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Antibacterial activity of isoproponalic extracts of selected Indian spices against isolated Urinary Tract Infectious *E. coli*

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ABSTRACT

E. coli is the most common organism responsible for UTI infections in both male and female individuals. *Escherichia coli* bacterium is present in the human intestine, which helps in preventing the entry of pathogenic microorganisms. *E. coli* are non-pathogenic in normal conditions, but if present in excess, will become causative agent of various diseases like urinary tract infections, diarrhoea, vomiting etc. (Shelef L.A., 1983). For treatment of UTI infections in humans various drugs are used against *E-coli*. Due to increasing resistance of microorganisms to antibiotics, there is a shift of choice from synthetic to ayurvedic and naturopathy, where herbs and spices are very common ingredients of medicines (Indu MN, Hatha *et al*., 2006). Herbs and spices are used in Indian recipes as they impart aroma and flavor to it. The six spice extracts namely turmeric, black pepper, cinnamon, tamarind, fennel and clove were tested against pathogenic *E.coli*. The isopropanolic extracts exhibited antimicrobial potential against uropathogen. The Black pepper extract showed maximum zone of inhibition (with 1.0,1.5,3.0 cm at 12.5,25,50 mg concentrations respectively) while Tamarind showed minimum zone of inhibition (with 0,0,1.2cm at 12.5,25,50 mg concentrations respectively)

Key words : *E. coli, Uninary Tract Infections, Spices, Inhibition, Antimicrobial*

Introduction

Urinary tract infections are the most common type of infection in the body Most of the urinary tract infections are caused by gram-negative bacteria like *Escherichia coli*, *Klebsiella* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Serratia*. Antibiotics are used in the treatment, but the pathogenic bacteria are becoming increasingly resistant to antibiotics (MA Chowdhury *et al*., 1994; Haque MA *et al*. 2001). The indiscriminate use of antibiotics has led to evolution of multi-drug resistant pathogens. This created the search for alternative compounds having antimicrobial property. Therefore emphasis has been laid over plants having medicinal value (Ahmad *et al*., 1998 and Iwu *et al*., 1999).

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves and other plant parts. Antimicrobial properties of spices have been documented in ancient literature and the interest continues to the present (Sibanarayan Rath, Rabindra N. Padhy 2014). The present study investigates the antimicrobial effect of selected spices against isolated UTI *E.coli* as an alternative to antibiotics and spread awareness of utility of natural antimicrobials.

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Table 1. List of used spices and their scientific name

Common Name	Scientific Name
Turmeric	*Curcuma longa*
Black pepper	*Piper nigrum*
Cinnamon	*Cinnamonum verum*
Tamarind	*Tamarindus indica*
Fennel	*Foeniculum vulgare*
Clove	*Syzygium aromaticum*

Material and Methods

Isolation of uropathogen

The *E.coli* was isolated from infected samples in **Chandan Diagnostics** at Biotech Park. Isolates were purified by streaking on nutrient agar and pure cultures were maintained.

Preparation of plant extracts

The samples were washed thoroughly 2-3 times with running tap water and once with sterile water, air-dried, powdered and used for extraction Extract of each spice was prepared by 10 g of dry spice in 50 ml of isopropanol at 30°C for 48 hours in shaking condition at 120 r.p.m. and then solvents were separated by rotatory evaporator respectively. Then concentrated extracts were dried on water bath. After complete solvent evaporation, extracts were dissolved in dimethylsulphoxide (DMSO) to a final concentration of 50 mg/ml and stored at 4°C in labelled sterile eppendroff for further use.

Effect of spice extracts on growth of *Escherichia coli*

The spices namely turmeric (*Curcuma longa*), black pepper (*Piper nigrum*), cinnamon (*Cinnamomum aromaticum*), fennel (*Foeniculum vulgare*), clove (*Syzgium aromaticum*), tamarind (*Tamarindus indica*) were used for the present study were procured from the local market.

Preparation of broth

Bacteria are generally cultivated in broth, the medium devoid of agar. In fact requirements of nutrients are met by supplementing beef extract (which is source of mineral salts, organic carbon and nitrogen, vitamins etc.) and peptone (a semi digested protein). Peptone (5.0 gm), beef extract (3.0 gm), distilled water (100 ml), pH (7).

Preparation of nutrient agar medium

Nutrient agar is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria. It is useful because it remains solid even at relatively high temperature. Also bacteria grown in nutrient agar grows on the surface and is clearly visible as small colonies. In nutrient broth, the bacteria grow in the liquid, and are seen as a soupy substance, not as clearly distinguishable clumps. Peptone (0.5 gm), beef extract (0.3 gm), NaCl (0.5 gm), distilled water (100 ml), and agar (1.5 gm) .

