

Evaluation of Antimicrobial Potential of Stems of *Cissus quadrangularis* (Linn) Using Two Gram Positive and Negative Bacteria

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ABSTRACT

Since prehistoric times plants have served human beings as a natural source of treatments and therapies, amongst them attention on medicinal herbs have grown because of their wide use and less side effects. In present times thrust on plant research has increased exponentially throughout the world and increasing amount of evidences are being collected and documented to show the immense potential of medicinal plants employed in various traditional systems, which is reflected from the fact that more than 15000 plants have been studied during the last 5-year period. The fact that most efficient drugs available are directly or indirectly derived from the plant kingdom has instigated scientists recently to focus their full attention on using these renewable resources to harvest new generation of therapeutic solutions. The frugal and pragmatic nature of production of plant-based pharmaceuticals has also attracted more capital investment in the research and development of new plant consequential therapeutics, giving patients access to new drugs faster. This review highlights some of the phytochemical and pharmacological aspects of various species of the *Cissus* family (Vitaceae) found throughout the world. It is a fleshy, cactus-like climbing plant, commonly found in tropical and subtropical xeric woods and extensively used as a food item in India. Folk literature suggests that the plant has a plethora of therapeutic uses in ameliorating pathological states of helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers and swellings amongst others. The present study is an attempt to explore antimicrobial activity of stems of *Cissus quadrangularis* (Linn) using different microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) by disc diffusion methods. The result shows that out of all extracts tested, methanol extracts were effective against all the four microorganisms when compared to standard 20 µg /disc ampicillin.

INTRODUCTION

Cissus quadrangularis (Linn) is used for its fracture healing power in common folk medicine in India. According to Ayurveda, it can be used in ear diseases, analgesic in eye, anthelmintic, dyspeptic, digestive, tonic and in the treatment of irregular menstruation and asthma. Whole plant is used in oral re-hydration, while the extracts of leaf, stem, and root of this plant are important

in the management of various ailments. Lupenone^[1], delta-amyrin^[2], kaempferol^[3], quercetin^[4] were reported in various parts of *C. quadrangularis*. The present study is an attempt to explore its antimicrobial activity.

MATERIAL AND METHODS

Collection of Plant Material

Dried stems of *C. quadrangularis* were collected and authenticated from Dr. Harish, Botanist, Alva's Education Foundation (R), Alvas Health Centre Complex, Moodbidri-574227, Karnataka, India.

Fifteen grams of powdered *C. quadrangularis* was charged in a 'thimble' made of cellulose. It was placed in a central compartment of Soxhlet assembly. By using Soxhlet extractor, exhaustive extraction with a series of solvents of increasing polarity was done. Solvents used with increasing polarity are petroleum ether, benzene, chloroform, acetone, methanol and water.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on successive extractions from petroleum ether, benzene, chloroform, ethanol and aqueous solvents.

Screening of Plant Extracts for Antimicrobial Activity

Antibacterial activities^[5-7] of different extracts were studied by the disc diffusion method.

Preparation of Extracts

The plant part was powdered and extracted successively with different solvents by increasing then polarity, in a Soxhlet extractor. The plant extracts were filtered through Whatman No. 1 filter paper into beaker. The filtrates were dried and constant dry weights of each extracts were obtained. The residues were stored at 4 °C for further use.

Test Organisms

The pure cultures of bacteria maintained in the college microbiology laboratory were used for the microbiological work. The test organisms^[8] were maintained on nutrient agar medium (NA). The test organisms used for screening are *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Preparation of Inoculums

Stock cultures^[9] were maintained at 4 °C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated without agitation for 24 h at 37 °C and 25 °C respectively. The cultures were diluted with fresh nutrient broth and Sabouraud dextrose broth.

Preparation of Media

Media was prepared by dissolving 3 gm of nutrient agar obtained from Himedia (Mumbai) in 100 ml water and autoclaved at 121 °C for 15 min.

Antibacterial Susceptibility Test

Standard disc diffusion method was carried out to screen the antibacterial activity^[6]. *In vitro* antibacterial activity was screened by using nutrient agar media. The NA plates were prepared by pouring 10 ml to 15 ml of molten liquid media into sterile Petri plates. The plates were allowed to solidify for a few minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 10 min. Test discs were prepared by loading different extract on 5 mm sterile disc till saturation, separately and dried on air. The loaded test discs were placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate by using standard drugs (20 µg /disc ampicillin).

RESULTS

Presence of sterols was detected in all the extracts through phyto-chemical screening. The methanolic, ethanolic and aqueous extracts showed the presence of phenols and flavanoids, whereas the acetone and aqueous extracts showed the presence of alkaloids. Antibacterial activity of the concerned extracts was comparatively studied against standard drug using different microorganisms (*B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*).

Extracts of petroleum ether, chloroform, acetone, methanol, aqueous and standard drug were chosen for the comparative study, which showed zones of inhibition of diameter 9 mm, 12 mm, 13 mm, 18 mm, 11 mm and 18 mm respectively in culture of *S. aureus*. The same extracts and standard drug exhibited zones of inhibition of 10 mm, 12 mm, 13 mm, 16 mm, 12 mm and 17 mm diameter respectively in case of culture of *B. subtilis*; zones of 10 mm, 14 mm, 14 mm, 18 mm, 13 mm and 16 mm shown correspondingly in case of culture of *E. coli* and 9 mm, 12 mm, 13 mm, 18 mm, 11 mm and 19 mm for the same order for culture of *P. aeruginosa*.

