ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

Preliminary, Phytochemical and Antibacterial Activity of medicinally important plant - *Cissus quadrangularis* L. against some Clinical pathogens

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ABSTRACT

The aim of the present study is to analyze the phytochemical and antibacterial activity of *Cissus quadrangularis* L. collected from different regions of Vadakkankulam, Tirunelveli District, Tamil Nadu. Aqueous extracts and n-butanol extracts along with the leaves of *C. quadrangularis* L. shows the presence of tannins, steroids, carbohydrates, saponins, terpenoids, alkaloids, phenols and flavonoids. Glycosides and Steroid compounds were found to be absent in the leaves extracted with aqueous. Flavonoids and phenols compounds were found to be absent in the leaves extracted with n-butanol. Aqueous and n-butanol were used for extraction and samples were tested against different group of microorganisms like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Enterobacter*. The results of the study indicated that the aqueous leaf extract possessed most potent antibacterial activity as compared to n-butanol leaf extract.

Keywords: Phytochemical; Antibacterial; *C. quadrangularis;* Alkaloids; Phenols; Flavonoids.

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INTRODUCTION

Ayurveda and Chinese medicinal systems are the most acceptable traditional system which has a considerable amount of research on pharmacogenomics, chemistry, pharmacology and clinical therapeutics (**Kiranjor** and **Kunwarjeet, 2010**). Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs. The local use of natural plants as primary health remedies, due to their pharmacological properties is quite common in Asia, Latin America and Africa. The effective plant constituents can combat human and plant pathogenic bacteria, fungi and virus without toxic side effect and environmental hazards. It is evident that several plants have been used in traditional ayurvedic medicine for treatment and management of distinct inflammatory disorders and wound healing activities (**Gacche** *et al*., **2011**).

C. quadrangularis is one of the medicinal plants which are widely distributed in warmer regions of India, Sri Lanka, Malaysia, Java and West Africa (**Unnati Shah, 2011**). It has been imported to Brazil and the southern United States (**Bhagath Kumar Potu** *et al.*, **2009**). It is commonly known as Veldt Grape, Devil's backbone, Asthisamharaka, Hadjod and Perandi. *C. quadrangularis*, a vine that belongs to the family Vitaceae. *C. quadrangularis* (CQ) is a succulent perennial climber. It is commonly called as 'Bone setter' used as common food.

Plant materials occur as pieces of varying lengths, stem quadrangular, 4-winged, internodes 4-15 cm long and 1-2 cm thick. The surface is smooth, glabrous, buff colored with greenish tinge, angular portion reddish-brown; no taste and odour. Leaves are simple 2.5-5 cm long, broadly ovate or reniform, sometimes 3-7 lobed, denticulate, glabrous, cordate, rounded, truncate or cuneate at the base; petioles 6-12mm long; stipules small broadly ovate, obtuse. Flowers are in shortly peduncle cymes with spreading umbellate branches. Calyx is cup shaped, truncate or very obscurely lobed. Petals are 4, ovate-oblong, short, stout. Berry is obovoid or globose, scarcely 6mm, long apiculate, red when ripe, 1-(very rarely 2) seeded (**Rajpal, 2005**). Plant flowers in the month of June - December. The whole plant including all parts such as stems, leaves, roots are documented to possess medicinal properties in ethnobotanical surveys conducted by ethnobotanists in traditional system of medicine (**Baby Joseph** *et al.*, **2013**).

According to WHO, medicinal plants would be the best source to obtain a variety of drugs. The natural products from plant may offer new agents for antimicrobial use. This might be due to the presence of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids. In the current research, studies based on extraction of biologically active compounds from plant species used for therapeutic purposes are intensively increased (**Obeidat, 2011**).

Phytochemical constituents present in the plants are phenols, tannins, saponins, vitamin and carotene. The plant also possesses phytochemicals such as flavanoids, steroids

ISSN PRINT 2319 1775 Online 2320 7876

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and tri-terpenoids (**Prabhavathi** and **Jayaramu, 2015**). A high amount of ascorbic acid, carotene A, calcium and anabolic steroidal compounds are present in the plant (**Mishra** *et al.*, **2010**). Based on the above information, in the current study, the antibacterial activity and phytochemical analysis of petroleum ether and ethyl acetate extract of three-sided *C. quadrangularis* (Muppirandai) were evaluated due to its medicinal importance.

