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Antimicrobial activity of *Parrotiopsis jacquemontiana* and *Caesalpinia decapetala* plant extracts against selected pathogens

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Abstract: Antimicrobial-resistant bacteria are a global health concern. Some gram-negative bacteria have acquired resistance to many notorious diseases induced by various pathogens. Therefore, new antibacterial agents are needed to combat these infections. We utilised the agar well diffusion method to find the antibacterial capabilities of *Caesalpinia decapetala* and *Parrotiopsis jacquemontiana* aqueous and methanolic extracts. We aimed to find the efficacy of these extracts and their various components against selected pathogens. Methanolic extract showed significantly higher antimicrobial activity against all tested pathogens compared to aqueous extracts, such as 20 mg/mL of MRE-CD, which showed 12.16 ± 1.04 mm inhibitions against *P. aeruginosa*. In contrast, 10.5± 0.5 mm against *S. dysenteriae* inhibition compared to 20 mg/mL of MRE-PJ showed 10.16±0.76 mm inhibition against *E. coli*. Meanwhile, only aqueous root extracts of *P. jacquemontiana* at 10 mg/mL showed the least 1.5 ± 1.32 against *S. dysenteriae* mm inhibitions, while *E. coli* appears to be the less sensitive strain at 10 mg/mL of methanolic stem extract of *P. jacquemontiana* compared to the aqueous extract of *C. decapetala* stems, significantly affecting the growth of gram-negative bacterial strains. Therefore, these plant extracts have great natural antimicrobials, and further evaluation would be necessary to use them.

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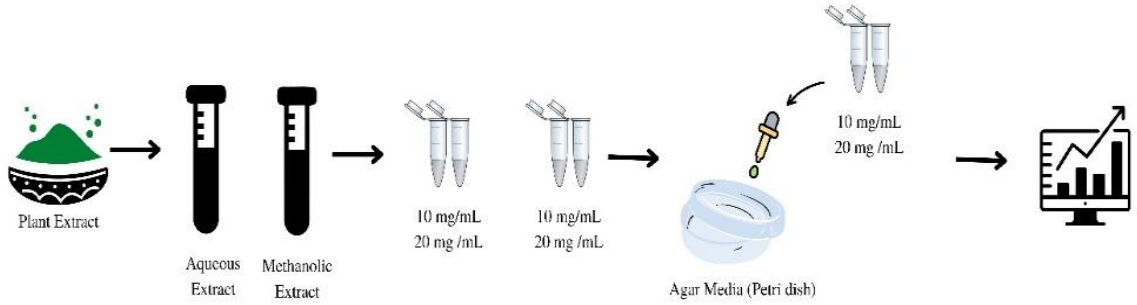

1. Introduction

Antibiotics are important in combating infection or elevating toxic microbes, in both cytotoxic and cytostatic manners which can further allow the body natural defenses. The immune system inhibits bacterial cell-related protein synthesis (Levy & Marshall, 2004; Abushaheen *et al*., 2020). Conversely, the higher demand for antibiotics and their irresponsible use across many sectors has significantly contributed to the advent of resistant strains (Sosa-Moreno *et al*., 2020). Previously, the World Health Organization (WHO) reported that antibiotic doses lost their ability to inhibit bacterial growth due to antibiotic-resistant bacteria effectively and are also considered an emerging public health problem. The imprudent usage of antibiotics has led to antibiotic resistance globally. Modified strains have been developed to reduce morbidity and prevent complications. These strains block the mechanisms or attack new targets to reduce resistance. However, gram-negative bacteria can cause hospital-related infections such as *P. aeruginosa* sometimes showing strong resistance to several antibiotics, which further might cause serious challenges in treating an infected individual and can lead to death (Levy, 1998), while *E. coli* can particularly cause urinary tract and bloodstream-related infections in humans. Although, regarding public health, *E. coli* is primarily causing foodborne diarrheal disease, and extraintestinal infections (Russo & Johnson, 2003). *S. dysenteriae,* usually causing foodborne epidemics related to gastroenteritis and can contribute to enteric infection as well as *S. dysenteriae* commonly known as verotoxins, which can contribute to induce several enteric pathogens such as hemorrhagic colitis and the hemolytic uremic syndrome (O'Loughlin *et al*., 2001). However, Gram-negative bacteria are a global priority due to their diverse pathogenic mechanisms, increasing their potential antibiotic resistance (Shrivastava *et al*., 2018; Varghese & Balachandran, 2021). Meanwhile, plants constitute vital sources of natural compounds. Since ancient times many plants have broadly been used as medicine (Shikov *et al*., 2021).

