Efficacy of medicinal plant extracts and antimicrobials on different serotypes of *Escherichia coli*

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ABSTRACT

Ethanolic extracts of 7 different medicinal plants used in folk medicine in Sikkim, India, and 16 different commercially available antimicrobial agents were investigated by using disc diffusion method for their antimicrobial activity against 25 different strains of *Escherichia coli* isolated from milk of dairy cattle and yak, and faeces of diarrhoeic calves and goats. *E. coli* were serotyped into 12 different somatic 'O' serogroups and three untypable strains. All these isolates showed sorbitol fermentation except O157 and 2 untypable strains. Enterohemorrhagic strains (O26 and O157) isolate both from diarrhoea and mastitis milk samples indicated that these might be the cause of intramammary inflammation as well. Amongst antimicrobials, highest sensitivity was recorded against sparfloxacin and ciprofloxacin (100% each) followed by nitrofurantoin (92%) and chloramphenicol (88%). Highest antimicrobial property was observed in the extract of *Eupatorium cannabium*, *Astilbe rivularis* and *Schima wallichi* in comparison to *Artemisia vulgaris*, *Aloe barbadensis* and *Kaempferia rotunda*. It is concluded that extracts of some medicinal plants and commercial antimicrobials showed sensitivity against different serotypes of *E. coli*.

Key words: Antibiogram, Diarrhoea, *Escherichia coli* serotypes, Mastitis, Medicinal plants

*Escherichia coli* is associated with severe diarrhoea with heavy mortality, particularly during the first week of life of piglets (Kumar and Soman 2002) and neonatal calves (Kaur et al. 2007). Further, it is the most common source of intramammary infection and inflammation during defecation which may leads to mastitis and thus presence of similar viable organisms in milk from infected quarter (Jones 1990). The most important group causing poisoning is EHEC, where serotype O157:H7 isolated from raw milk sample is of fecal origin and can cause severe disease in humans (Rugbjerg et al. 2003).

Development of microbial resistance to the available antibiotics has led to investigate the antimicrobial activity of medicinal plants (Bisignano et al. 1996, Hammer et al. 1999). However, very little information is available about the antimicrobial effects of the medicinal plants available in Sikkim. Therefore, the aim of the study was to determine

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MATERIALS AND METHODS

Milk samples (19 from yak and 15 from cattle) were collected aseptically from different locations of Sikkim. Dairy cows and yaks were examined clinically and by the California mastitis test (CMT). Also 28 rectal swabs from diarrhoeic calves and goats were collected aseptically.

All the CMT positive samples of milk and swab samples were subjected to bacteriological examination for the isolation and identification of *E. coli* (Edwards and Ewing 1986). The confirmed isolates were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh. All these isolates were tested for sorbitol fermentation by using Sorbitol Mac Conkey agar. These isolates were subjected to *in-vitro* antimicrobial sensitivity test by agar-disc diffusion method (Bauer et al. 1966) against 17 commercially available antimicrobial agents and extract of 7 different medicinal plants available in Sikkim.

Seven plant species commonly used in folk medicine in Sikkim, were selected and used in the present study (Table 1).
Mature plants and their parts were collected and used for extraction as per Dulger and Gonuz (2004) with little modifications. Each dry powdered plant material (10 g) was extracted with 80% ethanol (100 ml) for 12–14 h by using Soxhlet equipment. Extract was filtered using Whatman filter paper no. 1 and filtrate then evaporated to dryness at 55°C. Dried extract were stored in labeled screw capped bottles at –20°C. The dried plant extract were dissolved separately in 10% aqueous dimethylsulphoxide (DMSO) (method A) and in distilled water (DW) (method B) to a final concentration of 500 mg/ml. Empty sterilized antibiotic discs (6 mm), each were impregnated with 10 µl of extract (5 mg/disc) and used for the sensitivity test.

RESULTS AND DISCUSSION

Milk samples (6) each from yak and cattle were positive for California mastitis test (CMT) indicating intra-mammary infection. All the milk samples (CMT positive and negative) after subjecting to bacteriological examination revealed 7 E. coli isolates from yak (2 from subclinical and 5 from normal milk) and 1 from cow milk (CMT positive) samples. Seventeen strains of E. coli (11 from cattle and 6 from goats) from 28 rectal swabs and 8 strains from 34 milk samples could be serotyped into 12 different somatic ‘O’ serogroups while 3 were refractory to serotyping (Table 2).