The *C. quadrangularis* L. has been documented in Ayurveda and Siddha systems of medicine for the treatment of various ailments like syphilis, gouts, piles, leucorrhoea, veneral diseases, diarrhoea and dysentery. The entire plants have the medicinal properties like bone healing, anti-inflammatory, analgesic, antimicrobial, antiulcer, antiosteoporosis, antioxidant and antiobestity properties. The stem juice of the plant is used to treat scurvy, menstrual disorders, otorrhoea, and epistaxis (**Ashish Kumar, 2019**). The whole plant helps in oral rehydration, while the root extracts, leaves, and stem are supportive in the treatment of various ailments. It has various other unique properties such as antimicrobial, antiulcer, antioxidative, antiosteoporotic, gastro protective, and cholinergic activity (**Balasubramanian** *et al.*, **2010**).

The whole plant is considered to be edible while each part of the plant pharmacologically contributes to some activity. *C. quadrangularis* Linn. has potent fracture healing property, anti-microbial, anti-ulcer, anti-oxidative, anti-osteoporotic, cholinergic activity as well as beneficial effects on cardiovascular diseases (**Jainu** and **Devi, 2003**). The efficacy against different activities varies for different extract (**Jainu** and **Devi, 2005**). In Indian traditional medicine *C. quadrangularis* is used as a component of a plaster for treating swelling and bone fractures (**Annie Shirwackar** *et al.*, **2004**). The biogenic synthesis of metal nanoparticles reduces the hazards through the elimination / minimization of generated waste and the implementation of sustainable processes. Biological methods of nanoparticles synthesis using microorganisms (**Klaus,** *et al.*, **1999**; **Konishi**, *et al.*, **2007**) and plant or plant extracts have been studied as possible eco-friendly alternatives to chemical methods. Using plants for nanoparticle synthesis can be advantageous over other biological process of maintaining cell cultures and can be scaled up for large- scale nanoparticle synthesis (**Shankar** *et al.*, **2004**).

The present study was carried out to investigate the antimicrobial activity and phytochemical screening of leaves extracts of *C. quadrangularis* L. against some selected bacterial pathogens.

MATERIALS AND METHODS

Collection of Plant

C. quadrangularis L. plant specimen collected from Vadakkankulam, Tirunelveli District, Tamil Nadu, India. The leaves were thoroughly washed with tap water and then with sterile distilled water for the removal of dust and sand particles. The leaves were shade dried and

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powdered by hand crushing. The powdered samples were hermetically sealed in separate Polythene bags until the time of the extraction. This was used as the raw material for the extraction of antimicrobial compounds against the pathogenic microorganisms used.

Culture Medium

Nutrient Agar Medium (for bacteria) were used as growth media for these microorganisms in this study.

Plant Extraction by Solvent Extraction method

The shade dried leaf, were used for the solvent extraction procedure. About 25 g of this powder was soaked in 250 ml of aqueous and n-butanol for 72 Hrs. The contents were then filtered through Whatman filter paper no.1. This dried extract was then dissolved in DSMO for testing its antimicrobial potential. Extracts were stored at 4°C until further use.

ANTIMICROBIAL ACTIVITY

The antibacterial activity studies were carried out by disc diffusion technique (**Newall** *et al.*, **1996**). The nutrient agar media was sterilized at 121°C under 15 lbs pressures for 30 minutes. After cooling to about 65°C, 25 ml of the medium was poured in Petri-dish. The plates were kept at room temperature for solidification and stored at 4°C until using. Bacterial culture was spread over the nutrient agar plates by using separate sterile spreader. Holes were made in the medium by using 7 mm cork borer. The dried plant extract was dissolved in dimethyl sulfoxide (DMSO) to final extract of 100 mg/ml. The hole in each plate was filled with 50 µl of plant extract. DMSO was used as a negative control in one of the plates. The plates were incubated for 24-48 hours at 37°C along with negative controls. The antibacterial activity of each extract was recorded based on the inhibition of bacterial growth by the extract at the end of incubation period. The zones of inhibitions were measured to the nearest millimeter (**Andrews** *et al.*, **2001**). The inhibition zone is the area surrounded by the hole**,** shows no growth of inoculated microorganism. For confirmation of the results, each test was performed twice.

