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ANTIMICROBIAL ACTIVITY OF CINNAMON BARK OIL AGAINST URINARY TRACT INFECTION ASSOCIATED PATHOGENS

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ABSTRACT

Background: The aim of the present study is to determine the antimicrobial activity of cinnamon bark essential oil (*Cinnamomum zeylanicum*) against urinary tract infection (UTI) associated pathogens. **Methodology**: The antimicrobial activity of cinnamon essential oil was screened using disc diffusion method against four standard control bacteria, one Gram positive bacterium (*Enterococcus faecalis* ATCC 29212), three Gram negative control bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883 and *Pseudomonas aeruginosa* ATCC 27853) and 37 clinical isolates from previousely mentioned bacterial species along with *Proteus mirabilis* . The antifungal activity was investigated against one standard control fungus (*Candida albicans* ATCC 7596 and 7 clinical isolates). The minimum inhibitory concentrations (MICs) were determined by tube dilution method using concentrations ranging from 0.625% to 0.019% for bacteria, and from 1% to 0.015% for *Candida albicans*, and then MBCs were calculated for bacterial pathogens by subsequent sub-culturing of MICs on Mueller Hinton agar. **Results:** Cinnamon bark essential oil exhibited promising inhibitory activity against all pathogens tested. MICs ranged from 0.125% to 0.312%, MBC for *Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae* was 0.312%, and 0.156% for *Pseudomonas aeruginosa*. All (100%) clinical bacterial isolates were susceptible to cinnamon essential oil, which exhibited antibacterial potential better than that demonstrated by ciprofloxacin and vancomycin which were used as reference antibacterial drugs in except for *Proteus mirabilis* and *Pseudomonas aeruginosa* which showed lower activity to Cinnamon essential oil. **Conclusion:** Cinnamon bark essential oil showed pronounced antimicrobial activity against all the microorganisms tested. Therefore, it can be a good antibacterial and antifungal agent since it is not toxic at the MICs used.

KEYWORDS: Antimicrobial activity, *Cinnamomum zeylanicum*, Urinary Tract Infections.

1. INTRODUCTION

Urinary tract infection (UTI) has long been recognized as one of the commonest bacterial infection which is prevalent in both community and health care settings. Symptomatic infections are common and are associated with morbidity and rarely mortality; however asymptomatic infections are more common. It may infects individuals from different age groups and from both sexes[1], it can leads to poor maternal and perinatal outcomes[2], infectious complications in renal transplant recipients and potential poorer graft outcomes.[3]

Antibiotic resistance among uro-pathogens causing urinary tract infection (UTI) is increasing worldwide.[4] In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Antibiotics are sometimes associated with adverse effects on the host like hypersensitivity. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from other sources, such as plants. Natural products of higher plants may be a new source of antimicrobial agents possibly with novel mechanisms of action.[5] Plant essential oils are potential sources of antimicrobial compounds and have been used in traditional medicine for many years. Essential oils are natural compounds containing a complex mixture of odorous and volatile constituents. Essential oils have been observed to possess antibacterial, antifungal activities.[6] Cinnamon (*Cinnamomum zeylanycum* Boiss.), a member of the family Lauracaea, grows in southern Asia and it is used as a spice to improve flavor of local food preparations and cosmetics and pharmaceutical preparations,[7,8] It contains about 60-70% w/w of cinnamaldehyde, C6H5=CHCHO which is the active constituent, 4-10% of phenols (chiefly

eugenol), hydrocarbons (pinene, phellandrene, caryophyllene), and small quantities of ketones, alcohols and esters.[9]

This study has been undertaken to determine the susceptibility of Uro-pathogens to cinnamon oil.

2. MATERIALS AND METHODS

2.1 Plant material

The bark of cinnamon used in this study was purchased from local markets (Khartoum).

2.2 Preparation of Crude Extract

By steam distillation method; amount of 303 g of mashed cinnamon sticks were introduced into the distillation flask (1L) connected to the steam generator via a glass tube and to a condenser to retrieve the oil. The essential oils were volatilized with boiling water at temperature of 100°C for 6-8 hours. The mixture was allowed to settle and the oil was withdrawn. After the steam distillation process, the product was collected and separated using separatory funnel. The essential oils settled at the bottom layer of the separatory funnel and were separated several times until no oil was left in the separatory funnel.[10] The remaining water of condensation was removed with the addition of anhydrous sodium sulphate powder.

2.3 Tested microorganisms

The essential oil of cinnamon was tested against standard control organisms: *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 53657), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC7596). The tested organisms were obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional medicine Research Institute (MAPTMRI), National Center for Research, Sudan.

Forty four clinical isolates of *Escherichia coli* (12), *Klebsiella pneumonia* (10), *Proteus mirabilis* (2), *Pseudomonas aeruginosa* (3), *Enterococcus faecalis* (10) and *Candida albicans* (7) were collected during the period 20/3/2016-20/4/2016 from Soba University Hospital.

