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ORIGINAL ARTICLE

Screening for antibacterial activity of *Cissampelos pareira* L. root extract: an in-vitro study

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ABSTRACT

BACKGROUND
The recurrence of antibacterial infections after antibiotic treatment necessitates the investigation of alternative therapies against uropathogens. *Cissampelos pareira* is an Ethiopian medicinal plant that has been used for centuries by traditional healers to treat various diseases. The plant is selected on the basis of its traditional use in treating urinary tract infections by the local community. The objective of this study was to determine traditionally used anti-uropathogenic properties of *C. pareira* root extracts.

METHODS
C. pareira plant roots collected from Pawe Woreda were shade-dried, powdered, and extracted using chloroform, hexane, acetone, methanol, and ethanol, respectively. The antibacterial activities with different concentrations of the crude extracts were determined using the disc diffusion assay. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude extracts were determined using a two-fold broth dilution method.

RESULTS
The antibacterial activities of the root extracts against tested organisms as shown by inhibition zone diameters ranged from 7.0±0.1 to 20.8±1.0 mm. The highest inhibition was recorded from the ethanol extract while the lowest was from the chloroform extract. The MIC and MBC values ranged from 12.5 to 50 µg/mL and 25 to 100 µg/mL, respectively. Ethanolic and methanolic *C. pareira* root extracts showed the presence of antibacterial compounds (alkaloids, flavonoids, tannins, terpenoids and steroids).

CONCLUSION
This study showed that *C. pareira* root serves as a potential source for developing new antibacterial drugs against bacteriuria. However, nontoxicity evaluation is recommended for the use of herbals as therapeutic agents in pharmacy.

Keywords: *Cissampelos pareira*, antibacterial activity, human pathogenic bacteria, minimum inhibitory concentration, minimum bactericidal concentration, urinary tract infections

INTRODUCTION

Urinary tract infections (UTIs) are conditions in which microbes (bacteria, fungi, viruses) infect and colonize any part of the urinary tract (urethra, bladder, ureter, and kidney).[1] Worldwide, UTIs are among the most common infectious diseases affecting more than 150 million people each year.[2] A study involving 253 apparently healthy asymptomatic undergraduate female students between the ages of 17 and 26 years, showed that the prevalence rate of bacteriuria was 13.8% and that identified bacterial isolates comprised *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*.[3] Despite breakthroughs in numerous fields of medicine, UTIs are still regarded as serious public health issues that place a significant burden on healthcare facilities. High recurrence rates and rising antibiotic resistance among uropathogens are threatening to exacerbate the financial burden of these illnesses. Antibiotic resistance is spreading, necessitating the development of new antibiotics. Finding innovative tactics for selecting intriguing medicinal plants from the community is one strategy to boost the chances of discovering novel antibiotics.[4]

Since prehistoric times, medicinal plants have been identified and employed in traditional medicine. A large number of medications have been produced from medicinal plants in recent years. In many developing countries, including Ethiopia, herbal remedies are regarded as safe and extremely important by many indigenous peoples. Traditional medicine is a body of knowledge, skills, and practices based on people's views, beliefs, and experiences that protects the community against pathogens. Indigenous peoples have their own traditional medicine systems, which include a variety of therapeutic herbs as well as traditional treatments for incurable diseases. Due to their content of secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, steroids, and others, medicinal plants have been praised for their pharmacological effects since ancient times. Some of these secondary metabolites are rich in natural antioxidants that help to lower the risk of illnesses and slow their and progression.[5]

Cissampelos pareira (L.) or velvet leaf is a sub-erect or climbing dioecious plant belonging to the *Menispermaceae* family that is found in the tropics and sub-tropics.[6] According to Mukherjee and Mao [7] cough, abdominal pain, kidney stones, asthma, arthritis, diarrhoea, dysentery, kidney infection and fever are the most common ailments treated with this medicinal plant. Alkaloids (bisbenzylisoquinoline, hayatine, hayatidine, berberine, cissampareine, dicentrine, insularine, cycleanine, curine, and isomerubrine), flavonoids, tannins, volatile oils, and glycosides are among the secondary metabolites found in *C. pareira*.[4,8] Anti-inflammatory, analgesic, antipyretic, immunomodulatory, antivenom, memory-enhancing, anti-diarrhoeal, antidiabetic, hepatoprotective, muscle relaxant, antiurolithic, cardiovascular, antioxidant, anticancer, antiulcer, antiparasitic, antimalarial, antibacterial, anti-diuretic, and anti-dengue effects are some of the pharmacological properties of this plant.[9]

