

EVALUATION OF ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS AND PHYTOCHEMICAL ANALYSIS OF *CISSUS QUADRANGULARIS* L

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ABSTRACT

Plants have various medicinal properties. Medicinal plants are in use for thousands of years and are renowned for their effectiveness in various treatments. The medicinally usable plants were identified and extracted for biochemical profile and formulated for medical applications. *Cissus quadrangularis* is an important medicinal plant belonging to the family Vitaceae. In India, *C. quadrangularis* is widely used as a common food item. In this study the qualitative phytochemical Analysis, Antifungal activity and *in vitro* Antimicrobial screening *C. quadrangularis* were evaluated by using ethanol extracts, Anti-bacterial activity of *C. quadrangularis* was studied in this study the secondary metabolites such as phenol, alkaloids, sterols, tannins and Flavonoids were present in the ethanol extract of

C. quadrangularis. The anti-bacterial activity of various extracts of leaf at different concentration was evaluated against some human pathogenic bacteria and the results were discussed. The result of the *in-vitro* antifungal activity of *C. quadrangularis*.

KEYWORDS: *In-vitro* Anti-bacterial, Anti-fungal, *Cissus quadrangularis*, Phytochemistry.

INTRODUCTION

Cissus quadrangularis (CQ) which is succulent perennial climber. *Cissus quadrangularis* reaches a height of 1.5 m and has quadrangular-sectioned branches with internodes 8 to 10 cm long and 1.2 to 1.5 cm wide. Along each angle is a leathery edge. Toothed trilobe

leaves 2 to 5 cm wide appear at the nodes. Each has a tendril emerging from the opposite side of the node. Racemes of small white, yellowish, or greenish flowers; globular berries are red when ripe (Mallika Jainu and C.S. Shyamala Devi, 2005). scattered all over India particularly in tropical regions, usually called as Asthisamdhani in Sanskrit, Vajravalli in Sanskrit, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oria, Vedhari in Gujrati, Perandai in Tamil, Nalleru in Telugu and Veldgrap in Indian languages and in English it is Called as Edible-stemmed Vine. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics (Periyasamy *et al.*, 2010). It is probably native to India or Sri Lanka, but is also found in Africa, Arabia, and Southeast Asia. It has been imported to Brazil and the southern United States. (Mia, Md *et al*, 2009), (Oben, J *et al*, 2006, 2008), the active principles of many drugs found in plants are secondary metabolites. The stem of *Cissus quadrangularis* is also reputed in Ayurveda as alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, in the treatment of irregular menstruation and asthma, in complaints of the back and spine. Scientific studies have revealed the *Cissus* extract to possess cardiogenic and androgenic property (Chopra *et al.*, 1986). Due to availability in the local area of Chittagong, the plant *Cissus quadrangularis* was chosen for study. Hence, the present study was undertaken specifically to investigate the antimicrobial and antifungal activity of sequential extracts of *Cissus quadrangularis* L. Leaf.

MATERIALS AND METHODS

Collection of Plant Materials and Preparation of Plant Extracts

The fresh and disease free plant leaves of *Cissus quadrangularis* were collected from Vayalur, Tiruchirappalli District. The leaves of *Cissus quadrangularis* L were surface sterilized with running tap water (3 times) and soaked in 0.1% mercuric Chloride solution. Finally, the leaves were washed with distilled water and shade dried. About 2g of leaves were ground in mortar and pestle with 10ml of aqueous and organic solvents acetone, n-butanol and Sterile distilled water and centrifuged at 10,000 rpm for 15 minutes. The supernatant was obtained and used for *In Vitro* Antimicrobial screening.

Microorganisms and Media: The human pathogenic bacteria species (*Arsenophonus nasoniae*, *Salmonella enterica typhi* and *Clostridium pneumonia*) and fungal species (*Penicillium sps*, *Aspergillus flavus* and *Geotrichum candida*) were collected from the Microbial Culture Collection Unit (MCCU), Sri Gowri Biotech Research Academy, Nagai Road, Tanjavur, TamilNadu.