2.4 Preparation of the test organisms

A loopful of isolated colonies was inoculated into 2 ml of sterile normal saline and the turbidity of bacterial suspension was adjusted to match the turbidity standard of 0.5 MC-Farland units**.**

2.5 Tube dilution

Final twofold dilutions of essential oil volumetrically in the Brain Heart infusion broth (Hi Media, India) for bacteria, and Sabouraud Broth (Hi Media, India) for fungi as recommended in National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[11, 12]

2.6 In vitro testing of extracts for antimicrobial activity

2.6.1Testing for antibacterial activity

As a preliminary step, the antibacterial activity of cinnamon essential oil was determined using disc diffusion method to screen its efficacy among control organisms and clinical isolates. It was diluted with methanol at 20% concentration (v/v). A volume of 20 µl of 10^8 CFU/ml of organism suspension was impregnated into the paper disc with 6mm diameter and then placed onto Mueller Hinton agar plates (Hi Media, India) for bacteria and Sabouraud Dextrose Agar for fungi. Vancomycin (30µg) and ciprofloxacin (10µg) were used as commercial reference antibiotics for Gram positive and Gram negative bacteria respectively, then incubated at 37 ºC for 18-24 hours for bacteria and for 48 hours for fungus.[13]

3. RESULTS

The essential oil of cinnamon was tested for its antimicrobial activity against one Gram positive standard bacterium *Enterococcus faecalis*, three Gram negative standard bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and *Candida albicans.* The antimicrobial activity of cinnamon oil was better than that of the commercial antibiotics vancomycin and ciprofloxicin. The essential oil exhibited inhibitory activity against both the standard bacteria and *Candida albicans* (photo. 1, 2, Figures 1, 2 and 3). The minimum inhibitory concentrations (MICs) of the cinnamon oil were determined against the standard organisms (*Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*).The minimum bactericidal concentrations (MBCs) showed same results of MICs regarding.

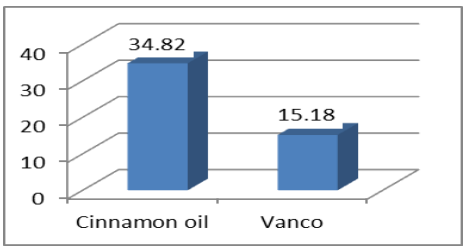

Figure (1): Inhibition diameter zones of cinnamon oil against *Enterococcus faecalis* using paper disc diffusion method. VA: vancomycin.

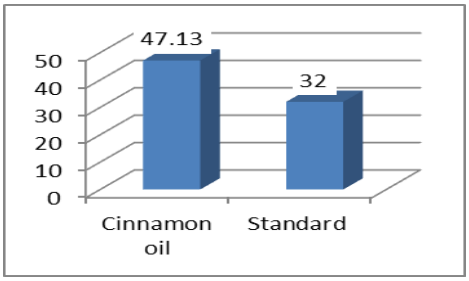

Figure (2): Inhibition diameter zones of cinnamon oil against *Candida albicans* using paper disc diffusion method.

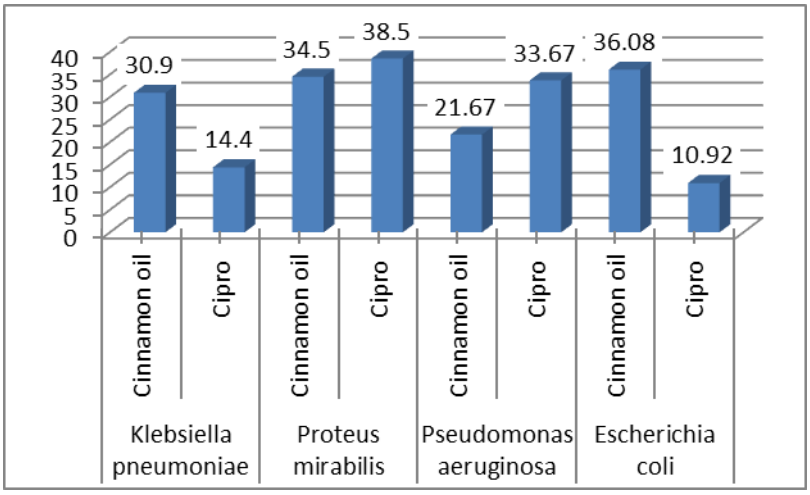

Figure (3): Inhibition diameter zones of cinnamon oil against Gram negative bacteria using paper disc diffusion method.

Photo 1: antifungal activity of cinnamon essential oil against *Candida albicans.*

Photo 2: antibacterial activity of cinnamon essential oil against *Klebsiella pneumoniae.*

Table (1): MICs and MBCs of cinnamon oil against UTI tested pathogens.

Standard Microorganisms	MIC of aqua's extract	MBC of aqua's extract
Enterococcus faecalis	0.312%	0.312%
Escherichia coli	0.156%	0.312%
Klebsiella pneumoniae	0.156%	0.312%
Pseudomonas aeruginosa	0.156%	0.156%
Candida albicans	0.125%	0.125%

Enterococcus faecalis, *Pseudomonas aeruginosa* and *Candida albicans* (Table1).

4. DISCUSSION

Research on medicinal plant has attracted a lot of attention globally.[14] Approximately, 7000 medical compounds in the modern pharmacopeia are derived from plants.[15] Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of infectious diseases.[16]

Therefore this study was conducted to investigate antimicrobial activity of *Cinnamoum zeylanicum* (cinnamon) essential oil against UTI associated pathogens, and to provide scientific evidence for its use.

In the present study revealed that the cinnamon essential oil exhibited a higher and stronger activity against *Candida albicans*, *Escherichia coli* and *Enterococcus faecalis* cinnamon essential oil exhibited strong activity than the commercial antibiotics discs of ciprofloxacin and vancomycin. *Klebsiella pneumoniae* showed high susceptibility to cinnamon than ciprofloxicin, in contrast to *Proteus mirabilis* and *Pseudomonas aeruginosa* in which essential oil exhibited lower activity when compared with ciprofloxacin. This is in agreement with the findings of Sharma *et al*, Prabuseenivasan *et al*, Wang *et al*, Ouafae Senhaji *et al* [17,18,19,20] when they used a variety of essential oils by different extraction methods against many pathogens and found that cinnamon essential oil had the highest antimicrobial activity among the other essential oils.

5. CONCLUSION

Cinnamon bark oil exhibited strong activity against the UTI associated microorganisms tested. Therefore it can be a good antibacterial and antifungal agent since it is not toxic at the low concentration.

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Conflict of interest
None declared.

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