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In vitro Combinational Effect of Bio-Active Plant Extracts on Common Food Borne Pathogens

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Abstract: The antibacterial effects *in vitro* of crude ethanol and aqueous extracts of Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Turmeric (*Curcuma longa*) and Neem (*Azadirachta indica*) were assayed against *Staphylococcus aureus*, *Salmonella typhi* and *E. coli* were positive. The antibacterial activity was determined only by agar disc diffusion method and it gave clear zone of inhibition. The aqueous and ethanolic plant extracts containing diffusion disc of diameter 5 mm with 167 µg/disc, gave the zone of inhibition. The zone diameter was less in single extracts but in combinations it was more effective. The highest inhibition zone of 15 mm was observed with synergistic combinations of Garlic and Turmeric (70% Ethanolic) extracts on *Staphylococcus aureus,* Garlic and Turmeric (Aqueous), Garlic and Turmeric (70% Ethanolic), Ginger and Turmeric (70% Ethanolic) on *Salmonella typhi*. The *E. coli* was more resistant than the other two organisms. Highest zone of inhibition observed with Garlic and Turmeric (Aqueous), was 13 mm. Results of these kind herald an interesting promise of designing a potentially active antibacterial synergized agent of plant origin. The control experiment was done with O-Trimoxazole, Ampicillin-A, Cloxacilin, Chloramphenicol of Hi-media Company.

Key words: Atibacterial activity, plant extract, food borne pathogen, disc diffusion method

INTRODUCTION

Plant and plant derived products have been used extensively throughout the history as anti-infective agents. It is an age old practice in traditional Africa and is a source of great economic value in the Indian subcontinent. It has been estimated that there are 250,000 to 500,000 species of plant on earth, relatively small percentage of these are used as food by both humans and other animal species and even more are used for medicinal purposes (Ekweney and Elegalam, 2005). Many plants extracts have shown the presence of antimicrobial properties, because of the presence of anti-infective agents, like emetine, quinine and berberine remain highly effective instruments in the fight against microbial infections. There are wide range of current antibiotics, that are used for the treatment of bacterial infections but are still some challenges to be met in microbial chemotherapy. One of the problems in the development of resistance of chemotherapeutic agent is due to abuse of these drugs (Reuter, 2005). Because of the side effects and the resistance that pathogens build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species. The use of plant extracts, as well as other alternative forms of medicinal treatments, is enjoying great popularity in late 1990s (Cowan, 1999).

The Gram negative bacteria show more resistant than Gram positive bacteria. Such resistance is due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan and Abu-Hasan, 1998).

The main objective of this study is to determine the phytochemicals effectiveness of the plants extracts against the microorganism which cause common intestinal infection in the human due to

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consumption of the contaminated food *in vitro*. The antibiotic sensitivity test was determined only by agar disc diffusion method and it gave clear zone of inhibition. These results were compared with the potent antibiotics that are effective against these diseases.

MATERIALS AND METHODS

The research were carried out at Administrative Management College, Bangaloare, India during March-October 2006.

Plant Material and Preparation of Extracts

The plant material used in this study consisted of Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Turmeric (*Curcuma longa*), Neem (*Azadirachta indica*) which were collected from local market, Bangalore, India in March 2006. Fresh plants material was washed under running tap water, air dried. After that ginger, garlic and turmeric were peeled aseptically, cut into pieces and kept at 60°C for three days in hot air oven and then grounded using blender.

Crude Extraction

Initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous or ethanol extractions and followed by various organic methods.

Aqueous Extraction

Ten grams of the ground material were soaked in 100 mL of the hot sterile water and allowed to stand for 72 h. The crude extract were obtained by filtration and stored at 4°C when not in use.

Solvent Extraction

Ten grams of dried plant was extracted with 100 mL of 70% ethanol in conical flasks sealed with foil and allowed to stand for 72 h. They were filtered to obtained crude ethanolic extracts and stored at 4°C when not in use.

Microorganisms

The microorganisms were obtained and identified in Vaidehi Medical College, Whitefield, Bangalore, India. The organisms are *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*.

Antibacterial Assay

The antibacterial activity of different plant species were evaluated by agar disc diffusion method using Nutrient agar (Hi-media) for the assay. The microorganisms were activated by inoculating a loopful of the strain in the nutrient broth (25 mL) and incubated at 37°C for 24 h.