An in-vitro study to investigate the antibacterial activity of *C. pareira* showed showed that it had maximum activity against *K. pneumoniae*, *P. aeruginosa*, *E. coli* and *S. aureus*.[4] Another study revealed that *Cissampelos pareira* L. has antibacterial activity to both Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Proteus vulgaris*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens*) bacteria.[10] However, a study by Njeru et al.[11] in Kenya showed that the ethyl acetate fraction of *C. pareira* L root extract at 2.5 µg/disc had no activity against *K. pneumoniae*. A new study is needed due to these conflicting results.

To the best of my knowledge, for generations, in Ethiopia, especially in the Gumuz community, *C. pareira* root has been used to treat muscle pain, snakebite, rheumatism, diarrhoea, and dysentery.[12] According to the local communities of Pawe Woreda [or Pawe District, in the northeastern part of Metekel Zone], the plant is also used to treat UTIs. This study documents and validates the anti-uropathogenic properties of the *C. pareira* plant, traditionally used in Pawe Woreda, to counteract the rapid loss of ethnobotanical knowledge regarding its medicinal uses.

METHODS

Research design

The study was conducted from October 2021 to June 2022, in Mekdela Amba University, South Wollo Zone of Amhara Region, North Ethiopia, at a distance of 480 km from Addis Ababa, the capital city of Ethiopia. Based on the 2016 Amhara Regional Health Bureau (ARHB) report, there were about 4,244 public health facilities (69

hospitals, 839 health centres, and 3,336 health posts).[13]

Sample collection

Accordingly, the fresh and healthy plant roots were collected randomly from Manjari settlement, village 17 [Pawe District, Metekel Zone, Benishangul-Gumuz Regional State] using polythene bags. The collected root samples were brought to the biology laboratory of Mekdela Amba University and kept for two weeks in the dark at room temperature to dry and for subsequent processing.

Collection of test microorganisms

Human pathogenic bacterial strains of *Staphylococcus saprophyticus* (ATCC® 15305), *Enterococcus faecalis* (ATCC® 29212), *Escherichia coli* (ATCC® 25922), *Klebsiella pneumoniae* (ATCC® 700603), *Proteus mirabilis* (ATCC® 35659), and *Pseudomonas aeruginosa* (ATCC® 27853) were used to check the in-vitro effectiveness of *C. pareira* medicinal plant. The bacterial test strains were collected from Amhara Public Health Institute.

Standardization and inoculum preparation for in vitro antibacterial activity evaluation

0.5 McFarland standard was prepared by mixing 0.50 mL of (1.175% w/v) barium chloride dihydrate ($BaCl_2.2H_2O$) solution with 99.50 mL of (1% v/v) sulfuric acid (H_2SO_4). The turbidity standard solution was aliquoted into identical test tubes that were used to prepare the inoculum suspension. To prevent evaporation, the standard solution tube was tightly sealed and stored at room temperature. Before being compared with the bacterial suspension, the turbidity standard tube was mixed using a vortex mixer to get a uniform turbid appearance. [14]

From a 24-hour pure agar culture, 4-5 morphologically identical bacterial colonies were suspended in 5 mL sterile nutrient broth (Oxoid, UK) and compared to that of a 0.5 McFarland standard, which is approximately equivalent to 1.0 –1.5×10^8 CFU/mL. After adjusting the turbidity, a sterile cotton swab was dipped into the suspension and streaked over the entire surface of the prepared Mueller-Hinton Agar (MHA) medium (Oxoid, UK) by rotating the plate at 60° to ensure even distribution of the inoculum.[15]

