

ANTIMICROBIAL ACTIVITIES OF *EMBLICA OFFICINALIS* AND *CORIANDRUM SATIVUM* AGAINST GRAM POSITIVE BACTERIA AND *CANDIDA ALBICANS*

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Abstract

Present investigation focused on antimicrobial potential of aqueous infusions and aqueous decoctions of *Emblica officinalis* (amla) and *Coriandrum sativum* (coriander) against 186 bacterial isolates belonging to 10 different genera of G +ve bacterial population and 2 isolates of *Candida albicans* isolated from urine specimens. The well diffusion technique was employed. Aqueous infusion and decoction of *Emblica officinalis* exhibited potent antimicrobial activity against *Staphylococcus aureus* (80), *S. haemolyticus* (8), *S. saprophyticus* (65), *Micrococcus varians* (12), *M. lylae* (6), *M. roseus* (3), *M. halobius* (1), *M. sedenterius* (2), *Bacillus subtilis* (8), *B. megaterium* (1) and *Candida albicans* (2). The aqueous infusion and decoction of coriander did not show any antimicrobial activity against G -ve urinary pathogens as well as against *Candida albicans*.

Introduction

Herbs and spices are the most important part of human diet. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value (DeSouza *et al.*, 2005; Saeed & Tariq, 2006), which forms one of the oldest sciences. It is only in recent years that modern science has started paying attention to the properties of spices (Chaudhry & Tariq, 2006). Because of the concern about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Ansari *et al.*, 2006).

The fruit of *Emblica officinalis* commonly known as amla is highly valued in traditional Indian medicine (Scartezzini *et al.*, 2006). In Unani medicine the dried fruits of amla are used to treat haemorrhage, diarrhoea and dysentery (Parrotta, 2001). In addition, the fruit of *E. officinalis* is diuretic (Anon., 2006), adaptogenic (Rege *et al.*, 1999), hepatoprotective (Jeena *et al.*, 1999; Jose & Kuttan, 2000), antitumor (Jose *et al.*, 2001), hypcholesterolemic (Kim *et al.*, 2005), antioxidant (Bhattacharya *et al.*, 1999) and antiulcerogenic (Sairam *et al.*, 2002). The fruits are also reported to be anti-inflammatory (Sharma *et al.*, 2003), analgesic and antipyretic. Several constituents of *E. officinalis* fruit has been identified, mainly the hydrolysable tannins, emblicanin A, emblicanin B, punigluconin and pedunculagin (Perianayagam *et al.*, 2005). Emblicanin A and B have been proposed to be the active constituents with significant *In vitro* antioxidant activity (Ghosal *et al.*, 1996).

Earlier studies have demonstrated potent antimicrobial properties of *E. officinalis* (Ahmed *et al.*, 1998) and it is used as antiviral for cold and flu. In the respiratory infections, it has an antibiotic activity against a wide range of bacteria, used traditionally in the treatment of lungs (Chopra & Simon, 2000). It also has shown antifungal activity *In vitro* (Dutta *et al.*, 1998).

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Coriandrum sativum (coriander) is considered both as an herb and a spice. Both its leaves and seeds are used as seasoning condiment. Coriander seeds have health-supporting reputation that is high on the list of healing spices. It has traditionally been referred to as antidiabetic (Gray & Flatt, 1999), anti-inflammatory and cholesterol lowering (Chithra & Leelamma, 1997). In addition, it is also used as carminative, diuretic, stimulant, stomachic, refrigerant, aphrodisiac, analgesic (Chaudhry & Tariq, 2006), antihelmintic (Equale *et al.*, 2006) and hypoglycemic (Waheed *et al.*, 2006).

The seeds of *C. sativum* contain 0.5-1 % essential oil and are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol and linalool. Coriander's flavonoides include quercitin, kaempferol, rhamnetin and epigenin. It also contains active phenolic acid compounds including caffeoic and chlorogenic acid. Research also suggests that the volatile oils found in the leaves of *C. sativum* plant may have antimicrobial properties against food borne pathogens such as *Salmonella* species (Isao *et al.*, 2004).

The present study was therefore conducted to evaluate the antibacterial potential of aqueous infusions and decoctions of *E. officinalis* and *C. sativum* against 345 different isolates belonging to 6 genera of Gram-ve negative bacilli isolated from urine specimens viz., *Escherichia coli* (270), *Klebsiella pneumoniae* (51), *K. ozaenae* (3), *Proteus mirabilis* (5), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (1), *S. paratyphi A* (2), *S. paratyphi B* (1) and *Serratia marcescens* (2).

Materials and Methods

Maintenance of isolates: A total of 186 isolates belonging to 10 different species of G +ve bacteria and 2 isolates of *C. albicans* isolated from urine specimens were maintained on tryptone soy agar (TSA) (Oxoid).

Preparation of aqueous infusions: Aqueous infusions of *E. officinalis* and *C. sativum* were prepared by steeping 20g in 100 ml sterile distilled water in separate sterile flasks. The flasks were kept for two days with occasional shaking. The contents of flasks were filtered.

Preparation of aqueous decoctions: Aqueous decoctions of *E. officinalis* and *C. sativum* were prepared by boiling 20g in 100 ml sterile distilled water for 15 minutes. The flasks were then plugged and removed from heat and allowed to cool. After cooling the contents of flasks were filtered.

Screening of antibacterial activity

Media: Mueller-Hinton agar (MHA) (Merck) was used as base medium for screening of antibacterial activity and Mueller-Hinton broth (MHB) (Merck) for preparation of inoculum.

