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PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL ACTIVITY OF CINNAMON SPICE AGAINST URINARY TRACT INFECTION AND FUNGAL PATHOGENS

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ABSTRACT

There is an increasing apprehension among scientists regarding the use of chemical preservatives and synthetic antimicrobials. These chemicals used to inactivate or inhibit growth of pathogenic organisms in turn make them resistant to these antimicrobials over the time. Cinnamon (*Cinnamomum verum*) is well known in India as a food spice but little do people know that Cinnamon has an enormous potential as an antimicrobial agent and is a powerful antioxidant. Its abundant value in treating disorders like diabetes (Khan A. et al. 2003), inflammation, ulcers (Jakhetia V. et al. 2010), Alzheimer's (Peterson DW et al. 2009) have already been proven. We wanted to find out, through our study, if Cinnamon (in any of its three forms – bark, leaf or oil) has any effect on urinary tract infection isolates and fungal isolates, in an attempt to replace chemical drugs with nature's products. Infected urine samples were used to isolate bacteria which were subjected to the antimicrobial assay of commercially available cinnamon oil and the extracts of cinnamon leaf and bark. The inhibitory effect was checked as a consequence on the growth curve of bacteria. The extracts were analyzed for phytochemicals which give cinnamon its antimicrobial property. Lastly, the antifungal property of cinnamon was tested on *Aspergillus niger*, a common food spoiling and disease causing fungus. The study showed that cinnamon oil is a more potent antimicrobial agent than any cinnamon extract and it has the potential for further research in drug development and as a food preservative.

KEYWORDS: ***Cinnamon, cinnamon oil, antimicrobial, growth curve, urinary tract infection***

INTRODUCTION

The use of plant based drugs for treating various ailments is known to humans since thousands of years. There is evidence of spice trading between the Indian and Roman empires for their use in medicines, foods, perfumes etc. Plants and plant based products are the basis of many of the modern pharmaceuticals we use today for our various ailments. Cinnamon has a long history both as a spice and as a medicine. Today, scientific research reveals that not only the chemicals from the plants have effects on diseases but also their antioxidant properties have a beneficial effect to humans (Asimi O.A. et al. 2013; Hoque M. M. et al 2008) Increased fungal and bacterial infections, toxicity of some antifungal and antibacterial drugs and development of resistance of some species of microbes against these drugs have led to many studies to search for new antimicrobial agents (Gupta C. et al 2008). Therefore, antimicrobial efficacies from plants have to be explored. One such infection prevailing in large parts of the world is the Urinary tract infection affecting millions of people every day. And they remain to be a major clinical problem even after introduction of antimicrobial chemotherapy. Urinary infections were caused by common uropathogens like enteric gram negative bacteria – *E.coli, Proteus mirabilis, Klebsiella sps. Enterococcus, Pseudomonas* etc. Kumar A. et al in 2012 showed that cinnamon oil is effective against *Shigella* and *Pseudomonas* isolated from UTI patients. In our study however, we have compared the results of cinnamon oil with the methanolic, chloroform and aqueous extracts of Cinnamon bark and leaf against pathogens isolated

from UTI patients. Alongside, we also tried to test if Cinnamon has an effect on *Aspergillus niger*, a common food borne pathogen and a causative agent of bladder aspergillosis (an infection of the urinary bladder). The aim of the present study was to determine the phytochemical composition of the cinnamon (*Cinnamonum verum*) and to assess the antibacterial and antifungal activities of cinnamon against various human pathogens. The consequence of the phytochemicals on bacterial growth was studied by observing the changes in the growth curve of bacteria in the presence and absence of the plant extract. Finally, the antioxidant activity of cinnamon was evaluated using standard assays. The effects of cinnamon extracts and cinnamon oil on these bacterial species were determined and in this way, have tried to come up with alternatives for the conventional therapies against such deadly diseases.

