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Research Article
Antibacterial Profile of *Cissus quadrangularis* Extracts Against Antibiotic-Resistant Bacteria Isolated from Roi Et Hospital

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Abstract

Background and Objective: The using the active antibiotic option that treats the infectious pathogenic bacteria in humans due to antibiotic-resistant strains is limited. The finding of the new antibiotic reagent source is required. This research aimed to investigate *in vitro* anti-human pathogenic bacterial activity of *Cissus quadrangularis* against seven human pathogenic bacteria isolated from clinical specimens of patients at Roi Et hospital. **Materials and Methods:** The *C. quadrangularis* were extracted with extraction solvents. The plant extracts were tested on seven human pathogenic bacteria using an agar disc diffusion assay. The broth microdilution assay and colorimetric assay were used to determine the Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of *C. quadrangularis* extracts. **Results:** The inhibition zone was measured in mm. The *C. quadrangularis* extracted with ethanol was presented the largest inhibition zone at 15 mm against *Enterococcus faecalis* and *Pseudomonas aeruginosa* (CoR-PA). The result of the lowest MIC value was at 0.19 mg mL^{-1} was presented in ethanol extraction against *A. baumannii*. The lowest MBC values were at 3.13 mg mL^{-1} were obtained from methanolic and ethanolic extracts against *S. maltophilia* and *E. faecalis*. **Conclusion:** This is the first report to present the novel information of antibacterial profile of *C. quadrangularis* extracts against human pathogenic bacteria isolated from clinical specimens of patients at Roi Et hospital which can be developed further as a natural antibiotic drug for treating bacterial infectious diseases.

Key words: Antibacterial profile, *Cissus quadrangularis* extracts, multidrug-resistant bacteria (MDR), natural antibacterial reagent, clinical specimens, mobile genetic elements, agar disc diffusion assay

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The increase of antibiotic-resistant bacteria is one of the most important medical problems worldwide. Especially, the resistance to the group of β-lactams, aminoglycosides, fluoroquinolones and sulfonamides[1]. The misusing of antibiotic is a major problem leading to antibiotic resistance in bacteria and antibiotic resistance genes could be transferred among microorganisms via Mobile Genetic Elements (MGEs), including integrons, insertion sequences and plasmids[2]. Many antibiotic-resistant bacterial strains are seriously threatens the lives of patients in the hospital such as *Acinetobacter baumannii*[2], *Stenotrophomonas maltophilia*[3], *Enterococcus faecalis*[4], *Burkholderia pseudomallei*[5], *Proteus mirabilis*[6], MDR *Klebsiella pneumoniae* (MDR-KP)[7], Colistin resistant *Pseudomonas aeruginosa* (CoR-PA)[8].

Plants are the source of natural substances including antibiotics, antineoplastic, analgesics, cardioprotective, among others[9]. Many publications about antibacterial activity of medicinal plants were published such as *Cissus incisa*[10], *Zamioculcas zamiifolia* (Lodd.)[11], *Litsea cubeba*[12], *Bidens sulphurea*, *Bidens pilosa*, *Tanacetum vulgare*[13], *Cissus quadrangularis* L.[14], *Lawsonia inermis*, *Azadirachta indica*, *Achyranthes aspera*[15], *Premna pubescens* and *Centella asiatica*[16] etc.

Cissus quadrangularis L. (family Vitaceae) is an indigenous medicinal plant of India and distributed throughout the tropical region in the world. This plant consists of various bioactive compounds such as alpha amyrin, beta amyrin, beta-sitosterol, friedelin, quercetin, genistein and daidzein. *Cissus quadrangularis* is used for diabetes[17], obesity, high cholesterol, digestive tonic, bone fractures, allergies, cancer[18], stomach upset, painful menstrual periods, analgesic, malaria, wound healing, peptic ulcer disease, weak bones, weak bones (osteoporosis) and as bodybuilding supplements as an alternative to anabolic anthelmintic, anti-dyspeptic and treatment for scurvy, asthma[14,19], preserving the cellular integrity during DMBA induced oral carcinogenesis[20] and antibacterial[21]. Kashikar and George[21] were reported about methanol and ethyl acetate extract showed high activity against the *Bacillus subtilis* ATCC 6633), *Pseudomonas aeruginosa* ATCC 27853), *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* and *Streptococcus pyogenesis*[21].