The nutrient agar medium was used for culturing bacterial species and the potato dextrose agar medium was used for culturing fungal species.

Composition of Nutrient Agar Medium Preparation

1. Beef extract-3gm, Sodium chloride-5gm, Peptone-5gm, Agar-15gm and Distilled water 1000 ml
2. The above ingredients were weighted and put into the conical flask contains 1000 ml distilled water. Then adjust the pH of the medium to 6.8, using a ph meter by the addition of either acid (or) alkali. The flask were sterilized by using an autoclave at 121° C for 15 lbs pressure for 15 minutes and allowed to cool.

Composition of Potato Dextrose Agar Medium

1. Potato-200 gm, Dextrose-20 gm, Agar-15gm and Distilled water-1000 ml.

Preparation of Potato Dextrose Agar Medium

The potato tubers were peeled and weighed for about 200g. The tubers were chopped into small pieces with the help of sterile knife. The chopped potatoes were transferred into a conical flask containing about 1000ml of distilled water. The content was boiled for 20min. The supernatant were decanted and filtered by muslin cloth and the filtrate was collected. Dextrose & Agar were transferred into the extract and shaken to dissolve the ingredients. The medium was made up to 1 liter by addition of distilled water.

The pH medium was observed and adjusted to 5.6 by using 1N Hydrochloric acid (or) Sodium hydroxide drop wise. Finally, the medium poured was into two conical flasks, cotton plugged and sterilized in pressure cooker for 20 minutes. The prepared medium was used for the experiment.

Preparation of Microbial Inoculums

The young microbial inoculums / culture was prepared and used in the entire research period. The Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were prepared and poured into test tubes and sterilized. The pure microbial cultures were collected from the institute either solid (or) liquid medium and inoculated in the tubes using inoculation loops (or) needles. After, these tubes were incubated at different temperatures and time duration (at 37°C for 24 - 48 hrs for bacteria; and at 37° C for 24 - 48 hrs for fungi) for testing the antibacterial and antifungal activity, the cultures were incubated into respective broth and incubated 18 – 24

hrs for bacteria and 48-72 hrs for fungi. After incubation the cultures were used for the experiment.

Antimicrobial assay, antifungal activity assay and antibacterial activity assay

The antimicrobial activity of the plant extracts were tested using agar well diffusion method (Perez *et al.*, 1990). In the freshly prepared and sterilized potato dextrose agar medium, a pinch amount of streptomycin was added and mixed well. Then these 20ml of medium was poured into each Petri plates and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swap. Then wells (5cm) were made in the medium by using sterile cork borer. 100 µl of each aqueous, acetone and n-butanol plant extracts were transferred into separate well. Then these plates were incubated at 27°C for 48 – 72 hrs. After incubation, the results were observed and measured the diameter of inhibition zone around the well. The standard antibiotics (positive) like Amphotericin B and respective solvents (negative) like acetone. N-butanol and water were used as controls. In the freshly prepared and Sterilized Nutrient agar medium. Then these 20ml of medium was poured into each Petri plate and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using sterile cotton swap. Then wells (5cm) were made in the medium by using sterile cork borer. 100µl of each aqueous, acetone and n-butanol plant extracts were transferred into separate well. Than these plates were incubated at 27° c for 24 – 72 hrs. After incubation, the results were observed and measured the diameter of inhibition zone around the well. The standard antibiotics (positive) like Amphotericin B and respective solvents (negative) like acetone. N-butanol and water were used as controls (Perez *et al.*, 1990).

