Evaluation of antibacterial activity of *Cinnamomum zeylanicum* and *Eclipta alba* (L) Hassk. on UTI pathogens.

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Abstract

This study was investigate to asses the Antibacterial Activity of *Cinnamomum zeylanicum* and *Eclipta alba* (L) Hassk. Used against UTI Causing Pathogens. Bacteria were isolate from the urine sample of UTI infected patients and characterized by biochemical methods. Chloroform and acetone extracts were prepared from test plant materials and the zones of inhibition were compared with the zone of inhibition of standard antibiotics. Results from the present study showed that *Cinnamomum zeylanicum* had more antibacterial activity compared to *Eclipta alba* (L) Hassk. and *Cinnamomum zeylanicum* was effective as commercially used antibiotics.

Keywords: *Cinnamomum zeylanicum*, *Eclipta alba* (L) Hassk, Antibacterial, UTI.

1. Introduction

A urinary tract infection (UTI) is an infection that initiate in the urinary system. The urinary tract consists of the kidneys, ureters, bladder and the urethra (Geetha et al., 2011). *E. coli* which is the most frequently infecting organisms causing 95% of UTI [Kebira et al., 2009]. However, many other bacteria can cause UTI for example Klebsiella, Pseudomonas, Enterobacter, Proteus, Staphylococcus, Mycoplasma, Chlamydia, Serratia and Neisseria spp. Different age group of people can affect by UTI. Sometimes, the UTI is symptomatic or asymptomatic and complicated or uncomplicated in nature. It is reported that female are more likely to get UTI and about 35% of healthy female suffer symptoms of Urinary tract infection and about 5% of female each year suffer with the problem of dysuria (painful urination) and frequency (Hootan, 2003). Due to increasing antibiotic resistance among bacteria has made therapy of UTI difficult.

Herbs and spices have been used since ancient times in the treatment of UTI, because of their antimicrobial properties. Cinnamon is one of the spices used to treat UTI and also act as a health-promoting agent for the treatment of diseases such as inflammation, gastrointestinal disorders (Brierley and Kelber, 2011 and Al-Jiffri et al., 2011), anti-oxidant (Hoque et al., 2008 and Asimi et al., 2013), antimicrobial (Nabavi et al., 2015 and Marchese et al., 2014), anti-diabetic and anti-tumor. Cinnamon is the bark of the evergreen tropical cinnamon tree. It may be in the form of quill or ground powder. So it not only adds aroma and taste to your food but also has profound health benefits. *Cinnamomum*, commonly used as spices, contain many antibacterial compounds and the term cinnamon commonly refers to the dried bark of *C. zeylanicum* and *C. aromaticum* (Jakhetia et al., 2010). *C. zeylanicum* could be considered a more valuable in the treatment of infection and mainly against MRSA bacteria (Mandal, et al., 2011).
Eclipta alba is commonly known as Bhringaraja belonging to the family Asteraceae/Compositae which is small branched annual herbaceous plant with a long history of traditional medicines used as antitymotic, analgesic, antibacterial, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, immunomodulatory properties (Manoj Kumar Pandey et al., 2011) and also used in catarhral jaundice and for skin diseases (Dalal et al, 2010). Chemical constituents of Eclipta alba (L.) Hassk are ecliptal, ecliptine, ecliptalbine, α-terthienylmethanol, β-amyrin and sigmasterol (Prasad et al, 2012). In this present study, the antimicrobial effect of the chloroform and acetone extracts of C. zeylanicum and Eclipta alba were tested against UTI causing pathogens Escherhia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Staphylococcus aureus. To evaluate the antibacterial activity of C. zeylanicum and Eclipta alba (L.) Hassk extracts in comparison with a known drug of antibacterial activity such as a few antibiotics.

2. Materials and Methods

2.1 Isolation of test pathogens:

Test pathogens were isolated from urine samples of UTI infected patients and were cultured in the nutrient broth.

