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Antibacterial activity of *Cissus quadrangularis* stem extract against *Bacillus subtilis* and *Proteus mirabilis*

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ABSTRACT

The aim is to evaluate the antibacterial activity of *Cissus quadrangularis* stem extract against *Bacillus subtilis* and *Proteus mirabilis*. Ethanol and chloroform was used for extraction. The extraction was done at room temperature. All the extracts were filtered through Whatmann No.1 paper to get filtrate as extracts and were dried to concentrate the samples. Qualitative analysis was tested. The Characterization of sample was done with Thin layer chromatography, Fourier Transform Infrared, and UV- visible spectroscopy. The antibacterial activity of CQ extract was evaluated by agar disc-agar method. The zones of inhibition formed against *Proteus mirabilis and Bacillus subtilis* were measured. *Cissus quadrangularis* showed zone of inhibition against both *Proteus mirabilis and Bacillus subtilis*. *Cissus* quadrangularis showed antibacterial activity against both *Bacillus subtilis* and *Proteus mirabilis.*

Keywords: *Cissus quadrangularis;* Phytochemical test; Characterization; Antibacterial activity.

1. INTRODUCTION

The important advantage of medicinal plants in various treatments is their safety besides being less expensive, efficacy and availability throughout the world [1, 2]. The ayurvedic system of medicine uses about 700 species of unani, sidda and modern medicine around 30 species [3]. The medications are gotten either from the entire plant or from various organs, similar to leaves, stem, dim, root, blossom, seed, and so on. a few medications arranged from excretory plant item, for example, gum, gums and latex [4]. Indeed, even the allopathic arrangement of medication has received various plant-got drugs from a significant section of the cutting edge pharmacopeia. Some significant substance intermediates requirements for assembling the cutting edge medications are additionally gotten from plants [5].

C.quadrangularis is a perpetual plant of the grape family. It is generally known as veldt grape or fallen angel's spine. *Cissus* is a genus of approximately 350 species of woody climberin the grape family (vitaceae)[6]. They are used as food plants by the larvae of some lepidopteran species including hyper compaeridanus and hypercompeicasia [7]. It grows to a height of 1.5 m and has quadrangular- sectioned branches with internodes 8 to 10 cm wide appear at the nodes. Each has a tendril emerging from the opposite side of the node fragments of small white, yellowish, or greenish flower [8]. It has additionally been utilized for bone cracks, frail bones (osteoporosis), scurvy, malignancy, steamed stomach, hemorrhoids, peptic ulcer sickness (PUD), excruciating menstrual periods, asthma, intestinal sickness, and agony [9-12]. It is said to have a antibacterial, antifungal, antioxidant, anthelmintic, antihemorrhoidal and analgesic activies [14-16]. It has been found to contain rich sources of carotenoids, triterpenoids and ascorbic acid.

2. MATERIAL AND METHODS

2.1. Sample Collection

Fresh & healthy *Cissus* quadrangularis stem were collected in a separate sterile polythene bags from the area in and around Manamai, Mahapalipuram, Kanchipuram (dist), Tamil Nadu.

2.1. Preparation of Solvent Extracts

The cleaned, vigorous plant materials are cut in to small pieces and dried under dark for 4 weeks. The dried material was crushed into fine powder in an electric mixer. Obtained powder was stored in desiccator's setup and used for extraction. Extraction was carried out using 1gm of each sample roughly powdered plant material with 25 ml of solvent and kept for 48 hrs with minor shaking. Here, ethanol and choloroform (HPLC grade) was used for extraction. The extraction was done at room temperature. All the extracts were filtered through Whatmann No.1 paper to get filtrate as extracts and were dried to concentrate the samples. The residual power was weighed and was re-dissolved in the individual solvents to get a final concentration 1mg/ml. The powder was stored in airtight containers under refrigeration condition.

2.2. Phytochemical Analysis

2.3. Qualitative Analysis

Following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones and Proteins.

Test	Observation
Flavonoids test: To 2 ml of each extract was added with few drops of 20% sodium hydroxide. To this, few drops of 70% dilute hydrochloric acid were added	Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract
Phenols Test: To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added	Development of blue colour indicates the presence of phenols in the sample extract.
Tannins Test: To 2 ml of each extract, 10% of alcoholic ferric chloride was added	Formation of brownish blue or black colour indicates the presence of tannins.
Alkaloids Test: To 1 ml of each extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed	Appearance of dark orange or purple colour indicates the presence of alkaloids.
Saponins Test: To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously	Formation of bubbles or persistent foam indicates the presence of saponins
Protein Test: To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added.	Formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.
Terpenoids Test: Take 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid.	Formation of reddish brown precipitate indicates the presence of terpenoids in the extract.
Carbohydrates Test: Take 1 ml of extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes.	Development of red or dull violet colour indicates the presence of carbohydrates in the sample extract.

2.4. Characterization of Plant extract

The chloroform and ethanol extract of stem extract were collected and characterized by Thin layer chromatography, UV-Visible spectroscopy and Fourier transform infrared spectroscopy. Thin layer chromatography was done in etheyl acetae: methanol: water ratio of 62:35:3 solvent as mobile phase. The UV-visible spectroscopy reading was read at 190 nm to 600 nm to find the peak absorbance and tabulated. FTIR reading were measured at 400 cm^{-1} to 4000 cm^{-1}.

