EVALUATION OF ANTIBACTERIAL ACTIVITY OF CISSUS QUADRANGULARIS AGAINST NASOCOMIAL INFECTANTS

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ABSTRACT
Cissus quadrangularis Linn is one of the medicinal plants which are found in India and this plant belongs to the family Vitaceae. In this present study, a comparative evaluation of the antimicrobial activity of the leaf, root, fruit and stem extracts of Cissus quadrangularis eluted using different solvents like chloroform, diethyl ether and petroleum ether were carried out. The nasocomial pathogens were isolated from clinical specimens from the hospital patients. The Chloroform extract of the samples showed antimicrobial activity against most of the clinically isolated pathogenic microorganism. Diethyl ether extract of the samples showed antagonistic effect against P. aeruginosa. The chloroform extracts of the leaf sample showed a better antimicrobial effect against the microorganisms whereas the Diethyl ether extract showed antimicrobial effect against only Enterobacteriaceae. Petroleum ether extract of the leaves did not show any antimicrobial activity. The Chloroform extract of the Root sample showed antimicrobial activity against five microorganisms, whereas the Diethyleneether extract showed activity against only 2 microorganisms. The Chloroform extract from the Fruit samples showed a prominent antimicrobial activity against only five microorganisms. The minimum inhibitory concentration was analysed for the Chloroform samples as most the solvent extract showed higher zone of inhibition. The MIC for most of the samples was found to be at 40µg except for Enterobacteriaceae against Root sample. The present study indicates the potential of Cissus quadrangularis as a source with medicinal applications.
KEYWORDS: *Cissus quadrangularis*, Medicinal plant, Antagonistic, clinical isolates, Chloroform.

INTRODUCTION

Medicinal plants have been identified and used throughout human history as plants have the ability to synthesize a wide variety of chemical compounds that are been used to perform important biological functions and to defend against insects, bacteria, fungi and other pests. More than 12,000 such compounds have been isolated so far.[1,2] The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. The annual global export value of pharmaceutical plants in 2011 accounted for over US$2.2 billion (http://www.traffic.org/medicinal-plants/).

Different cultures around the world have used herbs for thousands of years to treat several health conditions. One of the herbs that have shown beneficial effects belongs to the *Cissus* family of plants. *Cissus quadrangularis* is a medicinal herb used in Siddha and Ayurvedic medicine since ancient times in Asia, as a general tonic and analgesic, especially for bone fracture healing. [3] *Cissus quadrangularis* belongs to the vitaceae family and is found in South East Asia where it is edible and is used as a vegetable. This plant has been used from ancient times to enhance fracture healing and has several other health benefits including anti inflammatory[4], anti glucocorticoid[5], anti diabetic[6] antibacterial[7], and antioxidant properties.[8] This plant has tri terpenoids, steroids,[9, 10] stilbenes[11], flavanoids lipids and several catalpols.[12]

The plant has been documented in Ayurveda for the treatment of osteoarthritis, rheumatoid arthritis and osteoporosis.[13] The use of sap with tamarind has been reported in East Africa for the treatment of gonorrhoea.[14] A paste of stem is given in asthma, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels.[15]
MATERIALS AND METHODOLOGY

Sample collection

*Cissus quadrangularis* were collected from GKVK Campus, Hebbal, Bangalore and Hosur which were brought to the lab in ziplock bags and stored in shade. The samples were dried completely to remove the moisture and then blended into fine powder for further investigation.

**Solvent extraction:** 10 gm of each sample powder was added to 100ml of the solvents and extracted using soxhlet apparatus. Here, chloroform, diethyl ether and petroleum ether were used as a solvent for extraction as different solvents elute out different compounds from the sample. The extract were filtered by using whatmann no1 filter paper to get filtrate as extracts and were concentrated by evaporating the solvents at the boiling temperature of the solvents using distillation unit. The residue was weighed and they were re-dissolved with the same solvent to get a final concentration of 1mg/ml.

**Antimicrobial activity:** The residual powder was taken for the antimicrobial activity and the powder was re-dissolved in particular solvent to get a concentration of 1mg/ml. These concentrated samples were used for antimicrobial analysis. The antimicrobial analysis was carried out by agar diffusion method where in 80µl of the concentrated extract was put into the wells. The antimicrobial activity was determined against clinical isolates like *E.coli*, *S.aureus*, *Proteus spp*, *Morganella spp*, *Actinobacteria*, *S.haemolyticus*, *P.aeruginosa*, *Enterobacteriacea*, *S.epidermidis and K.pneumoniae*.

**Minimum inhibitory concentration:** The solvent extract which showed higher zone of inhibition against the clinical isolates was selected and the minimum inhibitory concentration was determined. The MIC was determined by checking different concentrations of the sample ranging from 40-80µg. The MIC is identified by determining at which concentration the zone of clearance is seen against the pathogenic isolates.

