetracycline, a broad-spectrum antibiotic, inhibits bacterial cell wall or protein synthesis. Also, the Gram-positive *L. monocytogenes* and *S. aureus* are two major foodborne pathogens, that occur at high frequencies, have been found in different foods, and cause many dangerous diseases.[37] The data obtained by many authors reported that plants from Saudi Arabia demonstrated antibacterial activity and many plants were found and needed to be studied.[4,38] In this study, methanol extract showed the best inhibitory results for the tested bacteria using the agar well-diffusion method. Many authors used methanol, ethanol, and chloroform for active material extractions but the best detected antibacterial activity was for methanol extract.[5,39] The most inhibited bacteria were *K. pneumonia, P. aeruginosa,* and *S. aureus.* Moussa et al. (2012)[40] reported that Gram-negative are more sensitive to antimicrobial agents than Gram-positive bacteria but Deans and Ritchie (1987)[41], found no clear difference in bacterial sensitivity. Organic solvents can dissolve and extract photochemical agents while water extracts only water-soluble substances. The antimicrobial activity may be due to Saponins, Tannins, Alkaloids, and Flavonoids which were recorded in the many plant extracts and may destroy bacterial cell walls and cell membranes, leading to cell lysis and death or affect cell DNA, RNA, proteins, and polysaccharides.[42] Also, plant extracts may exert antimicrobial effects through various mechanisms, including membrane disruption, enzyme inhibition, or modulation of bacterial signaling pathways. The diversity of bioactive compounds in plants, such as alkaloids, flavonoids, and terpenoids showed different antimicrobial properties. The location of a plant, time of collection, climate, soil, and propagation method are all factors that affect the active constituents of the used plant.[43,44] Nowadays, exponentially rising multidrug-resistant (MDR) bacteria are the predominant cause of treatment failure and increased percentage of mortality.[45,46]

Antimicrobial drugs play a crucial role in the management of infectious diseases. MIC is the lowest concentration of the tested material at which the bacterial counts were reduced to about 90% while MBC is the concentration which inhibits 99.9 or 100% of total bacterial counts. Polymixin B reduced the development of drug-resistant bacteria and maintained their effectiveness. Also, amoxicillin is a potent member of the β-lactam family that inhibits bacterial cell-wall biosynthesis and is highly effective against a wide range of gram-positive drug-resistant bacterial infections compared to penicillin. Tetracyclines are a class of broad-spectrum antibiotics, used to manage and treat various bacterial infections and act as protein synthesis inhibitors. Similarly, a synergistic effect has been reported between ethanol plant extract and gentamycin against some oral pathogenic bacteria.[47] *Acinetobacter baumannii* is emerging with resistance to many antibiotics, polymyxins B, amoxicillin, and tetracycline. It was poorly affected by the tested *Commiphora* extract or the combination of three different antibiotics. In contrast to these results, the combination of ampicillin-sulbactam with meropenem and polymyxin B achieved maximum reductions and additivity synergistic effects against 10^8 CFU/ml of two clinical isolates of *A. baumannii* which were resistant to the three drugs.[48] Also, a synergistic antibacterial effect was recorded between the ethanolic extracts of *Punica granatum*, *Commiphora molmol,* and *Azadirachta indica* in combination with amoxicillin, metronidazole, tetracycline, and azithromycin on oral pathogens, *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* and *Aggregatibacter actinomycetemcomitans* and the best synergism was exhibited by *P. granatum* with amoxicillin against *A. actinomycetemcomitans*.[49] Additionally, understanding the synergistic interactions between antibiotics and plant extracts may lead to combination therapies that enhance treatment outcomes while reducing the risk of resistance development. The synergistic effects of plant extracts in combination with antibiotics were comprehensively studied and gained significant attention due to their potential in combating antibiotic resistance. The results evaluated additive interactions between plant extracts and antibiotics, emphasizing their combined antimicrobial efficacy. The plant extracts increase antibiotic activity, potentially leading to the development of novel therapeutic approaches. Additionally, assays may include tests for synergistic effects when combining antibiotics and plant extracts, aiming to enhance antimicrobial activity or combat resistance mechanisms. The mechanisms of action underlying antibiotics and plant extracts often differ. Antibiotics typically target specific bacterial structures or processes, such as cell wall synthesis (e.g., penicillin) or protein synthesis (e.g., tetracycline), disrupting bacterial growth and replication. Studying the effect of antibiotics and plant extracts on bacteria plays a vital role in finding alternative antimicrobial strategies.

Some bacteria secrete a substance that helps them attach to other bacteria, cells, or objects. This substance combines with the bacteria to form a sticky layer called biofilm. Biofilms also help protect bacteria from antibiotics by making them difficult to kill. The tested bacterial isolates were screened and assayed for biofilm formation using the Crystal Violet method described by Christensen et al. (1995).[24] The percentage of bacterial biofilm inhibition by the *Commiphora* extract was excellent and similar to this result, Jastaniah et al., (2022)[15] recorded the the results for other plant extracts. The previous plant extract showed no toxicity In contrast, the high toxicity of *Citrullus colocynthis* extract was reported by Amin and Hussain (2018) and this toxicity depends on the dose, duration of exposure, and the used plant part.[50] Similar to the obtained results, high antibacterial, antitumor, and antioxidant activities were detected for the methanolic extracts of four medicinal plants, collected from Wadi Al-Karak, Jordan, due to their high phenolic contents.[51]

Some plants' potential anticancer, antioxidant, and anti-inflammatory effects may be attributed to flavonoids, alkaloids, and essential oils. Furanocoumarins extracted from *R. graveolens* recorded cytotoxic and antiproliferative activities against the cell line of breast cancer, colon cancer, and leukemia and these compounds induce apoptosis, impede cell growth, and perturb cell cycle progression in cancer cells.[52]

In this study, the tested plant extract showed good antioxidant activities, detected using two different protocols, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP). Al Qaisi et al. (2024) used the two previous methods to detect the antioxidant activities of three important plants.[53] Shimada et al. (1992) reported an antioxidant of plant product (xanthone) that prevents the oxidation of soybeans.[54] Also, the methanolic extracts of three medicinal plants, *Ruta graveolens, Peganum harmala,* and *Citrullus colocynthis* showed effective antioxidant and antitumor activities, especially *R. graveolens* extract which was recorded as a potential source of safe and excellent antioxidant and antitumor agents that improve human health.[53]

Conclusion

The ever-increasing rates of antibiotic resistance have prompted the exploration of alternative therapeutic strategies, including using plant extracts alone or in combination with antibiotics. By elucidating the efficacy and mechanisms of action of plant-derived compounds, these assays contribute to finding new therapeutic drugs and increase antibiotic effectiveness in the face of rising resistance. Moreover, variability in bacterial strains and the complex composition of plant extracts pose challenges in elucidating specific mechanisms of action. Additionally, while plant extracts offer potential as alternative antimicrobial agents, rigorous preclinical and clinical studies are necessary to evaluate their safety, efficacy, and pharmacokinetics for clinical use. Plant secondary metabolites are excellent sources of many antimicrobial agents with high activity and low toxicity that must be developed quickly to face the problem of multidrug-resistant bacteria and prevent persistent and recurrent biofilm-associated infections. However, further research is needed to overcome challenges and translate promising findings effectively into clinical practice.

Conflict of Interest

None.

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