

Short Communication

**PHYTOCHEMICAL PROFILES OF THREE TRADITIONAL MEDICINAL PLANTS AND ITS
ANTIMICROBIAL ACTIVITIES**

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ABSTRACT

Objective: The objective of the current study was to compare the phytochemical composition and to evaluate the antibacterial properties of *Cissus quadrangularis*, *Cinnamomum zeylanicum* and *Trigonella foenum-graecum* against the bacterial strains *Escherichia coli* and *Bacillus circulans*.

Methods: Qualitative analysis and quantitative estimation of various phytochemical components were done using standard protocols. Antibacterial activity against gram-negative *Escherichia coli* and gram-positive *Bacillus circulans* was evaluated using standard protocol of agar well diffusion and disc diffusion assay. The zone of inhibition was calculated.

Results: Preliminary phytochemical analysis showed the presence of alkaloids, saponins and tannins in all three plant extracts. In quantitative estimation, *Cinnamomum zeylanicum* showed high alkaloid content (22%), *Cissus quadrangularis* showed high saponin content (6%) *Trigonella foenum-graecum* showed a high concentration (4.65 mg/g) of tannin. All the three plants showed moderate antimicrobial activity. The water extract of *Cinnamomum zeylanicum* showed the highest zone of inhibition (13 mm) against *Escherichia coli* and the water extract of *Trigonella foenum-graecum* showed the highest zone of inhibition (11 mm) against *Bacillus circulans*.

Conclusion: The result of this study supports the use of all the selected three medicinal plants as a source of antibacterial substance for the possible treatment of human pathogenic organisms. These plants can be further subjected to isolation of the therapeutic phytochemicals and further pharmacological evaluation.

Keywords: *Cissus quadrangularis*, *Trigonella foenum-graecum*, *Cinnamomum zeylanicum*, Phytochemical, Antibacterial, Agar well diffusion assay, Disc diffusion assay

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Transformations in the ecological environment and the variation in the pathogens have led to a surge in the number of new emerging infectious diseases. Unprecedented environmental changes are increasingly becoming a concern as a factor in the emergence of many infectious diseases. A focused phytochemical screening backed by ethnomedicinal data often leads to the discovery of new lead compounds that can play a role in the global efforts against pathogens. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [1, 2]. *Cissus quadrangularis* L., succulent plant of the family Vitaceae is known for anthelmintic, dyspeptic, analgesic, eye and ear diseases, in the treatment of irregular menstruation and asthma. It is studied for its medicinal properties for the treatment of bone fractures [3], management of obesity, complications associated with metabolic disorders [4], antioxidant [5] and also its hepatoprotective activity [6]. *Trigonella foenum-graecum* L., commonly known as Fenugreek, is an annual legume crop belonging to the family Fabaceae, mainly grown for use as a spice in many parts of the world [7]. Fenugreek is known for its therapeutic effects such as pain relief, antidiabetes, antiatherosclerosis, anti-inflammation, carminative, laxative, antispasmodic, anticancer, sexual desire increasing, astringent, hypertension decreasing, triglyceride lowering, breast milk increasing, oxytocic and antithrombotic properties [8]. *Cinnamomum verum*, J Presl. (*Syn. Cinnamomum zeylanicum* Nees.), an evergreen tropical tree belonging to the Lauraceae family, is commonly used in the food industry because of its special aroma. This spice is known to have strong antioxidant, antibacterial, antipyretic and anti-inflammatory properties, which play an important role in tissue repair [9]. *Cinnamomum* is used as traditional medicine for treating sore throats, cough, indigestion, abdominal cramps, intestinal spasms, nausea, flatulence and diarrhoea [10]. It also displays an antifungal property that slows down food spoilage [11]. It also has an antiatherosclerotic effect [12]. Research concerning the efficiency

of *Cissus quadrangularis*, *Trigonella foenum-graecum* and *Cinnamomum zeylanicum* against the most common microbial pathogens are scanty. Hence, the study aimed to evaluate the phytochemical constituents and investigate the antibacterial activity of these traditionally important plants against *Escherichia coli* and *Bacillus circulans*.