Plant extraction

The plant root material was washed thoroughly 2-3 times with running tap water and once with sterile water and shade dried for two weeks at room temperature in the medical microbiology laboratory and ground to powder using a wooden mortar and pestle. The 100 g powdered plant material was soaked separately in 1000 mL each of chloroform, hexane, acetone, methanol, and ethanol and stayed in a shaker for 72 h until complete extraction of biologically active materials was achieved. After 72 h of extraction, each extract was filtered through Whatman No.1 filter paper (GE Healthcare Whatman™, USA) and the extraction solvent was evaporated under reduced pressure at 50˚C using a rotary evaporator to yield the crude extract. [16] The dry yields of the extracts were stored in screw-capped brown bottles and kept in the refrigerator at 4˚C for further antibacterial activity evaluation.

In-vitro antibacterial activity evaluation of the plant crude extract

The antibacterial activity of the plant crude extract was evaluated against bacterial pathogens using the disc diffusion method as described in the study by Sitotaw et al. [14] After adjusting the turbidity to that of 1.5×10^8 CFU/mL, bacterial strains namely, *S. saprophyticus* (ATCC® 15305), *E. faecalis* (ATCC® 29212), *E. coli* (ATCC® 25922), *K. pneumoniae* (ATCC® 700603), *P. mirabilis* (ATCC® 35659) and *P. aeruginosa* (ATCC® 27853) were swabbed uniformly on sterile MHA medium (Oxoid, UK) using a sterile cotton swab. Sterile 6 mm diameter paper discs were impregnated with 10 µL of each plant extract at concentrations of 100µg, 50µg, 25µg, 12.5µg, and 6.25µg. The inoculated plates and the impregnated discs were left for 5-10 minutes to absorb the moisture and to dry the disc. The impregnated discs were introduced to the upper layer of the seeded agar plate using sterilized forceps and the plate was incubated at 37 °C for 24 h. The antibacterial activities of the extracts were compared with the known antibiotic Gentamicin (10µg/disc) as a positive control and sterile water (10 µL/disc) as a negative control. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone (mm) and the results were reported as mean ± SD after three repeats of the experiment.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of the plant extract were determined by the broth two-fold serial dilution method as described in the study by Girma and

Aemiro.[17] The extract solution (100 µg/mL) was serially diluted with Mueller-Hinton broth (MHB) (Oxoid, UK) at 1:2, 1:4, 1:8, and 1:16 to obtain concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL, respectively. Each of the different extract concentrations and 100 µL of 0.5 McFarland standard adjusted test bacteria were aseptically introduced into the test tubes containing 1 mL sterilized MHB. The inhibition of growth was observed after 24 h incubation at 37˚C. The presence of growth was evaluated by comparing with the positive control, negative control and culture-containing test tubes. From the above test tubes with no growth (no turbidity), 0.1 mL was spread on the surface of MHA plates. After incubation at 37°C for 24 h, the colonies were observed and the MBC value was determined.

Phytochemical analysis

Major classes of phytochemicals (alkaloids, flavonoids, tannins, terpenoids and steroids) were determined qualitatively following the guidelines and standard protocols of Nortjie et al.[18]

Statistical analysis

The antibacterial activities of *C. pareira* root extracts were evaluated by measuring the diameter of the inhibition zone in millimeters (mm). The data collected were analyzed using descriptive statistics and reported as mean ± SD after three repeats of the experiment. The results were then presented in tables and figures.