Microbial Strains

The test microorganisms include Gram-Positive bacteria eg. *Bacillus subtilis*, *Staphylococcus aureus* and Gram-Negative bacteria e.g. *Escherichia coli*, *Enterobacter*. All the microbial cultures were maintained at 4°C on nutrient agar slants.

Assay for Antibacterial activity

Preparation of Inoculum

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The test bacterial strains were inoculated into nutrient broth and were incubated at 37°C on shaker. The inoculum size was maintained as per 0.5 McFarland standard (1x108 cfu/ml). The activated inoculum was used for antibacterial assay.

Agar well diffusion method

The screening of alcoholic extracts of different plant species for antibacterial activity was determined by agar well diffusion method (**Perez** *et al.*, **1990**). The molten Mueller Hinton Agar No. 2 media (Hi Media) was inoculated with 200 µl of the inoculum (1x108 cfu/ml). When the temperature of media reached 40-42°C, it was finally poured into the Petri plate (Hi-Media). After the media was solidified, a well was prepared in the plates with the help of a cup-borer (8.5 mm). The well was filled with 100 µl of the extract. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates were incubated at 37°C for 24 h. The result of antibacterial activity was obtained by measuring the diameter of the zone of inhibition.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of aqueous, alcoholic extracts of *Cissus quadrangularis* L was carried out by the standard methods provided for the presence and absence of metabolites such as alkaloids, glycosides, flavonoid, tannins, saponins. The preliminary phytochemical analysis was performed as per the method (**Kokate, 2001**).

Test for alkaloids:

2 ml filtrate was mixed with 1% HCl and about 6 drops of Mayor's reagents. A Creamish or pale-yellow precipitate indicated the presence of respective alkaloids.

Test for flavonoids:

2 ml filtrate was added to conc. HCl and magnesium ribbon. Pink-tomato red color indicated the presence of flavonoids.

Test for amino acids:

1 ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour shows the presence of amino acids.

Test for tannins:

1 ml of the extract was treated with few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration.

Test for saponins:

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Froth test for saponins were used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for steroids:

2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for phytosterol:

The extract was refluxed with the solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer got evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid, 3 ml of acetic anhydride was added, followed by few drops of Conc. H_2SO_4. Appearance of bluish green color showed the presence of phytosterol.

Test for reducing sugars:

The residue was re-dissolved in water on the water bath. With 2ml of the solution in the test tube, 1ml of each Fehling's solutions A and B were added. The mixture was shaken and heated in a water bath for 10min. The colour obtained was recorded. A brick-red precipitate indicates reducing sugar.

Test for terpenoids (Salkowski test):

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani Test):

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring appears below the brown ring, while in the acetic acid layer, a greenish ring was observed throughout as a thin layer.

Test for Chalcones:

2 ml of Ammonium hydroxide was added to 0.5 g ethanolic extract of each sample. Appearance of reddish colour showed the presence of chalcones.

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RESULTS

Aqueous and n-butanol extracts of *Cissus quadrangularis* L were subjected to suitable chemical tests to confirm the presence and absence of various phytoconstituents. Aqueous extracts of Cissus *quadrangularis* L. leaves showed the presence of Tannins, flavonoids, steroids, carbohydrates, saponins, terpenoids, alkaloids and phenols. Glycosides and Steroid compounds were found to be absent in the leaves extracted with aqueous. n-butanol extract of leaves of *Cissus quadrangularis* L exhibited the presence of compounds such as Tannins steroids, carbohydrates, saponins, terpenoids and alkaloids. Flavonoids and phenol compounds were found to be absent in the leaves extracted with n-butanol. (Table-1).

Table 1. Preliminary photochemical screening of *Cissus quadrangularis* L.

