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**Research Article** 

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## Antimicrobial and antioxidant properties of Cissus quandrangularis

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## ABSTRACT

Extracts of air dried stem of Cissus quadrangularis collected from Chennai were prepared by solvents extraction method using Soxhelet apparatus. Preliminary phytochemical studies were carried out to find out the presence of steroids and proteins. Antioxidant studies carried out by DPPH, ABTS, SOD, FRAP showed that the chloroform extract has highest activity.

Keywords: Cissus quadrangularis, phytochemical, antioxidant, antimicrobial, solvent extraction

## INTRODUCTION

Herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries. The synthetic products of the modern age surpassed their importance for a while. The important advantage of medicinal plants in various treatments is their safety besides being less expensive, efficacy and availability throughout the world [1]. The Ayurvedic system of medicine uses about 700 species of Unani, 700 of Sidda and modern medicine around 30 species. The drugs are derived either from the whole plant or from different organs, like leaves, stem, dark, root, flower, seed, etc. Some drugs prepared from excretory plant product such as gum, resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs from an important segment of the modern pharmacopoeia. Some important chemical intermediates needs for manufacturing the modern drugs are also obtained from plants [1].

*C. quadrangularis* is a perennial plant of the grape family. It is commonly known as Veldt Grape or Devil's Backbone. *Cissus* is a genus of approximately 350 species of woody climberin the grape family (Vitaceae). They are used as food plants by the larvae of some Lepidopteran species including hyper compaeridanus and hypercompeicasia. It grows to 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 fragments of small white, yellowish, or greenish flower [2]. It has also been used for bone fractures, weak bones (osteoporosis), scurvy, cancer, upset stomach, hemorrhoids, peptic ulcer disease (PUD), painful menstrual periods, asthma, malaria, and pain [2]. It is said to have antibacterial, antifungal, antioxidant, anthelmintic, antihemorrhoidal and analgesic activities. [3]. It has been found to contain a rich source of carotenoids, triterpenoids and ascorbic acid [4].

Extracts of *C. quadrangularis* were tested for antioxidant activity by  $\beta$ -carotene linoleic acid model and also by 1, 1diphenyl-2-picrylhydrazyl model. The ethyl acetate fraction of both fresh and dry stem extracts at a concentration of 100 ppm showed 64.8% antioxidant activity in the  $\beta$ -carotene linoleic acid system and 61.6% in the 1, 1-diphenyl-2-

## L. Jeyanthi Rebecca et al

picrylhydrazyl systems [5]. Phytochemical studies of *C. quadrangularis* the presence of various versatile constituents such as flavanoids, triterpenoids, Vitamin C, stilbene derivatives and many others, e.g. resveratrol, piceatannol, pallidolperthenocissin and phytosterols. Out of which ascorbic acid, triterpene,  $\beta$ -sitosterol, ketosteroid, two asymmetrical tetracyclic triterpenoids and calcium were identified as major constituents of this plant [6, 7, 8]. It has contains a high percentage of calcium ions (4% by weight) and phosphorous. Recently a study has been undertaken which showed that the plant extract when reacted with CO2, leads to formation of calcite crystals of highly irregular morphology, indicating that bioorganic molecules present in the extract modulate the crystal morphology [9].

### **EXPERIMENTAL SECTION**

#### Collection and preparation of plant sample

Fresh stem of *C. quadrangularis* were collected from a nursery in Chennai. The powdered leaves are used separately for the preparation of ethanol & chloroform extracts of known concentration by soaking in 100 ml for 24 hours. Then the extracts were filtered using sterile Whatman No 1. About 25 grams of each sample were taken and extracted separately with 250 ml ethanol and chloroform in a soxhelet apparatus. The extract were collected and dried. The condensed extract was then dissolved in ethanol and chloroform to the concentration of 100/ml.

#### Antimicrobial activity

The antibacterial and antifungal activities of *Cissus quandrangularis* were assessed using agar disc diffusion methods. The organisms used for screening were *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans*, *Cryptococcus neoformans*, *Penicillium sp* and *Aspergillus niger*.

## Antioxidant assay

#### **DPPH free radical scavenging**

The ability of the extract to scavenge DPPH radical was determined by the method described by NazninAra and HasanNur method. The absorbance was read at 517 nm.

Antioxidant activity (%) = Abs control – Abs sample/Abs controlx100

Where, Abs control = Optimal density of control; Abs sample = Optimal density of sample extract

#### **FRAP** assay

Total antioxidant activity is measured by ferric reducing antioxidant power assay of Benzie and stain (1999). The FRAP assay, is presented as a novel method for assessing antioxidant power. Absorbance at 593 nm was measured. FRAP value of sample ( $\mu$ M) = Change in absorbance of sample from 0 to 4 minute/change in absorbance of standard from 0 to 4 minutes ×FRAP value of standard (1000  $\mu$ M).