The study plants were growing at an altitude of 9-1 meters above sea level (9.98 °N 76.28 °E). The plants were identified at the herbarium section of the Department of Botany and Centre for Research, St. Teresa's College [Autonomous], Ernakulam. Crude extracts of plants were prepared using distilled water, ethanol and methanol. Aqueous extract was prepared by making an infusion in hot (95 °C) distilled water. The infusion was left overnight under refrigeration (4 °C) to prevent any possible contamination. After 24 h the extracts were kept in a rotary shaker at 1000 RPM for 1 h and filtered with Whatman No.1 filter paper and preserved at 4 °C for future use. Solvent extracts were made by mixing the pulverized plant material with a sufficient quantity of solvents viz. methanol, and ethanol. It was kept in a rotary shaker at 1000 RPM overnight and filtered with Whatman No.1 filter paper and preserved at 4 °C for future use. For the phytochemical screening standard protocols were adopted [13]. Determination of alkaloid was done according to the protocol prescribed in Harborne [14]. Tannin determination was done by Van-Burden and Robinson method [15]. Standard protocol was used for the quantitative determination of saponin [16]. Evaluation of the antimicrobial activity of the plant extracts was done against *Escherichia coli* and *Bacillus circulans* using standard well diffusion and disc diffusion methods [17, 18]. Amoxicillin was used as a positive control. All the experiments were performed in triplicates (n = 3).

The study showed the presence of alkaloids, saponins and tannins in all the three extracts of study plants. Table 1 summarizes the result of preliminary phytochemical analysis of aqueous, ethanol and methanol extracts of *C. quadrangularis*, *T. foenum-graecum* and *C. zeylanicum*.

Aqueous extract of all the three plants showed the presence of flavonoids and cardiac glycosides. The result was more pronounced in aqueous extract when compared to ethanol and methanol extracts. *C. quadrangularis* showed the best results among the three study plants.

The detected chemical groups such as flavonoids, alkaloids, saponins, tannins, phenols and cardiac glycosides proved the importance of *C. quadrangularis*, *T. foenum-graecum* and *C. zeylanicum* as a potential source of therapeutic drugs.

Table 1: Qualitative analysis of aqueous, ethanol and methanol extracts of *C. quadrangularis*, *T. foenum-graecum* and *C. zeylanicum*

Phytochemical	Aqueous extract			Ethanol extract			Methanol extract		
	<i>C. quadrangularis</i>	<i>T. foenum-graecum</i>	<i>C. zeylanicum</i>	<i>C. quadrangularis</i>	<i>T. foenum-graecum</i>	<i>C. zeylanicum</i>	<i>C. quadrangularis</i>	<i>T. foenum-graecum</i>	<i>C. zeylanicum</i>
Flavonoids	+	+	+	+	-	-	+	-	-
Alkaloids	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Phenols	-	-	ICG*	-	ICG	+	-	-	-
Cardiac Glycosides	+	+	+	-	+	+	-	-	-
Terpenoids	-	-	+	-	+	+	-	-	-

+indicates presence; -indicates absence and *ICG-Immediate colour change, n=3

Table 2: Quantitative estimation of selected phytochemicals in the study plants

Plant	Alkaloid	Saponin	Tannins
	---%---		---mg/g---
<i>Cissus quadrangularis</i>	20±1.12	6±0.57	1.38±0.97
<i>Trigonella foenum-graecum</i>	6±0.45	2±0.11	2.50±0.11
<i>Cinnamomum zeylanicum</i>	22±2.23	4±0.15	4.65±0.75

Results expressed as mean±SE, n=3

The quantitative estimation of alkaloids, tannins and saponins of *C. quadrangularis*, *T. foenum-graecum* and *C. zeylanicum* was estimated and the readings are recorded in the table 2. In the present study, *C. zeylanicum* showed the highest content of alkaloids and tannins, whereas high content of saponin was observed in *C. quadrangularis*.

