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Phytochemical Composition And Antimicrobial Activity of *Cinnamomum Verum* Bark Against UTI Causing Bacteria

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Abstract

Cinnamomum verum is a spice plant used widely known for its medicinal and pharmaceutical properties. The goal of the presented study was to evaluate phytochemical composition and antimicrobial activity of aqueous extract of *C. verum*. During study the antimicrobial activity of the extract was evaluated against 5 UTI causing bacteria while the extract was found to inhibit the growth of all these bacteria including *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, and *Escherichia coli*. Further the extract exhibited maximum relative percentage inhibition towards *K. pneumoniae* (95.06%) and best minimum inhibitory concentration towards *E. coli* (0.5 mg/ml). Phytochemical analysis revealed the presence of phenols, carbohydrates, tannins, flavonoids, oil and fats while saponins, proteins and alkaloids were found to be absent. The results of the study emphasized on the strong antibacterial property of this plants and its potential application in antimicrobial drug discovery.

Keywords:

Cinnamomum verum, Antimicrobial activity, Antibacterial activity, Urinary tract infections

Introduction

Urinary tract infection (UTI) is nowadays a disease of much concern due to a variety of factor that leads to recurrent bacteremia, drug resistance, it is regarded as second most common organ infection in humans that accounts for almost 8 million visits of medical professionals [1]. Different forms of urinary tract infections occurs which depends on the severity of the disease and functional abnormality of the urinary tract [2]. Women are showed to be more prone to UTIs due to structural anatomy of their reproductive and urinary organs, with many being at risk of acute non obstructive phyelopritis especially from those women that experienced recurrent infection [3]. Because of the high rate of antibiotic consumption, a number natural remedies are in place to help in curing both complicated and uncomplicated UTIs which may serve as a breakthrough in overcoming the current state of antibiotic resistance [1, 4].

Medicinal plants represent a very valuable source of medicine and since thousands of years a variety of medicinal plants are being used for developing medicine. *Cinnamomum verum* is a small tree, evergreen in nature and its bark commonly known as cinnamon is widely used as spice in India. Cinnamon is highly rich in nutrients and contains several

medicinal properties. It is widely used in traditional medicinal system antispasmodic, carminative, antidiarrheal, antiemetic, analgesic and also for the treatment of influenza and common cold [5]. In recent years *C. verum* bark extracts and essential oils have been reported to exhibit significant antimicrobial activity against a wide variety of bacteria and fungi [6-8]. These studies represent this plant as a valuable source of antimicrobial compounds therefore this study was designed to evaluate the antibacterial properties of the aqueous extract of *C. verum* bark against UTI causing bacteria.

Materials and Methods

Plant collection and extraction

The dried bark of *C. verum* was purchased from the local spice market in Phagwara Punjab. The pieces of bark were broken into small pieces and milled in a fine powder using a grinder. Ten gram of fine powder was added to a conical flask containing 100ml distilled water and kept on a rotary shaker (120 rpm) for 24 hours. Following this, the mixture was filtered and dried.

Antibacterial activity

Test organisms

Five bacterial cultures including *Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter aerogenes, Klebsiella pneumonia,* and *Escherichia coli* isolated from UTI patents were used during the study. The cultures were sub-cultured in nutrient broth and grown overnight at 37°C in an incubator.

Experimental controls

Amikacin (30mcg/disk) was used as positive control (PC) while Dimethyl sulfoxide was utilised a negative control (NC) during the study.

Sample preparation

Aqueous extract of *C. verum* was mixed in the DMSO (5mg/ml). The mixture was centrifuged at 5000rpm for 2 minutes to remove any insoluble particles.

Antibacterial assay

Antibacterial *C. verum* was evaluated by agar well diffusion method on Muller Hinton agar (MHA) plates. During the study, test bacteria were uniformly layered on the MHA surface by using sterilized cotton swab. Following this, the plates were kept in incubator at 37°C for 30 minutes. Using a gel borer, two wells were punched in the agar surface and a volume of 100µl of plant extract and 100µl of negative control was added to separate well using a micro pipette. A disk of positive control was also placed on the surface of plates and the plates were placed in incubator at 37°C for 24 hours. Zone of clearance was measured and recorded [9].

Relative percentage inhibition (RPI)

Following the antibacterial activity, a comparative analysis in the antibacterial potential of plant extract and standard drug was measure by below mentioned formula [10]:

$$\mathbf{RPI = [100 \times (x\text{-}y)] / (z\text{-}y)}$$

Where, x, y, z area of inhibition (AI) of sample, NC and PC respectively
AI= πr^2; where, r = radius of zone of inhibition

Minimum inhibitory concentration (MIC)

During the study, test bacteria were uniformly layered on the MHA surface by using sterilized cotton swab. Following this, the plates were incubated in an incubator at 37°C for 30 minutes. Using a gel borer, five wells were punched in the agar surface and a volume of 100µl of plant extract in declining concentrations (5 mg to 100µl) were added to separate well using a micro pipette and the plates were then incubated in an incubator at 37°C for 24 hours. Later plates were observed for the formation of zone of inhibition and the same was measured and recorded. Lowest concentration exhibited clear zone of clearance was recorded as MIC [11].

Phytochemical analysis

Aqueous extract of *C. verum* bark was subjected to qualitative phytochemical analysis by following standard procedures [12].

Statistical analysis

Each experiment was performed in three replicates and data is represented as mean ± standard deviation. All the calculation were performed by using MS Excel 2013.