MATERIALS AND METHODS

Collection of Sample

Fresh cinnamon bark and leaves were collected from the Konkan region of India. Cinnamon oil was purchased from a local market. The material was dried and ground to a fine powder in a blender (Figure 1).

Figure 1
Sample was collected, dried and ground to powder

Extract Preparation

10g of the bark and leaf powder was weighed and added in Soxhlet Apparatus. 200mL each of Methanol, Chloroform and Water were added separately to run each for 24 h (Ahmad S.I. et al 2013). The extracts were evaporated to dryness in a Rotary Evaporator or Water bath set at a desired temperature. The dried extract was dissolved in a known volume of DMSO (Dimethyl sulfoxide) and the concentration was noted.

Phytochemical Analysis

Phytochemical analysis was conducted for all extracts as per the protocol described by Khan A. et al, 2003 (Table 1)

- Alkaloids: Wagner's Test
- Tannins: Ferric Chloride Test
- Flavanoids: Lead acetate Test
- Saponins: Foam Test
- Terpenoids: Salkowski Test
- Cardiac Glycosides: Keller Kellani Test

Table 1
Results of phytochemical analysis of Cinnamon extracts

	ALKALOIDS	FLAVANOIDS	SAPONINS	TANNINS	TERPENOIDS	GLYCOSIDES
BARK METHANOL EXTRACT	+	+	+	+	+	+
BARK CHLOROFORM EXTRACT	+	-	+	+	+	+
AQUEOUS BARK EXTRACT	+	-	+	-	+	+
LEAF METHANOL EXTRACT	-	-	-	-	-	-
LEAF CHLOROFORM EXTRACT	+	+	+	+	+	+
AQUEOUS LEAF EXTRACT	-	+	+	+	+	-

Thin Layer Chromatography
Thin layer chromatography was carried out to analyze the compounds present in the crude extract. Normal phase silica gel pre-coated TLC plates were used. The solvent extracts were applied about 1.3cm from the edge using a 20µL capillary tube. The mobile phase, Ethyl acetate: Methanol: Water (4.4: 5.1: 10.2) was chosen by standardization. The plates were placed vertically into the mobile phase. After the mobile phase had moved to about 80% from the spotting line, the plate was removed from the developing chamber and dried. (Figure 2)

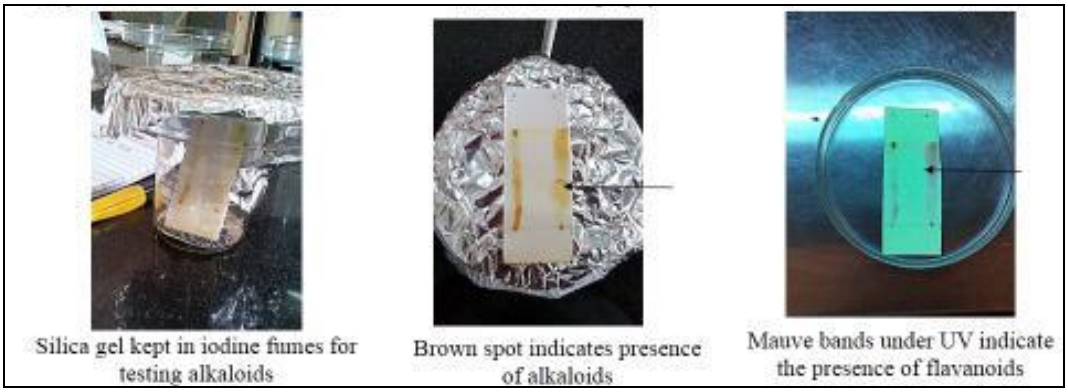

Figure 2
Bands as observed in Thin Layer Chromatography in the presence of iodine fumes (1 and 2) and under UV light (3). Here the solvent system used was of high polarity (ethyl acetate: methanol: water) and since most compounds in the cinnamon extracts show less Rf values in a polar solvent, it means that most phytochemicals in cinnamon are polar. This further validates our selection of solvents for extract preparation.