Isolation of Phyto Compounds from *Cissus quadrangularis*

Alkaloids, phenols, sterols and tannins

A known quantity of the sample material (dry/wet) was ground in pestle and mortar with 10 fold volume of 70% ethanol. The contents were shaken at 55° C (or) pooled twice the supernatant with equal volume of petroleum ether at 40-60° C and shaken vigorously. The petroleum ether layer containing chlorophyll was discarded. The ethanol fractions were evaporated to dryness under vacuum using either a water pump (or) rotary evaporator at 40° C. The residue was dissolved in a known volume of absolute ethanol (or) water for analysis (wager and 13/ adt, 1996). 2gms of powdered *Cissus quadrangularis* were lixiviated in methanol on rotary shakes (180thans/mins) for 24 hrs. Then these extract was filtered by

using what Mann No.1 filter paper. The condensed filtrate was used for TLC. (Harbarne, 1998) 2gms of powered *Cissus quadrangularis* were extracted with 10ml of methanol in water bath (80° c /15min).The condensed filtrate was used for TLC. (Wagner and Bladt, 1996) 2gms of powered *Cissus quadrangularis* were extracted and dissolved in 5ml of distilled water filtered and ferric chloride reagent added the condensed filtrate was used for TLC. (Trease and Evans, 1989).

Thin Layer Chromatography (TLC) Techniques

The phytocompound analysis of *Cissus quadrangularis* extracts using thin layer Chromatography was carried out as per the method of (Izanailov and Shraiver, 1938). Thin layer chromatography may be used with reagent such as H₂SO₄ which would react with paper and was especially useful for the separation and analysis of high molecular weight biochemical compounds. A wide variety of mixtures such as amino acids, dye and food storing drugs, sugars, natural products and insecticides may be separated and identified. TLC has also been applied to micro – analytical studies of inorganic ion and salts.

Thin Layer Plate Preparation, Sample Application

The stationary phase (Silica gel) was prepared as slurry with water or buffer at 1:2 and applied to a glass plate or an insert plastic (or) aluminium sheet as thin as using a glass rod or Pipette or using TLC applicator 0.25mm thickness for analytical separation and 2.5mm thickness for preparative are prepared. Calcium sulphate (Gypsum) (10.15%) was incorporated to the adsorbant was a binder and facilitates the adhesion of the adsorbant to the plate. After application of adsorbant, the plates are air dried for 10 – 15 minutes. This process was also known as activation of the adsorbant. The plates are used immediately or stored indicators.

Solvent Preparation: A line was drawn lightly with a pencil of about 1.5cm and 2.0cm from the bottom. If the thin layer was too soft to draw a pencil line, a scale was placed at the bottom and spotted at a distance of 1.5cm. The order was noted and the samples were spotted using capillary tubes at 1.5cm distance between them. For preparative TLC, the sample was applied as a band across the layer rather than as a spot.

Alkaloids, Phenols, Sterols, Tannins and Running of Sample in TLC

1. The alkaloids were extracted by following the method of Wagner and Bladt (1996). The alkaloids were separated by using N-butanol, Glacial acetic acid and water (4:1:2)

mixture.

2. The phenols were separated by following the method of Harborne (1998). The phenols were separated by using Chloroform and methanol (27: 3) solvent mixture.
3. The sterols were separated as per the method of Wagner and bladts in (1996). The sterols were separated by using acetone, glacial acetic acid, methanol and water (64:34:12:18) solvent mixture.
4. Tannins were separated as per the method. The tannins were separated by using n-butanol, glacial acetic acid and water (4:1:5) solvent mixture.
5. The chromatographic tank was filled with developing solvents to a depth of 1.5cm and equilibrated for about 5 hrs. The thin layer plate was placed gently in the tank and allowed to stand for about 60mins. It was taken care that the spots do not touch the solvent directly. The capillary action caused the solvent to ascend as in paper chromatography and the separation of compounds took place. When the solvent front reaches about 1.2cm from the top of the plate, the plate was removed. The solvent front was marked with a pencil immediately and allowed to air dry by placing the plate upside down.

Compound Detection

Several methods are available to detect the separated compounds. The different types of spraying reagents were used to detect the different components.

