

## Research Article



## Evaluation of Phytochemical, Anti-Bacterial and Anti-Cancerous Activity of *Cissus quadrangularis* from South-Western Ghats Regions of India

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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, Anti-bacterial and In-vitro Anti-cancer properties of *C. quadrangularis* were evaluated. The qualitative phytochemical screening was evaluated by using ethanol extracts, Anti-bacterial activity of *C. quadrangularis* was studied against some pathogenic bacteria and In-vitro Anti-cancer activity was screened by MTT assay against MCF cell line. In this study, the secondary metabolites such as phenol, alkaloids, tannins and flavonoids were present in the ethanolic 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 anticancer activity of *C. quadrangularis* revealed that ethyl acetate extract shows lowest percentage of viability which has significant activity against cancerous cells.

**Keywords:** Anti-bacterial, Anti-cancer, *Cissus quadrangularis*, In-vitro, Phytochemistry.

### INTRODUCTION

In India, medicinal plants provide raw material for all of indigenous systems of medicine namely Ayurveda, Siddha, Unani and Homeopathy.<sup>1</sup> Medicinal plant has traditionally occupied an important position in the cultural, spiritual and medicinal arena of rural and tribal lives of India.<sup>2</sup> Medicinal plants as a group comprise approximately 8,000 species and account for around 50% of all the higher flowering plant species of India.<sup>3</sup>

*Cissus quadrangularis* is one of the most common species scattered all over India particularly in tropical regions.<sup>4</sup> *C. quadrangularis* belongs to the family Vitaceae, which is a perennial plant commonly known as Veldt Grape or Devils backbone.<sup>5</sup> It is known to be an ancient medicinal plant, with optimal healing in white tissue area of the body (tendon, ligament, etc.).<sup>6</sup> Phytochemical analysis of *Cissus quadrangularis* indicates the presence of carotene, phytosterol, terpenoids,  $\beta$ -sitosterol,  $\delta$ -amyirin,  $\delta$ -amyrone and calcium.<sup>7</sup> The stem of *C. quadrangularis* is also an important medicinal plant 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.<sup>8</sup>

*C. quadrangularis* stem and leaves are used for the treatment of Hemorrhoid, Menstrual disorder, Scurvy and as Anti-oxidant, Anti-flatulence, Anti-bacterial, and Anti-fungal. The stem and leaves of *C. quadrangularis* is shown in Figure 1. It is very effective in strengthening the bones and joints. The plant resembles the shape of bones and joints in the body. The stem is fried in ghee and given

with milk as for curing fractures and osteoarthritis. The entire plant is being used in fractures, sprains, rheumatism and irregular growth of Teeth, Anthrax, Hematuria, Elephantiasis, Dislocation of Hip, various wounds and cracked tail. It can be cooked by the salt, dried and deep-fried food and can be eaten as a side dish.<sup>9</sup>



Figure 1: Stem and leaves of *Cissus quadrangularis*

### MATERIALS AND METHODS

#### Plant Collection and Identification

The plant species were collected from south Western Ghats regions of Kanyakumari District, Tamilnadu, India during the month of October – November in the year 2013. The plants were sent for proper identification. The plants were authenticated by Professor Dr.P.Nagendra Prasad, botanist of Noorul Islam Centre for Higher Education, Noorul Islam University, Tamilnadu, India.

#### Preparation of Extract

The aerial parts of shoots of *C. quadrangularis* were separated and cleaned well. Cleaned plant was then dried under shade. The drying was done until all the water



molecules evaporated and plants became well-dried for grinding. After drying, the plants were ground well using mechanical blender into fine powder and transferred into air-tight container with proper labeling for further use. The dried and powdered *C. quadrangularis* was extracted sequentially with Methanol, Ethanol, Chloroform, Ethyl acetate and Acetone using Soxhlet apparatus. Each 50 g of dried powdered plant was defatted with Petroleum ether by immersing the extracts in petroleum ether and kept for 24 hours incubation. After incubation excess petroleum ether was decanted and kept for drying. The dried samples were wrapped in muslin cloth and were kept for Soxhlet extraction in 300 ml of solvent at boiling point of increasing polarity. Solute thus separated were collected in a centrifuge tube and used for further studies.

### Qualitative Phytochemical screening

Ethanol extracts of *C. quadrangularis* were screened for different phytochemical constituents viz., carbohydrates, phenol, alkaloid, tannin, flavonoid and saponin. Phytochemical screening of the extracts was carried out by the standard methods.

