

Estimation of antibacterial activity of purified bioactive compounds separately and in combination with Chloramphenicol against Shiga toxin producing and Enterotoxigenic *Escherichia coli*

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Abstract

The study was carried out in order to investigate potential antibacterial efficacy of *p*-Coumaroyl-glucoside + *p*-Coumaroylquinic acid, Ferulic acid and Steroidal sapogenin individually as well as in combination with Chloramphenicol against Shiga toxin producing and standard Enterotoxigenic *E. coli* strain. Estimation of antibacterial effect was carried out by well-diffusion method. Strongest inhibitory result was observed from *p*-Coumaroyl-glucoside + *p*-Coumaroylquinic acid with zone of inhibition of 23.3 ± 0.6 mm for Shiga toxin producing *E. coli* and 24.0 ± 1.1 mm for standard Enterotoxigenic *E. coli* strain. The zone of inhibition recognized from Ferulic acid was of 14.6 ± 2.4 mm and 12.0 ± 0.0 mm respectively for Shiga toxin producing and Enterotoxigenic *E. coli* strain. From Steroidal sapogenin the recognized inhibitory zone diameter found in the two strains were of 16.6 ± 0.6 mm and 12.0 ± 1.1 mm. Combined administration of the bioactive compounds and Chloramphenicol strongest interaction was recognized with *p*-Coumaroyl-glucoside + *p*-Coumaroylquinic acid and Steroidal sapogenin whereas neither synergism nor antagonism was recognized on combined administration of Ferulic acid and Chloramphenicol determined on the basis of enhancement of inhibitory zone diameter.

Keywords: *p*-Coumaroylquinic acid, Ferulic acid, Steroidal sapogenin, Chloramphenicol

INTRODUCTION

Escherichia coli belong to the member of group referred as fecal coli form. *E. coli* associated infections are generally associated with inflammation of small intestine. EHEC is one of the major and most important classes of *E. coli* that infects almost all age group of people including young and adults. It is frequently observed in immunocompromised people. Infection occurs due to consumption of contaminated and uncooked meat, ingestion of unpasteurized milk as well as swimming in contaminated lakes and swimming pools. People infected with EHEC/ STEC strains can developed some range of symptoms including blood diarrhea, fever and abdominal cramps. In some cases, Hemolytic Uremic Syndrome (HUS) was observed in children causes serious destruction of RBC and causes kidney failure. All EHEC strains produces shiga toxin (stx 1) or shiga toxin 2 (stx 2) which are also referred as verotoxin 1 (VT1) and verotoxin 2 (VT 2). Three different non- O 147:H7 serotypes of STEC producing Stx1 or Stx2 was reported by Vali *et al.* (2007) which includes O26, O103 and O145. One of the major factors responsible for Traveler's diarrhea in human is ETEC which produces two different types of enterotoxins- heat stable enterotoxins (ST) and heat- labile enterotoxins (LT). ETEC was reported to be one of the major cause of colibacillosis in piglets and calves in developing countries as well as it was reported to cause similar infections in

various domesticated animals (Nagy and Feket, 2005). ETEC was also recognized to be the major cause of diarrheal diseases in infants and young children less than 5 years of age group (Sizemore *et al.*, 2004, Clemens *et al.*, 2004, Steinsland *et al.*, 2010). The serotypes reported in ETEC strains includes O114 (Mirnejad *et al.*, 2010), O86:H(-), O128: H2, O127: H21, O111: H(-), O126: H(-) (Al-Galas *et al.*, 2006).

Medicinal plants were principally used by human through out the world for treatment of various ailments for long back. About 250, 000 species of higher plants exists on earth. India is exceptionally rich in plant diversity and for long history use of plants was carried out for various ailments in Ayurvedha. About 7000 of plants are used in Ayurvedha, 600 in Siddha , 700 in Unani and 30 in modern medicines. Plants have been used for various purposes including food, drugs and perfumery (Mukherjee and Wahile, 2006). Local healers use traditional plants for treatment of various ailments without any scientific knowledge but by their personal experience since some plants can be highly effective to cure a particular disease. Therefore, it is necessary to develop a scientific approach for better treatment and determine drug doses.

The present study deals with antibacterial activity of purified bioactive compounds detected from leaf and bark extracts of *Acacia arabica* against Shiga toxin producing and Enterotoxigenic *E. coli* strains.