S. No	Name of the Phytoconstituent	Aqueous Extract	n-butanol extract
1	Tannins	+	+
2	Flavonoids	+	-
3	Glycosides	-	+
4	Steroids	-	+
5	Carbohydrates	+	+
6	Saponins	+	+
7	Terpenoids	+	+
8	Alkaloids	+	+
9	Phenols	+	-

+ Presence; - Absence

Antibacterial activity was assayed invitro by well diffusion method against four bacterial strains. The concentration of 100µg/ml of aqueous extract shows good antibacterial activity for gram positive bacteria. The antibacterial activity of the aqueous extract of leaves of *C. quadrangularis* L. was more effective against *Staphylococcus aureus*, *Bacillus subtilus*, *Escherichia coli* and *Enterobacter* with varying inhibitory zones. At the concentration of 25µg/ml, antibacterial activity was observed against *S. aureus* (11 mm), *B. subtilus* (9 mm), *E. coli* (8 mm) and *Enterobacter* (7.5 mm). At the concentration of 50 µg/ml, antibacterial activity was observed against *S. aureus* (15 mm), *B. subtilus* (11.5 mm), *E. coli* (9.5 mm) and *Enterobacter* (8mm). At the concentration of 75µg/ml, antibacterial activity was observed against *S. aureus* (19 mm), *B. subtilus* (13.5 mm), *E. coli* (12.5 mm) and *Enterobacter* (10.5 mm). At the concentration of 100 µg/ml, antibacterial activity was observed against *S. aureus* (21 mm), *B. subtilus* (18.5 mm), *E. coli* (14.5 mm) and *Enterobacter* (12 mm) (Table-2; Fig-1).

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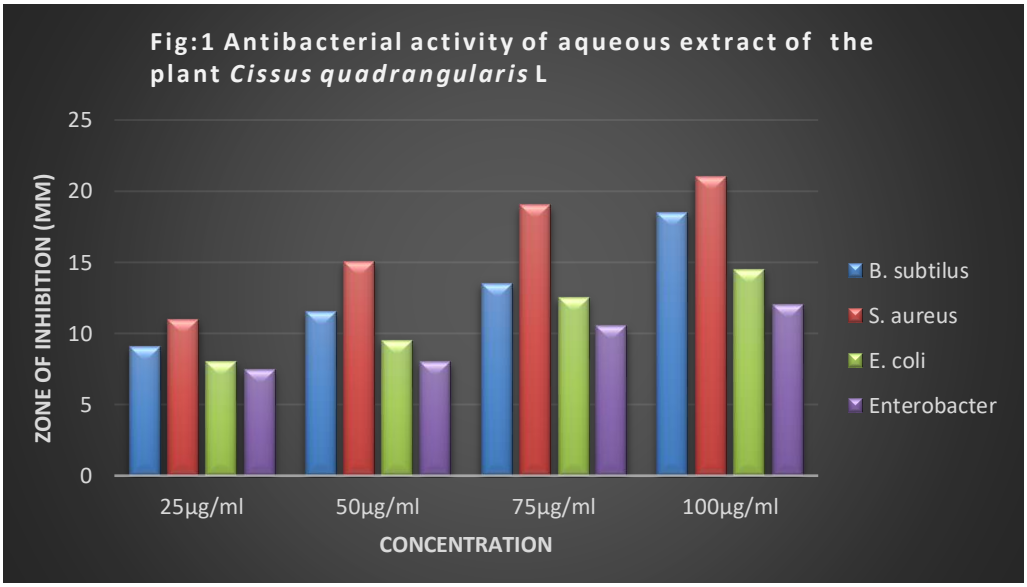
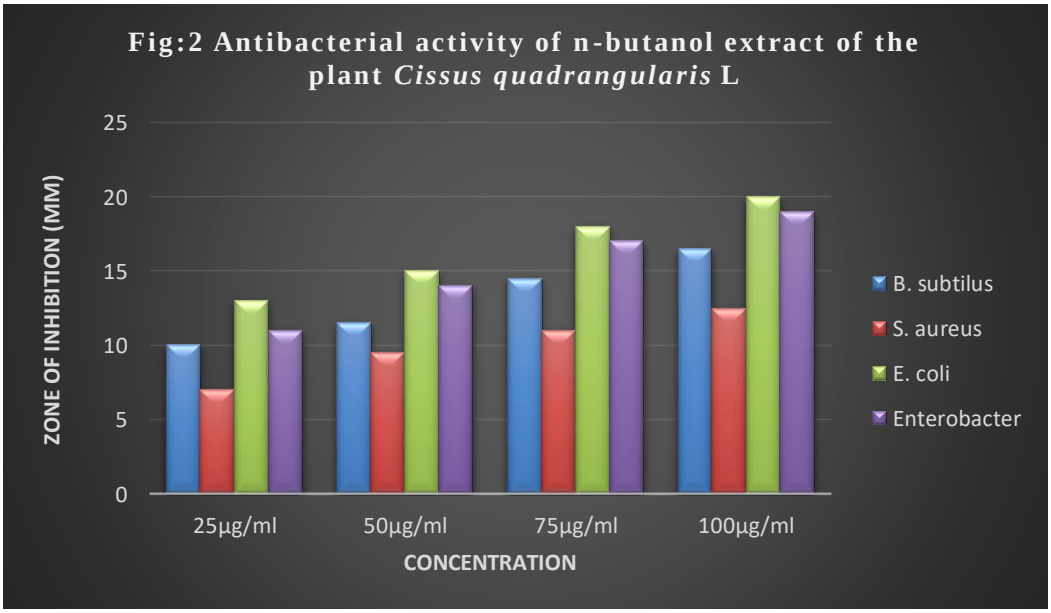


