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Comparative investigation of antibacterial activity of cold and hot extracts of *Acacia arabica* against *E. coli*

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Abstract

Both cold and hot fruit extracts of *Acacia arabica* were prepared in methanol, acetone, chloroform and water and their antibacterial activity was detected against five different diarrheagenic *E. coli strains* by broth microdilution method. MIC of cold methanolic fruit extract was found to be 0.6 mg/ml whereas MIC of hot methanolic fruit extract was observed to be 0.12-0.15 mg/ml. MIC of cold and hot acetone extract was observed to be ranged from 1.23-3.3 mg/ml and 0.4 -1.25 mg/ml whereas MIC of cold and hot chloroform fruit extract was found to be same of 0.3 mg/ml. No antibacterial activity was observed from aqueous extract. Results revealed that hot acetone and methanolic extract were more effective over cold extracts.

Keywords: Broth microdilution method

INTRODUCTION

The study deals with antibacterial effectiveness of fruit extracts of *Acacia arabica* against five different diarrheagenic *E. coli* strains. The strains used in the research purpose were Shiga toxin producing *E. coli* (STEC). In addition one standard Enterotoxigenic *E. coli* strain was used for the same purpose. STEC is one of the major and most important classes of *E. coli* that infects most of the ruminants and domesticated animals world wide. *STEC* O26: H 1 was reported to cause sporadic outbreak of diarrheal infection (Ananthanarayana and Panikar, 2009). Orden *et al.* (2002) reported that healthy cattles are also carriers of STEC strains and humans infected by direct and indirect contact with animals.

Medicinal plants in the form of extract proved to be an effective source to cure *E. coli* associated diarrheal infections in comparison to western drugs to cure domesticated animals as plants and plant derived products are easily available and cheaper with lower toxicity. But limited practice was conducted by live stock keepers to cure animals using herbs. Mc Gaw and Eloff (2008) reported that ethanoveterinary system is one of the cheapest alternative over western veterinary system.

Voravuthikunchai *et al.* (2004) analyzed MIC of ethanolic and aqueous extracts of *Quercus infectoria* and *Punica grantum* against *E. coli* O157: H7 and other non-O157 VT + *E.coli* strains like O26:H11, O111: NM, O22 and *E. coli* ATCC 25922 by broth microdilution technique. Duarte *et al.,* (2007) studied antibacterial properties of essential oils of 29 different medicinal plants against 13 different serotypes of *E. coli* including three enterotoxigenic, two enteropathogenic, three enteroinvasive and two shiga-toxin producers.

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MATERIALS AND METHODS

Plant material: Collection of fruit pods of *Acacia arabica* were done during the month of November. The pods were finely chopped after washing and shade dried for 4-5 weeks. Dried plant material was finely pulverized with the help of mixer grinder for extract preparation.

Extract Preparation: For preparation of crude extracts four solvents, methanol, acetone, chloroform and distilled water were used. Cold extracts were prepared as per methods recommended by Nair *et al.* (2005) whereas hot extracts were prepared by the technique followed by Johnson *et al.* (2008).

Culture Isolation and Identification: Four different strains of *E. coli* O22, O11, O89 and O61 were isolated. The two clinical isolates; strain O22 and O11 were isolated from diarrheagenic calf and buffalo stools obtained from Veterinary Hospital, Supela, whereas, strains O89 and O61 were isolated from environmental samples (from unorganized farms and animal husbandry drinking water sources). A standard strain, *E. coli* MTCC 723 was obtained from IMTECH, Chandigarh. Serotyping and identification of all isolated strains was done at National Salmonella and Escherichia Center, CRI, Kasauli (H.P) and ribotyping was done at DBRI, Hyderabad (A.P).

Minimum Inhibitory Concentrations (MIC): Minimum Inhibitory Concentrations of crude extracts were investigated by broth micro dilution method with concentration ranged from 10 mg/ml to 0.019 mg/ml (Kashikar and George, 2006) with some modifications. The controls used for broth micro dilution method were Chloramphenicol, Ampicillin (concentration ranged from 10 mg/ml to 0.07 mg/ml) and distilled water.