2.5. Anti-bacterial activity:

Antibacterial activity was screened by the disc agar diffusion method. The stem extracts were tested for antibacterial activity, against bacterial pathogens such as *Bacillus subtilis* and *Proteus mirabilis.* LB broth and LB agar were used for microbial culture and microbial assays. Fresh colonies were picked and inoculated in the LB broth. Then kept at 37°C for 24 h. Cotton swaps were used to swap the sterile petri plates with both the bacterial cultures. Then 6mm sterile disc were prepared with the stem and leaf extract at

different concentration were loaded. Kept sample in incubator for 24 h at 37°C.

3. RESULT AND DISCUSSION

3.1. Phyotochemical analysis

Alkaloids were mainly seen in most of the samples except methanolic extract of stem and methanolic extract of fruit. Tannins, proteins, carbohydrate and phenol were present in all the 4 samples extracted by both methanol and ethanol. Flavonoids were seen only in leaf samples where as cardiac glycosides was seen only in stem extracted when extracted with methanol. Saponins were mainly present in the samples extracted from methanolic solvent.

Plant part	Stem	
Solvents	**Methanol**	**Choloroform**
Alkaloids	+	-
Flavonoids	-	-
Tannins	+	+
Trepenoids	+	+
Saponin	+	-
Proteins	+	+
Carbohydrates	+	+
Phenols	+	+

3.2. Characterization by thin layer chromatography (TLC):

A chromatographic method TLC was also based on the principle of separation (Figure 1). The separation depends on the relative resemblance of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) will travel over the surface of stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture was achieved. Once separation occurs individual mechanism are visualized as spots at own level of travel on the plate. Their nature was identified by means of suitable detection techniques. The chloroform extract and ethanol extract of stem was placed on the TLC sheet and kept in the solvent for 15 min. Later it was visualized under the UV light and picture taken. The solvent composition chloroform 62 %, methanol 35 % and water 3 % were used. Further NMR studies needed to elucidate the molecules.

The partition relies upon the general proclivity of mixes towards stationary and portable stage. The mixes affected by portable stage (driven by fine activity) will go over the outside of stationary stage. During this development the mixes with higher fondness to stationary stage travel gradually while the others travel quicker. In this way partition of segments in the blend was accomplished. When partition happens singular segments are imagined as spots at separate degree of movement on the plate. Their tendency or character are recognized by methods for appropriate identification systems.

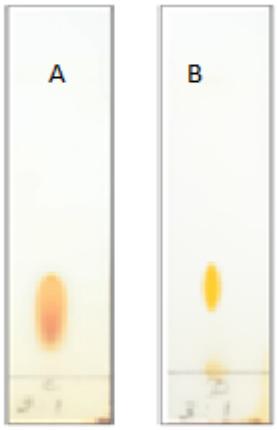


Figure - 1: Thin layer chromatography of chloroform and ethanol extract of *Cissus* quadrangularis stem.

3.3. Characterization by UV-visible spectroscopy:

Atoms containing π-electrons or non-holding electrons (n-electrons) can ingest the vitality as bright or obvious light to energize these electrons to higher enemy of holding sub-atomic orbitals. The more effectively energized the electrons (for example lower vitality hole between the HOMO and the LUMO), the more extended the wavelength of light it can absorb. The chloroform and ethanol extract of leaf/stem wavelength were measured by UV visible spectroscopy. Results are summarized in the table 1.

Table - 1: The chloroform and ethanol extract of leaf/stem wavelength were measured by UV visible spectroscopy

Extracts	Peak Wavelength (nm)	Absorbance
Stem ethanol	300	0.6
Stem chloroform	302	0.5

3.4. Characterization of Fourier transform infrared (FTIR)

The FTIR spectrum was obtained from both the leaf extract/stem extract of chloroform

and ethanol. The IR spectrum of leaf extract showed that major peaks were observed in the lower frequency regions at 1543, 1652 and 1237cm-1 which were assigned for CQC, carbonyl (4CQO) stretching vibrations of amide I and amide II linkages which is due to the functional group that clearly implies the presence of protein/peptide serves as stabilizing as well as reducing agent for the formation of AgNPs. Infraredactive modes attributed to side chain vibrations include C–Hstretching symmetric and anti-symmetric modes at 2923 and 2854cm-1 which corresponds to aliphatic and aromatic compounds, respectively. The intense peak absorbance at 3287cm-1 is the characteristic of the hydroxyl functional group in alcohols and phenolic compounds. Figure are summarized in (Figure 2, 3).

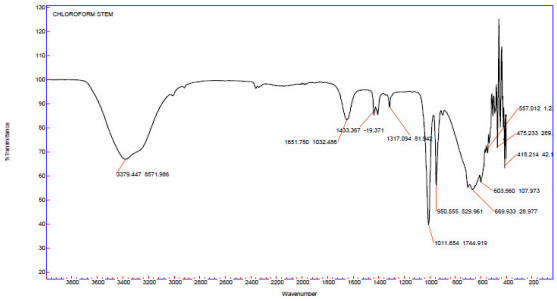


Figure. - 2: Chloroform extract of Stem.