Alkaloids, Phenols, Sterols and Tannins

1. The presence of alkaloids in the developed chromatogram was detected by spraying the freshly prepared Wagner's reagent (0.67g iodine and 1g of potassium iodide dissolved in 2.5ml distilled water and the solution was made up to 50ml with distilled water). The formation of brown colour spot showed positive result.
2. The presence of phenols in the developed chromatogram was detected by spraying the Folin – ciocalteu's reagent, after the plates were heated at 80° c for 10mins. A positive reaction was the formation of blue colour spot.
3. The presence of sterols in the developed chromatogram was detected by spraying the Folin – Ciocalteu's reagent, after the plates were reacted at 80 c for 10mins. A positive reaction was the formation of blue colour spot.
4. The presence of tannins in the developed chromatogram was detected by spraying the 10% ferric chloride dissolving ethanol solution was reacted at 80 c for 10mins. A positive

reaction of was the formation of blue colour spot.

Determination of Rf Value

The RF values of the various phytochemical compounds were calculated using the following formula. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates. $RF = \text{Distance traveled by solute (measured to centre of the spot)} / \text{Distance traveled by solvent}$.

Cissus quadrangularis L.

Phytochemicals	Color of the spot	Present(+) Absent(-)	Rf value
Alkaloids	Brown color	+	11/12
Sterols	Blue color	+	9/10
Phenols	Blue color	+	6/8
Tannins	Blue color	+	7/9

Preliminary Phytochemical Analysis:

The aqueous, N-butanol and acetone extracts of leaves of *Cissus quadrangularis* obtained by infusion were analyzed by qualitative method (thin layer chromatography and silica gel/UV detection at 365 nm) for the presence of alkaloids, tannis, flavonoids, sterols etc.

RESULTS AND DISCUSSION

Phytochemical screening of *Cissus quadrangularis* is given in the Table .1 *In vitro* antibacterial activities of aqueous, N-Butanol and acetone extracts of *Cissus quadrangularis* are shown in figure1. Among the solvent extracts N-Butanol extracts showed maximum inhibitory (18.1 ± 0.2) activity against *Arsenophonus nausoniae*, Aqueous extract showed activity against *Arsenophonus nausoniae* (7.1 ± 0.1), *Salmonella enterica typhi* (7.8 ± 0.1), *Clostridium pneumonia* (7.9 ± 0.1); N-Butanol extracts revealed antibacterial activity against *Salmonella enterica typhi* (17.1 ± 0.2), *Clostridium pneumonia* (16.1 ± 0.2); Acetone extract showed ineffective against *Arsenophonus nausoniae*, *Salmonella enterica typhi*, *Clostridium pneumonia*; N-Butanol extract confirmed antibacterial activity against *Arsenophonus nausoniae* (18.1 ± 0.2), *Salmonella enterica typhi* (17.1 ± 0.2), *Clostridium pneumonia* (16.1 ± 0.2).

Anti Microbial and Antibacterial Activity

Methanol extract (90%) and dichloromethane extract of stems possess antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* and mutagenicity against *Salmonella microsome*. (Luseba D *et al.* 2007,) Antimicrobial activity has also been reported from stem and root

extract (Murthy KNC *et al.* 2003,) the alcoholic extract of aerial part was found to possess antiprotozoal activity against *Entamoeba histolytica* (Rajpal.V and Standardization, 2005) Alcoholic extract of the stem showed activity against *E. coli* (Rao BS, and Deshpande. V 2005) Methanol and dichloromethane extract of whole plant were screened for *in vitro* antiplasmodial activity (Paulsen BS, Sekou *et al.* 2007).

In vitro antifungal activity of Aqueous.N-Butanol and Acetone extracts of *Cissus quadrangularis* are shown in Table 2. Among the three extracts N-Butanol showed maximum antifungal activity against *Geotricum candidum* (19.8 ± 0.1),Aqueous extracts showed antifungal activity in *Penicillium* sps (9.1 ± 0.2),*Aspergillus flavus* (9.0 ± 0.1), *Geotricum candidum* (10.2 ± 0.1) N-Butanol extract confirmed activity against *Penicillium* sps (19.5 ± 0.1) Acetone extract showed in effective against *Penicillium* sps, *Aspergillus flavus*, *Geotricum candidum*(0)

Preliminary Phytochemical Screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary / secondary metabolites. Table 3. Qualitative analysis of the phytochemical of *Cissus quadrangularis*.