Isolation of pathogenic strains of bacteria from infected urine samples
Two infected urine samples were collected from a dialysis center in Pune, India and brought to the lab for isolation of pathogens. Differential media were used for identification of different organisms– Mac Conkey agar (*E.coli and Staphylococcus aureus),* Eosin methylene blue agar (*E.coli),* Nutrient Agar (all microbes present in the sample, eg. *Klebsiella pneumoniae),* Dettol agar (*Pseudomonas aeruginosa),* and Blood agar (*Proteus mirabilis)* (Kumar A. et al, 2012).After repeated subcultures, a pure culture of bacteria was obtained which was subjected to colony characterization, gram staining and motility tests. Further, biochemical tests were performed to determine the species of bacteria – Nitrate reducing test, Catalase test, Carbohydrate fermentation, IMViC, Urease test. The results were confirmed from Bergey's Manual of Systematic Biology.

Determination of Anti-bacterial activity

Nutrient agar was plated with 0.1ml bacterial suspension by spreading the inoculum on the medium. Wells of 5mm diameter were punched in the agar with a cork borer and filled with approximately 0.1ml of the plant extract. Control wells contained neat solvents and standard antibiotic solution, Streptomycin (100µg/ml) (Ramya B. S. and P. Ganesh, 2012). The antibacterial activity was assessed by measuring the zone of inhibition (Fig. 3, 4).

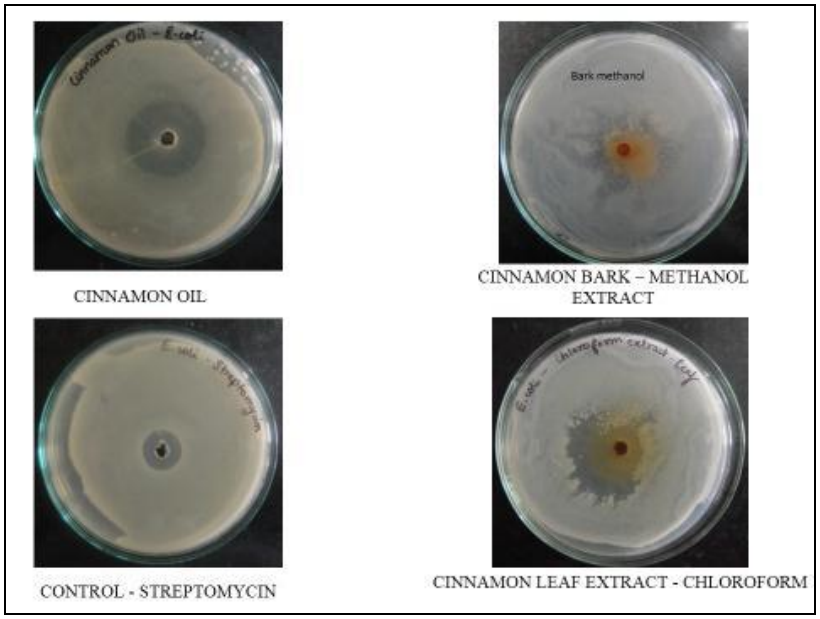

Figure 3

Activity of Cinnamon oil vs activity of solvent extracts of cinnamon against E.coli isolated from Urine samples spread on nutrient agar plate. Cinnamon oil gave a zone of inhibition of 29mm while Cinnamon leaf chloroform extract produced a zone of 36mm.