#### ABTS radical scavenging assay

The total antioxidant activity of the extract was measured by the ABTS radical cation - decolonization assay. Absorbance was measured at 734 nm after 5 min of incubation.

#### Lipid peroxidation inhibition assay

In this assay the estimation of thiobarbituric acid reactive substance (TBARS) is estimated by the Okhawa method. The % inhibition= Abs (control) – Abs (sample)/Abs control x100.

#### **RESULTS AND DISCUSSION**

The present study was carried out to find out the antioxidant and antimicrobial properties of *Cissus quadrangularis*. Plant sample were collected and the processed for the extracts. Findings of complete extract process revealed the highest yield for ethanol extract than chloroform. It is clear that chloroform is more polar than ethanol. Many solvent are used as bioactive compounds and the most commonly used solvent is ethanol. The use of non polar solvent completely indicates that active compound for solvent dissolve in polar solvent only. The yield of crude extract from the stem of *Cissus quadrangularis* was ethanol 1.39 g and chloroform 1.50 g.

## L. Jeyanthi Rebecca et al

#### Antioxidant assay

DPPH radical scavenging activity of various phenolics like, resveratrol, quercetin, myricetin ,catechin ,fistein, kaemferol, ellagic acid and naringenin at different concentration were compared with respect to trolox and tocopherol. Quercetin, myricetin were found to have the strongest antiradical activity. Each molecule of quercetin and myricetin scavenged 10 molecules of DPPH; catechin and first in scavenged 5 molecules; resveratrol scavenged 3.6 molecules and ellagic acid could scavenge 3.3 molecules of DPPH. Quercetin, myricetin, catechin, fistein, resveratrol and ellagic acid were stronger then trolox and tocopherol which scavenged 2 molecules of DPPH. Kaempferol and naringenin were found to be the weakest antiradicals, which scavenged 1.7 and 0.5 molecules of DPPH per molecule respectively. The results showed highest radical scavenging activity was absorbed in chloroform extract (Table-1). The lowest radical scavenging activity was observed in ethanol extract (29.4 %) when Butylated hydroxyl toluene (BHT) used as standard showed 86.3%.

Extract was reacted with  $Fe^{3+}$  to reduce and convert into  $Fe^{2+}$ . The degree of colouration indicated the potential of the extracts. The change in the absorbance produced at 510 nm as been used as a measure of ferric ions reducing activity. The result of various extracts showed that the highest at 23% for chloroform extract, 17% for ethanol extract. This result indicates that the *Cissus quadrangularis* extracts reduced the ferric ions when compared to the standard Gallic acid (Table-1).

## Table-1 DPPH and FRAP results for ethanol and chloroform extracts of Cissus quadrangularis

SOLVENT	DPPH (%)	FRAP (%)
Ethanol	29.4	17.0
Chloroform	65.2	23.0

Generation of ABTS radical actively forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of various extracts. Both ethanol and chloroform extracts showed inhibition at  $200\mu$ g/ml,  $500\mu$ g/ml,  $1000\mu$ g/ml respectively (Figure-1).



Figure-1 Percentage inhibition of ABTS against the crude extract of Cissus quadrangularis

The occurrence of malondialdehyde, a secondary byproduct of the oxidation of polyunsaturated fatty acids is considered a useful index of general lipid peroxidation. A common assay for measuring malondialdehyde is thiobarbituric acid reactive substances (TBARS) assay. It reacts with thiobardituric acid and is recorded based on the absorbance at 532 nm. The inhibition concentration was highest for  $1000\mu$ g/ml (69 µg/ml) and lowest for  $10\mu$ g/ml (2µg/ml) for chloroform extract. The inhibition concentration was highest for  $1000\mu$ g/ml (33 µg/ml) and lowest for  $10 \mu$ g/ml (3 µg/ml) for ethanol extracts (Figure-2).

## L. Jeyanthi Rebecca et al



Figure 2- Lipid Peroxidase against the crude extract of Cissus quadrangularis

## Antimicrobial activity

The study of antimicrobial activity revealed that both ethanol and chloroform extracts of *Cissus quadrangularis* showed antimicrobial activity (Table-2).

S. No	Organism	Zone of inhibition (mm)	
	Organishi	Ethanol	Chloroform
1	Staphylococcus aureus	12	10
2	Escherichia coli	10	9
3	Pseudomonas aeruginosa	8	10`
4	Salmonella typhi	10	9
5	Candida albicans	8	11
6	Cryptococcus neoformance	10	13
7	Aspergilus niger	9	10
8	Pencillium species	6	9

Table 2-Antimicrobial activity of Cissus quadrangularis

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