The antibacterial activity of the n-butanol extract of leaves of *Cissus quadrangularis* L. was more effective against *S. aureus, B. subtilis, E. coli* and *Enterobacter* with varying inhibitory zones. At the concentration of 25µg/ml**,** *antibacterial* activity was observed against *S. aureus* (7 mm), *B. subtilus* (10 mm), *E. coli* (13 mm) and *Enterobacter* (11 mm). At the concentration of 50 µg/ml, antibacterial activity was observed against *S.* aureus (9.5 mm), *B. subtilus* (11.5 mm), *E. coli* (15 mm) and *Enterobacter* (14 mm). At the concentration of 75µg/ml, antibacterial activity was observed against *S.* aureus (11mm), *B. subtilus* (14.5 mm), *E. coli* (18 mm) and *Enterobacter* (17 mm). At the concentration of 100µg/ml, antibacterial activity was observed against *S.* aureus (12.5 mm), *B. subtilus* (16.5 mm), *E. coli* (20 mm) and *Enterobacter* (19 mm) (Table-3; Fig-2).



ISSN PRINT 2319 1775 Online 2320 7876

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Cissus quadrangularis extracted with aqueous and n-butanol, shows more or less similar impact over clinical pathogens. Both extracts showed a similar activity on *S. aureus, B. subtilis, E. coli* and *Enterobacter*, whereas aqueous extract showed a better antibacterial activity against *S. aureus, B. subtilis* and n-butanol extract showed a better antibacterial activity against *E. coli* and *Enterobacter* (Table-2 & 3; Fig-3).