P. jacquemontiana is a family member of Hamamelidaceae and the best-known redolent remedial plant, which has widely been employed in both conventional and current medicines. Recently reported studies of *P. jacquemontiana* have been shown to alleviate oxidative stress and inflammation (Ali *et al*., 2017; Malik *et al*., 2011). Moreover, the natural compounds of *P. jacquemontiana* crude extract can inhibit proliferation in cancer cells (Ali *et al*., 2021), as well as can also treat inflammation and antipyretic (Parveen *et al*., 2013). Recently, systematic studies of *C. decapetala* showed antitumor activities (Wei *et al*., 2013) and this extract can be used against neurodegenerative-related diseases (Bhadoriya *et al*., 2012). The *C. decapetala* extract in a half-maximal inhibitory concentration manner can potentially exhibit human pancreatic cancer cell line activities (Qiao *et al*., 2012). However, this potential and their different activities might play a significant role in improving antibiotic quality and actions toward the selected pathogens *i.e., E. coli, S. dysenteriae,* and *P. aeruginosa*. Therefore, we

aimed to study the antibacterial capabilities of *C. decapetala* and *P. jacquemontiana* plant species and their two parts including stem and root in methanolic and aqueous-based mediums in a concentration-dependent manner including 10, 20 mg/mL.

2. Materials and methods

2.1. Preparation of plants materials

The *C. decapetala* (CD) and, *P. jacquemontiana* (PJ) plant specimens were collected in Buner district, of Khyber Pakhtunkhwa, Pakistan (PK). Briefly, these plants dried under sunlight for 15 days (about 2 weeks) and milled into a fine powder by using a milling machine and lastly, categorized into two different groups based on solvent medium such as methanolic and aqueous extracts and plant parts such as stem and root. The methanolic stem extract of *C. decapetala* (MSE-CD), and *P. jacquemontiana* (MSE-PJ), while the methanolic root extract of *C. decapetala* (MRE-CD) and *P. jacquemontiana* (MRE-PJ) were prepared separately, as well as the aqueous stem extract of *C. decapetala* (ASE-CD), and *P. jacquemontiana*, (ASE-PJ), while aqueous root extract of *C. decapetala*, (ARE-CD), and *P. jacquemontiana* (ARE-PJ), respectively.

2.2. Preparation of bacterial strains

The following bacterial strains were studied: *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *S. dyesenteriae* IOA-108 (M, Tc, Do). These isolates were further identified in the laboratory using the standard biochemical test and subculture in Mueller Hinton agar (MHA) media in slants, incubated for 24 hours at 35 °C in nutrient agar.

2.3. Preparation of *C. decapetala* and *P. jacquemontiana* extracts

The stem and root powder of plant species were prepared by using water and methanol as described previously (Jan *et al*., 2012; Matu & van Staden, 2003). Briefly, *C. decapetala* and *P. jacquemontiana* stem and root powder (2 g) were further suspended in 20 mL of distilled water or methanol (2 g / 20 mL) and robustly vortexed for 5 minutes. The soluble parts of extracts in both solvent mediums were further collected by centrifuging at 15,000 rpm/30 minutes and filtered. The aqueous *C. decapetala* root and stem and *P. jacquemontiana* were further stored at 5 ˚C. The methanolic *C. decapetala* root and stem and *P. jacquemontiana* were evaporated and dried under reduced pressure. Further, the aqueous and methanolic plant extracts were redissolved in distilled water (DW) and 90 % methanol, respectively, and a 0.2 mm membrane filter to sterilized for further use. Meanwhile, the final concentration of plant extracts was prepared by following (Masola *et al*., 2009). Briefly, each plant specimen extracts, such as root and stem were dissolved in 2 mL of dimethyl sulfoxide (DMSO) as a neutral solvent to prepare a stock solution. Eventually, two fractions' concentrations (*i.e.,* 10 and 20 mg/mL) of both methanolic and aqueous extracts of *C. decapetala* and *P. jacquemontiana* were prepared, similarly to standard antibiotics of Amoxicillin and Ciprofloxacin, respectively.

2.4. Preparation of standard antibiotics

The process involves preparing the stock antibiotic solution using different solvents including sterile distilled water, phosphate-buffered saline (PBS), diethyl alcohol, ethanol, and Dimethyl

sulfoxide (DMSO). The antibiotic solutions were prepared by using DMSO as a solvent, a 1 mL microtube was selected, and 400µL of DMSO was added to each tube. An appropriate amount of selected antibiotic powder was then added to each tube, resulting in stock solutions of antibiotics. Different concentrations of antibiotics were then prepared from the base antibiotic stock solution using DMSO as a solvent (Jan *et al*., 2012; Bennett *et al*., 1996).

2.5. Gram-negative bacterial strains and growth media preparation

The bacterial strains *E. coli, P. aeruginosa,* and *S. dyesenteriae* were used in this study. Briefly, Mueller-Hinton agar (MHA) media was prepared by dissolving 19.0 g in 500 mL of distilled water. The content was boiled in a conical flask until the powder completely dissolved. The bottle was always well-corked and autoclaved for 15 minutes at 121 ˚C (Mbatchou *et al*., 2015).

