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Validation of antibacterial activity of Saponin against diarreagenic *E.coli* isolated from leaves and bark of *Acacia arabica*

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Abstract
The leaf and bark extract of *Acacia arabica* constitute large amount of saponin (sapogenin aglycon) along with other polyphenolic compounds. The isolated sapogenin aglycon was tested against *E.coli* O22 associated with diarrheal infections in domesticated animals along with *E.coli MTCC 723* strain. Both well-diffusion and broth micro dilution technique was used to detect antibacterial activity. Synergistic interaction was observed on combined administration of saponin with Chloramphenicol to inhibit tested organism.

Keywords: Sapogenin aglycon, Broth micro dilution technique

INTRODUCTION

Bioactive compounds are the safest and more effective alternative than synthetically produced antimicrobial agents (Banso, 2009, Tang *et al.,* 2010). In recent years, the use of plants and plant based products gain much importance for treatment of various infections (Bagyalakshmi *et al.,* 2009). The use of plant derived bioactive analogues serves as the safest agent to cure bacterial infections associated with multi drug resistant strains. Saponins are the glycosides present in almost all plants belonging to family Leguminosae. It is also present in other plants belonging to different families. The present study deals with antibacterial and synergistic activity of sapogenin aglycon isolated from *Acacia arabica* against *E.coli*.

MATERIALS AND METHODS

Culture collection and identification

E.coli O22 was obtained from stool sample of diarrheagenic calf and standard strain *E.coli* MTCC 723 was purchased from IMTECH, Chandigarh

Collection and preparation of crude extract

Leaves and bark of *Acacia arabica* was collected during November in specimen voucher and thoroughly washed and shade dried. All plant materials were separately pulverized for preparation of extracts. Crude methanolic extract was prepared by the method recommended by Johnson *et al.,* (2008).

Separation and purification of saponin

Crude methanolic extract of leaf and bark was fractioned using column chromatography as per method recommended by Saleem *et al.* (2009) Sundaram and Mitra, (2006). Purified bioactive compounds were obtained by subjecting the fractions to Preparative HPLC (LC-18, Shimadzu, Japan) with C-18 column (20mm x 250mm). The mobile phase used was acetonitrile: water (7:3) at a flow rate of 10mL/min. The injection volume of fractions of leaf and bark extract was of 10 µl. The isolated saponin was evaporated to dryness under vacuum for detection of antibacterial activity (Kyung *et al.,* 2007). The mass spectrum of isolated saponin was analyzed using LC analysis Perkin Elmer Series (Japan) through positive electrospray ionization mass spectrometer (ESI-MS). The isocratic mobile phase was 100% methanol with flow rate of 10mL/min (Theerasin and Baker, 2009).

Antibacterial activity

Antibacterial activity was determined by well diffusion method (Cock, 2008) and broth microdilution method (Kashikar & George, 2006, Mann *et al.,* 2009) with some modifications.

Synergistic activity

In vitro synergistic activity between saponin and chloramphenicol was performed using well diffusion method (Odunbaku *et al.,* 2008).

RESULTS AND DISCUSSION

Acacia arabica is used widely for treatment of various infections including treatment of diarrhea and dysentery (Chopra *et al.,* 1992). There are reports on antibacterial activity of leaf and bark extract of *Acacia arabica* on *E.coli* (Dabur *et al.,* 2007). However, a very limited work was carried out in field of ethanoveterinary medicines to cure diarrheal infections in animals. Lans *et al.,* (2007) documented ethanoveterinary medicine (EVM) is the scientific term used for treatment of animals by using plants. In addition, very limited work was carried out on concurrent administration of both herbal drugs and convectional antibiotics to cure infections. The present study was adopted to assess antibacterial activity of leaf and

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bark extract derived steroidal sapogenin aglycon using well diffusion and broth micro dilution technique against diarrheagenic *E.coli* responsible for infection in animals. Among both methods broth microdilution method was the most reliable method for accurate quantification of antibacterial activity of sapogenin. In addition, another aim of the study was to investigate interaction of saponin with convectional antibiotics for more effective inhibition of tested organism. Table: 1 represents antibacterial activity of compound by well diffusion method on the basis of diameter of zone of inhibition and Table: 2 represent the activity of compound on the basis of MIC performed by broth microdilution assay using INT dye. The findings on antibacterial effectiveness of saponin is in agreement with the findings of other researchers (Arabski *et al.,* 2012, Hassan *et al*., 2010) who documented effectiveness of saponin against *E.coli*.