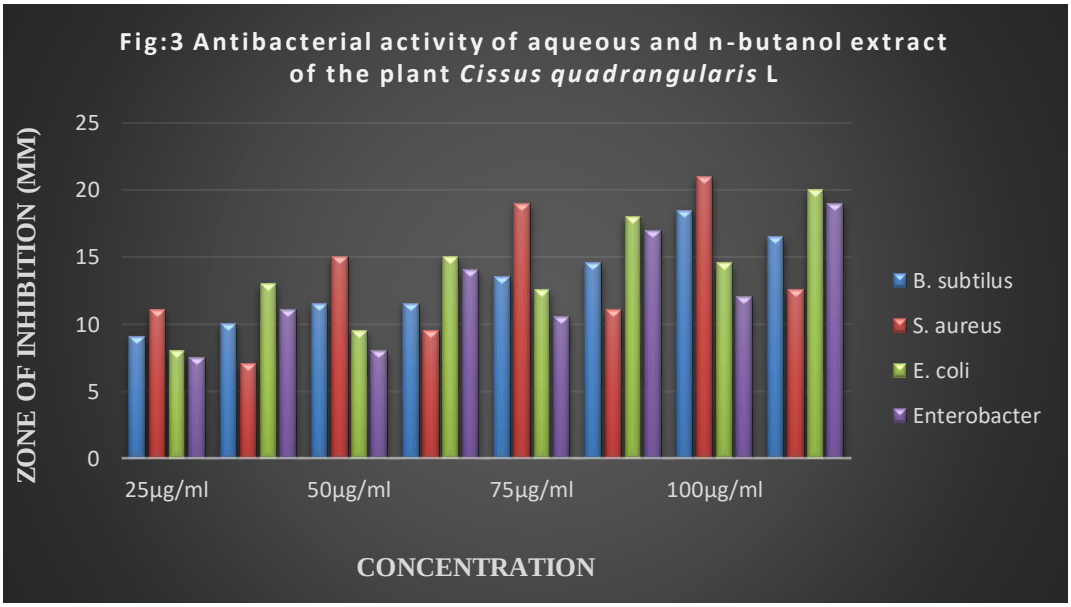

Table: 2 Antibacterial activity of aqueous extract of *C. quadrangularis* L. gram positive and gram negative bacterial species

S.No	Test Organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µl	50µl	75µl	100 µl
1	*B. subtilus*	C	09	11.5	13.5	18.5
2	*S. aureus*	C	11	15	19	21
3	*E. coli*	C	08	9.5	12.5	14.5
4	*Enterobacter*	C	7.5	08	10.5	12

Table: 3 Antibacterial activity of n-butanol extract of *C. quadrangularis* L. gram positive and gram negative bacterial species

S.No	Test Organisms	Zone of inhibition(mm) in different concentration				
		DMSO Control	25µl	50µl	75µl	100 µl
1	*B. subtilus*	C	10	11.5	14.5	16.5
2	*S. aureus*	C	07	9.5	11	12.5

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3	*E. coli*	C	13	15	18	20
4	*Enterobacter*	C	11	14	17	19

DISCUSSION

In the present investigation, phytochemical screening of leaves of *C. quadrangularis* L extracted with aqueous and n-butanol solvent, indicated the presence and absence of phytochemcial consitituents. Presence of bioactive compounds from *C. quadrangularis* L. with various solvents were reported (Table-1). The phytochemical analysis revealed that *C. quadrangularis* L contains bioactive substances which reflected on the antibacterial properties of the plant against clinical bacterial pathogens**.** The presence of such phytochemicals may be correlated with the facts that aqueous extracts showed maximum activity against the bacterial strains. The active constituents of plants usually interfere with growth and metabolism of microorganisms in a negative manner.

The difference in the antibacterial activity with the same source when extracted with different solvent has been proved that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent. Hence solvents of different polarity should be employed as discussed in this study (polar: water, acetone, ethanol; non-polar: ethyl acetate, petroleum ether). Sequential or successive solvent extraction is a good option for better solubility of many of the phytochemicals but it is always necessary to know the phytochemicals extracted by each individual solvent so as to avoid the inclusion of unnecessary solvents for extraction process as well as to understand the role of each solvent in the extraction of an individual or class of phytochemicals (Ashok Kumar and Vilayalakshmi, 2011).

Among the two extracts studied, the aqueous extract was found to have better antibacterial activity than the leaves extracted with n-butanol. Organic extracts provided more potent antibacterial activity when compared to aqueous extracts**.** It was found that the extract of leaves were effective against *S. aureus*, *Eschheria coli*, *B. subtilis* and *Enterobacter*. *C. quadrangularis* L leaves extract possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. This extract opens the possibility of finding new clinically effective antibacterial compounds (Kabra *et al.*, 2019).

Many research works has been carried out on the different extracts of *C. quadrangularis*. Some solvents which are used predominantly to extract the crude compounds include methanol, petroleum ether, ethanol, aqueous, ethyl acetate. The whole plant is considered to be edible while each part of the plant pharmacologically contributes to some activity. The efficacy against different activities varies for different extract (**Jainu and Devi, 2003**).

The plant exhibited antibacterial potential against organisms such as *S. aureus*, *E. coli* and *Pseudomonas aeroginosa*. It also has antiprotozoal activity against *Entamoeba histolytica* and anti-plasmodial activity (**Unnati Shah, 2011**). Phytochemical constituents

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present in the plants are phenols, tannins, saponins, vitamin and carotene. The plant also possesses phytochemicals such as flavanoids, steroids and tri-terpenoids (**Prabhavathi** *et al.,* **2015**). A high amount of ascorbic acid, carotene A, calcium and anabolic steroidal compounds are present in the plant (Mishra *et al.*, 2010).