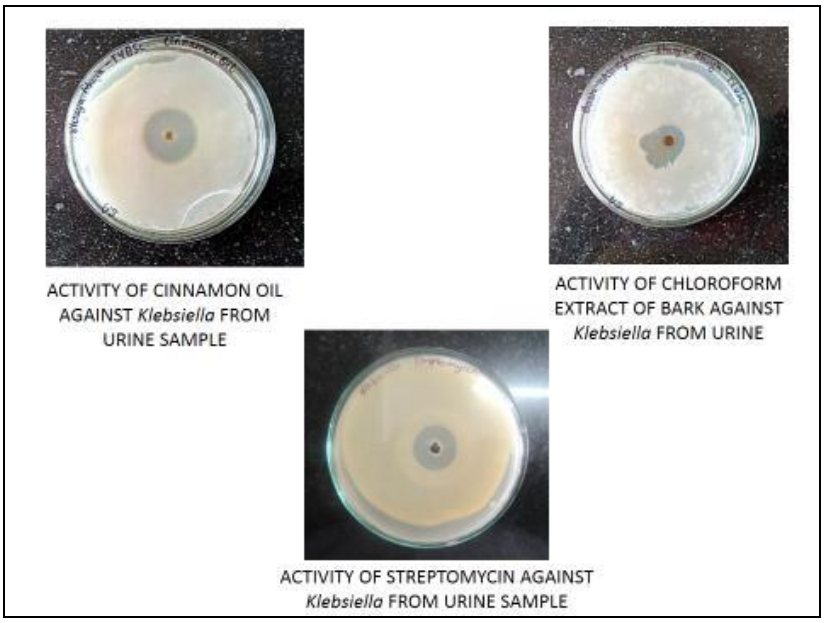

Figure 4

Activity of Cinnamon oil vs activity of cinnamon solvent extracts against Klebsiella isolated from infected urine samples. While cinnamon oil gives a zone of 22mm, streptomycin gives 21mm zone and the chloroform extract of cinnamon bark too gives an appreciable diameter.

Calculation of the minimum inhibitory concentration of the extracts [6]

The plant extract was prepared to the highest concentration of 100mg/ml (stock solution) in DMSO (Dimethyl Sulphoxide) and was serially diluted to a working concentration ranging from

25mg/ml to 100mg/ml. Agar diffusion method was used to determine the MIC value as the lowest concentration showing a zone of inhibition (Fig. 5).

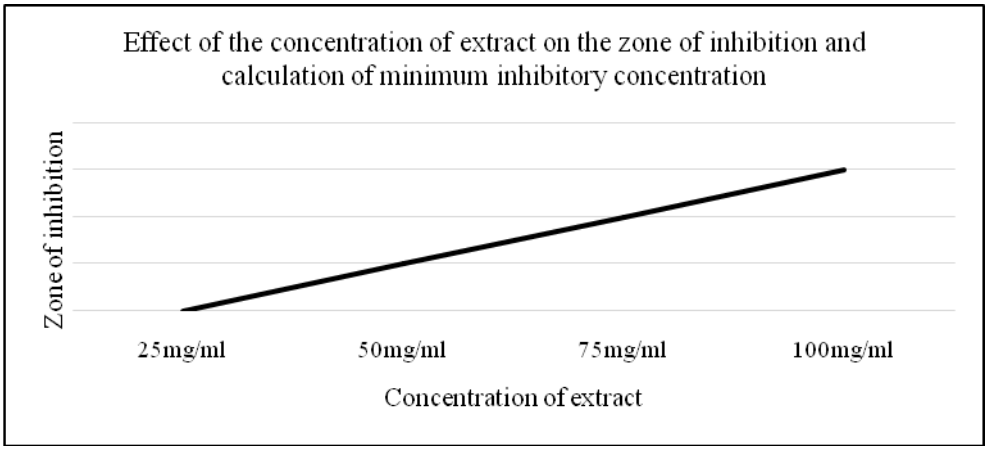

Figure 5

Graph showing increasing diameter of the zone of inhibition with increasing concentration of extract. Minimum inhibitory concentration was found to be 50mg/ml.