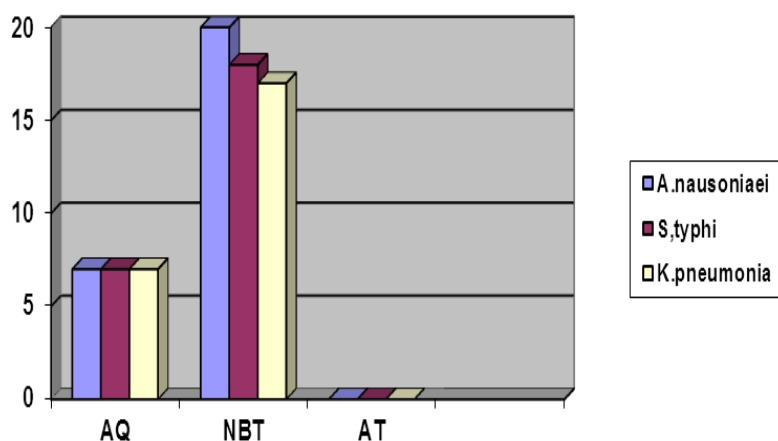


Table 1.The antibacterial activity of leaves extracts of *Cissus quadrangularis*.

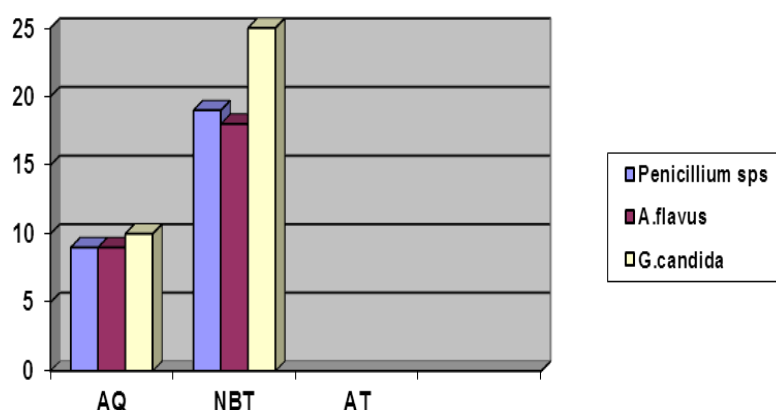
Micro organisms	AQ	NBT	AT
<i>A.nausoniaei</i>	7mm	20mm	-
<i>S.typhi</i>	7mm	18mm	-
<i>K.pneumonia</i>	7mm	17mm	-

Keys: AQ-Aqueous extract, NBT –N-butanol extract, AT-Acetone extract

Table-2. The antifungal activity of leaves extracts of *Cissus quadrangularis*

Micro organisms	AQ	NBT	AT
<i>Penicillium sps</i>	9mm	19mm	-
<i>A.flavus</i>	9mm	18mm	-
<i>G.candida</i>	10mm	25mm	-

AQ-Aqueous extract, NBT –N-butanol extract, AT-Acetone extract

**Table 3. Qualitative analysis of the phytochemicals of *Cissus quadrangularis***

Phytoconstituents	AQ	N-B	A
Alkaloids	+	+	+
Tannins	+	+	+
Flavonoids	+	-	-
Steroids	+	+	+
Phenols	+	+	+
Amino acids	-	-	-
Carbohydrates	+	+	+
Terpenoids	-	-	-
Gum and mucilages	+	+	+
Sponins	-	+	-

Phytochemical screening of extracts of leaves of *Cissus quadrangularis* by infusion:

Tested Material	Positive tests for	Negative tests for
Aqueous extract	Alkaloids, Flavonoids, Phenols, Carbohydrates. Tannins sterols gum and mucilages.	Amino acids, Terpenoids, Sponins
N-butanol extract	Alkaloids, Tannins, sterols, Phenols, Carbohydrates, gum and mucilages, Sponins.	Flavonoids, Amino acids, Terpenoids
Acetone extract	Alkaloids, Tannins, sterols, Phenols, Carbohydrates, gum and mucilages.	Flavonoids, Amino acids, Terpenoids, Sponins.

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