Preparation of Inoculum

The isolated *E.coli* culture was inoculated in nutrient broth and incubated at 35° C for 48 hours. This culture was used for antimicrobial assay.

Antibacterial activity assay

Antibacterial activity of aqueous and extracts was determined by agar well diffusion method. Seeded agar was prepared with inoculum (*E.coli*) containing 10^6 CFU/ ml. With the help of sterile cork borer three wells were made per plate in the well solidified nutrient agar plates. Different extracts were added to these wells (25µl , 50µl & 100µl) and plates were incubated in B.O.D at 35°C. After 24 hours diameter of clear zones produced surrounding the wells were measured to the nearest mm with the help of scale and were presented in the graph.

Result and Discussion

The isopropanolic extracts of the spices have shown strong antibacterial activity against urinary tract infectious *E.coli*. It was observed that {Figure 1 (1.1 - 1.6)} Black pepper have shown maximum zone of inhibition while Tamarind have shown minimum, only at 5 mg concentration. The antibacterial activity may be attributed to not only a single active principle but to a cocktail of a variety of active principles or alkaloids (Britto and Senthilkumar, 2001).

In the previous studies has been found to be a best solvent for extraction of the active ingredient (β asarone) from spices. Though, solvents such as isopropanol and methanol is used in the most of the previous studies were also found to be suitable for extraction of active ingredients. It is well established that the β-asarones found in leaf and spices, root, rhizomes tissues are responsible for almost all of the antimicrobial activities of the spices (MC Graw-Hill companies Inc 2004).

The active ingredients of spices may exhibit anti-

Fig.1.1 Extracts of turmeric against UTI *E.coli*

Fig.1.2. Extracts of black pepper against UTI *E.coli*

Fig. 1.3. Extracts of cinnamon UTI *E.coli*

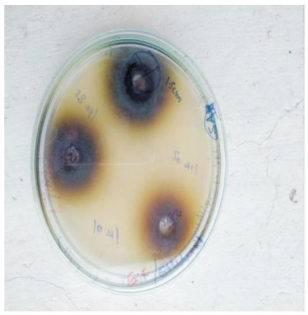


Fig. 1.4. Extracts of tamarand against UTI *E.coli*

Fig. 1.5. Extracts of fennel against UTI *E.coli*

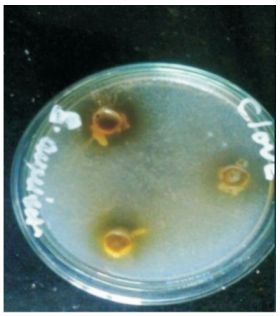


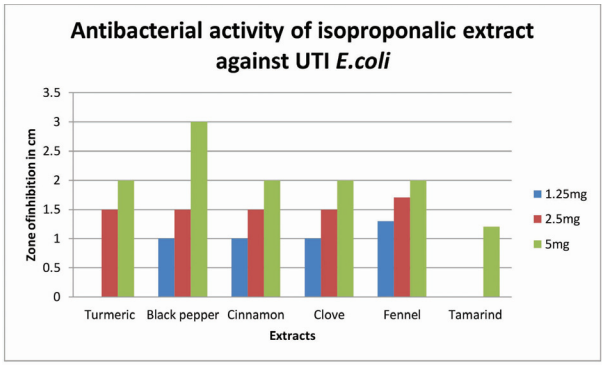
Fig. 1.6. Extracts of clove against UTI *E.coli*

Fig. 1 (1.1-1.6). Effect of different spices extract on growth of *E. coli*

Table 2. Antibacterial activity of isopropanolic extracts of turmeric diameter of inhibition zone with different concentration (µg) of turmeric extract

Extract	1.25mg	2.5mg	5.0mg
Turmeric	Nil	1.5cm	2.0cm
Black pepper	1.0	1.5	3.0
Cinnamon	1.0	1.5	2.0
Fennel	1.0	1.3	1.7
Clove	1.0	1.5	2.0
Tamarind	Nil	Nil	1.2

* Reading is mean of extract samples



Graph 1. Zone of inhibition at different concentrations

microbial potential either by degradation of cell wall, disruption of cytoplasmic membrane, leakage of cellular components, damage protein, interfere with the enzymatic activities inside cell (Seema Rawat and Anurag Rawat, 2015). Several recent papers reported that the presence of antibacterial activity are due to flavonoids (Harborne, 1973.) Alkaloids are important defense of the plants against pathogenic organisms and herbivores.

Conclusions

In view of the above results, it can be inferred that addition of spices to the food preparations helps to keep a check on the concentration of *Escherichia coli* in the body. These spices are commonly used for taste and aroma in the food. However compounds can be isolated from these spices and formulations can be prepared as natural and novel antimicrobials.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

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Conflict of Interest

The authors declare that no conflict of interst exists.

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