Growth curve studies

24 h old bacterial suspension was inoculated in 100mL nutrient broth in side-arm flasks. One flask was used as a control and the others were inoculated with 1ml of the test extracts and cinnamon oil. Optical density was recorded using a colorimeter set at 595nm and the flasks were then placed in a shaking incubator at 37ºC. Readings were taken after every 30mins and the graph of time vs optical density was plotted for all extracts (Ahmad S. I. et al, 2013). Deviations from the standard curve were observed and interpreted (Figure 5).

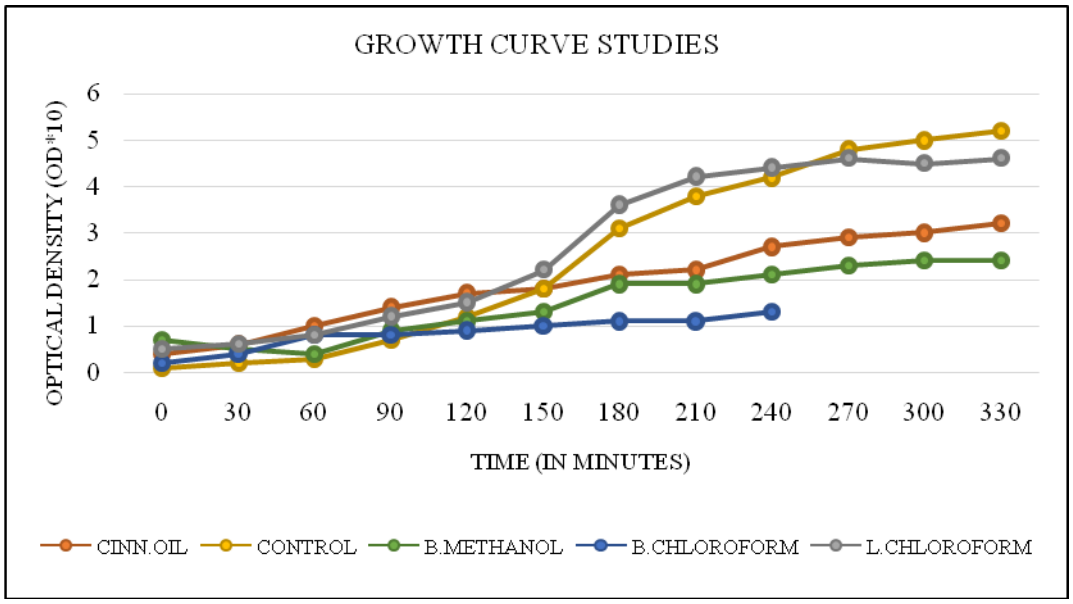

Figure 6

Graph of the growth curve (OD at 595nm vs time) of bacteria in the presence and absence of Cinnamon. Cinnamon oil shows significant decrease in growth with suppressed and delayed exponential phases with respect to control. Other extracts too show a drastic reduction in the exponential phases of growth with a prolonged lag phase.

Antifungal tests

5ml Potato Dextrose molten agar inoculated with *Aspergillus niger* and this suspension was poured on 15ml solidified Potato Dextrose Agar plates. The plates were allowed to cool and solidify. 5mm diameter wells were punched into the agar and 0.1ml of the test extract and cinnamon oil were added in these wells. The plates were kept in the

refrigerator for half an hour for the extracts to diffuse through the agar. The plates were kept for incubation at 37ºC for 48-72 hrs. (Ahmad S. I. et al, 2013). The fungal growth was observed and results were interpreted (Figure 6).