Preparation of McFarland Nephelometer standard: McFarland tube number 0.5 was prepared by mixing 9.95 ml 1% Sulphuric acid in MHB and 0.05 ml 1% Barium chloride in distilled water in order to estimate bacterial density (Saeed & Tariq, 2006). The tube was sealed and used for comparison of bacterial suspension with standard whenever required.

Table 1. Antimicrobial activities of aqueous infusion and decoction of *Emblica officinalis* and *Coriandrum sativum* against G +ve bacteria and *Candida albicans*.

| S. No. | Organisms | No. of isolates | Mean zone of inhibition \pm Standard deviation | | | |
|--------|------------------------------|-----------------|--|------------------|---------------------------|-----------|
| | | | <i>Emblica officinalis</i> | | <i>Coriandrum sativum</i> | |
| | | | Infusion | Decoction | Infusion | Decoction |
| 1. | <i>Staphylococcus aureus</i> | 80 | 18.32 \pm 2.15 | 22.45 \pm 4.12 | 0 | 0 |
| 2. | <i>S. haemolyticus</i> | 8 | 18.30 \pm 3.95 | 23.32 \pm 3.15 | 0 | 0 |
| 3. | <i>S. saprophyticus</i> | 65 | 17.63 \pm 1.32 | 19.59 \pm 3.22 | 0 | 0 |
| 4. | <i>Micrococcus varians</i> | 12 | 20.15 \pm 1.75 | 20.95 \pm 2.53 | 0 | 0 |
| 5. | <i>M. lylae</i> | 6 | 16.23 \pm 3.15 | 18.43 \pm 3.23 | 0 | 0 |
| 6. | <i>M. roseus</i> | 3 | 15.32 \pm 3.96 | 19.24 \pm 3.33 | 0 | 0 |
| 7. | <i>M. halobius</i> | 1 | 18.3 | 22.5 | 0 | 0 |
| 8. | <i>M. sedenterius</i> | 2 | 17.3 \pm 0.00 | 21.59 \pm 1.92 | 0 | 0 |
| 9. | <i>Bacillus subtilis</i> | 8 | 20.46 \pm 2.51 | 20.26 \pm 2.29 | 0 | 0 |
| 10. | <i>B. megaterium</i> | 1 | 17.3 | 18.3 | 0 | 0 |
| 11. | <i>Candida albicans</i> | 2 | 10.56 \pm 1.25 | 12.32 \pm 1.15 | 0 | 0 |

Preparation and standardization of inoculum: Four to five colonies from pure growth of each test organism were transferred to 5 ml of MHB. The broth was incubated at 35-37°C for 18-24 hours. The turbidity of the culture was compared with 0.5 McFarland Nephelometer standard to get 150×10^6 CFU/ml. The standardized inoculum suspension was inoculated within 15-20 minutes.

Well diffusion technique: Screening of antibacterial activity was performed by well diffusion technique (Saeed & Tariq, 2005). The MHA plates were seeded with 0.1 ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 100 μ l of each infusion and decoction of *E. officinalis* and *C. sativum* was introduced in the well.

Incubation: The inoculated plates were incubated at 35-37 °C for 24 hours and zone of inhibition was measured to the nearest millimeter (mm).

Statistical analysis: Mean zone of inhibition and standard deviations were calculated.

Results and Discussion

One hundred and eighty six urinary pathogens belonging to 10 different genera of G+ve bacteria isolated from urine specimens viz., *Staphylococcus aureus* (80), *S. haemolyticus* (8), *S. saprophyticus* (65), *Micrococcus varians* (12), *M. lylae* (6), *M. roseus* (3), *M. halobius* (1), *M. sedenterius* (2), *Bacillus subtilis* (8), *B. megaterium* (1) and *Candida albicans* (2), were used in the present study. The results of *in vitro* antibacterial activity of aqueous infusions and decoctions of *E. officinalis* and *C. sativum* are presented in Table 1.

The aqueous infusion of *E. officinalis* exhibited maximum activity against *B. subtilis* with 20.46 mm mean zone of inhibition \pm 2.51 standard deviation and aqueous decoction exhibited maximum activity against *S. haemolyticus* with 23.32 mm mean zone of inhibition \pm 3.15 standard deviation. The minimum activities of both aqueous infusion and decoction of *E. officinalis* were found against *C. albicans* with 10.56 mm \pm 1.25 SD and 12.32 mm \pm 1.15 SD respectively. Aqueous infusion and decoction of *E. officinalis*

also exhibited potent antibacterial activities against all bacterial isolates tested. The results of the present study are similar to those reported by Khanna & Nag (1973) that constituents of *E. officinalis* have been found to be active against a range of bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium tuberculosis*, *S. typhosa* and *Candida albicans*.

In the present study, the antibacterial activities of aqueous infusion and decoction of *C. sativum* were also evaluated. All tested isolates were found resistant to aqueous infusion and decoction of *C. sativum*. These findings are in fair correlation with the study carried out by Chaudhry & Tariq (2006) who found that decoction of *C. sativum* does not have antibacterial potential against G +ve and G -ve bacteria. Similarly, aqueous decoction of coriander was found to have no bactericidal activity against *Helicobacter pylori* (O'Mahony *et al.*, 2005). In contrast, some workers have found that *C. sativum* has strong antibacterial activity against both G +ve and G -ve (Al-Jedah *et al.*, 2000). Similarly, the compounds aliphatic 2E-alkenals and alkanals, isolated from the fresh leaves of *C. sativum* were found to possess bactericidal activity against *Salmonella choleraesuis* (Isao *et al.*, 2004).

The present study has revealed the importance of natural products to control antibiotic resistant bacteria which are a threat to human health and can serve as an important platform for the development of inexpensive, safe and effective medicines.

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(Received for publication 2 December 2006)