Table 1. Antibacterial activity of root extracts of *C. pareira* obtained using chloroform, hexane, acetone, methanol, and ethanol as solvent at five different concentrations using the disk diffusion method

Extract solvent	Extract conc. µg/100µL test bacteria	Diameter of inhibition zone (mm) represented as mean ± SD, (n=3)					
		Gram-positive		Gram-negative			
		S. saprophyticus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. mirabilis***	***P. aeruginosa***
Chloroform	6.25	7.0 ± 0.4	7.3 ± 0.4	7.0 ± 0.1	7.2 ± 0.5	7.2 ± 0.6	7.0 ± 0.6
	12.5	7.2 ± 0.8	7.5 ± 0.9	7.0 ± 1.0	7.5 ± 0.5	7.3 ± 0.8	7.3 ± 0.3
	25	7.6 ± 1.0	8.3 ± 0.7	7.0 ± 1.5	8.0 ± 1.0	7.0 ± 1.5	7.5 ± 0.5
	50	8.0 ± 0.6	9.7 ± 0.5	8.0 ± 0.7	8.0 ± 0.3	8.0 ± 1.4	7.7 ± 1.0
	100	9.0 ± 0.5	10.0 ± 0.8	9.0 ± 1.0	8.0 ± 0.5	8.0 ± 0.2	8.0 ± 0.3
Hexane	6.25	8.1 ± 0.4	8.1 ± 0.4	7.0 ± 0.8	8.2 ± 0.5	8.0 ± 0.6	7.2 ± 0.5
	12.5	8.2 ± 0.8	9.5 ± 0.9	8.1 ± 1.0	8.5 ± 0.5	8.2 ± 0.8	8.3 ± 0.3
	25	8.4 ± 1.0	9.3 ± 0.7	8.2 ± 1.5	8.7 ± 1.0	9.4 ± 1.5	8.5 ± 0.5
	50	9.6 ± 0.6	10.0 ± 0.5	8.4 ± 0.7	9.2 ± 0.3	9.5 ± 1.4	8.7 ± 1.0
	100	9.7 ± 0.5	10.4 ± 1.0	9.0 ± 1.0	10.5 ± 0.5	10.8 ± 0.2	9.0 ± 0.3
Acetone	6.25	7.0 ± 0.3	7.0 ± 0.6	7.0 ± 0.9	9.0 ± 0.6	7.0 ± 0.4	7.0 ± 0.7
	12.5	10.4 ± 0.4	11.0 ± 0.8	11.5 ± 1.4	11.0 ± 1.4	10.0 ± 0.7	11.0 ± 0.6
	25	11.0 ± 0.7	12.0 ± 1.0	12.7 ± 0.5	12.0 ± 1.0	12.0 ± 1.4	11.0 ± 0.8
	50	13.0 ± 0.3	13.3 ± 0.2	13.0 ± 1.0	13.0 ± 0.6	13.0 ± 1.0	12.0 ± 1.4
	100	14.7 ± 1.0	15.0 ± 0.8	14.6 ± 0.5	14.3 ± 0.8	14.0 ± 0.6	13.2 ± 1.3
Methanol	6.25	7.7 ± 1.0	7.0 ± 0.8	7.0 ± 0.7	7.3 ± 1.0	7.5 ± 0.4	7.0 ± 0.3
	12.5	11.6 ± 0.5	11.0 ± 1.4	10.4 ± 1.0	11.4 ± 0.5	11.1 ± 0.7	12.0 ± 0.8
	25	11.8 ± 1.0	12.0 ± 0.6	11.0 ± 0.8	12.3 ± 0.6	12.7 ± 1.0	12.0 ± 0.6
	50	12.5 ± 1.0	13.0 ± 0.5	12.0 ± 0.6	13.0 ± 0.7	13.6 ± 0.5	13.0 ± 0.2
	100	17.7 ± 0.5	17.0 ± 0.3	18.0 ± 1.0	17.0 ± 0.1	16.0 ± 0.9	17.0 ± 0.4
Ethanol	6.25	7.4 ± 0.5	7.0 ± 0.2	8.0 ± 0.1	7.0 ± 0.1	7.0 ± 1.4	7.0 ± 0.3
	12.5	11.4 ± 0.7	12.3 ± 0.3	11.0 ± 0.6	12.5 ± 0.9	12.0 ± 0.7	11.0 ± 1.0
	25	13.6 ± 0.4	13.0 ± 0.6	12.4 ± 0.5	13.0 ± 0.8	13.6 ± 1.0	12.6 ± 1.4
	50	14.8 ± 0.5	14.3 ± 1.0	13.8 ± 0.4	15.3 ± 0.9	16.0 ± 1.4	14.8 ± 1.0
	100	16.0 ± 0.7	19.5 ± 1.0	16.0 ± 0.6	19.7 ± 1.0	20.8 ± 1.0	19.4 ± 0.8
Gentamicin	10µg/disc	25.3 ± 1.0	26.4 ± 1.4	23.4 ± 0.4	25.5 ± 0.5	24.0 ± 0.4	23.0 ± 0.7
Sterile water	10µl/disc	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

