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ETHANOLIC EXTRACT OF *CINNAMOMUM TAMALA* LEAVES ENHANCES SENSITIVITY ZONES OF CEPHALOSPORINS IN ESBL PRODUCING *ESCHERICHIA COLI*

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ABSTRACT
Objectives: Cephalosporin group of antibiotics are resistant to Extended Spectrum Beta Lactamases (ESBL) producing *E. coli* (ESBL Ec). As natural products are important sources of different bioactive agents it is important to find out newer antimicrobial agents from natural sources. **Materials and methods:** Ethanolic etracts of the leaves of *Cinnamomum tamala* was used to find out any synergistic action of cephalosporins and the extract against ESBL Ec. **Results:** In this study we observed an enhanced sensitivity zones of three cephalosporins – cefotaxime, ceftazidime and cefuroxime in ESBL Ec in presence of ethanolic extract of *Cinnamomum tamala* leaves. **Conclusion:** This finding appears very important regarding the emerging drug resistance of most pathogenic bacteria. The synergistic action appears due to tannins, terpenoids or flavonoids which are normally present in *C. tamala*. This finding may help in new formulations of combined antibiotic-plant extract antimicrobials which may be effective in drug resistant bacteria.

KEYWORDS: *Cinnamomum tamala*, cephalosporins, ESBL producing *E. coli*.

INTRODUCTION
In 2000, World Health Organization (WHO) and the European Commission interested in the study of emerging global drug resistance to find out the determinants responsible for such alarming drug resistance causing high morbidity and mortality rates.[1,2,3] Later in 2011 detailed meetings were completed in different regions of the globe. In South-East Asia, the health ministers of the South East Asia region's Member States cleared their obligation to combat antimicrobial resistance (AMR) through the Jaipur Declaration on AMR.[4] Since then, there has been growing alertness throughout the region. All eleven Member States have approved to add information for a regional database and to participate in a regional counseling process. A more detailed description of the present situation in each country is available in a report from a recent regional workshop which shows alarming condition.[5] Extended spectrum beta-lactamases (ESBLs) are enzymes which are produced by many bacteria by which they can hydrolyze extended spectrum cephalosporins. They can inactivate beta lactam antibiotics like ceftazidime, cefotaxime etc. and oxyimonomonobactam. It has been noted that, the ESBL-producing *Escherichia coli* (ESBL Ec) are emerging worldwide.[6] The ESBL-producing strains are particularly dreaded as they are resistant to all penicillins, to cepahlosporins, and to aztreonam. Additionally, they are often cross-resistant to trimethoprim/sulfamethoxazole and quinolones. This combination of properties can appreciably affect the course and outcomes of infections, both in the community and in the hospital setting.

Cinnamomum tamala plant, leaves of which are used in this experiment, also known as Indian bay leaf, tejpat, tejpatta, tejpata, Malabar leaf, Indian cassia etc. Its leaves emit clove-like smell due to presence of aromatic oils. It is an Angiosperm plant which may grow 20m tall The three veined leaves are olive green in colour and are twice as long as their width. The dried leaves are typically khaki in colour.

In this experiment we selected three cephalosporins which are resistant to ESBL Ec and compared any change of the sensitivity zones after combining *Cinnamomum* extract with the resistant antibiotic.

MATERIALS AND METHODS

The bacterial strains

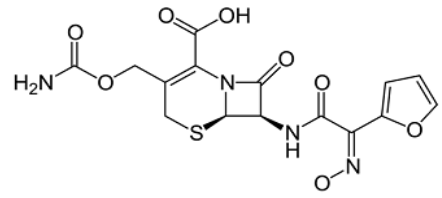
The five ESBL Ec bacterial strains used in this experiment were isolated from clinical samples. All the strains were identified by Vitek 2 compact automated system and were found ESBL producers and were resistant to cephalosporins. The culture strains were maintained in the laboratory and were used in the experiment from fresh subcultures.