The plant extracts were examined to see if their traditional uses for the treatment of infectious diseases could be linked to the presence of these classes of compounds. Antimicrobial study was carried out in all the three extracts using both well and disc diffusion assay against gram-

negative *E. coli* and gram-positive *B. circulans*. All the three plants showed appreciable antimicrobial activity. Well diffusion study showed a better result than disc diffusion study [19]. The aqueous extract of *C. zeylanicum* showed the highest zone of inhibition against *E. coli* and aqueous extract of *T. foenum-graecum* showed the highest zone of inhibition against *B. circulans* [table 3]. In disc diffusion study, water extract of *C. quadrangularis*, ethanol extract of *T. foenum-graecum*, methanol and ethanol extract of *C. zeylanicum* showed the highest zone of inhibition against *B. circulans* and methanol extract of *C. zeylanicum* showed the maximum zone of inhibition against *E. coli* [table 4].

Table 3: Antimicrobial screening of plant extracts against bacterial pathogen using well diffusion assay

Organism	<i>C. quadrangularis</i>				<i>T. foenum-graecum</i>				<i>C. zeylanicum</i>			
	Zone of Inhibition(mm)											
	Methanol	Ethanol	Water	Control	Methanol	Ethanol	Water	Control	Methanol	Ethanol	Water	Control
<i>E. coli</i>	10±0.1	10±0.1	6±0.3	22±0.1	10±0.1	10±0.1	10±0.2	25±0.1	7±0.1	7±0.1	13±0.1	22±0.1
<i>B. circulans</i>	6±0.2	10±0.1	10±0.1	24±0.2	10±0.1	3±0.4	11±0.3	23±0.1	10±0.1	10±0.1	10±0.1	25±0.5

Results expressed as mean±SE, n=3

Table 4: Antimicrobial screening of plant extracts against bacterial pathogen using disc diffusion assay

Organism	<i>C. quadrangularis</i>				<i>T. foenum-graecum</i>				<i>C. zeylanicum</i>			
	Zone of Inhibition(mm)											
	Methanol	Ethanol	Water	Control	Methanol	Ethanol	Water	Control	Methanol	Ethanol	Water	Control
<i>E. coli</i>	5±0.1	2±0.1	1±0.01	26±0.9	5±0.03	1±0.1	1±0.1	25±0.7	10±0.1	3±0.01	1±0.09	26±1.03
<i>B. circulans</i>	5±0.1	7±0.1	10±0.1	25±0.8	3±0.02	10±0.7	3±0.01	25±0.8	10±0.4	5±0.4	10±0.1	25±0.9

Results expressed as mean±SE, n=3

The quantitative phytochemical estimation specifies that the study plants contain a significant amount of alkaloid, saponin and tannin content [table 2]. The alkaloids, saponins and tannins are one of the largest groups of secondary metabolites to have exhibited antimicrobial activity. Antimicrobial property of saponin is that it works with the phosphate groups of the phospholipids of cell

membranes and enters cells [20]. This causes leakage of proteins and certain enzymes from the cell [21]. Tannins bind to proline-rich proteins and interfere with protein synthesis [22]. Alkaloids are reported to have an antibacterial activity where it inhibits transcription and toxin production of bacteria [23], also inhibits the enzyme dihydrofolate reductase resulting in the inhibition of nucleic

acid synthesis [24]. The active chemical principles of all the study plants need to be analyzed with an organized scientific outlook in times to come through specific experimental animal models and clinical trials to decipher their molecular mechanism of action.

The present study covers the phytochemical and antimicrobial study of *Cissus quadrangularis*, *Trigonella foenum-graecum* and *Cinnamomum zeylanicum*. The study revealed the presence of major secondary metabolites such as flavonoids, alkaloids, tannins, phenol, cardiac glycosides and saponin and showed potential antibacterial activity on *Escherichia coli* and *Bacillus circulans*. The results of this study support the use of these plants as traditional medicines for the treatment of infectious diseases. Further work needs to be done to isolate the specific chemical/chemicals that show potential acceptable efficacy against these bacterial species.

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AUTHORS CONTRIBUTIONS

Reshma Maria Joseph carried out the experiments and drafted the manuscript. Pressy. P. Prakasia supervised the work, co-authored and edited the manuscript.

CONFLICT OF INTERESTS

Declared none

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