RESULTS

In-vitro antibacterial activity evaluation

The root extract of *Cissampelos pareira* showed effective antibacterial activity against all tested bacteria (Table 1). The chloroform extract at 6.25µg showed the least inhibition zone of 7.0±0.1 mm against *E. coli* pathogenic bacterial strain, whereas the highest inhibition zone (10.0±0.8 mm at 100µg extract) was recorded against *E. faecalis.* The hexane extract showed the least inhibition zone (7.0±0.8 mm at 6.25 µg) towards *E. coli*, while the highest zone of inhibition (10.8±0.2 at 100 µg) was recorded for the *P. mirabilis* strain. The acetone extract showed the least inhibition zone of 7.0±0.3 mm at 6.25 µg and the highest inhibition zone of 15.0±0.8 mm at 100 µg towards *S. saprophyticus* and *E. faecalis*, respectively. The maximum inhibition zone for methanol extract was seen against *E. coli* (18.0±1.0 mm at 100 µg) and the minimum was against *P. aeruginosa* (7.0±0.3 mm at 6.25 µg). Ethanol extract showed the highest inhibition zone against *P. mirabilis* strain (20.8±1.0 mm at 100 µg) and the least growth inhibition zone (7.0±0.1 mm at 6.25 µg) against the *K. pneumoniae* strain. The negative control (sterile water) did not show any inhibition zone, while the positive control (Gentamicin) showed greater antibacterial activity as compared to the root extract of *C. pareira*, but varying slightly with bacterial strain (**Table 1 and Figure 1**).