RESULTS AND DISCUSSION

The present work deals with antibacterial activity of *Acacia arabica* against various serotypes of *E. coli* associated with diarrheal infections. The strains used for the purpose were *E. coli* O22, O11,

O89, O61 and one standard MTCC strain. Fratamico *et al.* (2009) isolated *E. coli* serogroup O22 from diarrheagenic stool samples of cows, animals and human and reported it as extra intestinal pathogenic strain. Wang *et al*. (2006) reported *E. coli* O11 as Shiga toxin producing *E. coli* associated with hemorrhagic colitis and hemolytic-uremic syndrome (HUS) in humans. Dutta *et al*. (2011) isolated three serogroups of *E. coli* O89 along with other serogroups of *E. coli* associated with diarrheal outbreak in poultry birds and detected the serogroup as STEC. Dharani *et al.* (2003) isolated multi drug resistant *E. coli* O61 strains associated with colibacillosis in Chicken.

MIC of cold methanolic fruit extract (Table-1) was found to be 0.6 mg/ml for *E. coli* O22, O11, O89, O61 and 0.81 mg/ml for *E. coli* MTCC 723 respectively. MIC of cold acetone fruit extract ranged from 1.23-3.3 mg/ml whereas MIC of cold and hot chloroform fruit extract was found to be same of 0.3 mg/ml for all strains. MIC of hot methanolic and acetone fruit extracts ranged from 0.12-0.15 mg/ml and 0.4 -1.25 mg/ml respectively. Several authors reported MIC values of various plant extracts by using broth microdilution technique Minimum Inhibitory Concentration (MIC) of acetone and aqueous extract of *Quercus infectoria* was studied against *E. coli* NCTC 12079 O157:H7 and various other disease cause standard strains by broth microdilution technique using 96-well microtiter plates (Basri and Fan, 2005). Iscan *et al.* (2002) studied antibacterial activity of bioactive compounds of *Mentha piperita* essential oils against *E. coli* ATCC 25922 using microdilution techinique.

In our present study strongest inhibitory result was observed in hot methanolic fruit extract which therefore suggests that diarrheal infections associated with E. coli strains can be cured using fruit extract derived drugs of *Acacia arabica* in ruminants.

Table 1. MIC (in mg/ml) of fruit extracts (Mean ±SEM of triplicates)

E. coli	CMFE	CAFE	CCFE	CAQFE	HMFE	HAFE	HCFE	HAQFE	C	A & D.W
022	0.6± 0	1.23± 0.6	0.3± 0	0	0.12± 0.02	0.5± 0.09	0.3± 0	0	2.9± 0.5	0± 0
011	0.6± 0	1.23± 0.6	0.3± 0	0	0.12± 0.02	0.4± 0.09	0.3± 0	0	0.32± 0.15	0± 0
089	0.6± 0	3.3± 0.83	0.3± 0	0	0.15± 0	0.6± 0	0.3± 0	0	1.03±0.21	0± 0
061	0.6± 0	1.23± 0.6	0.3± 0	0	0.15± 0	1.03± 0.6	0.3± 0	0	1.23± 0.6	0± 0
MTCC 723	0.81± 0.2	2.08±0.41	0.3± 0	0	0.12± 0.02	1.25± 0	0.3± 0	0	0.4± 0.09	0± 0

CMFE: Cold Methanolic Fruit Extract, CAFE: Cold Acetone Fruit Extract, CCFE: Cold Chloroform Fruit Extract, CAQFE: Cold Aqueous Fruit Extract, HMFE: Hot Methanolic Fruit Extract, HAFE: Hot Acetone Fruit Extract, HCFE: Hot Chloroform Fruit Extract, HAQFE: Hot Aqueous Fruit Extract, C: Chloramphenicol, A: Ampicillin, D.W: Distilled Water

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