Phytochemical Analysis and Antimicrobial Activity of *Cinnamomum Verum*

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Abstract: **The microorganisms have developed resistance to many commercial antibiotics due to indiscriminate use of antimicrobial drugs. Therefore, investigation of medicinal plant with new chemical compounds and its antimicrobial action have become a necessity. As spices have the antimicrobial potential, in the present study, the antimicrobial activity of *Cinnamomum verum* have been investigated as alternative to antibiotics. In search of new chemical compound, methanolic and chloroform extract of *Cinnamomum verum* was screened for antimicrobial property and phytochemical analysis.Antimicrobial activities of these extracts were tested against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Bacillus megatarium, Klebsiella mobilis, Bacillus cereus, Proteus vulgaris, Aspergillus niger* and *Penicillium stolonifer*. The agar well diffusion was used for initial screening, while minimum inhibitory concentrations (MICs) were determined usingserial agar macro-dilution method for active extracts on the initial method. Chloroform extract of spice showed highest antibiotic activity compared to methanolic extract. Phytochemical analysis was used for detection of alkaloids, flavonoids, glycosides, terpenoids, steroids, tannin etc. In conclusion, the Chloroform extract of spice could be used as potential sources of new antimicrobial agent.**

Keywords: **antimicrobial activity, phytochemical screening, *Cinnamomum verum***

I. INTRODUCTION

The development of microbial resistance towards antibiotics has heightened the importance of the search for new potential effective plants and plant constituents against pathogenic microorganisms. The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Biologically active widespread from plant sources have always been of great interest to scientists working on infectious diseases. Over the past decade there has been an explosion of interest in the antimicrobial, particularly antibacterial and antifungal, activity of natural products. The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery. The effect of plant extracts on microorganisms has been studied by a very large number of researches in different parts of the word.

Spices have been an irreplaceable part of cuisines all over the world and for the last couple of centuries, beginning from the founders of Ayurveda, these very spices and their constituents used not only for flavor and aroma of the foods but also to provide antimicrobial properties Spices may contribute piquancy of foods and beverages (Praveen *et al*., 2006). In addition to these spices are some of the most commonly used natural antimicrobial agents in foods. Some of the natural compounds found in various spices possess antimicrobial activity. Keeping in view this fact the present study was conducted to find out the antimicrobial activity of *Cinnamomum verum.* Cinnamon *(Cinnamomum verum)* is commonly used in the food industry because of its special aroma. Additionally, it has strong antibacterial properties, anticandidial, antiulcer, analgesic, antioxidant and hypo cholesterolaemic activities (Lin *et al,*. 2003).It is an ever green tropical tree, belonging to the *Lauraceae* family. Different parts of the plant (bark, roots and leaves) essential oils are used as a medicine. Due to its distinct odour, it is widely used as an important ingredient of many mouth watering dishes of the world. Cinnamon has been reported to have remarkable pharmacological effects in the treatment of type II diabetes and insulin resistance (Hassan *et al*., 2012). Cinnamon is indicated as an analgesic and antipyretic agent against cold, fever, headache, myalgia (mascular pain), arthralgia (arthritic pain) and amenorrhea (failure of menstruation). In Indian traditional literature including Ayurveda, many other valuable actions are attributed to cinnamon bark (Pandey *et al.*, 2014). Many scientific pharmacological investigations are also reported on anti-inflammatory potential of the bark of cinnamon. The anti-inflammatory action has been attributed to a series of tannins. The antinociceptive (analgesic) and antipyretic (fever reducing) activity were also been reported (Sachinv et al., 2013). In present study spice extracts were used to study the phytochemical and antimicrobial properties.

II. MATERIALS AND METHOD

Collection of plant material

Cinnamomum verum bark was collected from local marketin Vapi, Gujarat, India. The spice were ground into powder in a mixer-grinder and sieved into fine powder to be used for extraction. The powder was stored in an air tight container and kept at 4 °C for further use.