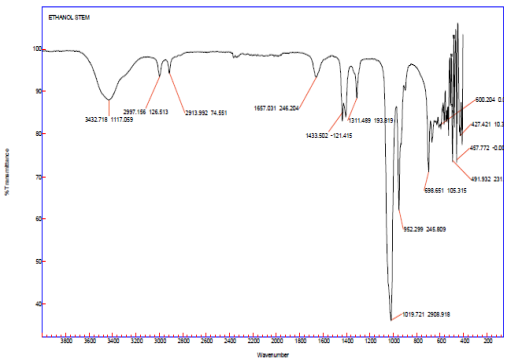
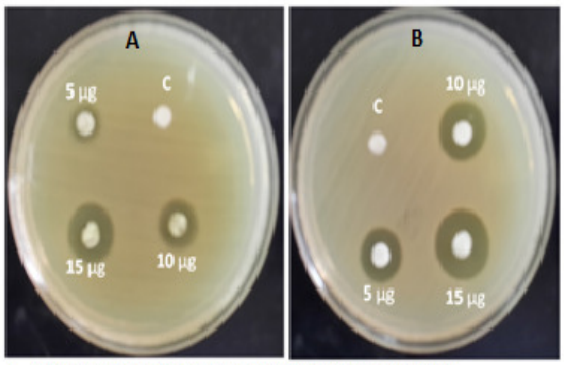


Figure - 3: Ethanol extract of stem.

3.5. Antibacterial Activity

In the present study, antibacterial activity of *cissus quadrangularis* was analysed. The effect of stem extracts on *Proteus mirabilis* was shown in figure 4. According to the plate assay the chloroform and ethanol extract of stem showed a minimum inhibitory concentration at 5 µg and maximum zone of inhibition at 15 µg against *Proteus mirabilis*. As shown in figure 5. According to the plate assay the chloroform and ethanol extract of stem showed a minimum inhibitory concentration at 5 µg and maximum zone of inhibition at 15 µg against *bacillus subtilis*. Zone of inhibition was measured by Himedia scale in mm and summarized in Table 2 and Table 3. Austin et al. prepared aqueous and organic solvent extracts (chloroform, acetone and methanol) of *Cissus* and showed their activity against *Helicobacter pylori* [17]. Selvamaleeswaran et al., showed that the ethyl acetate and methanolic extracts also exhibited antimicrobial activity against Gram-positive organisms [18]. Baker et al reported that different geological location cissus quandrangularis exits different antibacterial activity [19].

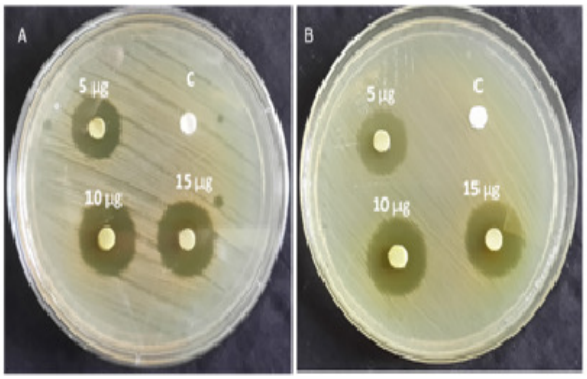


A - Ethanol Stem Extract against *Proteus mirabilis*
B - Chloroform Stem Extract against *Proteus mirabilis*

Figure - 4: The effect of chloroform and ethanol *cissus quadrangularis* stem extracts on *Proteus mirabilis*

Table - 2: Zone of inhibition of chloroform and ethanol *Cissus quadrangularis* stem extracts on *Proteus mirabilis*

Bacterial pathogen *Proteus mirabilis*	Zone of inhibition (mm)		
	5µg	10µg	15 µg
Ethanol Stem extract	6	9	12
Chloroform Stem extract	9	11.5	13



A - Ethanol Stem Extract against *Bacillus subtilis*
B- Chloroform Stem Extract against *Bacillus* subtilis

Figure – 5: The effect of chloroform and ethanol cissus quadrangularis stem extracts on bacillus subtilis.

Table - 3: Zone of inhibition of chloroform and ethanol *Cissus quadrangularis* stem extracts on *Bacillus subtilis*

Bacterial pathogen *Bacillus subtilis*	Zone of inhibition (mm)		
	5µg	10µg	15 µg
Ethanol Stem extract	9	11	14
Chloroform Stem extract	9	11.5	14

4. CONCLUSION

Conventional medication is a significant wellspring of conceivably valuable new mixes for the improvement of chemotherapeutic agents. The antibacterial activity of cissus quadrangularis stem chloroform and ethanol extract was tested against the *proteus mirabilis and bacillus subtilis.* Result showed some promising result for developing a new antibiotics compound from the plant. Hence, further NMR studies need to confirm the antibacterial compound of stem ethanol and chloroform extract against the bacterial pathogens.

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