2.2 Identification of test pathogens:

Gram staining procedure was adopted to differentiate between Gram positive and Gram negative organisms. Selective agar medium was used for further identification as follows:

Mac Conkey’s Agar : E. coli
PABM : Pseudomonas aeruginosa
EMB Agar : Klebsiella pneumoniae
MSA : Staphylococcus aureus

Identification was carried out based on the biochemical reactions. Nine biochemical tests were performed for each organism. They were Motility, Indole production test, MR (Methyl Red), VP (Vogus-Proskauer) test, Citrate utilization test, Catalase test, TSI (triple sugar iron) agar test, Urease activity test and Hydrogen Sulphide Production.

2.3 Chloroform and acetone extraction:

125 gms of bark of C. zeylanicum and areal part Eclipta alba were taken in separate containers and add 250 ml chloroform and acetone in individual containers. Extracts were prepared using Soxhlet apparatus (Tanira, et al, 1994). The extract collected was concentrated by exposing them in a laminar air flow and stored 4°C until further use (Caceres et al, 1995). These extracts were further used to study the antibacterial activity against the urinary tract pathogens.

2.4 Concentration of antibiotics discs

Amikacin - 10 mcg/disc
Ciprofloxacin - 5 mcg/disc
Norfloxacin -10 mcg/disc
Gentamycin- 5 mcg/disc

2.5 Antibacterial assay:

Petri plates containing 20 ml of Nutrient Agar medium were seeded with a 24 hrs old culture of the bacterial strains. The extracts were dissolved in Di Methyl Sulfoxide (DMSO) as 100 g of extract with 100 l DMSO (100%), 25 µl, 50 µl, 75 µl and 100 µl concentrations of bark extracts were impregnated into sterile 6mm diameter discs. Discs are dried and dispensed on the solidified Nutrient Agar, inoculated with test pathogens. Incubation was made at 37°C for 24hrs. The assessment of antibacterial activity was based on the measurement of diameter of the inhibition zone formed around the discs (Hudzicki, 2009). Inhibition zones with diameter less than 12 mm were considered as non antibacterial activity, diameters between 12 and 16 mm considered as moderately active and these with more than 16mm were considered as highly active (Indu, et al, 2006).

3. Results and Discussion

UTIs are considered as the most severe health problems facing the world. The present study has exposed the importance of natural herbs to control antibiotic resistant in bacteria which are being a threat to human health. Colony morphology were studied on nutrient agar medium and then on the selective media. Based on biochemical analysis, four different bacteria Escherhia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Staphylococcus aureus were isolated from urine of UTI infected patients. The results of final identification on the basis of biochemical analysis were shown in the Table 1. The Antibiotic sensitivity pattern of test pathogens showed several susceptible and resistant (Table 2). Antibacterial activity of C. zeylanicum and Eclipta alba on test pathogens was given in Table 3 and Table 4 respectively. In both C. zeylanicum and Eclipta alba no results were found in 25 µl concentration on both extracts except chloroform extract of C. zeylanicum on Klebsiella pneumonia and maximum size of zone of inhibition were observed in 100 µl concentrations. The zone of inhibition increased with increased concentration in both test plant materials. Cinnamon exhibited antibacterial activity against all the test pathogens. These results were in agreement with the findings of Anandharaj and Saju Varghese, 2015 and Jyothiprabha and Venkatachalam, 2015. To all the four test pathogens, C. zeylanicum had highest activity than Eclipta alba. According to Manoj Kumar Pandey et al., 2011 hexane extract of showed Eclipta alba high antibacterial activity against S.aureus, B.cereus, E.coli, S.typhi, K.pneumoniae, S.pyogenes and P.aeruginosa.
whereas acetone, ethanol, methanol and aqueous extracts showed intermediate activity against *S.aureus*, *B.cereus*, *E.coli*, *S.typhi*, *K.pneumoniae*, *P.aeruginosa*, *P.mirabilis* and *S.pyogenes*. Similar studies (Uddin *et al.*, 2010, Chitravadivu *et al.*, 2009) elsewhere also recorded that the ethanol aerial parts extract of *Eclipta alba* revealed high antibacterial activity for *S.aureus*, *E.coli*. Figure 1 to 4 showed the comparison of antibacterial activity of *C. zeylanicum* and *Eclipta alba* against *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus* with zone of inhibition of standard antibiotic respectively. The results of the present study showed that the selected plants *C. zeylanicum* and *Eclipta alba* extracts was effective against the test bacteria. The results of the present study also support the medicinal value and usage of the studied spice and herb extracts can be used as antimicrobial agents and *C. zeylanicum* showed more or less equal zone of inhibition when compared with commercially used drugs to treat UTIs caused by these test pathogens.