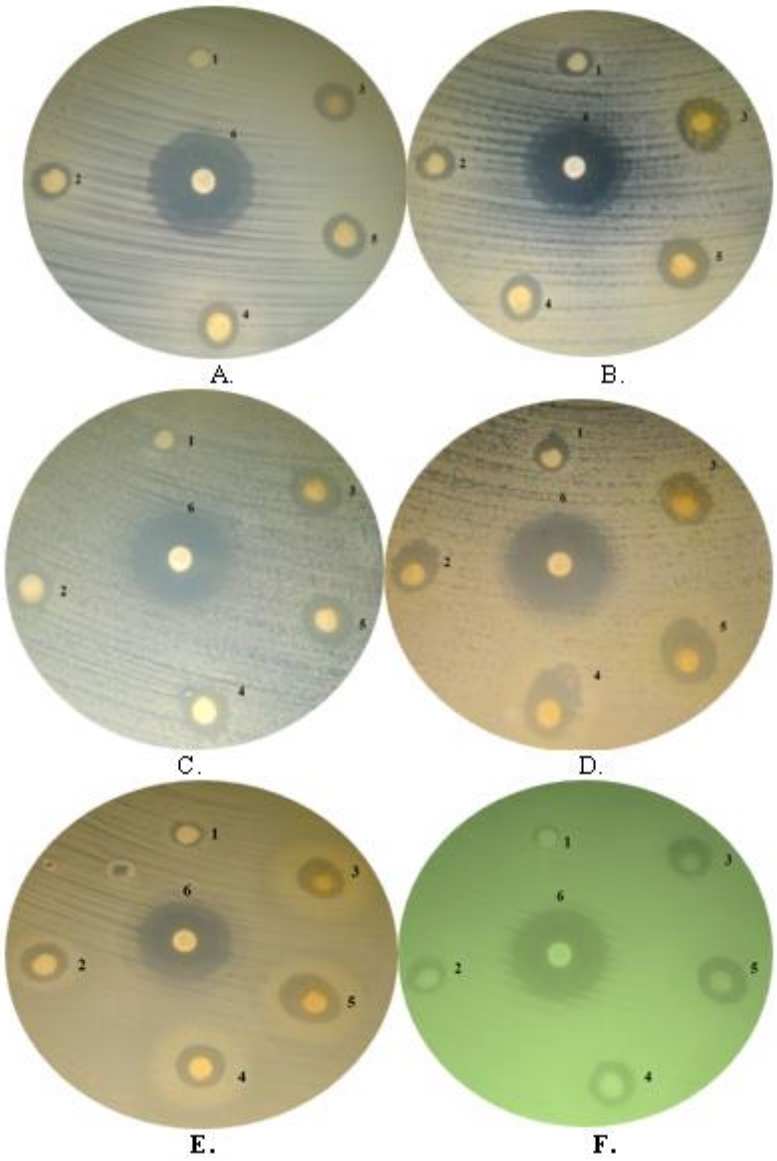

Figure 1. Antibacterial activity screening of 10 µL *C. pareira* root extract with ethanol at different concentrations (**5**=100, **4**=50, **3**=25, **2**=12.5, **1**=6.25 µg/mL and **6**=Gentamicin (10µg/disc) against human pathogenic bacteria **A.** *S. saprophyticus*, **B.** *E. faecalis*, **C.** *E. coli*, **D.** *K. pneumoniae*, **E.** *P. mirabilis* and **F.** *P. aeruginosa*

Determination of MIC and MBC of human pathogenic bacteria

The MIC and MBC of different concentrations of chloroform, hexane, acetone, methanol and ethanol solvent extracts were assessed against six human pathogenic bacterial strains (Table 2). The MIC values of plant extracts against tested pathogenic bacterial strains showed a range of 12.5-50 µg/mL while the MBC values ranged from 25 to 100 µg/mL. More than half of the tested organisms had an MIC of 25 µg/mL and an MBC of 50 µg/mL. The lowest MIC was with ethanol solvent against *P. mirabilis* (12.5 µg/mL) (**Table 2 and Figure 2**), whereas the lowest MBC was with methanol against E. *faecalis* (25 µg/mL).

Table 2. The MIC and MBC values of five root extracts of *Cissampelos pareira* (µg/mL) against UTI causing bacterial pathogens

	MIC						MBC					
Extract	**Ss**	**Ef**	**Ec**	**Kp**	**Pm**	**Pa**	**Ss**	**Ef**	**Ec**	**Kp**	**Pm**	**Pa**
Chloroform	50	50	50	50	50	50	100	100	100	100	100	100
Hexane	50	50	50	50	50	50	100	100	100	100	100	100
Acetone	50	25	25	25	25	50	100	50	100	50	50	100
Methanol	25	25	50	25	25	50	50	50	100	50	50	100
Ethanol	25	25	25	25	12.5	25	50	25	50	50	50	50

Note Ss: *S. saprophyticus*, **Ef:** *E. faecalis*, **Ec:** *E. coli*, **Kp**: *K. pneumoniae*, **Pm:** *P. mirabilis*, and **Pa:** *P. aeruginosa*, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