The cephalosporin antibiotics

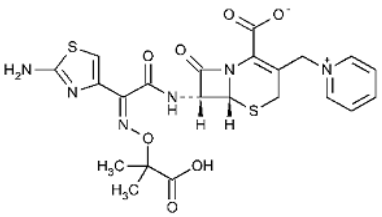
The cephalosporins are β lactam antibiotics which were originally isolated from *Acremonium* fungus, which was previously known as *Cephalosporium*. They prevent synthesis of peptidoglycan of bacterial cell wall, and are bactericidal in nature. So far there are five generations of cephalosporins. ESBL producing bacteria can inactivate third generation antibiotics easily. In this experiment we have used two third generation cephalosporins namely Ceftazidime and Cefotaxime. We have also used cefuroxime which is a second generation cephalosporin. The structures of three cepharosporins used in this experiment are given in Fig. 1.

The extraction of *Cinnamomum* leaves

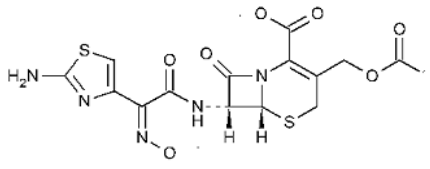
The dried *Cinnamomum* leaves were crushed in a mixer, the powder part was separated. One gram of the powder was weighed and mixed with 10 ml ethanol in a vortex mixture for five minutes. After that the supernatant was separated by centrifugation and further diluted to 500 µg/ml in ethanol. The extracted material was used fresh.



Cefuroxime



Ceftazidime



Cefotaxime

Fig.1 : Molecular structures of the three cephalosporins used in the experiment

Preparation of filter paper discs containing the *Cinnamomum* extract

The *Cinnamomum* at 500 µg/ml crude extract concentration was used to soak 6mm size sterile Whatman no. 1 filter paper discs, which were then dried in incubator and used in the experiment.

Final experiment

The bacteria was inoculated on the Mueller Hinton agar as an uniform layer with Macfarland 0.5 standard concentration. Then antibiotic discs and *Cinnamomum* discs were placed in three sets – Cinnamomum with antibiotic in one set, while only *Cinnamomum* and antibiotics in two different sets. After placement of the discs the plates were placed in the incubator at 37^{O}C and observed after overnight incubation. Sensitivity zones if present were measured and recorded, followed by statistical analysis for validation of the results.

RESULTS

The results are given in Table 1, Fig.2 and Fig.3. The differences between the sensitivity zones of cephalosporins alone and cephalosporins with the extract was found highly significant statistically with a p value of 0.0046 (t value 3.152), which clearly indicates beneficial effect of the *C. tamala* extract against drug resistance of antibiotics.

ESBL producing *E. Coli* (strain number and source)	Sensitivity zones with cephalosporins (mm) Mean±SD±SEM	Sensitivity zones with cephalosporin and Extract together (mm) Mean±SD±SEM	Sensitivity zones with extract only (mm) Mean±SD±SEM
327 Ascitic fluid	10.67±1.15±0.67	13.33±2.31±1.33	6.67±0.58±0.33
304 Urine	6.67±0.58±0.33	9.33±2.52±1.45	6.33±0.58±0.33
373 Urine	9.33±3.06±1.76	8.08±2.08±1.15	6.33±0.58±0.33
370 Urine	7.33±0.58±0.33	9.67±0.58±0.33	7.006.33±0.58
391 Urine	7.35±0.58±0.33	10.00±2.00±1.15	6.33±0.58±0.33
All strains*	7.83±1.85±0.53	10.58±2.39±0.69**	6.58±0.67±0.19

* strain no. 373 was a capsulated bacteria and the extract shows no enhancement of sensitivity zones indicating probably no activity of the extract in capsulated bacteria. The data of this strain was not used in final calculation.

**The enhancement of the sensitivity zones in cephalosporin with the extract in comparison to cephalosporin alone is statistically highly significant (p value 0.0046), while the difference of sensitivity zones between only cephalosporins and only extract was just significant with a p value of 0.0385, indicating only cephalosporin is slightly better than the crude extract regarding their antimicrobial activities.

