



Research Article

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## Study of antibacterial potential and phytochemical constituents of three sided *Cissus quadrangularis*

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### ABSTRACT

Plant based products are healthier, safer and much more reliable than the synthetic products. According to WHO, approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care. It was reported that during the past 20 years, at least one novel compound from higher plants has been marketed every 2.5 years. This proves the importance of ethno pharmacy in today's research. The current study explains about the antibacterial activity of the petroleum ether and ethyl acetate plant extract of three sided *Cissus quadrangularis*. The antibacterial potential of the crude plant extract obtained by soxhlet extraction was determined by disc diffusion and well diffusion assays using both gram positive organisms (*Bacillus cereus* and *Staphylococcus aureus*) and gram negative organisms (*Escherichia coli* and *Salmonella typhi*). The results showed that the ethyl acetate plant extract was highly effective against all the organisms compared to petroleum ether extract. Among the bacterial strains, both the extracts showed maximum zone of inhibition against *Staphylococcus aureus*. Phytochemical analysis proved the presence of constituents such as flavanoids, carbohydrates, tannins, phenols and saponins. Thus, this study proved that the extracts of three sided *Cissus quadrangularis* possess valuable phytochemicals and it could act as an effective antibacterial agent.

**Keywords:** Plants extract, solvents, antibacterial, inhibition, phytochemical.

### INTRODUCTION

Plants are a reliable source for the treatment of diseases. According to WHO, approximately eighty percentage of the people depend on traditional medicines for their primary healthcare. The drug industries also depend on plants for new drugs because synthetic medicines produce side effects [1]. Hence, researchers show interest in identifying the plants that are used in traditional remedy and evaluating their beneficial activities. In the recent times, valuable scientific support has been given in order to authenticate the use of the plants in disease treatment and also to detect the mechanism of action of compounds present in the plants [2]. One such therapeutically important plant considered is three sided *Cissus quadrangularis*. There are different types of *Cissus quadrangularis*. According to siddha literatures, the types of *Cissus quadrangularis* are 'Muppirandai' (three sided), 'Sadurappirandai' (four sided), 'Olaippirandai' (two sided), 'Pulippirandai' (acid taste), 'Teempirandai' (sweetish) and 'Uruttuppirandai' (round) [3]. More research works have been undergone in the plant four sided *Cissus quadrangularis* (Pirandai) while the three sided *Cissus quadrangularis* (Muppirandai) is comparatively less explored or underutilized. In general, *Cissus quadrangularis* is a perennial plant belonging to the family Vitaceae. It is mostly found in India [4]. Traditionally, the plant is used in healing fractured bones. It also acts as an antimicrobial, antihelminthic, antiulcer and antihemorrhoidal agent. Ayurvedic studies state that the plant is useful for treating osteoporosis and rheumatoid arthritis. The plant exhibited antibacterial potential against organisms such as *Staphylococcus aureus*, *Escherichia*

*coli* and *Pseudomonas aeruginosa*. It also has antiprotozoal activity against *Entamoeba histolytica* and antiplasmodial activity [5]. Phytochemical constituents present in the plant are phenols, tannins, saponins, vitamin and carotene [1]. The plant also possesses phytochemicals such as flavanoids, steroids and tri-terpenoids [6]. A high amount of ascorbic acid, carotene A, calcium and anabolic steroidal compounds are present in the plant [7]. Based on the above information, in the current study, the antibacterial activity and phytochemical analysis of petroleum ether and ethyl acetate extract of three sided *Cissus quadrangularis* (Muppirandai) were evaluated due to its medicinal importance.

## EXPERIMENTAL SECTION

### Collection and extraction of plant sample

The plant three sided *Cissus quadrangularis* was collected from Kolli hills, Namakkal District, Tamil Nadu, India. The stem part of the plant was washed several times with distilled water and kept for drying under shade. After drying, it was powdered using a domestic grinder. The plant powder was then stored in an airtight container. 10 grams of plant powder were weighed and was extracted with 100ml petroleum ether using soxhlet apparatus. The temperature maintained for extraction was 50°C. The duration of soxhlet extraction was 3 to 4 hours. 10 grams of plant powder were weighed and was extracted with 100ml ethyl acetate using soxhlet apparatus. The temperature for extraction was 67°C. Both the extracts were concentrated by pouring them into clean petri plates and were allowed for evaporation of solvents. Then the concentrated crude extract was stored in eppendorfs [6].

### In vitro antibacterial activity

The *in vitro* antibacterial potential of the plant three sided *Cissus quadrangularis* was evaluated by disc diffusion and well diffusion assays.

### In vitro antibacterial activity by disc diffusion assay

The ethyl acetate and petroleum ether extracts of the plant three sided *Cissus quadrangularis* were subjected to disc diffusion assay against the microorganisms *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The strains were inoculated onto plates with the Muller Hinton agar medium by spread plate technique. The plant extracts were prepared at a concentration of 100µg/10µl/disc. The sterilized discs of 6mm were dipped into the plant extracts and were then placed onto the inoculated plates. Tetracycline was used as positive control while sterile water was used as negative control. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition of bacterial growth by the plant extract was measured in millimeters. The assay was done in triplicates [8].