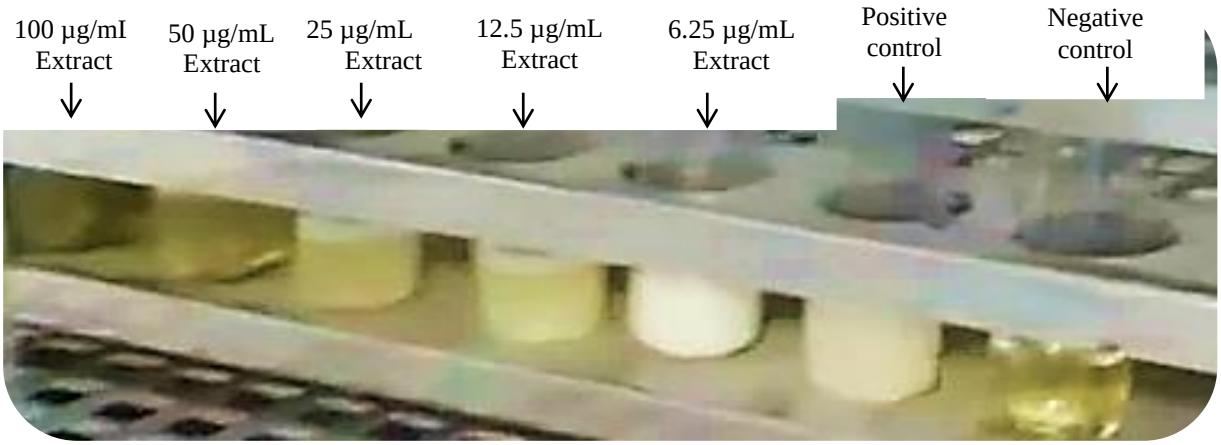

Figure 2. Minimum inhibitory concentration of *C. pareira* root extracts in ethanol solvent against human pathogenic bacterial strain *P. mirabilis* (ATCC® 35659).

Table 3. Phytochemical analysis results of *C. pareira* root extracts in different solvents

Extracts	**Alkaloids**	**Flavonoids**	**Tannins**	**Terpenoids**	**Steroids**
Chloroform	+	+	-	-	-
Hexane	-	+	+	+	-
Acetone	+	+	-	+	-
Methanol	+	+	+	+	+
Ethanol	+	+	+	+	+

+ Present, -Absent

Phytochemical analysis of *C. pareira* (L.) root extracts

The phytochemical content of *C. pareira* root extracts in different solvents (chloroform, hexane, acetone, methanol and ethanol) was determined (**Table 3**). The root was evaluated for different chemical compounds (alkaloids, flavonoids, tannins, terpenoids and steroids) having antibacterial activities. Methanol and ethanol extracts had all compounds while variations among the chemical compounds were observed in chloroform, hexane and acetone extracts.

DISCUSSION

The expanding multidrug bacterial resistance to the most commonly used antibiotics has continued as a major global health problem, especially in developing countries such as Ethiopia. Currently, some bacterial infections have acquired resistance to almost all antibiotics.[19] Therefore, new antibacterial agents from natural sources with diverse chemical structures and novel mechanisms of action are urgently needed to combat resistant organisms. Medicinal plants have long been used to treat infectious and non-infectious diseases in both rural and urban areas of Ethiopia. Since ancient times, *C. pareira* has been a medicinal plant that has continued to play a significant role in the maintenance of human health by serving as a source of diverse medicinal compounds. However, the effects of most botanicals have not been confirmed experimentally to develop traditionally used medicinal plants into modern drugs.[20]

The present study has shown that *C. pareira root* extracts have potential in the fight against human pathogenic bacterial strains and that all strains were sensitive to most of the extracts prepared in different concentrations. This result agreed with the previous study of Shrestha and Gupta [21] from Nepal who demonstrated antibacterial activity of *C. pareira* root extract towards both Gram-positive and Gram-negative bacterial strains such as *Escherichia coli* and *Staphylococcus aureus*. Njeru et al.[11] also investigated the antibacterial activities of *C. pareira* root extracts against standard strains (*S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853)) and clinical strains (*K. pneumoniae*; methicillin resistant *S. aureus*; *S. sonnei*, and *S. typhi*). This antibacterial activity is associated with the fact that root extracts of this plant may contain a variety of phytochemicals with broad-spectrum antibacterial activities such as alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, anthraquinones, glycosides and reducing sugars. However, a study by Njeru et al.[11] in Kenya showed that the *C. pareira* root ethyl acetate fraction at 2.5 µg/disc had no activity against *Klebsiella pneumoniae*. This difference may be attributed to the diversity of bioactive compounds found in this plant, which is influenced by genetic characteristics, environmental factors, the time in the plant's life history when collection occurred, treatment after collection, and the presence of a distinct phenotype of a specific species.[22]

In comparing ethanol, chloroform, hexane, acetone, and methanol extracts, the ethanol extracts showed a higher inhibition zone against both Gram-positive and Gram-negative pathogenic bacterial strains. This was similar with a report from Ethiopia by Gadisa and Tadesse.[23] This difference can either be in regard to the presence and absence of certain phytochemicals or the concentration difference of the secondary metabolites found in the extract.