Preparation of Plant Extracts

Extraction was carried out using Maceration technique. Two types of solvents were used for the extraction of various components.

Methanol Extract

For the extraction, 15g of each powdered spice were macerated in separate conical flasks containing 80ml methanol. flasks were allowed to stand for 3-7 days with occasional shaking. The liquid was then strained off, the solid material was pressed and then liquid was clarified by using muslin cloth. The filtrates were air dried at room temperature and residual moisture was removed in a vacuum oven at 50 to52°C. Then dried extract was dissolved in DMSO (Dimethyl sulphoxide) and kept at 4ºC until use.

Chloroform Extract

For the extraction, 15g of each powdered spices were macerated in separate conical flasks containing 80ml of chloroform. The further extraction procedure was carried out same as described earlier.

Maintenance and preservation of culture

The bacterial species used in present study were gram negative *(Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Protease vulgaris*), gram positive (*Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Staphylococcus aureus*). The fungus species used for the test were *Aspergillus niger, Penicillium stolonifer.* Various non pathogenic organisms were procured from MTCC. All the cultures were maintain by sub culturing on nutrient agar slant and PDA slants and stored at 4˚C in refrigerator.

Phytochemical Analysis

Various extracts of the spice sample were tested for Tannins, Steroid,Phenols, Saponins, Cardiac glycosides, Flavonoids and Alkaloids.

Test for Alkaloids (Mayer's test)

0.5 ml of extract was added with a drop or two of Mayer's reagent by the side of test tube. Formation of white or creamy precipitate indicates positive test for alkaloids.

Test for Flavonoids (Ammonia test)

1 ml of the extract was taken in the test tube and ammonia solution was added (1:5) followed by the addition of concentrated sulphuric acid. Appearance of yellow color and its disappearance on standing indicates the positive test for flavonoids.

Test for Glycosides (Keller Kiliani test)

5 ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1 ml of concentrated sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Test for Phenols (Ferric chloride test)

0.5 ml of the extract was added with few drops of neutral ferric chloride (0.5%) solution. Formation of dark green color indicates thepresence of the phenolic compounds.

Test for Saponins (Froth test)

1 ml of the extract was taken in a test tube and distilled water (2 ml) was added to it. The test tube was then kept in boiling water bath for boiling and was shaken vigorously. Existence of froth formation during warming confirms the presence of saponins.

Test for Tannins (Ferric chloride test)

1 ml of the extract was added with 5 ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this 0.1% ferric chloride solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.

Test for Steroids and Triterpenoids (Salkowki test)

About 0.5g of Crude extract was mixed with 2ml of Chloroform. Then concentrated H_2SO_4 added to form two layers. Formation of green colour at the upper layer and reddish brown colour at the lower layer indicates the presence of Steroids and Triterpenoids respectively.

Determination of Anti-bacterial Activity

Agar-well diffusion method was employed for the determination of antibacterial activities of methanolic and chloroform extracts of spice.The test organisms were grown on nutrient broth prior to start the experiment. The organisms were kept in incubator at 37°C for 24 hours. The inoculums for each test culture were prepared which have approximately 10^6 CFU/ml (0.5 Mac-Farland Standard). 100µL of standardized inoculums of each bacterium was spread uniformly on sterile Muller-Hinton Agar plate. Wells(6mm in diameter) were cut from the agar with a sterile borer. 150µL (100mg/ml) volume of the extract was introduced in to the well. Standard antibiotic Tetracycline (50µg/ml) was used as positive control and DMSO was used as negative control. The agar plates were then incubated at 37°C for 24 hours. The zone of inhibition was recorded to the nearest size in mm.

