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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 'Ö' 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 intra-mammary 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 serotypes of *E. coli* isolated from diarrhoea and mastitis milk, and to investigate the antimicrobial activity of commercially available antimicrobials and the extracts of different medicinal plants locally available in Sikkim.

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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).

Table 1. Ethnobotanical data of studied plants

Botanical name	Local name	Plant's parts used	Traditional medicinal use
Artemisia vulgaris	Titepati	Whole plant*leaves	As a appetizer, prevent convulsion, insecticidal, antibacterial and antifungal
Eupatorium cannabium L.	Banmara	Roots and *leaves	Emetic, diuretic, purgative and antiseptic
Astilbe rivularis	Buriokhati	Leaves, *roots/Rhizome	Uterine prolaps, hemorrhages, diarrhoea, dysentery and blood purifier
Aloe barbadensis	Ghewkumari	*Leaves	Purgative, carminative, opthalmia, piles, muscular pain, inflammation, constipation, fever, spleen and liver complaints, skin ailments and arthritis
Urtica parviflora	Sisnu	Whole plant*and leaves	Fever
Kaempferia rotunda L.	Bhui Champa	Roots, whole plant *leaves	Wound, promote suppuration, remove coagulated blood and purulent matter when taken internally
Sehima wallichii	Chilaune	Bark and *leaves	Irritant, vermicide and gonorrhoea

Source: Gurung (2002). * Plant parts used in the present study.

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 sheep and camel. Serotype O157 was successfully isolated from diarrhoeic calves by Gunes *et al.* (2004). However, Chachra *et al.* (1999) found 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.

Table 2. Antibiotic resistance pattern and frequency of serotypes of *E. coli* isolated from bovine mastitis, and cattle and goat diarrhoea

Serotype	Serotype frequency	Antibiotic resistance pattern
O 3	1	AAmCpMtPSz
O 13	1	AAmCbMtP
O 25	4	AAmCtMtPPd
O 26	1	AAmMtOPPd
O 28	3	AAmCbMtPSmSz
O 86	1	AAmMtPPd
O 88	2	AAmMtPPbPd
O 104	2	AAmCtMtPSmSz
O 132	1	AAmCpMtP
O 157	3	MtSmSzT
O 162	2	CpMtOPPbPd
O 172	1	MtOPPb
Untypable	3	AAmMtPPb

A-Ampicillin, Am-Amoxycillin, Cb-Carbencillin, Cp-Cephalexin, Ct-Chlortetracycline, Mt-Metronidazole, O-Oxytetracycline, P-Penicillin-G, Pb-Polymyxin-B, Pd-Pendistrin, Sm-Sulfamethizole, Sz- Sulfadiazine and T-tetracycline

Maximum number of serogroups recovered from diarrhoea belonged to serogroup O3, O25, O28, O86, O88,

Table 3. Antimicrobial activity of medicinal plant extracts on different serotypes of *E. coli* isolated from bovine mastitis, and cattle and goat diarrhoea

Serotype	Inhibition zone (mm)													
	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B	7A	7B
O 3	8	8	20	22	14	12	-	-	-	-	-	-	10	9
O 13	-	-	9	8	11	10	-	-	8	-	-	-	9	9
O 25	-	-	16	16	12	10	-	-	-	-	-	-	15	11
O 26	6	6	15	16	13	11	-	-	-	-	-	-	10	11
O 28	6	7	16	18	11	12	-	-	6	6	6	7	11	11
O 86	-	-	11	6	11	12	-	-	-	-	-	-	11	11
O 88	-	-	9	13	12	12	-	-	8	6	-	6	10	13
O 104	-	-	10	10	12	12	-	-	-	-	-	-	10	9
O 132	-	-	9	8	11	10	-	-	8	-	-	-	9	9
O 157	-	-	15	14	11	11	-	-	-	-	-	-	13	12
O 162	7	7	20	20	11	11	-	-	8	6	7	-	10	10
O 172	-	-	13	20	10	12	-	-	-	-	-	-	12	10
Untypable	-	-	20	13	12	13	-	-	-	-	6	-	9	11

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

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 diarhoeic 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 and Mudul (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 *Urtica parviflora* 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 cannabium, Astilbe rivularis* and *Sehima 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 cannabium* L. 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 cannabium, *Astilbe rivularis* and *Sehima 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 (100%) followed by nitrofurantoin (92%) and chloramphenicol (88%) and, extracts from *Eupatorium cannabium*, *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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