Table 1: Sensitivity zones in different experimental sets with different ESBL Ec

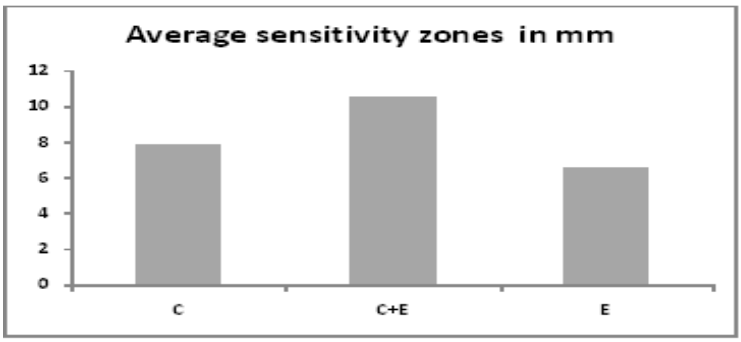


Fig.2: Average sensitivity zones in mm in different experimental sets. C-only cephalosporin, C+E- Cephalosporins + *C. tamala* extract, E- Only *C. tamala* extract.

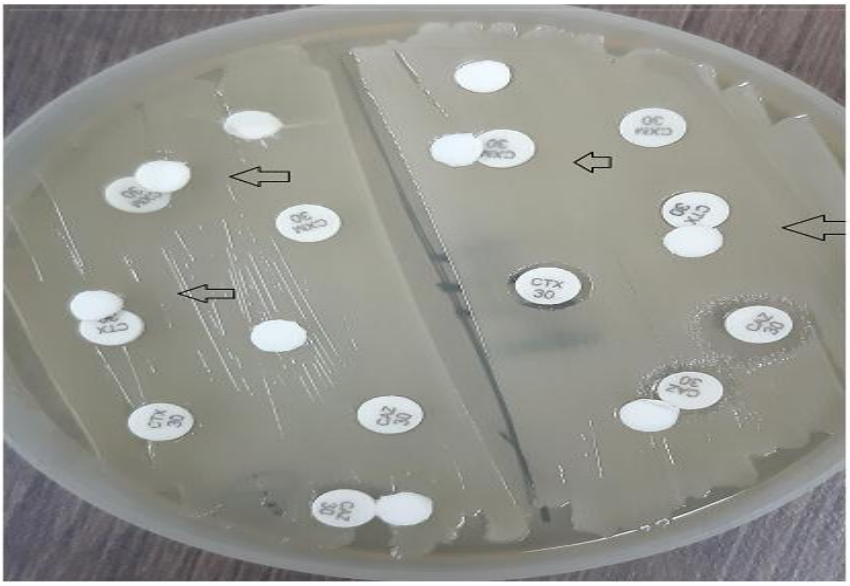


Fig. 3: Enhanced sensitivity zones of cephalosporins with the extract. CTX – Cefotaxime, CAZ-Ceftazidime, CXM-Cefuroxime. Some enhanced zones are indicated with arrow heads.

DISCUSSION

Antimicrobial properties of various plants have been used to treat many infectious diseases for centuries. Although there are more than 1,70,000 structural diversity of phytochemicals or secondary metabolites of plants, but they originate from only a few biochemical pathways – the isoprenoids and terpenoids pathway (60%); the polyphenols, phenylpropanoids or polyketides pathway (30%); and alkaloid pathway (10%). Other biochemical pathways like the glucosinolates pathway are extremely minor. The phytochemicals of *C. tamala* leaves mainly comprised of alkaloid, tannin, terpenoids, flavonoids, sterol, saponins.[7,8,9] Antibacterial[10], and antifungal[11] activities of this plant extract have been described in recent past. The extract of the leaves were found effective both in gram positive and gram negative bacteria.[12] It is well known that flavonoids show anti-inflammatory activities[13], tannins and terpenoids are antioxidants or free radical scavengers[14] and the antimicrobial property of *C. tamala* leaves may be attributed to these chemicals.

CONCLUSION

This finding appears very important regarding the emerging drug resistance of most pathogenic bacteria. The synergistic action appears due to tannins, terpenoids or flavonoids which are normally present in *C. tamala*. This finding may help in new formulations of combined antibiotic-plant extract antimicrobials which may be effective in drug resistant bacteria

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