0.1 mL of inoculum was inoculated into the pre solidified agar plates and spread plate technique was applied using L-shaped spreader. Sterile filter paper disc (The Whatman No. 1) were impregnated with different crude extracts and mixtures and dried in a hot air oven at 60°C for five min. The combination of extracts was summarizing in Table 1.The concentration of the disc was found to be 167 µg/disc.

The control experiment was carried out with O-Trimoxazole Co^{25} (Sulpha/trimethoprim), Ampicillin-A, Cloxacilin and Chloramphenicol (30 mg/disc).

RESULTS

The antibacterial activities of the extracts obtained from the plants under study by diffusion method were shown in the Table 2.

The antibacterial activity of the control antibiotic disc (Hi-Media) was shown in the Table 3.

Table 1: Extracts and its combination

Sl No.	Extract/ Combination
E1.	Ginger (Aqueous extract)
E 2.	Ginger (Ethanolic extract)
E 3.	Garlic (Aqueous extract)
E 4.	Garlic (Ethanolic extract)
E 5.	Turmeric (Aqueous extract)
E 6.	Turmeric (Ethanolic extract)
E 7.	Neem (Aqueous extract)
E 8.	Neem (Ethanolic extract)
E 9.	Ginger + Garlic (Aqueous extract)
E 10.	Ginger + Garlic (Ethanolic extract)
E 11.	Turmeric + Neem (Aqueous extract)
E 12.	Turmeric + Neem (Ethanolic extract)
E 13.	Garlic + Turmeric (Aqueous extract)
E 14.	Garlic + Turmeric (Ethanolic extract)
E 15.	Ginger + Turmeric (Aqueous extract)
E 16.	Ginger + Turmeric (Ethanolic extract)
E 17.	Ginger + Neem (Aqueous extract)
E 18.	Ginger + Neem (Ethanolic extract)
E 19.	Garlic + Neem (Aqueous extract)
E 20.	Garlic + Neem (Ethanolic extract)

Table 2: Inhibitory properties (inhibition zone diameter in mm) of plant extract on different bacteria

Organisms	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20
S. aureus	9	8	7	13	11	11	8	10	11	9	12	9	12	15	12	11	13	12	9	9
S. typhi	10	13	8	10	-	8	6	9	10	8	11	8	15	15	8	8	8	8	8	10
E. coli	7	8	-	6	7	7	-	-	9	8	8	7	13	-	8	8	8	8	8	-

The inhibition zones including disc (5 mm)

Table 3: Inhibitory properties (inhibition zone diameter in mm) of std. antibiotic disc on different bacteria

Microorganisms	O-Trimoxazole Co^{25}	Ampicillin-A	Cloxacilin	Chloramphenicol
S. aureus	11	12	13	18
S. typhi	42	24	35	22
E. coli	-	9	8	-

The inhibition zones including disc (6 mm)

Successful prediction of botanical compounds from plant material was largely dependent on the type of solvent used in the extraction procedure and also the plant used. Garlic ethanolic extract was more consistent than Garlic aqueous. But in combination of two, aqueous extracts give good result. The zone diameter of different plant extracts was ranging from 8-15 mm in the case of *S. typhi* and *S. aureuss* where as *E. coli* shows more resistance showing diameter 7-9 mm.

DISCUSSION

Kela and Kufeji (1995) reported that antibiotics are not the only antimicrobial agents. In this study the antibacterial enhancement (synergistic effects) of *Allium sativum* with *Curcuma longa* may partly explain the use of these combinations traditional plant medicines in India against a number of infections for generations (India Ayurveda) *Azadirachta indica* was less effective in the case of said infection.

In contrast the water extract of this plant is potent this shows that water is a better extractant of the active component of this plant. Water is the major solvent mostly used for the preparation of infusions and decoctions from herbs.

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections and in food spoilage.

Result of this kind herald an interesting promise of designing a potentially active synergized agent of plant origin. Many plants have become sources of important drugs and the pharmaceutical industries have come to consider traditional medicine as a source of bio-active agents that can be used in the preparation of synthetic medicine (Aboaba *et al*., 2006).

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