### Table 1: Biochemical reactions.

| Tests                | Organism confirmed | *E. coli* | *Pseudomonas aeruginosa* | *Klebsiella pneumoniae* | *Staphylococcus aureus* |
|----------------------|--------------------|-----------|--------------------------|-------------------------|-------------------------|
| Motility             |                    | +         | +                        | -                       | +                       |
| Indole               |                    | +         | -                        | -                       | -                       |
| MR                   |                    | +         | -                        | -                       | +                       |
| VP                   |                    | -         | -                        | +                       | +                       |
| Citrate              |                    | -         | +                        | +                       | -                       |
| Catalase             |                    | +         | +                        | +                       | +                       |
| TSI                  |                    | +         | +                        | +                       | +                       |
| Urease               |                    | +         | +                        | +                       | -                       |
| Hydrogen Sulphide Production |          | -         | -                        | -                       | +                       |
| Gram staining        |                    | -         | -                        | -                       | +                       |

### Table 2: Antibiotic sensitivity pattern of urinary tract pathogens.

| Name of test pathogens | Antibiotics (Zone of inhibition in mm) |
|------------------------|----------------------------------------|
| *E. coli*              | Norfloxacin 22                         |
| *Pseudomonas aeruginosa* | Ciprofloxacin 25                     |
| *Klebsiella pneumoniae* | Amikacin 20                           |
| *Staphylococcus aureus* | Gentamycin 19                         |

### Table 3: The *C. zeylanicum* sensitivity pattern of urinary pathogens

| Name of Extracts | Pathogens                  | Zone of inhibition (mm) |
|------------------|----------------------------|-------------------------|
|                  |                            | Concentration (µl)      |
|                  |                            | 25  | 50  | 75  | 100 |
| Chloroform       | *Pseudomonas aeruginosa*   | -   | 12  | 16  | 23  |
|                  | *Klebsiella pneumoniae*    | 12  | 15  | 16  | 16  |
|                  | *Staphylococcus aureus*    | -   | 12  | 17  | 26  |
| Acetone          | *E. coli*                  | -   | -   | 17  | 21  |
|                  | *Pseudomonas aeruginosa*   | -   | 12  | 12  | 20  |
|                  | *Klebsiella pneumoniae*    | -   | 16  | 16  | 16  |
|                  | *Staphylococcus aureus*    | -   | -   | -   | 18  |
Table 4: The *Eclipta alba* sensitivity pattern of urinary pathogens.

| Name of Extracts | Pathogens               | Zone of inhibition (mm) | Concentration (µl) |
|------------------|-------------------------|-------------------------|--------------------|
|                  |                         |                         | 25                 |
| Chloroform       | *E. coli*               |                         | 50                 |
|                  | *Pseudomonas aeruginosa*|                         | 75                 |
|                  | *Klebsiella pneumoniae* |                         | 100                |
|                  | *Staphylococcus aureus* |                         |                    |
| Acetone          | *E. coli*               |                         | 25                 |
|                  | *Pseudomonas aeruginosa*|                         | 50                 |
|                  | *Klebsiella pneumoniae* |                         | 75                 |
|                  | *Staphylococcus aureus* |                         | 100                |

![Fig. 1: Antibacterial activity of *C. zeylanicum* and *Eclipta alba* against *E.coli*.](image-url)

*Fig. 1:* Antibacterial activity of *C. zeylanicum* and *Eclipta alba* against *E.coli.*
Fig. 2: Antibacterial activity of *C. zeylanicum* and *Eclipta alba* against *Pseudomonas aeruginosa*.

Fig. 3: Antibacterial activity of *C. zeylanicum* and *Eclipta alba* against *Klebsiella pneumoniae*.
Fig. 4: Antibacterial activity of *C. zeylanicum* and *Eclipta alba* against *Staphylococcus aureus*

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