METHODOLOGY USED

Plant material collection and extract preparation

Leaves and barks of the tree were collected, thoroughly washed and shade dried for 4 weeks. Finely powdered plant material

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were subjected for extract preparation as per method recommended by Johnson *et al.* (2008)

Microbial strains and culture collection

E. coli O22 (Shiga toxin producing *E. coli* strain) was obtained from diarrheagenic calf stool sample from Veterinary Hospital, Supela and standard enterotoxigenic *E. coli* strain was purchased from IMTECH, Chandigarh.

Bioassay guided fractionation of crude extracts

Methanolic crude extracts were fractioned by methods recommended by Saleem *et al.* (2002) and Sundaram and Mitra (2006). The purified bioactive compounds were obtained by subjecting the fractions to Preparative HPLC (LC-18, Shimadzu, Japan) with C-18 column (20mm x 250mm) and mobile phase acetonitrile: water (7:3) as isocratic solvent system at a flow rate of

10 ml/min with injection volume of 10 μ l. Sub fractions obtained were collected for detection of antimicrobial activity. The mass spectrum of isolated bioactive compounds were obtained by combination of Liquid Chromatography- Mass Spectrophotometry (Perkin Elmer Series, Japan) (Theerasin and Baker, 2009).

Assessment of Antibacterial activity of sub fractions

Individual peaks obtained from Preparative HPLC were tested against *E. coli* O22 and *E. coli* MTCC 723 by well diffusion method (Cock, 2008).

Assessment of synergistic /antagonistic activity between extracts and antibiotics

In vitro synergistic /antagonistic activity between bioactive compounds and Chloramphenicol was performed by well diffusion method (Odunbaku *et al.*, 2008).

Table 1. Mean Inhibitory Zone Diameter (in mm \pm SEM) of sub fractions of leaf extract

<i>E. coli</i>	<i>p</i> -Coumaroyl - glucoside + <i>p</i> - Coumaroyl quinic acid	Ferulic acid	Steroidal sapogenin	Chloramphenicol positive control
O22	23.3 \pm 0.6	14.6 \pm 2.4	8.6 \pm 0.6	0
MTCC 723	24 \pm 1.1	16.0 \pm 0	6.0 \pm 0	17.6 \pm 0.6

Table 2. Synergistic interactions between bioactive compounds and Chloramphenicol (Inhibitory Zone Diameter in mm \pm SEM)

<i>E. coli</i>	Chloramphenicol positive control	<i>p</i> -Coumaroyl - glucoside + <i>p</i> - Coumaroyl quinic acid	<i>p</i> -Coumaroyl- glucoside + <i>p</i> - Coumaroylquinic acid + Chloramphenicol positive control	Ferulic acid	Ferulic acid+ Chloramphenicol positive control	Steroidal sapogenin	Steroidal sapogenin + Chloramphenicol positive control
O22	0	23.3 \pm 0.6	27.3 \pm 0.6	14.6 \pm 2.4	15.0 \pm 0.0	8.6 \pm 0.6	16.6 \pm 0.6
MTCC 723	17.6 \pm 0.6	24.0 \pm 1.1	26.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0	6.0 \pm 0.0	12.0 \pm 1.1

RESULTS AND DISCUSSION

The inhibitory zone diameter observed from *p*-Coumaroyl glucoside + *p*- Coumaroylquinic acid, Ferulic acid and Steroidal sapogenin was of 23.3 \pm 0.6 mm, 14.6 \pm 2.4 mm and 8.6 \pm 0.6 mm respectively for *E. coli* O22 whereas the inhibitory zone diameter observed in *E. coli* MTCC 723 from *p*-Coumaroyl-glucoside + *p*- Coumaroylquinic acid and Ferulic acid and Steroidal sapogenin was of 24.0 \pm 1.1 mm, 16 mm and 6.0 mm respectively (Table-1). Panizzi *et al.* (2002) documented antibacterial efficacy of *Rubus ulmifolius* extract derived Ferulic acid which supports the present findings on antibacterial effectiveness of Ferulic acid. Sobolewska *et al.* (2006) documented antimicrobial activity of plant extracts derived from Steroidal saponin isolated from underground parts of *Allium ursinum* L.

On combined administration of Ferulic acid with Chloramphenicol no synergism was noticed whereas enhancement was recognized between *p*-coumaroyl derivatives with Chloramphenicol. Strongest *in vitro* synergistic interaction was recognized with Steroidal sapogenin with Chloramphenicol and enhancement of zone was observed in both *E. coli* O22 and *E. coli* MTCC 723 (Table-2). The findings of Neyestani *et al.* (2007) on synergistic interaction between tea extracts and its derived purified bioactive elements with antibiotics to inhibit *Streptococcus pyogenes* justifies the present findings on synergism between extracts and its

derived bioactive compounds with antibiotics to enhance inhibitory results.

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