Kesavaraj *et al.*[22] were reported *C. quadrangularis* extracted with ethanol were showed a zone of inhibition against both *Streptococcus mutans* and *Lactobacillus acidophilus*. Chenniappan *et al.*[23] were presented the methanol extract of *C. quadrangularis* showed potential antimicrobial activity against *Escherichia* sp. There is no previous report about the antibacterial activity profile of *C. quadrangularis* extracts against antibiotic-resistant bacteria isolated from Clinical Specimens of Patients at Roi Et Hospital.

This research aimed to investigate *in vitro* antihuman pathogenic bacterial activity of *C. quadrangularis* against seven human pathogenic bacteria isolated from clinical specimens of patients at Roi Et hospital. The result from this research will value-added of *C. quadrangularis* and suggested that can be used *C. quadrangularis* extracts can be used for new antibiotic drug production which can heal the patients.

MATERIALS AND METHODS

Study area: All the experiments were performed during June-December, 2020 in the Microbiology Laboratory, Major of General Science, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

Chemicals and reagents: Hexane, dichloromethane, ethyl acetate, ethanol and methanol were purchased from QRëC™ (Republic of New Zealand), Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA), Nutrient Broth (NB) and Bacterial Agar were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd., India).

Plant materials and extraction: The *Cissus quadrangularis* were collected from Ban That subdistrict, Phen District, Udon Thani Province, Thailand. The plant sample was washed 3 times using tap water and cut into a small piece before dried using a hot air oven (POL-EKO-APARATURA company, Wodzisław Śląski, Poland) at 50°C for 48 hrs. The dried plant sample was grounded into powder form using a grinding machine. Twenty grams of plant powder were extracted with 100 mL of different solvent (ethanol, methanol hexane, ethyl acetate and dichloromethane) by shaking at room temperature for 3 hrs. The extracts solution was filtered through Whatman Filter paper No. 1 and was evaporated using a rotary vacuum evaporator (BÜCHI Labortechnik AG, Switzerland). The percentage yield was calculated[11]. Dimethyl sulfoxide (DMSO, Sigma) was added into each plant extract to the final concentration at 500 mg mL^{-1} before used:

$$\text{Yield (\%)} = \frac{\text{Dry weight of plant extract}}{\text{Dry weight of plant material}} \times 100$$

Test human pathogenic bacteria: *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Enterococcus faecalis*, *Burkholderia pseudomallei*, *Proteus mirabilis*, MDR *Klebsiella pneumoniae* (MDR-KP), Colistin resistant *Pseudomonas aeruginosa* (CoR-PA) were obtained from the Department of Clinical microbiology, Roi Et Hospital, Roi Et, Thailand. Active bacterial cultures were generated by inoculating a single colony of each bacteria in separate 50 mL Nutrient Broth (NB) and incubating with shaking at 37°C for overnight. The bacterial cells were harvested by centrifugation at 10000 rpm for 30 sec and were adjusted the cell concentration at OD_{600} to 0.1 before use.

Screening of antibacterial activity of *Cissus quadrangularis* extracts using disc diffusion assay: The antibacterial activity of *C. quadrangularis* extracts was primary screened using disc diffusion assay[24]. One hundred microliters of each human pathogenic bacteria were spread onto Nutrient Agar (NA) and a sterile paper dish (0.6 mm) was placed on NA. Ten microliters of each *C. quadrangularis* extract were dropped onto the centre of the paper disc. The DMSO was used as a negative control. The *C. quadrangularis* extracts were allowed to diffuse for 15 min at room temperature before the plates were incubated at 37°C for 24 hrs. The inhibition zone formation around the paper disc was measured.

***In vitro* antibacterial activity determination using a microdilution assay:** The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of *C. quadrangularis* extracts were determined using a microdilution assay[25]. The *C. quadrangularis* extracts which presented the inhibition zone from primary screening were twofold serial diluted in a 96-well plate containing NB to obtain various concentrations. One hundred microliters of the overnight human pathogenic bacterial inoculum (OD_{600} = 0.1) were added into each well. DMSO was used as a negative control. The 96-well plates were incubated at 37°C for 24 hrs. The colorimetric assay was done by using Iodonitrotetrazolium Chloride (INT) as an indicator of bacterial growth. Fifty microliters of INT were added into each well of the 96-well plate and were incubated at 37°C for 1 hr. The well containing the bacterial growth turned into pink colour whereas the well without bacterial growth remained yellow colour. The MIC was referred to as the lowest concentration of the *C. quadrangularis* extract that inhibits bacterial growth. The MBC was considered as the lowest concentration of *C. quadrangularis* extract that eliminates the bacteria that did not produce a colour change after the addition of INT[26].

