

***Invitro* screening of antimicrobial potentials of *Cissus quadrangularis* L.**

***Merinal, S. and Viji Stella Boi, G.**

Department of Botany, Kunthavai Nachiyaar Government Arts College (W) Autonomous, Thanjavur

ABSTRACT

Cissus quadrangularis L. is a succulent plant of family Vitaceae commonly found in tropical and subtropical xeric wood. It is a fleshy, cactus like liana widely used as a common food item in India. The present study was designed to evaluate the antimicrobial activity of ethanol, diethyl ether and aqueous leaf extracts of *Cissus quadrangularis* L. against bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, methicillin resistant *Staphylococcus aureus* (MRSA) and fungal pathogens such as *Aspergillus flavus*, *Candida albicans* and *Fusarium solani* by in vitro agar well diffusion assay. The ethanol extract of the plant was found to possess strong antimicrobial activity against tested pathogens.

Key Words: *Cissus quadrangularis* L., antimicrobial activity, in vitro, pathogens.

INTRODUCTION

Cissus quadrangularis (CQ), a succulent vine native to India, also found in Sri Lanka, Africa, Arabia, and Southeast Asia. It is very commonly known as asthisamharaka. 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. The plant is prescribed in the ancient ayurvedic literature as a general tonic and analgesic, with specific bone fracture healing properties.

The roots and stems are most useful for healing of fracture of the bones. The stem is bitter, it is given internally and applied topically in broken bones, used in complaints of the back and spine. Leaves and young shoots are powerful alternatives, dried and powdered; they are administering in certain bowel infections connected with indigestion [1]. A paste of stem is useful for muscular pains. The stem juice of plant is used to treat scurvy, menstrual disorders, otorrhoea and epistaxis. Decoction of shoots with dry ginger and black pepper is given for body pain the infusion of plant is anthelmintic. The herb is fed to cattle to induce flow of milk. The ash of plant is useful as a substitute for baking powder [2].

The plant has been documented in Ayurveda for the treatment of osteoarthritis, rheumatoid arthritis and osteoporosis [3, 4]. The use of sap with tamarind has been reported in East Africa for the treatment of gonorrhoea [5]. A paste of stem is given in asthma, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels [6].

Hence in the present investigation to evaluate the *in-vitro* antimicrobial potentials of ethanol, diethyl ether and aqueous leaf extracts of *Cissus quadrangularis* L. by agar well diffusion method.

MATERIALS AND METHODS

Plant Collection

The plant was collected from the areas in and around the villages of Thanjavur (DT), Tamilnadu, India. Collected plants were carefully examined and identified with the help of regional Floras; Gamble [7], Mathew [8], Nair and Hendry [9]. Specimens were further confirmed with reference to Herbarium sheets available in the Rabinat Herbarium, St. Joseph's College, Thiruchirappalli, Tamilnadu, India.

Sterilization of Plant Materials

The disease free and fresh plant was selected for this investigation. About 2 grams of fresh and healthy leaves were taken for each solvents including aqueous. These are washed with tap and distilled water. Then surface sterilized with 0.1% mercuric chloride for few seconds. Again the plant materials were washed thoroughly with distilled water (Three times).

Extract Preparation

The plant leaves were surface sterilized and then macerated with 10ml of each solvent separately using Mortar and Pestle. The solvents such as ethanol, diethyl ether and distilled water were used.

After maceration the extracts were centrifuged for 15 minutes at 5000 rpm. After centrifugation, the supernatant was collected and tested for their potentials of antimicrobial activity.

Antimicrobial activity

Antimicrobial activity was screened by agar well diffusion method (10). The leaf extracts were tested for antimicrobial activity against bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and fungal pathogens such as *Aspergillus flavus*, *Candida albicans*, and *Fusarium solani*.

The microorganisms were collected from the Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory by periodic subculture.

Microbial inoculum Preparation

The young microbial inoculums culture was prepared and used in the entire research period. The nutrient broth (NB) and potato dextrose broth (PDB) were prepared and poured into several tubes and sterilized. The pure microbial cultures were inoculated in the tubes using inoculation needles or loops. The bacterial tubes were incubated at 37°C for 24-48 hours consequently and the fungal tubes were incubated at 27°C for 48 – 72 hours.

Composition of Nutrient Agar Medium

| Chemicals | Composition |
|-----------------|-------------|
| Beef extract | - 3 g |
| Peptone | - 5 g |
| NaCl | - 5 g |
| Agar | - 15 g |
| Distilled water | - 1000 ml |
| pH | - 7 |

Nutrient Agar Medium Preparation

The ingredients were weighed and put into conical flask containing 1000ml distilled water. Then, pH of the medium was adjusted to 7.2, using a pH meter by the addition of either acid or alkali. The flasks were sterilized by using autoclave at 121°C for 15 lbs pressure for 15 minutes and allowed to cool.

Composition of Potato Dextrose Agar Medium

| Chemicals | Composition |
|-----------------|-------------|
| Potato | - 200 g |
| Dextrose | - 20 g |
| Agar | - 15 g |
| Distilled water | - 1000 ml |
| pH | - 5.6 |

Potato Dextrose Agar Medium Preparation

The potato tubers were peeled and weighed for about 200 gms. The tubers were chopped into small pieces with the help of a sterile knife. The chopped potatoes were transferred into a conical flask containing about 1000 ml of distilled water. The contents were boiled for 20 minutes. The supernatant was decanted and filtered through muslin cloth and the filtrate was collected. To this filtrate dextrose and agar were added and shaken well to dissolve the ingredients and made up to 1000 ml by addition of distilled water. Finally, the medium was autoclaved at 121°C for 20 mins at 15 lbs pressure. Streptomycin sulphate (50µg/ml) was added and mixed well to prevent the bacterial contamination.