### Antimicrobial activity

#### Culture and Media Preparation

Antimicrobial activity was screened by agar well diffusion method. The shoot extracts were tested for antimicrobial activity against bacterial pathogens such as *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas species*, and *Staphylococcus aureus*. The microorganisms were collected from the Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory by periodic subculture.

#### Antibacterial Assay

Sterilized discs were soaked in shoot extracts (Ethanol, Ethyl Acetate and Methanol) and kept overnight in room temperature. Then soaked discs were dried aseptically to ensure evaporation of solvents. The prepared Muller-Hinton Media was poured in each petridish and allowed to cool. Cotton swabs charged with each test bacterial suspension were inoculated on Muller-Hinton agar plates and were spreaded over agar surface to make a lawn. Then the plates were allowed to dry for 20 minutes. The sterile, dried antimicrobial discs impregnated with crude plant extracts of 25mg/disc were carefully dispensed with uniform distances placed on Muller-Hinton agar plates and incubated for 18-24 hours at 37°C. The assay was carried out in triplicate. Here a normal filter paper is used as a negative control. The zone of inhibition was measured from the centre of disc to the clear zone in millimeter and the results were recorded.

#### In vitro studies of Anti-cancer activity against MCF cell line (Breast cancer cell line)

MCF (Michigan Cancer Foundation) procured from NCCS Pune was maintained at 10% heat inactivated FBS (Foetal

Bovine Serum) in carbon dioxide incubator. The cells may be in confluent stage. So the cells were trypsinised using 0.025% trypsin (cell culture grade HIMEDIA) upon reaching confluences. Following which, the cells were sub-cultured on to micro culture plates and used for further studies. Anticancer effect of *C. quadrangularis* ethanolic extracts was determined on MCF cell lines. A standard concentration of 500 µg/ml was added and incubated. The Anti-proliferative effect was determined by standard MTT assay.

### MTT Cell Viability Assay

The cell culture suspension was washed with 1 X PBS (Phosphate Buffered Saline) and then added with 200 µl MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5-diphyhyl tetrazolium Bromide] solution to the culture flask (MTT 5 mg/volume dissolved in PBS). It is then filtered through a 0.2 µm filter before use. It is then incubated at 37°C for 3 hours, removed all MTT solution, washed with 1 X PBS and added with 300 µl DMSO to each culture flask and incubated at room temperature for 30 minutes until all cells get lysed and homogenous color was obtained. The solution was then transferred to centrifuge tube and centrifuged at top speed for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD was measured at 540 nm using DMSO blank. Then the percentage viability was calculated using the percentage of viability formulated.

### Calculation

$$\text{Percentage of viability} = \frac{\text{Mean absorbance of sample}}{\text{Mean absorbance of control}} \times 100$$

## RESULTS AND DISCUSSION

**Table 1:** Qualitative phytochemical screening of ethanolic extract of *C. quadrangularis*

Parameters	Result
Carbohydrates	-
Phenol	+
Alkaloid	+
Tannin	+
Flavonoids	+
Saponin	-

“+” represents the presence of compound and “-” represents the absence of compound.

### Qualitative Phytochemical Screening

Qualitative phytochemical screening was used to determine the presence of some secondary metabolites. The results of screening test revealed the presence of medically active compounds. From the Table (1), it could be seen that phenol, alkaloids, tannins and flavonoids were present in the ethanolic extract of *C. quadrangularis*, while saponin and carbohydrates were absent.

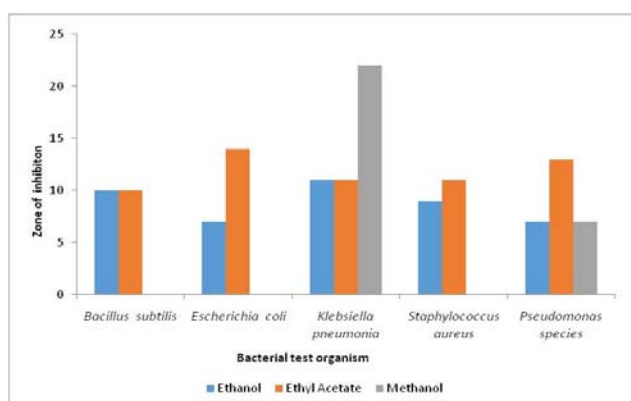


**Table 2:** *In vitro* Studies (MCF) of Anticancer Activity of *Cissus quadrangularis*

Extracts	Optical Density	Percentage of viability
Control	0.772	100
Acetone	0.466	60.36
Chloroform	0.416	53.86
Methanol	0.367	47.5
Ethanol	0.386	50.38
Ethyl acetate	0.399	41.5