The observations clearly depicted remarkable anti-bacterial activity of the methanolic extract amongst others in cultures of all the four organisms. The extracts of acetone, chloroform, aqueous and petroleum ether showed anti-bacterial activity in decreasing order (Figure 1 and Tables 1-3).

Table 1. Phytochemical screening of *C. quadrangularis*.

Chemical Constituent	Tests	Pet. ether Extracts	Benzene Extracts	Chloroform Extracts	Acetone Extracts	Methanol Extracts	Ethanol Extracts	Aqueous Extracts
Alkaloids	Mayer's test	-	-	-	+	-	-	+
	Dragendorff's test	-	-	-	-	-	-	-
	Wagner's test	-	-	-	-	-	-	-
	Hager's test	-	-	-	+	-	-	+
Carbohydrates	Molisch's test	-	-	-	-	-	-	-
	Benedict's test	-	-	-	-	-	-	-
	Fehling's test	-	-	-	-	-	-	-
Glycosides	Modified Borntrager's	-	-	-	-	-	-	-
	Legal test	-	-	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-	-
Phytosterols	Salkowski test	-	-	+	-	+	+	-
	Liebermann Burchard	+	+	+	+	+	+	-
Phenols	Ferric Chloride test	-	-	-	-	+	+	+
Tannins	Alkaline Reagent	-	-	-	-	-	-	-
Flavanoids	Gelatin test	-	-	-	-	-	-	-
	Lead acetate test	-	-	-	+	+	+	+
	Shinoda test	-	-	-	-	-	-	-
Proteins	Xanthoproteic test	-	-	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-	-	-
	Biuret test	-	-	-	-	-	-	-

Table 2. Antibacterial activity of different extracts of stem of *C. quaderangulris* against gram positive organisms.

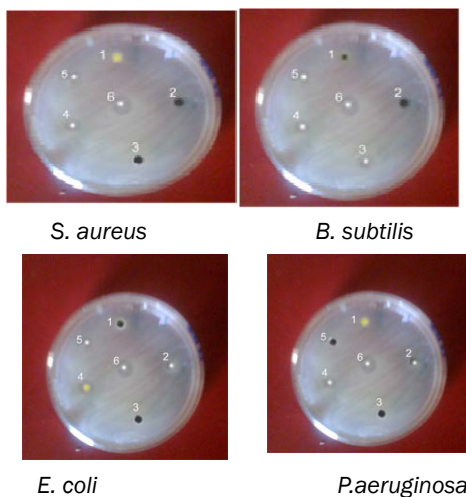
Gram positive organisms	Zone of inhibition of extract (mm)					STD
	PEE	CE	AE	ME	AQE	
<i>S. aureus</i>	9	12	13	18	11	18
<i>B. subtilis</i>	10	12	13	16	12	17

PEE: Petroleum Ether Extract; CE: Chloroform Extract; AE: Acetone Extract; ME: Methanol; AQE: Aqueous Extract; STD: Ampicillin

Table 3. Antibacterial activity of different extract of stem of *C. quaderangulris* against gram negative organisms.

Gram negative organisms	Zone of inhibition of extract (mm)					STD
	PEE	CE	AE	ME	AQE	
<i>E. coli</i>	10	14	14	18	13	16
<i>P. aeruginosa</i>	9	12	13	18	11	19

PEE: Petroleum Ether Extract; CE: Chloroform Extract; AE: Acetone Extract; ME: Methanol; AQE: Aqueous Extract; STD: Ampicillin



1=Petroleum Ether Extract; 2=Chloroform Extract; 3=Acetone Extract; 4=Methanol Extract; 5=Aqueous Extract, 6=Ampicillin as Standard.

Figure 1. Antimicrobial activity.

DISCUSSION

Recent years have witnessed escalating ethnobotanical and traditional uses of natural compounds, especially of plant origin. This inclination towards herbal remedies has been reinforced by a sharp rise in the number of evidences regarding lethal side effects of synthetic medicine and studies supporting the “at par” efficacy and enhanced safety of plant derived principles regarding human use. Increased incidences of epidemic outbreaks with no effective containment strategy and therapeutic interventions conjugated with increased awareness of the populace pertaining to miraculous remedial capabilities of plant based constituents has also contributed dramatically to the era of the “herbal remedies”. They obviously deserve more documentation and systemic scientific study such as phytochemical and pharmacognostical investigation, biological screening on experimental animal models, toxicological studies, investigation of mechanism of action of isolated phytochemical moieties on molecular level and their clinical trials. It is the best classical approach to discover new lead molecules for management of various ailments. Our detailed scrutiny of literature available on *C. quadrangularis* showed an interesting fact that though the plant has been documented extensively for a wide range of ailments, still only a tip of the iceberg has been touched regarding the immense therapeutic potential of plant. The active chemical principles of *C. quadrangularis* needs to be analyzed with an organized scientific outlook in times to come through specific experimental animal models and clinical trials to decipher their molecular mechanism of action, in search of lead molecule from natural resources.

Present study has also attempted to explore the antimicrobial potential of *C. quadrangularis* in both gram positive and gram negative bacteria. Results explained that the methanolic extract was the most effective compared to other extracts like pet ether, acetone, and aqueous extract.

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