Determination of Anti-fungal Activity

Agar-well diffusion method was employed for the determination of antifungal activities of different extract of spice. Tested fungi was subcultured on malt extract plates and incubated at 28°C for 3-5 days. Wells (6mm in diameter) were cut from the agar with a sterile borer. 150µL(100mg/ml) volume of the extract was introduced into the well. Standard

antifungal Fluconazole (50µg/ml) was used as positive control and DMSO was used as negative control. The activity was determined after 72 hrs of incubation at 28°C. Appearance of zones of inhibition was regarded as presence of the antifungal action in the test substance.

Minimum inhibitory concentration

MIC for bacterial strains (Serial agar macro-dilution method)

The strains of microorganism obtained were inoculated in conical flask containing 50 ml of nutrient broth. These conical flasks were incubated at 37ºC or 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. once the agar was solidified, it was punched with six millimeters diameter well. Serial two-fold dilution of each extracts were added in each well in a concentration ranging from 1,00,000µg/ml to 97.65 µg/ml. The plates were incubated aerobically for 24 hours at 37°C. MIC was defined as the lowest concentration ofextract in which no colony was observed after incubation (Mattana *et al.*, 2010).

MIC for fungal strains (serial agar dilution method)

The inoculum was prepared by flooding the agar slants with the sporulated fungi with Tween 80, scraping the surface with a teasing needle, and withdrawing the resulting suspension with a sterile Pasteur pipette. The suspension was vigorously vortexed for 15 s and spreaded on Sterile malt extract agar plates. Plates were punched with six millimeters diameter well. Serial two-fold dilution of each extracts were added in each well in a concentration ranging from 1,00,000µg/ml to 97.65 µg/ml. The plates were incubated at 30 °C until growth was seen in the growth control subculture. A growth control of each tested strain was included. Minimal Inhibitory Concentration (MIC) is defined as the lowest concentration capable to inhibit any visible fungal growth (PUJOL *et al.*, 1996).

III. RESULTS AND DISCUSSION

Phytochemical test for methanolic and chloroform extracts of the spice carried out.The results of phytochemical analysis are presented in Table 1 and Table 2. The Phytochemical analysis of the various extracts from, *Cinnamomum verum* showed presence of Phenols, Glycosides, and tannins all the extracts. Alkoloids, flavonoid and saponins were absent in all the extract.

Table 1 Phytochemical Analysis of methanol extract of *Cinnamomum verum*

S. No.	Compound	Solvent
		Methanol
1	Alkaloids	+
2	Flavonoids	-
3	Glycosides	+
4	Phenols	+
5	Steroids	+
6	Saponins	-
7	Tannins	+
8	Terpenoids	-

Note: + indicates = Positive results, - indicates = Negative results

Table 2 Phytochemical Analysis of Chloroform extract of *Cinnamomum verum*

S. No	Compound	Solvent
		Chloroform
1	Alkaloids	-
2	Flavonoids	-
3	Glycosides	+
4	Phenols	+
5	Saponins	-
6	Steroids	+
7	Tannins	+
8	Terpenoids	+

Note: + indicates = Positive results, - indicates = Negative results

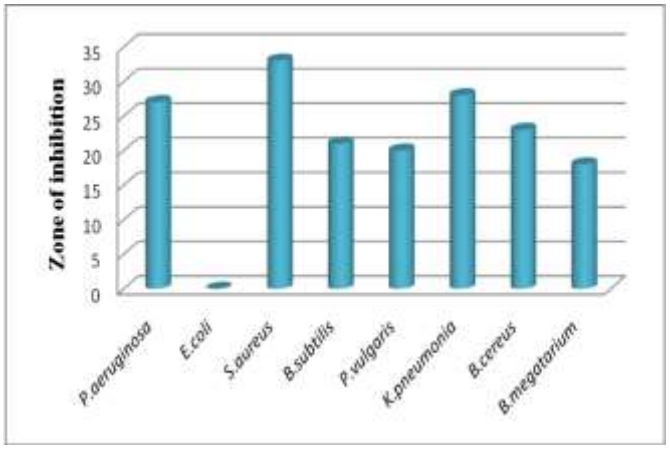
Anti-bacterial activity

In the present study, the antibacterial activity of three different spices extracts towards clinically significant microbes is reported. The extracts obtained from spices were found to be effective against the bacteria *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris,* and *Peudomonas aeruginosa.*