**Table 3:** Antibacterial Activity of *Cissus quadrangularis* Extract against Bacterial Test Organisms

Bacteria	Diameter of zone of inhibition (mm)		
	Ethanol	Methanol	Ethyl acetate
<i>Bacillus subtilis</i>	10	-	10
<i>Escherichia coli</i>	7	-	14
<i>Klebsiella pneumonia</i>	11	22	11
<i>Pseudomonas species</i>	7	7	13
<i>Staphylococcus aureus</i>	9	-	11

**Figure 2:** Antibacterial activity of ethyl acetate, ethanol and methanol extract of *Cissus quadrangularis* against bacterial test organisms

### Anti-Bacterial Activity

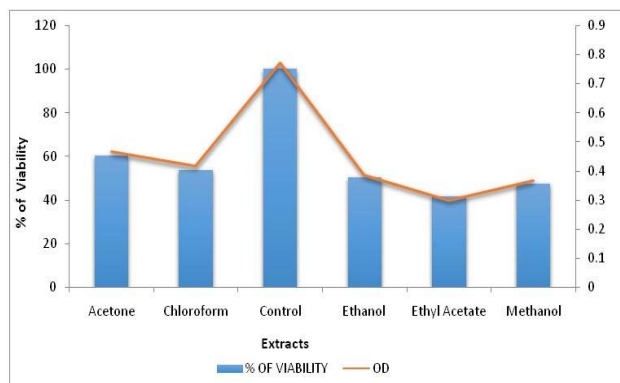
The extracts of *C. quadrangularis* such as ethanol, methanol and ethyl acetate showed varying levels of antibacterial species. The result showed that the ethanol, methanol and ethyl acetate extract of *Cissus quadrangularis* exhibited maximum activity against *Klebsiella pneumonia* with the zone of inhibition of 22, 11 and 10mm respectively. The result obtained was given in Table 2, Figure 2 and Figure 3.

### Anti-Cancer Activity

The MCF cell line (Breast cancer cell line) was used for in vitro studies of anticancer activity. The percentage of viability of MCF cell line for control is 100%. The values below 50% show significant anticancer activity. The

percentage of viability for Acetone is 60.36%, 47.5% for Methanol, 50.38% for Ethanol, 53.86% for chloroform and 41.5% for Ethyl acetate. This was shown in Table 3 and Figure 4.

Ethyl acetate has lowest percentage of viability and shows significant anticancer activity. All the other solvent extracts such as Acetone, Ethanol, Methanol and Chloroform have cytotoxic effect but not much that of significant anticancer activity.

**Figure 3:** *In vitro* Studies (MCF) of Anticancer Activity of *Cissus quadrangularis*

In the present study, the result of qualitative phytochemical screening of ethanolic extract of *C. quadrangularis* reveals the presence of phenol, alkaloids, tannins and flavonoids in the shoots of *C. quadrangularis*, while saponin and carbohydrates were absent.<sup>10</sup> For MCF cell line (Breast cancer cell line), among the extract of *C. quadrangularis* such as ethanol, methanol, ethyl acetate, chloroform and acetone showed varying levels of viability, in which ethyl acetate has lowest percentage of viability (41.5%).<sup>11</sup> The value below 50% shows significant anticancer activity. This is due to the sensitivity of the cell line to the active compounds in the extract.<sup>12</sup> The extract of *C. quadrangularis* such as ethanol, methanol and ethyl acetate showed varying levels of antibacterial activity with different bacterial species.<sup>13</sup> The result showed that the ethanol, methanol and ethyl acetate extract of *Cissus quadrangularis* exhibited maximum activity against *Klebsiella pneumoniae* with a zone of inhibition of 11mm, 22mm and 10mm respectively.

### CONCLUSION

The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines mostly plant drugs.<sup>14</sup> The experimental material selected for the study was *Cissus quadrangularis*. It belongs to the family Vitaceae. The present study was carried out to determine the qualitative phytochemical, anti-tumor, and anti-bacterial activity of *C. quadrangularis* extracts.<sup>15</sup> The result reveals that the ethanol, methanol and ethyl acetate extract of *C. quadrangularis* exhibited maximum activity against *Klebsiella pneumoniae* with a zone of inhibition of 11mm, 22mm and 10mm. On the basis of results it can be

concluded that the ethyl acetate of *C. quadrangularis* possess significant anticancer activity against in vitro studies.<sup>16</sup> The study also provide a strong evidence for the use of *C. quadrangularis* stem extract to treat anticancer activity. The activity may be due to the presence of one or more phytochemical constituents present in the extract.<sup>17</sup>

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