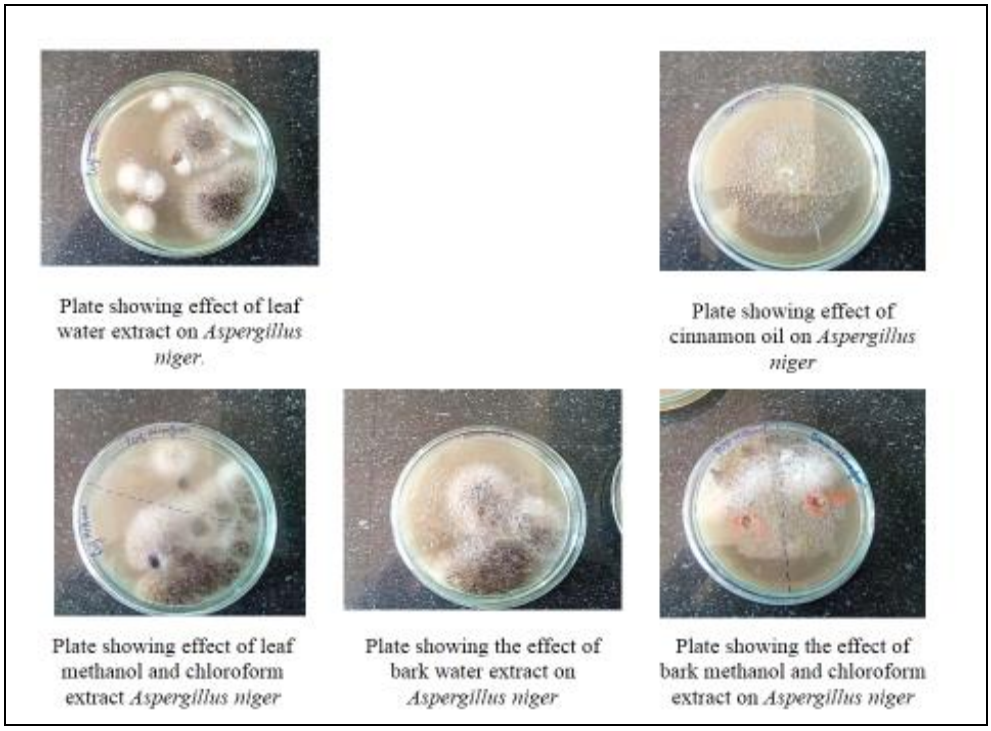

Figure 7

Results of the antifungal property of Cinnamon oil vs antifungal property of cinnamon extracts. No growth was observed on the plate containing cinnamon oil. In the case of chloroform extracts of cinnamon leaf, there was no growth within 20mm of the well indicating a potential antifungal activity

Antioxidant activity

FRAP and Peroxidase assays were carried out to determine the antioxidant potential of the test extracts as described by Tandon et al [12, 14].

Table 2

Results of FRAP Assay. Methanol extracts of both the bark and leaf show the greatest FRAP values indicating their higher antioxidant power as compared to the chloroform extracts. The FRAP values of most extracts exceeded 2 which is the FRAP value of Ascorbic acid. This indicates a high potential of Cinnamon as an antioxidant.

SAMPLE	OD OF SAMPLE AT 0mins	OD OF SAMPLE AFTER 4mins	CHANGE IS ABSORBANCE	FRAP VALUE (µM/100g sample)
Bark Aqueous	0.354	0.682	0.328	1.17
Bark methanol	1.766	2.500	0.734	2.62
Bark chloroform	1.905	2.500	0.595	2.125
Leaf methanol	1.849	2.500	0.651	2.325
Leaf chloroform	0.737	1.185	0.448	1.60

RESULTS AND DISCUSSION

Cinnamomum verum was tested for its bactericidal, fungicidal and antioxidant activity. Cinnamon extracts were prepared using methanol, chloroform, and water. The extracts were subjected to phytochemical analysis to ascertain the secondary metabolites present in cinnamon which govern its antimicrobial properties. Phytochemicals i.e. secondary metabolites of plants are an essential component of our diet. Phytochemicals are responsible for the beneficial properties which plants possess like anti-microbial, anti-inflammatory, cholesterol lowering, blood sugar lowering, insecticidal and fungicidal properties. Even the anti-oxidant properties of fruits,