The ethanol and methanol extracts of the plant was found to possess strong antimicrobial activity against tested bacteria. Research work has been carried out to examine the presence of antimicrobial and anti-oxidant activity of *C. quadrangularis* L. The effects of plant extracts on bacteria have been studied by many researchers in different parts of the world (**Nair** *et al.,* **2004**). Earlier, a number of reports on the antimicrobial activity of *C. quadrangularis* L. were reported. (**Chidambara Murthy** *et al.*, **2003; Garima Mishra** *et al.* **2009**).

Pharmacological studies of fresh leaves and roots showed that the plant possesses antioxidants, antibacterial, analgesic and neurosedative activities (**Amos 2001**). **Adegoke** *et al.,* **2000**, investigated the antimicrobial activity of aqueous, methanol and chloroform leaf extracts of *C. multistriata* L. against 8 bacterial and 2 fungal test organisms, using the tube dilution and agar ditch diffusion methods.

Kashikar and Indu George 2006, carried out the in vitro antibacterial activity of different extracts of *C. quadrangularis* L. (Vitaceae) against some Gram-negative and Gram-positive bacteria and their investigation proved that the ethyl acetate, acetone, and methanol extracts, showed antimicrobial properties and that *B. subtilis, P. aeruginosa S. typhi, S. aureus,* and *S. pyogenes,* were susceptible to at least two extracts. Petroleum ether, ethanol, and water extracts, failed to inhibit the bacterial growth of the strains tested. *E. coli* did not respond to any of the extracts used.

Both the methanol and chloroform leaf extracts inhibited all the test organisms with chloroform leaf extract showed the highest zone of inhibition against *E. coli* (25 mm) and least against *S. aureus* (13 mm). The methanol leaf extract shows least inhibition against *Salmonella typhi* (diameter 8 mm) and more inhibitory against *S. aureus* (diameter 15 mm). The methanol leaf extract of *C. multistriata* L. shows more antifungal activity compared with chloroform leaf extract, with *Candida albicans* being more susceptible than *Aspergillus niger* to both methanol and chloroform leaf extracts.

C. quadrangularis stem extracted with five solvents (Petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract) were tested for antimicrobial activities against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Penicillium sp, Mucor sp, Aspergillus niger and Candida albicans* by disc diffusion method. Among the five extracts, aqueous extract showed maximum antibacterial and antifungal

ISSN PRINT 2319 1775 Online 2320 7876

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activity against *Pseudomonas aeruginosa* and *Mucor spp*. Chloroform and ethyl acetate extracts were ineffective against Proteus mirabilis and Penicillium spp. (**Anitha** *et al.*, **2010**).

The ethyl acetate extract and methanol extract of both fresh and dry stems further exhibited antimicrobial activity against Gram-positive bacteria, including *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*, and *Streptococcus species*. The results of the study have some implications in the use of *Cissus quadrangularis* as an antibacterial agent and as antioxidant in several applications (**Chidambara Murthy** *et al.*, **2003**).

The isolated chemical constituents from *C. quadrangularis* extract, plays major role including gallic acid derivatives, steroids, iridoids, flavonoids, stilbenes and triterpenes. This review is concentrated on the different extracts of Cissus quadrangularis and its activity against numerous pathophysiological effects (**Subhashri** *et al.*, **2013**).

CONCLUSION

Based on the phytochemical analysis, the leaves of *C. quadrangularis* L. contains more bioactive compounds which in turn elicit the antibacterial efficiency of the plant against the tested clinical bacterial pathogens of the present study. Further studies on phytochemical characterization on the leaves of *C. quadrangularis* L. might throw light on the specific component responsible for such antibacterial activity. According to their observation n-butanol leaf extract had less activity against both the gram positive and gram-negative bacterial tested organisms, aqueous leaf extracts showing the highest zone of inhibition against *B. subtilus* and least against *S. aureus*. The results of this study have shown that the extract of *C. quadrangularis* L. leaves have great potential as antibacterial agents in clinical organisms. Further detailed investigation of the active components of the plant for the exact mechanism of action will contribute greatly to the development of new pharmaceuticals.

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