Antibacterial activity of Methanol extract

Methanolic extract of all three spice showed antibacterial activity with varying magnitudes against gram positive and gram negative bacteria. The results observed revealed that methanolic extract of *Cinmomum verum*against the test organisms and the zone of inhibition observed was ranging from 21-33 mm and does not shown an inhibition against *Escherichia coli.* Brindha*et al.* (2014)showed that *Cinnamomum zeylanicum* bark extracts had inhibited both gram-positive bacteria and gram-negative bacteria indicating broad spectrum inhibitory effect. Gram positive bacteria were more susceptible than gram negative bacteria by the action of *Cinnamomum zeylanicum* bark extracts, demonstrating antibacterial effect which was comparable with that of the standard drug amphicillin.

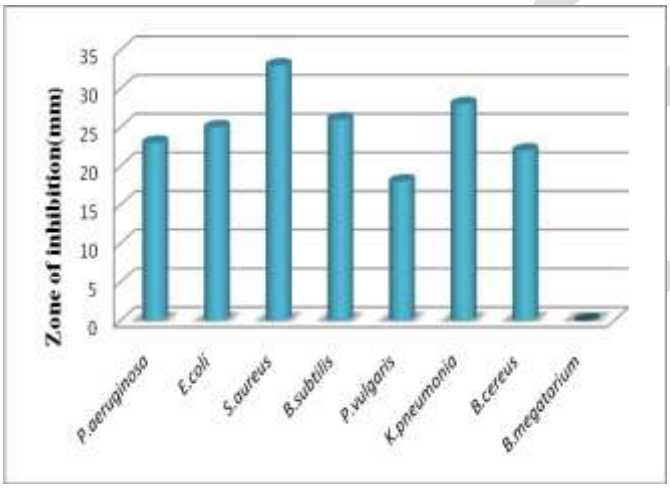
Figure 1: Antimicrobial activity of methanolic extract of *Cinnamomumverum*



Antibacterial activity of Chloroform extract of Spice:

The chloroform extract of *Cinmomum verum* shows zone of inhibition against both gram negative and gram positive organims in the range of 18-33 mm but the extract was inactive against *Bacillus megatarium*. The chloroform extract of the bark of *Cinnamomum verum* had strong antimicrobial activity showed that the active components have been extracted in chloroform. This results are contrary to the report given by Liwei*et al* ., (2004).

Figure 2: Antimicrobial activity of chloroform extract of *Cinnamomum verum*



MIC (Minimum Inhibitory Concentration) determination

Determination of MICs of the spice extracts was done by well diffusion technique and the concentrations of the extracts used were 100,000, 50,000, 25,000, 12,500, 6,250, 3,125, 1562.5, 781.25, 390.62, 195.31, 97.65 µg/ml. The zone diameter of inhibition was determined for the different concentrations tested.

MIC of methanolic extract of Cinnamomum verum for bacterial strain

The MIC value of methanol extract of *Cinnamomum verum* (Table 3.5) was found to be 12,500 µg/ml against *Bacillus cereus*, 25,000 µg/ml against *Bacillus subtilis*, *Staphylococcus aureus*, *E.coli*, *B.megatarium* and 50,000 µg/ml against *Klebsiella pneumonia*, *Pseudomonas aeruginosa and Protease vulgaris.* MIC of water, methanol, ethanol and hexan extract of *Cinnamomum verum* has been previously reported by Senhaji *et al.*, (2004).