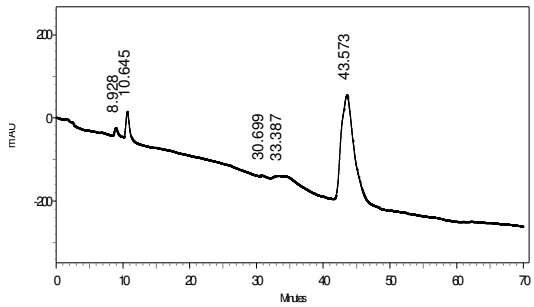

Fig 1. Preparative HPLC Chromatogram of leaf extract fraction

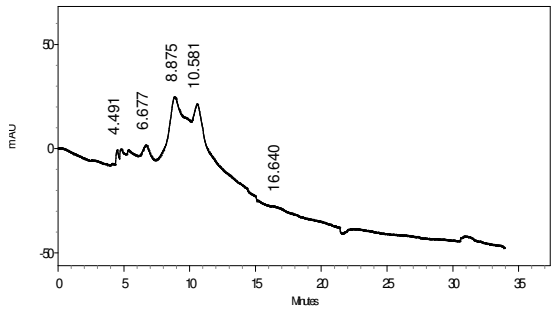

Fig 2. Preparative HPLC Chromatogram of bark extract fraction

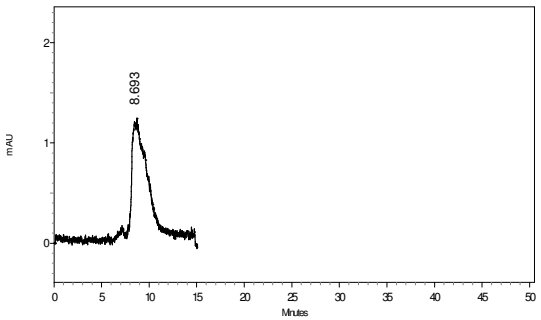

Fig 3. HPLC Chromatogram of Standard Saponin

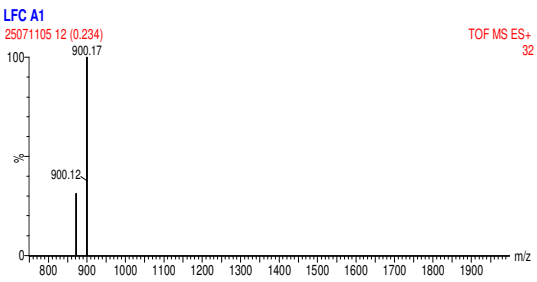

Fig 4. Mass spectrum of leaf extract derived Saponin

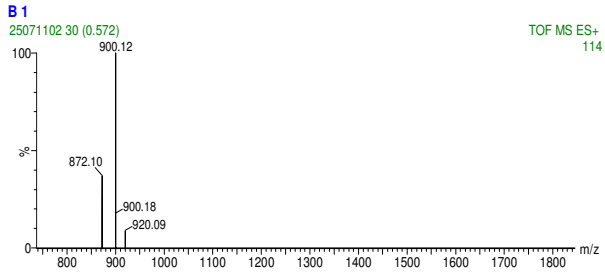

Fig 5.Mass spectrum of bark extract derived Saponin

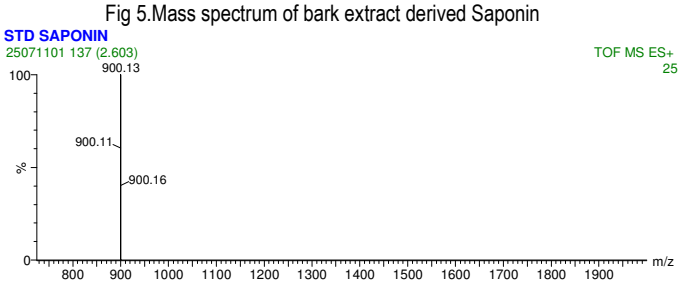

Fig 6. Mass spectrum of Standard Saponin

Table 1. Mean IZD± SEM (in mm) of saponin from leaf and bark extracts of *Acacia arabica*

E.coli	Leaf extract derived saponin	Bark extract derived saponin	Chloramphenicol
O22	8.6±0.6	8	0
MTCC 723	6	4	17.6± 0.6

Table 2. MIC of leaf &bark extract derived Saponin (in µg)

E.coli	Leaf extract derived saponin	Bark extract derived saponin
O22	8.3 ±1.6	1.26±0.3
MTCC 723	6.6±0.6	1.57±0.3

Table 3. Mean IZD± SEM (in mm) of combined effect of leaf &bark extract derived Sapogenin with Chloramphenicol

E.coli	Leaf extract derived saponin + Chloramphenicol	Bark extract derived saponin + Chloramphenicol
O22	16.6±0.6	11.6±0.6
MTCC 723	12± 1.1	26.6±0.6

The effect of combination of sapogenin with Chloramphenicol was presented in Table: 3. Combination of Chloramphenicol with leaf extract derived sapogenin showed synergistic effect on *E.coli* O22. However, on combination of Chloramphenicol to bark extract derived sapogenin additive effect on the same strain was noticed (Table: 3). In addition, strongest *in vitro* synergistic interaction was observed on *E.coli* MTCC 723 on combined administration of Chloramphenicol and leaf and bark extract derived sapogenin. Kim *et al*., (1987) documented synergistic interaction between ginseng saponin and antibiotics to inhibit various pathogens which supports our findings on interaction

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