2.6. Agar well diffusion assay

The modified agar-well diffusion method of Shefali *et al*. (2011) was employed to study the antibacterial activities of these plant specimen's extract. Briefly, the solidified plates of the microbial broth culture suspension of about 0.1 mL of a colony of microbial culture were prepared. Further, the media of Mueller-Hinton agar (MHA) was spread by an L-shaped sterilized glass, under aseptic conditions by using laminar airflow. In each plate the wells were made with the 08 mm diameter borer, and in these wells, approximately 0.1 mL of each extract was loaded, individually. Overall, the method depends upon the extract's diffusion from the hole through the solidified petri dish agar layer to the extent the microorganism grew around the extract in the 08 mm diameter hole and was stored overnight in the incubator for 24 h at 37 ^{o}C. Lastly, the clear zone inhibition at different plant concentration around the hole was formed and measured in mm. Overall, the inhibition activity of these plants at different concentrations was compared to the standard antibiotic Amoxicillin and Ciprofloxacin, respectively.

2.7. Statistical Analysis

The three independent experiments ($n = 3$), data were tabulated and shown in the Mean ± Standard deviation (SD), meanwhile, the differences between the means were evaluated by using Tukey's test and simple variance analysis (ANOVA) for the multiple comparisons have been applied and determined with $p < 0.05$ significance by using the SPSS (v18.0) at a confident interval of 95 %.

3. Results

3.1. The methanolic root extract of *P. jacquemontiana* has higher in vitro antimicrobial activity against *E. coli*

To determine whether stem and root extracts of *C. decapetala* (CD) and *P. jacquemontiana* (PJ) plants in both aqueous and methanolic mediums show resistance to *E. coli*, we observed the growth of incubated *E. coli* cells with different concertation of standard antibiotic Amoxicillin and Ciprofloxacin and plant parts extract (*i.e.,* stem and root) in aqueous and methanolic conditions are shown in Table-1. We found that the methanolic root extract of *P. jacquemontiana* (MRE-PJ) supplementation markedly showed resistance to *E. coli* in a concentration-dependent manner (*i.e.,* 10, 20 mg/mL), whereas the aqueous root extract of *P.*

jacquemontiana (ARE-PJ) slightly showed the resistance to *E. coli* (Figure A1). These results suggested that the MRE-PJ can show resistance against *E. coli*.

3.2. The methanolic stem and root extract of *C. decapetala* has higher in vitro antimicrobial activity against *S. dysenteriae*

Given the above *E. coli* resistance methanolic root extract of *P. jacquemontiana* signifies that these plants' crude extract has protective capabilities. Therefore, we further examined the growth of *S. dysenteriae* cells incubated with different concertations of standard antibiotic Amoxicillin and Ciprofloxacin are shown in Table-1. and plant parts extract of *C. decapetala* (CD) and *P. jacquemontiana* (PJ) against *S. dysenteriae* (*i.e.,* stem and root) in aqueous and methanolic conditions. We have found that the methanolic stem extract of *C. decapetala* (MSE-CD) and methanolic root extract of (MRE-CD) supplementation distinctly exhibited *S. dysenteriae* resistance in a concentration-dependent manner are shown in Table-1.

Table-1: Antimicrobial Activity of *P. jacquemontiana* (PJ) and *C. decapetala* (CD): A comparison with antibiotics