Table 3 MIC of Methanol extract of *Cinnamomum verum* for various bacterial strains

Concentration (µg/ml)	*B. cereus*	*B. subtilis*	*S. aureus*	*E. coli*	*B.megatarim*	*K.pneumonia*	*P. vulgaris*	*P. aeruginosa*
100,000	+	+	+	+	+	+	+	+
50,000	+	+	+	+	+	+	+	+
25,000	+	+	+	+	+	-	-	-
12,500	+	-	-	-	-	-	-	-
6,250	-	-	-	-	-	-	-	-
3,125	-	-	-	-	-	-	-	-
1562.5	-	-	-	-	-	-	-	-
781.25	-	-	-	-	-	-	-	-
390.62	-	-	-	-	-	-	-	-
195.31	-	-	-	-	-	-	-	-
97.65	-	-	-	-	-	-	-	-

Positive Sign = Presence of zone;**Negative Sign**= Absence of zone

MIC of chloroform extract of Cinnamomum verum for bacterial strains

The MIC value of chloroform extract of *Cinnamomum verum* (Table 3.6) was found to be 3,125 µg/ml against *Staphylococcus aureus*, 6,250 µg/ml against *Bacillus cereus* and *Bacillus subtilis*, and 25,000 µg/ml against *E.coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa and Protease vulgaris.*

Table 4 MIC of Chloroform extract of *Cinnamomum verum* for various microorganisms

Concentration (µg/ml)	*B. cereus*	*B. subtilis*	*S. aureus*	*E. coli*	*B.megatarium*	*K.pneumonia*	*P. vulgaris*	*P. aeruginosa*
100,000	+	+	+	+	-	+	+	+
50,000	+	+	+	+	-	+	+	+
25,000	+	+	+	+	-	+	+	+

12,500	+	+	+	-	-	-	-	-
6,250	+	+	+	-	-	-	-	-
3,125	-	-	+	-	-	-	-	-
1562.5	-	-	-	-	-	-	-	-
781.25	-	-	-	-	-	-	-	-
390.62	-	-	-	-	-	-	-	-
195.31	-	-	-	-	-	-	-	-
97.65	-	-	-	-	-	-	-	-

Positive Sign = presence of zone; **Negative Sign**= Absence of zone

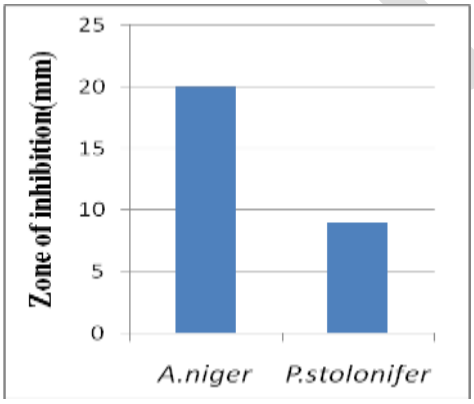
Antifungal activity

Anti-fungal activity was determined using agar - well diffusion technique. Two tested fungi *Aspergillus niger* and *Penicillium stolonifer* were used to study the effects of methanol and chloroform extracts of *Cinnamomum verum*.

Antifungal activity of methonolic extract of Cinnamomum verum

In the antifungal study of methenolic extract of spice, maximum antifungal activity was shown by *Cinnamomum verum* extract against *Aspergillus niger* but it exhibited minimum activity against *Penicillium stolonifer*. This finding is comparable with the finding of Pandey*et al*., (2013) who reported methanol extracts of spices (clove, ajwain, turmeric, black pepper and dalchini) given high antifungal activity against different fungi (*Aspergillusniger* and *Trichoderma spp*.). The efficacy of cinnamon oil as an antifungal agent was reported by Soliman*et al*., (2002); Vellutie*t al*., (2003); Singh *et al*., (2007).