Out of 12 different ‘O’ serotypes of E. coli, serotype O25 (4 isolates) in goats, O28 (3 isolates) in cattle and O157 (3 isolates, 1 each from cow milk, cow and goat rectal swabs) were most commonly isolated. Singh et al. (2007) also isolated O25 from diarrhoeic neonatal calves, while Chatterjee et al. (2007) isolated the same O25 serotype from cyclosporin-resistant E. coli from diarrhoeic calf. Singh et al. (2007) isolated various E. coli serotypes from diarrhoeic calves, sheep and goats except serotypes O25, O28 and O157. All the three O157 serotypes and 2 untypable serotypes did not ferment sorbitol.

Maximum number of serogroups recovered from diarrhoea belonged to serogroup O3, O25, O28, O86, O88,
O104 and O162 and were reported to be enterotoxigenic and enteropathogenic (Wolf 1997, Evans and Varnam 1991). However, Chachra et al. (1999) noted the serogroups O2, O12, O20, O8 and O9 were more prevalent in diarrhoeic calves. In the present study, isolation of enterohemorrhagic strains O26 and O157 both from diarrhoea and mastitis milk samples indicated that they might be the cause of intramammary inflammation as well. However, Turutogle (2002) observed that intramammary inflammation as well. However, Turutogle (2002) observed that E.coli O157:H7 did not cause bovine mastitis.

Details of the antibiotic sensitivity tests and resistance pattern are depicted in Table 2. Highest sensitivity was recorded against sparfloxacin and ciprofloxacin (100%) followed by nitrofurantoin (92.00%), chloramphenicol (88.00%) and tetracycline (80.00%). These findings were similar to that of Kumari et al. (2002) who reported 93.33% sensitivity to both cephotoxime and chloramphenicol. Sikdar et al. (1994) reported 78.90 and 67.89% sensitivity of E. coli strains to chloramphenicol and nitrofurantoin, respectively, whereas, Hui and Das (2000) reported 69.70% sensitivity to chloramphenicol. High sensitivity of the strains to these antimicrobial agents was attributed to the fact that these drugs were seldom used in the farms under study.

All plants expect Aloe barbadensis and Kaempferia rotunda showed antimicrobial activity against the test organisms (Table 3). The antimicrobial activity of Aloe barbadensis and Kaempferia rotunda was marginal. The highest antimicrobial property was observed in the extract of Eupatorium cannabinum, Astilbe rivularis and Schima wallichii as it inhibited all the strains of E. coli followed by Artemisia vulgaris which inhibited 4 stains. The findings are in accordance with Dulger and Gonuz (2004) who did not find antimicrobial property with Urtica dioica and Artemisia vulgaris. Amongst all, serotype O28 was highly sensitive as it was inhibited by all the 6 plant extracts tested followed by serotype O162, which was inhibited by 5 plant extracts. Serotype O3 was more susceptible to the extract of Eupatorium cannabinum and Astilbe rivularis as compared to standard antibiotics. Serotypes O28 and O104 were resistant to maximum number of standard antibiotics and serotypes O25, O86, O157 and O172 to a maximum number of plant extracts (Tables 2, 3).

Eupatorium cannabinum, Astilbe rivularis and Schima wallichii extracts (dissolved in aqueous DMSO or distilled water) showed maximum antimicrobial activity. However, there was insignificant difference in the antimicrobial sensitivity by the extract dissolved in both the methods thereby indicating that distilled water can be used for dissolving the dried extract of plant materials. It is concluded that enterohemorrhagic strain O157 of E. coli might be a cause of intramammary inflammation in cows, sparfloxacin and ciprofloxacin showed highly effective antimicrobials thereby indicating that distilled water can be used for dissolving the dried extract of plant materials. It is concluded that enterohemorrhagic strain O157 of E. coli might be a cause of intramammary inflammation in cows, sparfloxacin and ciprofloxacin showed highly effective antimicrobials (100%) followed by nitrofurantoin (92%) and chloramphenicol (88%) and, extracts from Eupatorium cannabinum, Astilbe rivularis and Schima wallichii showed higher antimicrobial properties against E. coli in comparison to Artemisia vulgaris, Aloe barbadensis and Kaempferia rotunda L.

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A. DMSO; B. DW; 1, Artemisia vulgaris; 2, Eupatorium cannabinum L.; 3, Astilbe rivularis; 4, Urtica parviflora; 5, Aloe barbadensis; 6, Kaempferia rotunda L.; 7, Schima wallichii
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REFERENCES