Extracts	Conc	Zone of Inhibitions (Mean ± SD)		
		E. coli (mm)	*S. dysenteriae* (mm)	*P. aeruginosa* (mm)
Methanolic Stem Extract (MSE-PJ)	10 mg/mL	1.5 ± 1.3	3.83 ± 0.28	3.66 ± 0.57
	20 mg/mL	3.33 ± 0.57	6.3 ± 1.52	6.16 ± 1.25
	DMSO	0 ± 0	0 ± 0	0 ± 0
Methanolic Root Extract (MRE-PJ)	10 mg/mL	6.67 ± 1.52	4.16 ± 0.76	6.5 ± 1.32
	20 mg/mL	10.16 ± 0.76	7 ± 0.5	8.33 ± 2.08
	DMSO	0 ± 0	0 ± 0	0 ± 0
Aqueous Stem Extract (ASE-PJ)	10 mg/mL	2.75 ± 0.9	4 ± 1	2.83 ± 0.288
	20 mg/mL	5.16 ± 0.76	6.3 ± 0.76	3.83 ± 0.76
	DMSO	0 ± 0	0 ± 0	0 ± 0
Aqueous Root Extract (ARE-PJ)	10 mg/mL	2.5 ± 0.5	1.5 ± 1.32	3.5 ± 0.5
	20 mg/mL	4.16± 1.25	3.16 ± 0.76	6 ± 1
	DMSO	0 ± 0	0 ± 0	0 ± 0
Methanolic Stem Extract (MSE-CD)	10 mg/mL	5.83 ± 1.25	5.67 ± 1.15	4.5 ± 1.32
	20 mg/mL	8.5 ± 1.32	9.5 ± 1.5	5.66 ± 0.57
	DMSO	0 ± 0	0 ± 0	0 ± 0
Methanolic Root Extract (MRE-CD)	10 mg/mL	4 ± 1	7 ± 1	6.66 ± 1.52
	20 mg/mL	6.5 ± 0.5	10.5 ± 0.5	12.16 ± 1.04
	DMSO	0 ± 0	0 ± 0	0 ± 0
Aqueous Stem Extract (ASE-CD)	10 mg/mL	3.83 ± 0.76	5.5 ± 1.32	6.66 ± 1.52
	20 mg/mL	5.33 ± 0.57	6.6 ± 1.52	11.16 ± 1.25
	DMSO	0 ± 0	0 ± 0	0 ± 0
Aqueous Root Extract (ASE-CD)	10 mg/mL	5 ± 1	5.16 ± 0.28	3.33 ± 1. 25
	20 mg/mL	9.33 ± 1.52	7.6 ± 1.52	5 ± 1
	DMSO	0 ± 0	0 ± 0	0 ± 0
Amoxicillin	10 mg/mL	4.16 ± 0.28	4 ± 1	3 ± 1
	20 mg/mL	4.83 ± 0.76	7.33± 0.57	5.66 ± 0.57
Ciprofloxacin	10 mg/mL	3.6 ± 0.57	5.33 ± 0.57	4 ± 1
	20 mg/mL	6 ± 1	8 ± 1	8.33 ± 1.15

In addition, the methanolic root extract of *P. jacquemontiana (*MRE-PJ) had shown slight resistance to *S. dysenteriae* (Figure A2). As a result, these findings suggested that the MSE and MRE-CD have resistance abilities against *S. dysenteriae*.

3.3. The aqueous stem and methanolic root extract of *C. decapetala* has higher in vitro antimicrobial activity against *P. aeruginosa*

It is more commonly observed that the plant crude extract has different secondary metabolites which can act in different sites to protect a cell thereby contributing to the overall protective activity of plant crude extract. Furthermore, the anti-microbial activity of these plant extracts may be exerted due to their secondary metabolites which may not only be eliminated but can potentially affect their pathogenic functions (Gupta & Birdi, 2017). Therefore, we further determine the resistance capability of stem and root extracts of *C. decapetala* (CD) and *P. jacquemontiana* (PJ) plants in both aqueous and methanolic mediums against *P. aeruginosa*. In a concentration-dependent manner (10, 20 mg/mL) of standard antibiotic Amoxicillin and Ciprofloxacin and plant crude extract (*i.e.,* stem and root) in aqueous and methanolic conditions. We concluded that the aqueous stem extract *of C. decapetala* (ASE-CD) and methanolic root extract *of C. decapetala* (MRE-CD) crude extracts have shown a potential resistance to *P. aeruginosa*, meanwhile, the MRE-PJ slightly showed resistance to *P. aeruginosa* are shown in Figure A3. These results suggested that the ASE and MRE-CD can show resistance against *P. aeruginosa* cells due to the existence of phytochemical such as phenols, alkaloids, and flavonoids, and secondary metabolites including tannins, coumarins, betacyanins, triterpenoids, phlobatannins, glycosides, saponins, quinones, and vitamin C (Ali *et al*., 2018).

4. Discussion

The pathogenic bacteria especially antibiotic-resistant bacteria have been seriously posing a global public health concern and antimicrobial drugs are no longer potentially curing bacterial-related infections including *E. coli*, *S. dysenteriae*, and *P. aeruginosa* (Lowy *et al*., 2003). In addition, drug resistance studies had previously reported that these bacteria are causing infections within the hospital environment (Pendleton *et al*., 2013). Meanwhile, scientists have been understanding the mechanism of antimicrobial resistance (AMR) in a wide range, including inactivation, modification of drug targets via the enzyme, and biofilm protection (Gupta *et al*., 2017). Plants have a wide existence of phytochemicals and get attention alongside these phytochemical-derived defense mechanisms in living organisms. Previous studies suggested that the antimicrobial capabilities of plants and their phytochemicals including phenols, and flavonoids can be used as therapeutic agents (Ali *et al*., 2022; Dixon, 2001). Importantly, the recent research on antibacterial agents is considering and escalating the antibiotic-resistance at certain levels of pathogenic bacteria. This study was mainly aimed at the abilities of therapeutic plants, and their globally available resources. Furthermore, the therapeutic approach of these medicinal plants and their antimicrobial are well recognized, particularly for antibiotic development. Previous studies confirmed that the methanolic extract of *P. jacquemontiana* contained octadecanoic acid and Octadecanoic acid methyl-ester which are reported as antibacterial agents (Donia & Hamann, 2003; Hsouna *et al*., 2011).