### In vitro antibacterial activity by well diffusion assay

The well diffusion assay was performed with the ethyl acetate and petroleum ether extract against both gram positive organisms (*Bacillus cereus* and *Staphylococcus aureus*) and gram negative organisms (*Escherichia coli* and *Salmonella typhi*). The bacterial culture was inoculated onto the plates containing Muller Hinton agar medium by the spread plate method. Wells were bored onto the plates using a well borer, into which different concentrations of plant extract (250µg/ml, 500µg/ml, 750µg/ml, and 1000 µg/ml) were added. Erythromycin and sterile water were used as positive and negative control respectively. The plates were then kept for incubation at 37°C for 24 hours. After incubation, the plates were observed for the zone of inhibition formed around the wells. The zone of inhibition was measured in millimeters. The assay was performed in triplicates [6].

### Statistical analysis

Statistical analysis was done with the zone of inhibition values of different concentrations of plant extract against the two gram positive and two gram negative organisms. The mean and standard deviation were calculated. The level of significance was determined using one way ANOVA technique [9].

### Phytochemical analysis

The phytochemical constituents were screened by qualitative tests with petroleum ether and ethyl acetate extracts of the plant three sided *Cissus quadrangularis* [10].

## RESULTS AND DISCUSSION

**In vitro antibacterial activity**

The petroleum ether and ethyl acetate extracts of the plant showed antibacterial activity against all the tested microorganisms. In both disc diffusion and well diffusion assays, the maximum zone of inhibition was formed against *Staphylococcus aureus* and the minimum against *Bacillus cereus*. The petroleum ether plant extract was statistically significant for all the tested organisms, in the well diffusion assay. Ethyl acetate extract also produced significant results for the gram positive and gram negative organisms. Comparison of antibacterial activity of petroleum ether and ethyl acetate extracts by disc diffusion and well diffusion were represented in Table 1 and Table 2 respectively. Researchers have mentioned that the four sided *Cissus quadrangularis* extract was found effective against *Escherichia coli* and *Staphylococcus aureus* [8]. The plant extract also exhibited antibacterial efficacy against the organisms *Escherichia coli* and *Salmonella typhi* [11].

Table 1: Antibacterial activity of three sided *Cissus quadrangularis* by disc diffusion assay

EXTRACT	DIAMETER OF ZONE OF INHIBITION (mm)			
	Gram positive		Gram negative	
	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
Petroleum ether	-	5	3	5
Ethyl acetate	3	7	5	6

Table 2: Antibacterial activity of three sided *Cissus quadrangularis* by well diffusion assay

Microorganism	Concentration of plant extract (µg/ml)	Mean ± SD (For Petroleum ether extract)	Level of Significance	Mean ± SD (For Ethyl acetate extract)	Level of Significance
<i>Bacillus cereus</i>	250	-	P > 0.05	-	P > 0.05
	500	1.13 ± 1.02		-	
	750	2.36 ± 1.09		3.8 ± 1.20	
	1000	3.66 ± 0.57		6.06 ± 1.20	
<i>Staphylococcus aureus</i>	250	1.26 ± 1.10	P > 0.05	2.76 ± 1.20	P > 0.05
	500	2.36 ± 1.09		5.23 ± 1.00	
	750	4.16 ± 1.04		9.76 ± 1.56	
	1000	7.4 ± 1.50		12 ± 1.56	
<i>Escherichia coli</i>	250	-	P > 0.05	1.26 ± 1.10	P > 0.05
	500	1.66 ± 0.57		3 ± 1.11	
	750	3.06 ± 1.00		6.33 ± 1.15	
	1000	4.03 ± 1.12		7.33 ± 1.52	
<i>Salmonella typhi</i>	250	2.2 ± 1.05	P > 0.05	2.26 ± 1.10	P > 0.05
	500	1.93 ± 1.10		4.1 ± 1.08	
	750	4.03 ± 1.00		6.83 ± 1.04	
	1000	5.06 ± 0.90		10.4 ± 1.03	

**Phytochemical analysis**

Compounds such as carbohydrates, phenols, proteins and amino acids were detected in the petroleum ether extract of the plant. The phytochemical constituents found to be present in the ethyl acetate extract of plant three sided *Cissus quadrangularis* were carbohydrates, flavanoids, tannins, saponins and phenols. The results obtained were supported by Sathyaprabha [12]. The result also correlates with previous research work that have been reported the existence of compounds such as phenol, tannins and flavanoids [8]. Researchers reported the presence of tannins, saponins and flavanoids in the four sided *Cissus quadrangularis* [13].

## CONCLUSION

In the recent times, utilization of plants for treatment of various ailments without any side effects has been increasing. The plants possess unique therapeutic efficacy which can be revealed through research findings. In the current study, the antibacterial potential of the plant three sided *Cissus quadrangularis* was evaluated and the phytochemical compounds were detected qualitatively. The extracts were found to be effective against both the tested gram positive and gram negative organisms. Comparing the bacterial strains, *Staphylococcus aureus* was highly inhibited by both petroleum ether and ethyl acetate extracts of the plant three sided *Cissus quadrangularis*. Phytochemical constituents such as flavanoids, tannins, saponins, phenols carbohydrates, proteins and amino acids

were present in the plant. Hence it is evident from this study that the plant extract of three sided *Cissus quadrangularis* possess antibacterial property and could act as an alternate antibacterial agent.

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