Results and Discussion

UTIs is one of the highly prevalent infection worldwide while the incidence rate is high in developing countries than that of developed countries. In developing countries like India, UTIs are responsible for causing a large number of febrile illness cases with high incidence in younger population [13]. In last few decades, a large number of uropathogens have been reported to exhibit drug resistance and multi drug resistance towards the commonly used antibiotics [14]. Therefore this study was undertaken for studying the antimicrobial activity of aqueous extract of *C. verum* against 5 bacteria isolated form pathents suffering UTIs including *E. coli, P. aeruginosa, K. pneumoniae, P. mirabilis* and *E. aerogenes*.

During this study aqueous extract of *C. verum* was found to be effecting in inhibiting the growth of all test bacteria while the highest activity was found to be towards *K. pneumoniae* (19.33±1.52) followed by *E. aerogenes* (18.66±2.08), *E. coli* (18.33±1.52), *P. aeruginosa* (14.66±2.08), and *P. mirabilis* (13.66±0.57) (Table 1). Further the relative percentage inhibition of the plant extract towards UTI causing bacteria was evaluated (Table 2). Aqueous extract of *C. verum* exhibited maximum RPI towards *K. pneumoniae* (95.06%) followed by *E. aerogenes* (91.8%), *E. coli* (80.88%), *P. mirabilis* (69.49%) and *P. aeruginosa* (68.75). following this the MIC of the extract was found to be towards *E. coli* (0.5 mg/ml), *E. aerogenes* (0.7 mg/ml), *K. pneumoniae* (1 mg/ml), *P. mirabilis* (2 mg/ml) and *P. aeruginosa* (2.5 mg/ml) (Table 2).

Results of present study are well supported by previous available literature where varies solvent extracts collected from plant had been reported to exhibit significant antimicrobial activity against a variety of microorganisms. In one of the study, methanol and chloroform extract of *C. verum* bark exhibited broad spectrum antibacterial activity toward both Gram Positive (*Staphylococcus aureus*, *Bacillus subtilis*, B. cereus, *B. megatarium*) and Gram negative (*E. coli, P. aeruginosa, Klebsiella mobilis, Proteus vulgaris*) bacteria [8]. Other than solvent extracts, essential oil collected from the *C. verum* bark has also been reported to possess significant antimicrobial activity. In one of the study, essential oil from the *C. verum* bark in combination with piperacillin against multi drug resistant *E. coli* (β-lactamase TEM-1 plasmid-conferred *E. coli* J53 R1) [6]. In one other study essential oil from the *C. verum* bark exhibited significant antibacterial activity against *Porphyromonas gingivalis* while cinnamaldehyde was found to be the active constituent of the oil [7].

It is evident from earlier studies that the medicinal properties of any plant is always due to the presence of phytochemicals in them, which are primarily produced by plants for protecting themselves from pathogens. Preliminary phytochemical analysis of aqueous extract of *C. verum* exhibited the presence of flavonoids, phenolic compounds, carbohydrates, oil, tannins, and fats while saponins, proteins and alkaloids were found to be missing (Table 3). Above observations are in aggriment with the previous reports where aqueous extract of *C. verum* bark showed the presence of flavonoids, tannins, carbohydrates and glycosides [15] while methanol extract of *C. verum* bark has been reported to show the presence of alkaloids, glycosides, phenols, steroids, and tanins [8]. Theses phytochemicals in addition to the other unstudied phytochemicals are the principle candidates towards the bioactive nature of the *C. verum*.

Conclusion

During the presented study aqueous extract of *C. verum* bark was screened for antibacterial activity towards causative organisms of bacterial urinary tract infection. It is evident from the results that the test extract possess good antibacterial property against UTI causing bacteria and future studies could be conducted for the isolation and characterization of the active molecule and subsequent studies could be planed for identification of inhibitory mechanism.

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Tables:

Table 1: Antimicrobial activity of aqueous extract of *Cinnamomum verum* against UTI causing bacteria

Test organisms	Zone of inhibition (mm)		
	Mean ± SD (n=3)		
	Plant extract	PC	NC
E. coli	18.33±1.52	22.66±1.52	0±0
P. aeruginosa	14.66±2.08	21.33±2.51	0±0
K. pneumoniae	19.33±1.52	20.33±1.52	0±0
P. mirabilis	13.66±0.57	19.66±1.52	0±0
E. aerogenes	18.66±2.08	20.33±0.57	0±0

PC (Positive control): Amikacin (30mcg/disk), NC (Negative control): DMSO

Table 2: Relative percentage inhibition and minimum inhibitory concentration of aqueous extract of *Cinnamomum verum* against UTI causing bacteria

Test organisms	RPI (%)	MIC (mg/ml)
E. coli	80.88	0.5
P. aeruginosa	68.75	2.5
K. pneumoniae	95.06	1
P. mirabilis	69.49	2
E. aerogenes	91.8	0.7

RIP: Relative percentage inhibition, MIC: Minimum inhibitory concentration

Table 3: Phytochemical composition of aqueous extract of *Cinnamomum verum*

Test	Methods	*Cinnamomum verum*
Phenolic test	Ferric chloride test	+++
Flavonoids	Aluminium chloride	+++
Tannins	0.1% ferric chloride test	+
Saponins	Foam test	_
Protein	Nin hydrin test	_
	Biuret test	-
Alkaloids	Wagner's test	-
	Mayer's test	-
Carbohydrates	Benedicts	+
	Molisch test	+
	Fehling test	+
Oil and fats	Spot test	++

Key: += presence, - = Absence of tested phytochemical