RESULTS AND DISCUSSION

The antagonistic effect from the parts of the plant samples extracted by different solvents was checked against different pathogenic microorganisms like *E.coli*, *S.aureus*, *Proteus spp*, *Morganella spp*, *Actinobacteria*, *S.haemolyticus*, *P.aeruginosa*, *Enterobacteriacea*, *S.epidermidis and K.pneumoniae*. 
The Chloroform extract of the samples showed antimicrobial activity against most of the clinically isolated pathogenic microorganism. Diethyl ether extract of the samples showed antimicrobial activity against only few of the microorganisms and Petroleum ether extract did not show much of the activity against any of the microorganisms.

![Figure 1: Antimicrobial Activity of the stem sample of Cissus quadrangularis](image1)

The stem extracts from all the solvent showed antagonistic effect against *P. aeruginosa*. The chloroform, diethyl ether and petroleum ether extract showed antimicrobial activity against 8 pathogens, and the other two solvents against 1 pathogenic microorganism each respectively pathogenic microorganisms respectively. The highest zone of inhibition was seen against Actinobaie by chloroform extract with a zone of 15mm in diameter (Fig 1).

![Figure 2: Antimicrobial Activity of the Leaf sample of Cissus quadrangularis](image2)

The chloroform extracts of the Leaves sample showed a better antimicrobial effect against the microorganisms whereas the Diethyl ether extract showed antimicrobial effect against only Enterobactericeae. The highest zone of inhibition was formed against *Enterobactericeae* by
the Chloroform extract with a zone diameter of 23mm. Chloroform extract showed a least zone of inhibition against *E.coli* with a zone diameter of 12mm (Fig 2). Petroleum ether extract did not show any antimicrobial activity.

![Cissus quadrangularis-Root](attachment:image1)

**Figure 3: Antimicrobial Activity of the Root sample of *Cissus quadrangularis***

The Chloroform extract of the Root sample showed antimicrobial activity against five microorganisms, whereas the Diethylether extract showed activity against only 2 microorganisms and Petroleum ether extract did not show any activity (Fig 3).

![Cissus quadrangularis-Fruit](attachment:image2)

**Figure 4: Antimicrobial Activity of the Fruit sample of *Cissus quadrangularis***

The Chloroform extract from the Fruit samples showed a prominent antimicrobial activity against only five microorganisms. The Diethylether and Petroleum ether extract of the Fruit sample did not show any activity. The highest zone of 16mm diameter size was seen against *P.aeruginosa* by the chloroform extract (Fig 4).
Minimum Inhibitory Concentration

The minimum inhibitory concentration was analysed for the Chloroform samples as most the solvent extract showed higher zone of inhibition. The MIC for most of the samples was found to be at 40µg except for Enterobacteriaceae against Root sample, this showed a MIC at 50µg. The zone of inhibition increased with the increase in the concentration of the samples (Fig 5).

Figure 5: Minimum inhibitory concentration

Cissus quadrangularis: MIC- Chloroform Extract

The minimum inhibitory concentration was analysed for the Chloroform samples as most the solvent extract showed higher zone of inhibition. The MIC for most of the samples was found to be at 40µg except for Enterobacteriaceae against Root sample, this showed a MIC at 50µg. The zone of inhibition increased with the increase in the concentration of the samples (Fig 5).[16] investigated antimicrobial activity of aqueous, methanol and chloroform leaf extracts of Cissus multistriata against 8 bacterial and 2 fungal test organisms, using the tube dilution and agar ditch diffusion methods. According to their observation aqueous leaf extract had no activity against both the bacterial and fungal test organisms, methanol and chloroform leaf extracts inhibited all the test organisms with chloroform leaf extract showing the highest zone of inhibition against Escherichia coli and least against Staphylococcus aureus. The methanol leaf extract was least inhibitory against Salmonella typhi and most inhibitory against S. aureus.

Cissus quadrangularis Linn. has potent fracture healing property and antimicrobial, antiulcer, antioxidative, antosteoporotic, gastroprotective, cholinergic activity as well as beneficial effects on cardiovascular diseases.[17]

Kashikar and Indu George 2006, carried out the in vitro antibacterial activity of different extracts of Cissus quadrangularis Linn (Vitaceae) against some Gram-negative and Gram-positive bacteria and their investigated proved that the ethyl acetate, acetone, and methanol extracts, showed antimicrobial properties and that B. subtilis, P. aeruginosa, S. typhi, S. aureus, and S.pyogenes, were susceptible to at least two extracts. Petroleum ether, ethanol,
and water extracts, failed to inhibit the bacterial growth of the strains tested. *E.coli* did not respond to any of the extracts used.

**REFERENCES**