vegetables and spices are a result of the biochemical process which these phytochemicals undergo. Whereas Flavonoids and Tannins impart anti-oxidant properties, Saponins are anti-cancerous and lower cholesterol levels. Alkaloids are used as anti-malarial compounds and as analgesics. Terpenoids are used for Cardiac related disorders for being a source of Vitamin A. From the results of the phytochemical analysis, it was found that methanolic extract of Cinnamon bark contained all the six phytochemicals which were tested (Table 1). The extracts were then tested against isolates from infected urine obtained from UTI infected patients. The isolates were characterized by various biochemical tests and proved to be sensitive to cinnamon extracts. The order of bactericidal activity was cinnamon oil> cinnamon chloroform extracts> cinnamon methanol extracts> cinnamon aqueous extracts, with the minimum inhibitory concentration to be 50mg/mL (Figure 3,4,5). In fact, Cinnamon oil showed a similar or sometimes even larger inhibitory zone than the conventional antibiotic – Streptomycin. The effect of the extracts and oil was studied by their influence on the growth rate of bacteria. It was found that the presence of cinnamon in the medium had a noticeable effect on the log phase of an actively growing culture, i.e. the log phase duration was significantly reduced. Also, the lag phase was prolonged which is evident from the difference seen between the control and test sample curves. The stationary phase was also reached faster in the presence of Cinnamon (Figure 6). Cinnamon extracts were then tested for their antioxidant power using FRAP assay and Peroxidase enzyme activity (265.96/liter). Cinnamon showed a high value of FRAP (2.0±4) and Peroxidase making itself a potent natural source of antioxidants (Table 2).Lastly, in the presence of Cinnamon oil in the Potato Dextrose Agar, *Aspergillus niger* showed no growth as opposed to other extracts, in the presence of which, little sparse growth was observed. Thus, Cinnamon spice proves to be a potential antimicrobial agent and must be subjected to further analysis of its properties. There were many more fungal and bacterial pathogens which need to be tested for and this calls for further experimentation in this area. Another area which requires further exploration is bacterial infections prevailing in humans worldwide. As the work for development of herbal medicines is in progress worldwide, the present report will help in the discovery of naturally available products/drugs which are as potent as synthetic drugs and at the same time palatable, causing fewer secondary infections. It can be concluded that the active compounds present in *Cinnamonum* species should be studied more extensively to explore its potential in the treatment of infectious diseases and can be promoted worldwide as a potent antimicrobial agent that can substitute a number of chemicals, drugs and artificial preservatives.

CONCLUSION

Urinary tract infections are a serious health problem affecting millions of people every day. And they remain to be a major clinical problem even after introduction of antimicrobial chemotherapy. Urinary infections are caused by common uropathogens like enteric gram negative bacteria – E.coli, Proteus mirabilis, Klebsiella sps. Enterococcus, Pseudomonas etc. We tried to look for the effects of the various extracts of cinnamon and it's oil on isolated bacteria and fungi which are known to cause UTIs. It was found that Cinnamon shows appreciable activity against the bacterial isolates. In terms of antifungal activity, cinnamon oil has a vast potential as a fungicidal agent and must be considered for further research. The cinnamon extracts, especially cinnamon oil showed an appreciable decline in the growth of bacteria which further confirms the proposed antimicrobial activity of cinnamon. The phytochemical analysis shows that cinnamon contains a variety of compounds such as alkaloids, tannins, flavonoids, phenols etc. which might be responsible for the bacteriostatic and bactericidal activity. Lastly, the results obtained using FRAP assay showed that cinnamon could be considered as a potential source of natural compounds with significant antioxidant property. As the work for development of herbal medicines is in progress worldwide, the present report will help in the isolation of new products/drugs. It can be concluded that the active compounds present in *Cinnamomum verum* species should certainly find place in treatment of various bacterial infections and indicate that this spice should be studied more extensively to explore its potential in the treatment of infectious diseases.

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