Additionally, active compounds of plant extracts with antibacterial capability can be further transformed into possible medication, such as the antibacterial activities of *Paullinia cupana* seed possesses a strong antimicrobial capability against *E. coli* (Majhenič *et al*., 2007). Although, the most copious compound is gallic acid in methanolic-derived extracts of *P. jacquemontiana*, which are antimicrobial, and anti-inflammatory agents (Nayeem *et al*., 2016). Similarly, the antimicrobial-designed study of the *Mentha longifolia* ssp. *longifolia*-derived oil

in a methanolic medium showed antimicrobial activity against 30 microorganisms (Gulluce *et al*., 2007). Therefore, we concluded that the anti-bacterial activity of these plant species including *C. decapetala* and *P. jacquemontiana* plant extracts both in aqueous and methanolic, can have potential use against *E. coli*, *S. dysenteriae,* and *P. aeruginosa*-induced pathogens due to existence phytochemical in ample range, comprising α-linolenic, methyl-ester (Kumar *et al*., 2023). Mostly, bacteria are resistant to antibiotics, as a result showing less effectiveness, and pharmaceutical-based corporations are focusing on their manufacturing to produce new generations of antibiotics that can be proficient in curing such antibiotic-resistant bacterial strains and other health-related applications such as nanoparticle-based study revealed the antibacterial capability against *P. aeruginosa* by combining a plant-based Zinc oxide and antibiotics against pathogenic *P. aeruginosa* (MadhumitaGhosh *et al*., 2022).

Our study revealed that these plant crude extracts posed antibacterial capabilities against *E. coli, S. dysenteriae,* and *P. aeruginosa* in a concentration-dependent manner. These activities might be induced by a diverse range of phytochemicals alongside a solvent medium as well while studying these bioactive compounds' activities in resulting exhibited antibacterial activities of tested microorganisms. Meanwhile, the methanolic extract showed higher significant antibacterial activity against all tested pathogenic microorganisms, as compared to aqueous extracts. Similarly, a previous study reported a higher antibacterial activity in methanolic derived extract of *Ajuga iva*, *Marrubium vulgare*, *Mentha pulegium*, and *Teucrium polium* against *E. coli* and *S. aureus* (Khaled-Khodja *et al*., 2014). On the contrary, only the aqueous stem extract exhibits the least antibacterial action against all pathogenic microorganisms, while *E. coli* appears to be the least sensitive strain to the aqueous extract of *C. decapetala* stem. Certainly, methanol has significantly showed a higher antibacterial activity during this work and is considered the better solvent due to its solubility properties. Similarly, a previous antimicrobial study of methanolic extract of *Thymus vulgaris* had shown a maximum number of phenolic compounds that can have antimicrobial activities (Al-Bayati, 2008).

Nevertheless, many studies on herbal-derived flavonoids are often correlated with antimicrobial activity by forming a complex between residing extracellular proteins of the bacterial cell wall (Mahboubi *et al*., 2015). Our study showed that methanol extract has maximum antimicrobial activity as compared to the aqueous solvent due to the presence of diverse secondary metabolites such as gallic acid, which concludes that the previous studies of *Bidens pilosa* Linn. var. *Radiata* extracts have more potential against gram-negative than gram-positive microorganisms (Deba *et al*., 2008). In addition, a *Juniperus oxycedrus* methanolic extract has robust antibacterial activity as compared to aqueous extract, which might be due to the medium/solvent of extraction and the methanolic solvent can have more bioactive constituents (Karaman *et al*., 2003), especially inducing antimicrobial agents compared to other solvents including aqueous-derived extract.

5. Conclusion

In our experiments, the *C. decapetala,* and *P. jacquemontiana* plant extracts and their parts stem and leave in both aqueous and methanolic mediums have antimicrobial potential and can be further used for plant-based medicines against *E. coli*, *S. dysenteriae,* and *P. aeruginosa* induced diseases and disorders. These plant crude extracts can be a promising source of natural antimicrobials. Although the analysis of antimicrobial activity and concentration-based results of both plant extracts provide an assessment of these plant extracts in both mediums that can

significantly affect the growth of bacteria, especially methanolic extract, the only aqueous extracts of the stem showed the least antimicrobial effects against *E. coli* and appeared less sensitive strain to the aqueous extract of *C. decapetala* stem, resulting in these plant extracts significantly affected gram-negative bacteria growth. Meanwhile, these plant extracts have great value as natural antimicrobials and can be used safely.