Data analysis: In this study, it was used experimental design followed by descriptive analysis.

RESULTS AND DISCUSSION

***C. quadrangularis* extraction yields:** Five extraction solvents were used for *C. quadrangularis* extraction. The extraction was done by shaking for 3 hrs of the mixer the *C. quadrangularis* powder and each extraction solvents. The result indicated that the highest extraction yield at 7.25% was obtained from methanolic extraction, follow by ethyl ethanol, acetate and dichloromethane extraction was at 5.44, 1.55 and 1.52%. The lowest extraction yield at 1.28% was presented when extracted with hexane (Table 1). The extraction yield of ethanolic extraction from this research was lower than previously reported by Alain *et al.*[27] that presented the extraction yield of *C. quadrangularis* was at 6.8%. The suitable solvents for antibacterial reagent extraction from *C. quadrangularis* were methanol and ethanol.

Disc diffusion assay: Disc diffusion assay was used for the primary screening of antibacterial activity of *C. quadrangularis* extracts. The results showed that the highest diameter inhibition zone at 15 mm were presented when *C. quadrangularis* was extracted using ethanol against *E. faecalis* and CoR-PA (Table 2). The result was similar to Almawlah *et al.*[28] which presented that 100 mg mL^{-1} of *Allium cepa* bulbs extract has the inhibition zone at 14-30 mm against multidrug resistant *P. aeruginosa* isolated from burn wound. The inhibition zone from this research was larger than the report from Duailibe *et al.*[29] which found that *Bixa orellana* L. extract did not present the antibacterial activity against *E. faecalis* ATCC 292012 and *E. faecalis* 44 AB. Rachuonyo *et al.*[30] reported that extracts of *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopus* were presented as the zone of inhibition at 18.67, 17.76, 18.50 and 18.99 mm, respectively.

The smallest inhibition zone from this research at 7 mm was obtained from methanolic extraction against *S. maltophilia* and *E. faecalis* and ethanolic extraction against *A. baumannii*. This research presented the antibacterial activity against *S. maltophilia* lower than the report from Shah and

Table 1: Extraction yield of the *C. quadrangularis*

Extraction solvent	Yields (%)
Ethanol	5.44
Methanol	7.25
Ethyl acetate	1.55
Dichloromethane	1.52
Hexane	1.28

Table 2: Diameter of inhibition zones (mm) of *C. quadrangularis* extracts against seven human pathogenic bacteria at 500 mg mL^{-1} concentration

	Minimum inhibitory concentrations (mg mL^{-1})						
Extraction solvent	*A. baumannii*	*S. maltophilia*	*E. faecalis*	*B. pseudomallei*	*P. mirabilis*	MDR *K. pneumoniae*	Colistin resistant *P. aeruginosa*
Hexane	-	-	-	-	-	-	-
Dichloromethane	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-
Methanol	3.13	1.56	1.56	3.13	3.13	3.13	3.13
Ethanol	0.19	1.56	1.56	-	-	-	6.25
DMSO	-	-	-	-	-	-	-

-: No antibacterial activity, DMSO: Dimethyl sulfoxide, MDR: Multi-drug resistance

Table 3: MIC value of *C. quadrangularis* extracts against seven human pathogenic bacteria (mg mL^{-1})

	Inhibition zones (mm)						
Extraction solvent	*A. baumannii*	*S. maltophilia*	*E. faecalis*	*B. pseudomallei*	*P. mirabilis*	MDR *K. pneumoniae*	Colistin resistant *P. aeruginosa*
Hexane	-	-	-	-	-	-	-
Dichloromethane	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-
Methanol	8.0	7.0	7.0	8.0	8.0	8.0	8.5
Ethanol	7.0	8.0	15.0	-	-	-	15.0
DMSO	-	-	-	-	-	-	-

-: No antibacterial activity, DMSO: Dimethyl sulfoxide, MDR: Multi-drug resistance

Table 4: MBC value of *C. quadrangularis* extracts against seven human pathogenic bacteria (mg mL^{-1})

	Minimal bactericidal concentration (mg mL^{-1})						
Extraction solvent	*A. baumannii*	*S. maltophilia*	*E. faecalis*	*B. pseudomallei*	*P. mirabilis*	MDR *K. pneumoniae*	Colistin resistant *P. aeruginosa*
Hexane	-	-	-	-	-	-	-
Dichloromethane	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-
Methanol	6.25	3.13	3.13	6.25	6.25	6.25	6.25
Ethanol	6.25	3.13	3.13	-	-	-	12.5
DMSO	-	-	-	-	-	-	-