Antimicrobial Assay

The nutrient agar and PDA medium were poured into the sterile petri plates and allowed to solidify. The test bacterial and fungal cultures were evenly spreaded over the media by sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer. 200 µl plant extracts were transferred into the separate wells. The standard antibiotics (tetracycline and fluconazole) and solvents (distilled water, diethyl ether and ethanol) were used as positive and negative controls respectively. Then the plates were incubated at 37°C for 24 hrs and 37°C for 72 hrs for bacteria and fungi respectively. After the incubation the plates were observed for formation of clear inhibition zone around the well indicated the presence of antimicrobial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

RESULTS

In the present investigation, antimicrobial activity of *Cissus quadrangularis* (L.) was analysed. The effect of plant extracts on different organisms was shown in (Table 1 & 2).

Antibacterial activity

The ethanol extract showed maximum inhibition against *Escherichia coli* (10 mm) followed by *Staphylococcus aureus* (8 mm) and *Klebsiella pneumoniae* (7mm). Diethyl ether and distilled water extract exhibited minimum to moderate activity against tested pathogens.

Antifungal activity

The ethanol extract of *Cissus quadrangularis* showed maximum inhibition against *Candida albicans* (11 mm) followed by *Aspergillus flavus* (10 mm) and *Fusarium* sp. (8 mm). Diethyl ether extract exhibited promising activity against *Aspergillus flavus* (12mm). There was no effect on *Fusarium solani*. The aqueous extract did not produce inhibitory zone against tested pathogens. The standard antibiotic tetracycline exhibited maximum inhibition against *Staphylococcus aureus* (13 mm). The fluconazole antibiotic showed maximum activity against *Candida albicans* (Table 3). Negative control did not produce any inhibitory zone against all the tested bacteria and fungi.

Table 1. Antibacterial activity of *Cissus quadrangularis*

| S. No. | Plant Extracts | Inhibition of Growth (diameter in mm) | | |
|--------|-----------------|---------------------------------------|------------------------------|------------------------------|
| | | <i>Escherichia coli</i> | <i>Klebsiella pneumoniae</i> | <i>Staphylococcus aureus</i> |
| 1. | Diethyl ether | 7 | 6 | 4 |
| 2. | Ethanol | 10 | 7 | 8 |
| 3. | Distilled Water | 6 | 6 | 6 |

Table 2. Antifungal activity of *Cissus quadrangularis*

| S. No. | Plant Extracts | Inhibition of Growth (diameter in mm) | | |
|--------|----------------|---------------------------------------|-------------------------|------------------------|
| | | <i>Aspergillus flavus</i> | <i>Candida albicans</i> | <i>Fusarium solani</i> |
| 1. | Diethyl ether | 12 | 10 | - |
| 2. | Ethanol | 10 | 11 | 8 |
| 3. | Water | - | - | - |

Table 3. Effect of antibiotics on Microbes (Positive control)

| S. No | Name of the Microorganisms | Zone of inhibition (diameter in mm) | |
|-------|------------------------------|-------------------------------------|-------------|
| | | Tetracycline | Fluconazole |
| 1 | <i>Escherichia coli</i> | 11 | - |
| 2 | <i>Klebsiella pneumoniae</i> | 8 | - |
| 3 | <i>Staphylococcus aureus</i> | 13 | - |
| 4 | <i>Aspergillus flavus</i> | - | 12 |
| 5 | <i>Candida albicans</i> | - | 15 |
| 6 | <i>Fusarium solani</i> | - | 9 |

DISCUSSION

Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. The first step towards this goal is screening of plants used in popular medicine. Thus antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents. Plant drugs are frequently considered to be less toxic and free from side effects than the synthetic ones.

The antimicrobial activity of *Cissus quadrangularis* leaf extracts was tested against three pathogenic bacteria, viz, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and three pathogenic fungi viz., *Aspergillus flavus*, *Candida albicans* and *Fusarium solani*.

The ethanolic extract of *Cissus quadrangularis* possessed maximum zone of inhibition against *Escherichia coli* (10mm) and moderate activity against *Klebsiella pneumoniae* (7mm).

Antifungal activity of Diethyl ether extract of the plant possessed good activity against *A. flavus* (12 mm). In case of *Fusarium solani* no result was observed. Ethanolic extract showed good activity against *Candida albicans* (11 mm). Water extracts of *Cissus quadrangularis* did not have antifungal effect against tested fungal pathogens.

Luseba et al. [11] reported that the methanol extract and dichloromethane extract of stems of *Cissus quadrangularis* possess antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. Antimicrobial activity has also been reported from stem and root extracts of *Cissus quadrangularis* [12]. Similarly Rao and Deshpande [13] reported that the alcoholic extract of the stem of *C. quadrangularis* showed activity against *E. coli*.

Evidently the aqueous extracts of *A. vogelii* Planch, *M. lucida*, *T. scleroxylon*, *A. cordifolia*, *N. latifolia*, and *C. papaya*, were active against *E. coli* (14). Alcoholic extracts of *Punica granatum* appeared to be the most effective with the zone of inhibition sizes ranging from 15 to 30 mm against *Vibrio cholerae*, *Enterotoxigenic E. coli*, *Enteropathogenic E. coli* and *Enteraggregative E.coli*. (15)

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