The MIC and MBC results respectively showed the bacteriostatic and bactericidal effects of the plant extract against human pathogenic bacteria. In the present study, the MIC and MBC values of all tested extracts range from 12.5 to 50µg/mL and 25 to 100µg/mL respectively. This difference might be due to the different nature of the solvents and genetic differences between the bacteria. Furthermore, the highest MIC and MBC values in this study indicate that *C. pareira* root extracts were less effective against some pathogenic test organisms or that the bacterial strains may have acquired antibiotic resistance genes for this plant extract, whereas low MIC and MBC values indicate the extract's efficacy.[24]

With regard to phytochemical analysis, this study has shown that *C. pareira* root extracts contain bioactive compounds such as alkaloids, terpenoids, tannins, flavonoids and steroids. Also, the bioactivity of the plant against both Gram-positive and Gram-negative bacteria is an indicator of the presence of broad-spectrum antibiotic compounds. Bala et al.[8] isolated a new bioactive isoquinoline alkaloid along with the six known isoquinoline cmpounds from *C. pareira,* namely, magnoflorine, magnocurarine, cissamine, curine, hayatinine and cycleanine. According to Arip et al.[25] alkaloids function by increasing the role of immune cells and interfering with microbial DNA and cell-wall formation. The study of Lobiuc et al.[26] found that flavonoids and tannins act by complexing with microbial proteins, interfering with bacterial adhesion and further inactivating bacterial enzymes. Rahman and Borah [27] also reported that terpenoid and phytosteroid compounds act by disrupting microbial membranes. Therefore, the presence of different phytochemical compounds may contribute to the antibacterial activity of *C. pareira*.

The limitation of this study is that high performance liquid chromatography (HPLC) analysis and purification of metabolite antagonists were not performed. Because of the unavailability of a scanning electron microscope (SEM), the

effects of extracts on cell morphology and intracellular organization of the test organisms were not investigated.

Medicinal plants have been used for centuries in traditional medicine to treat various ailments, including bacterial infections. Their clinical implications in the context of antibacterial testing are significant for several reasons, such as identification of new antibacterial agents (discovery of novel compounds and management of antibacterial drug resistance), validation of traditional uses (confirmation of efficacy, safety and dosage), development of herbal medicines (formulation, regulation and quality control), complementary and alternative medicine (integrative approaches and patient preference), pharmacological insights (mechanisms of action and synergistic effects), and economic and accessibility considerations (cost-effective treatments and sustainability).

This is a preliminary study and requires many more processes and stages to be used in pharmacy. To know the efficiency, novelty, medical and commercial benefit of this antibiotic, further extraction, purification, structural elucidation, and characterization should be employed. This includes detailed compound identification, safety and dosage, formulations, and mechanism of action. Therefore, rigorous testing and research are essential to ensure that these plant-based treatments are effective, safe, and contribute meaningfully to managing bacterial infections in various healthcare settings.

CONCLUSION

The present study confirmed that *Cissampelos pareira* (L.) root extracts possessed an *in-vitro* broad-spectrum antibacterial activity against Gram-positive and Gram-negative human pathogenic bacterial strains. Methanol and ethanol extracts are preferable for extraction of antibacterial compounds from *C. pareira* root as compared to chloroform, hexane and acetone extracts. Thus, the results of this investigation revealed that the *C. pareira* root extracts collected from Pawe Woreda might be a potent source of chemically diverse antibacterial compounds with therapeutic potentials for the treatment of human pathogenic bacteria.

Conflicts of Interest

The author declares that he has no conflicts of interest.

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Data Availability Statement

The data used to support the findings of this study are included within this article.

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Declaration of Use of Ai In Scientific Writing

None.

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