-: No antibacterial activity, DMSO: Dimethyl sulfoxide, MDR: Multi-drug resistance

Shelar[9]. They reported that *Piper betel* leaf extract using 70% ethanol showed the zone of inhibition against *S. maltophilia* at 27 mm. Uğur *et al.*[31] was reported the *Senecio sandrasicus* extracted with ethanol had an inhibition zone at 13 mm against *S. maltophilia* MU 23. Kidane *et al.*[32] also reported about the aqueous extract of *Lannea fruticosa* showed the highest activity against *P. mirabilis* which was 19.5 mm of inhibition zone. No inhibitions were observed when *C. quadrangularis* was extracted using hexane, dichloromethane and ethyl acetate.

MIC and MBC values: The MIC and MBC values *C. quadrangularis* extracts were determined using broth microdilution assay. The results indicated that the lowest MIC value at 0.19 mg mL^{-1} was presented in ethanol extraction against *A. baumannii*. The highest MIC values at 6.25 mg mL^{-1} were observed when extracted using ethanol against CoR-PA (Table 3). The MIC value of *C. quadrangularis* extracts against *A. baumannii* from this research was lower than the MICs value of *Rosa rugosa* extracts, *Terminalia chebula* extracts which reported by Miyasaki *et al.*[33]. Montagu *et al.*[34] were measured the MIC value using eugenol, carvacrol and cinnamaldehyde against *A. baumannii*. The result indicated that eugenol, carvacrol and cinnamaldehyde had MIC value at 1.25, 0.31 and 0.31 mg mL^{-1}, respectively. Zhang *et al.*[35] reported the *Mentha arvensis* extract had MIC value against *A. baumannii* at 23.5 µg mL^{-1}. The methanolic C*. quadrangularis* extracts from this research had a MIC value lower than *Barringtonia acutangula* (L.) extract (4 mg mL^{-1}) which reported by Panomket *et al.*[36]. The MIC values of aqueous extracts of *Lannea fruticosa* against *P. mirabilis* was at 1.953 mg mL^{-1} which lower than methanolic *C. quadrangularis* extracts[32]. The

lowest MBC values at 3.13 mg mL^{-1} were obtained from methanolic and ethanolic extracts against *S. maltophilia* and *E. faecalis*. The highest MBC value at 12.5 mg mL^{-1} was presented in ethanolic extract against CoR-PA (Table 4). Zhang *et al.*[12] were reported 0.125% *Litsea cubeba* oil can be inhibited the growth of *S. maltophilia*. The MBC values of *C. quadrangularis* extracts from this research was lower than *Tanacetum vulgare* and *Bidens sulphurea* extracts which presented MIC values against *E. faecalis* at 62.50 and 31.25 mg mL^{-1} which reported by Chiavari-Frederico *et al.*[13]. This research demonstrated that *C. quadrangularis* presents anti-human pathogenic bacteria activity that was never reported before and should be used for new natural drug development or combined with antibiotics used for treating bacterial infectious diseases. *C. quadrangularis* is not widely cultivated so the imperative to promote the cultivation of this plant is require.

CONCLUSION

The *C. quadrangularis* was extracted using 5 solvents. The suitable extraction solvents were methanol and ethanol. The lowest MIC value at 0.19 mg mL^{-1} was obtained from ethanol extraction against *A. baumannii*. The lowest MBC values at 3.13 mg mL^{-1} were obtained from methanol and ethanol extraction against *S. maltophilia* and *E. faecalis*. The new information from this research gives a novel report on antihuman pathogenic bacterial activity profile of *C. quadrangularis* extracts against infectious pathogenic bacteria in humans. This research is useful and beneficial for new antibiotic drug development and can be combined with antibiotics to increase the efficiency of the disease treatment.

SIGNIFICANCE STATEMENT

This study discovers the anti-human pathogenic bacterial activity of *C. quadrangularis* extracts that can be beneficial for drug development. This study will help the researcher to uncover the critical areas of the antibacterial profile of *C. quadrangularis* extracts against human pathogenic bacteria that many researchers were not able to explore. Thus, a new application using the *C. quadrangularis* extracts for treating the bacterial infection may be arrived at.

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