Declaration of conflict of interest

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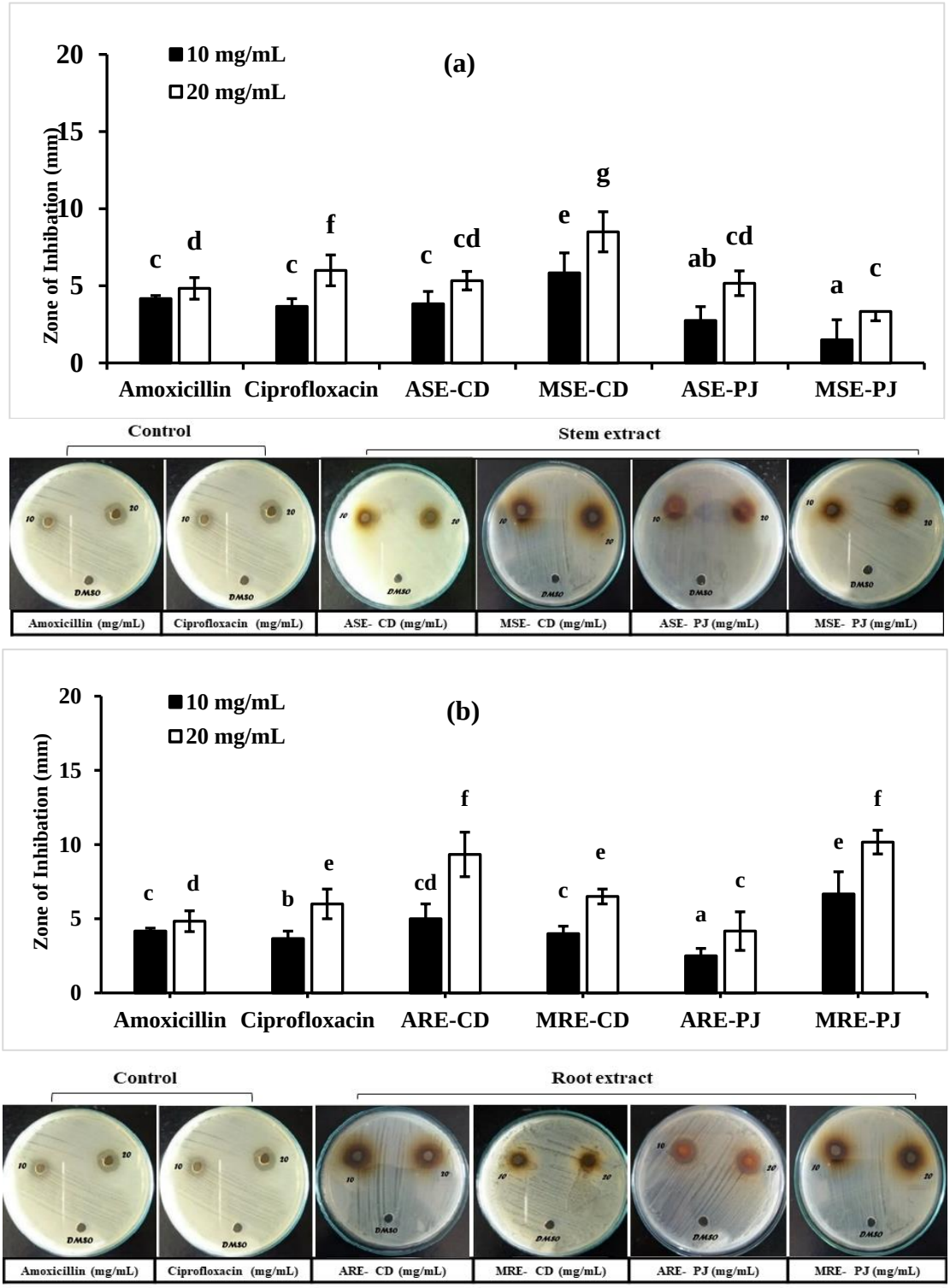
Appendix

Figure A1: Antibacterial activity of the *C. decapetala* (CD) and *P. jacquemontiana* (PJ) extracts against antibiotic resistance gram-negative bacteria