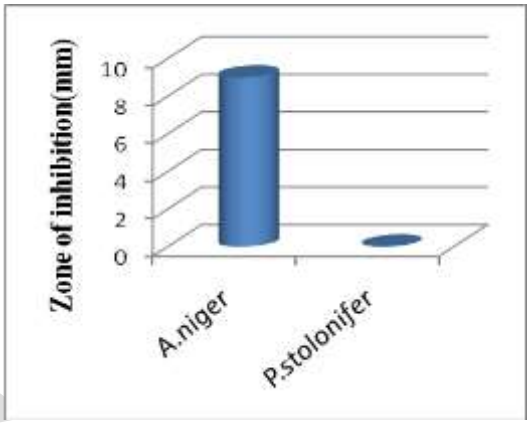
figure 3: Antifungal activity of methenoli extract of *Cinnamomum verum*



Antifungal activity of Chloroform extract

The chloroform extract of *Cinmomumverum* shows zone of inhibition of about 9mm against *Aspergillusniger* and the extract was inactive against *PenicilliumstoloniferT*he main constituent of cinnamon is cinnamaldehyde, which is the compound containing an aldehyde group and conjugated double bond outside the ring. This compound possesses much stronger antifungal activity (Wang et al., 2005) and it may be a potential lead compound for the development of antifungal drugs through the control β-(1,3)glucan and chitin synthesis in yeasts and molds (Bang et al., 2000).

Figure 4: Antifungal activity of chloroform extract of *Cinnamomumverum*



MIC of Methanolicand chloroform extract of Cinnamomum verum for various fungal strains

Penicilliumstolonifer and *Aspergillusniger* found to be sensitive to the both of the extracts by showing the MIC of 1,00,000µg/ml (Table 5).Several studies have been shown that extracts and oils of cinnamon demonstrated antifungal activities reported by several researchers (Li *et al*., 2014; sukatta*et al*., 2014; pandey*et al*., 2013).

Table 5 MIC of Methanolic extract of *Cinnamomumverum* for various fungal strains

Concentration (µg/ml)	***Methenolic extract***	
	Aspergillus niger	***Penicillum stolonifer***
100,000	-	-
50,000	+	+
25,000	+	+
12,500	+	+
6,250	+	+
3,125	+	+
1562.5	+	+
781.25	+	+
390.62	+	+
195.31	+	+
97.65	+	+

Table 6 MIC of chloroform extract of *Cinnamomum verum* for various fungi

Concentration (µg/ml)	Chloroform extract	
	Aspergilus niger	*Pellicilium stoloniferum*
100,000	-	-
50,000	+	+
25,000	+	+
12,500	+	+
6,250	+	+
3,125	+	+
1562.5	+	+
781.25	+	+
390.62	+	+
195.31	+	+
97.65	+	+

IV. SUMMARY AND CONCLUSION

The study was carried out for studying antimicrobial activity and phytochemical analysis of *Cinnamomum verum*. *Cinnamomum verum* was tested for its antimicrobial activity. Cinnamon extracts were prepared using methanol and chloroform. The extracts were subjected to phytochemical analysis to ascertain the secondary metabolites present in cinnamon which govern its antimicrobial properties. Phytochemical analysis of methanolic extract of all the three plants revealed presence of Terpenoids, Glycosides, Phenol, Steroids, Tannins, and the of both extracts found to be effective against both gram +ve and gram –ve bacteria as well as pathogenic fungi. The highest zone of inhibition was obtained by chloroform extract of spice. Minimum Inhibitory Concentration of the chloroform extract of spice was found to be effective on bacterial strains S.aureus at the concentration of 3,125µg/ml Whereas the bacterial strain *B.cereus*, *B.subtilis* was found to be at the concentation of 6,250µg/ml. *Aspergillusniger* showed more sensitivity as compared to the *Penicilliumstolonifir.*

Even though traditionally, spices are used as food preservatives and antiseptics, Further studies are required to investigate the mechanism of interaction of different phytochemicals,animal studies and human clinical trials to determine the safety profile, therapeutic window and optimum dosage schedule. The knowledge on efficacy of extracts can be extended from culinary food applications to pharmacology and food chemistry.

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