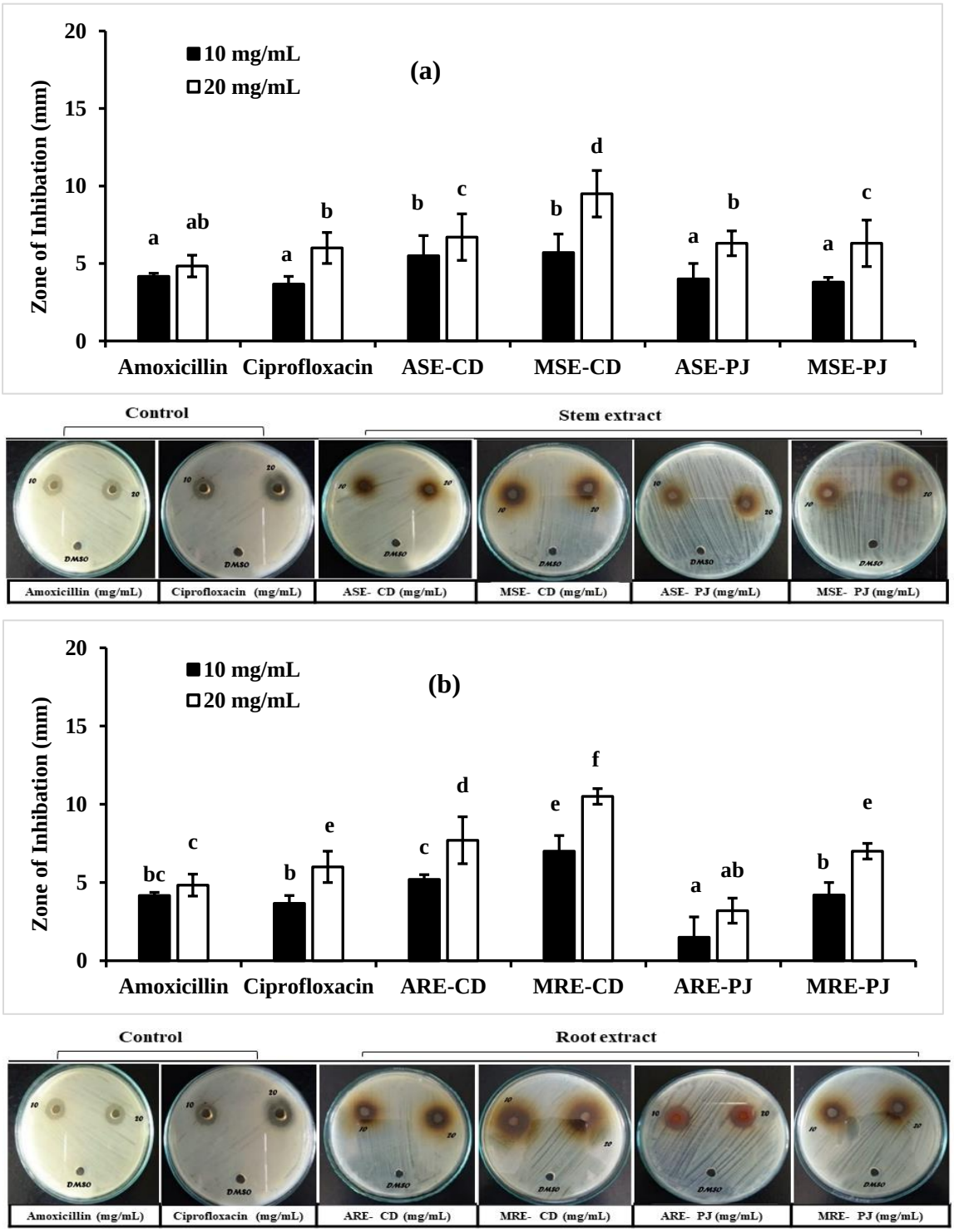


Note: The *E. coli* was grown under standard antibiotics (i.e., amoxicillin and ciprofloxacin) and plant extract conditions. The *E. coli* were grown in a Muller Hinton agar plate and exposed to 10 and 20 mg crude extract mL-1 of each plant extract, separately.

(a) The aqueous and methanolic stem extract of *C. decapetala* and, *P. jacquemontiana* (ASE-CD, MSE-CD, ASE-PJ, and MSE-PJ).

(b) The aqueous and methanolic root extract of *C. decapetala* and, *P. jacquemontiana* (ARE-CD, MRE-CD, ARE-PJ, and MRE-PJ) at 37 ◦C for 24 h. The measured zone of inhibition (mm) of each sample. Mean ± SD values of experiments (n=3). The statistical significant differences at $p < 0.05$, were indicated by different letters of superscript.

Figure A2: Antibacterial activity of the *C. decapetala* (CD) and *P. jacquemontiana* (PJ) extracts against antibiotic resistance gram-negative bacteria

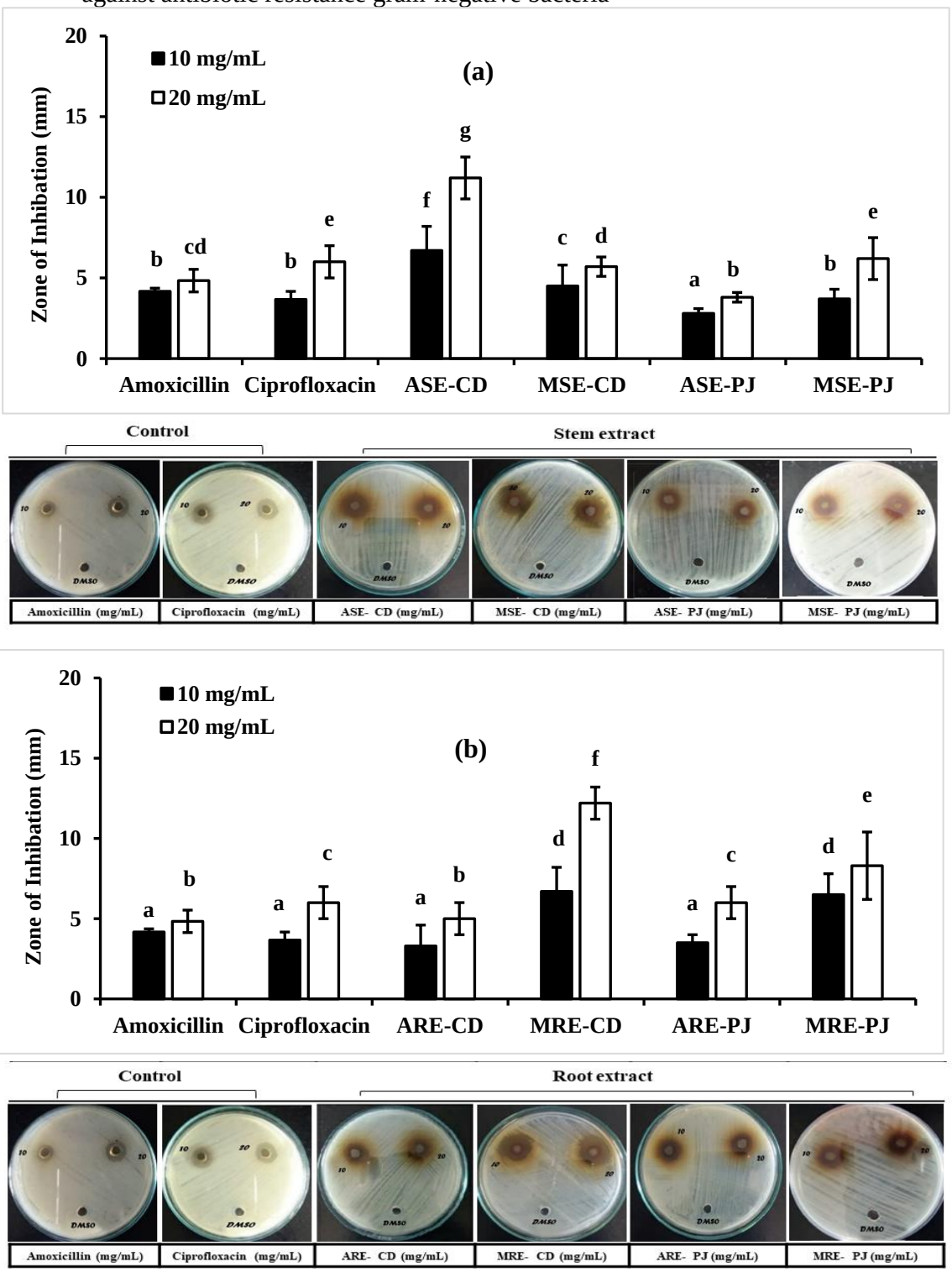


Note: The *S. dysenteriae* was grown under standard antibiotics (i.e., amoxicillin and ciprofloxacin) and plant extract conditions. The *S. dysenteriae* were grown in a Muller Hinton agar plate and exposed to 10 and 20 mg crude extract mL-1 of each plant extract, separately.

(a) The aqueous and methanolic stem extract of *C. decapetala* and, *P. jacquemontiana* (ASE-CD, MSE-CD, ASE-PJ, and MSE-PJ).

(b) The aqueous and methanolic root extract of *C. decapetala* and, *P. jacquemontiana* (ARE-CD, MRE-CD, ARE-PJ, and MRE-PJ) at 37 ◦C for 24 h. The measured zone of inhibition (mm) of each sample. Mean ± SD values of experiments (n=3). The statistical significant differences at $p < 0.05$, were indicated by different letters of superscript.

Figure A3: Antibacterial activity of the *C. decapetala* (CD) and *P. jacquemontiana* (PJ) extracts against antibiotic resistance gram-negative bacteria



Note: The *P. aeruginosa* was grown under standard antibiotics (i.e., Amoxicillin and ciprofloxacin) and plant extracts conditions. The *P. aeruginosa* were grown in a Muller Hinton agar plate and exposed to 10 and 20 mg dry extract mL-1 of each plant extract, separately.

(a) The aqueous and methanolic stem extract of *C. decapetala* and, *P. jacquemontiana* (ASE-CD, MSE-CD, ASE-PJ, and MSE-PJ).

(b) The aqueous and methanolic root extract of *C. decapetala* and, *P. jacquemontiana* (ARE-CD, MRE-CD, ARE-PJ, and MRE-PJ) at 37 ◦C for 24 h. The measured zone of inhibition (mm) of each sample. Mean ± SD values of experiments (n=3). The statistical significant differences at $p < 0.05$, were indicated by different letters of superscript.