

# Search for *in vitro* antibacterial efficacy of phytoconstituents of *Acacia arabica* leaf extracts against various serogroups of *E. coli* associated with diarrheal infections in ruminants

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## Abstract

The investigation was carried out in order to detect antibacterial activity of crude methanolic, acetone, chloroform and aqueous leaf extracts of *Acacia arabica* against four different *E. coli* strains *E. coli* O22, O11, O89 and O61 associated with diarrheal infections in ruminants. MIC of methanolic extract was observed to be ranged from 0.09 to 0.17 mg/ml where as acetone leaf extract ranged from 0.5 to 5 mg/ml whereas chloroform and aqueous leaf extract showed no inhibitory results against any tested organism.

**Keywords:** Phytoconstituents, *Acacia arabica*, *E.coli*

## INTRODUCTION

*E. coli* is one of the most important cause of diarrheal infections in young ruminants are generally very limited work is carried out in field of Ethnoveterinary medicines (EVM) to cure such type of infections. EVM is the term used to treat animals using medicinal plants. Hussain *et al.* (2011) strongly advocated the use of medicinal plants to cure domesticated animals due to its easy and cheap availability with out any side effects over western medicines. The present study deals with *in vitro* antibacterial efficacy of *Acacia arabica*, against four different diarrheagenic stool isolated *E. coli* stains.

## MATERIALS AND METHODS

### Bacterial Cultures

Cultures were obtained from stool samples and drinking water sources from veterinary hospitals and also from animal husbandry. All cultures were sent for identification and serotyping at CRI, Kasauli (H.P). Further 16 s-IRS-23 s ribotyping of the same tested strains were done at DBRI, Hyderabad (A.P).

### Plant material

Leaves of *Acacia arabica* were collected in specimen voucher during November season and thoroughly washed under tap water and shade dried.

### Extract preparation

Both cold and hot extracts were prepared as per methods

recommended by Nair *et al.* (2005) and Johnson *et al.* (2008) respectively.

### Bioassay

Crude (cold and hot) methanolic, acetone, chloroform and aqueous extracts of *Acacia arabica* were tested against four different stains by broth microdilution technique (Kashikar and George, 2006) with some modifications.

### Preliminary phytochemical analysis of bioactive compounds

Preliminary estimation of bioactive compounds (alkaloids, flavonoids, saponin, tannin and glycosides) were done by methods described by Aderotimi & Samuel (2006).

## RESULTS

Of the 8 different solvent (Table-1) extracts the observed MIC was recorded in 5 different solvent extracts where as cold and hot aqueous leaf extract as well as hot chloroform leaf extracts showed no inhibitory response against any tested strains. MIC of cold methanolic leaf extract was 0.6 mg/ml for *E. coli* O22, O11 and O89 strains and it is of 0.09 mg/ml for *E. coli* O61. MIC of cold acetone leaf extract ranged from 0.6-4.16 mg/ml where as MIC of cold chloroform leaf extract was of 0.3 mg/ml. MIC of hot methanolic leaf extract ranged from 0.09-0.17 mg/ml whereas MIC of hot acetone leaf extract ranged from 0.5-4.16 mg/ml. Preliminary analysis of phytochemical constituents (Table-2) showed presence of tannins, saponins and glycosides in both cold and hot methanolic and acetone extracts whereas flavonoids were present in very limited amount. Cold and hot chloroform extracts showed presence of moderate amount of saponins and traces of glycosides whereas flavonoids, saponins, tannins and glycosides were present in very limited amount in cold and hot aqueous extracts.

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Table 1. MIC (in mg/ml) of leaf extracts (Mean  $\pm$ SEM of triplicates)

<i>E. coli</i>	CMLE	CALE	CCLLE	CAQLE	HMLE	HALE	HCLE	HAQLE
O22	0.6 $\pm$ 0	4.16 $\pm$ 1.01	0.3 $\pm$ 0	0	0.17 $\pm$ 0.06	4.16 $\pm$ 1.01	0	0
O11	0.6 $\pm$ 0	0.6 $\pm$ 0	0.3 $\pm$ 0	0	0.12 $\pm$ 0.02	0.5 $\pm$ 0.09	0	0
O89	0.6 $\pm$ 0	2.5 $\pm$ 1.2	0.3 $\pm$ 0	0	0.09 $\pm$ 0.02	0.81 $\pm$ 0.17	0	0
O61	0.09 $\pm$ 0.02	1.25 $\pm$ 0	0.3 $\pm$ 0	0	0.15 $\pm$ 0	5 $\pm$ 0	0	0

CMLE: Cold Methanolic Leaf Extract, CALE: Cold Acetone Leaf Extract, CCLLE: Cold Chloroform Leaf Extract, CAQLE: Cold Aqueous Leaf Extract, HMLE: Hot Methanolic Leaf Extract, HALE: Hot Acetone Leaf Extract, HCLE: Hot Chloroform Leaf Extract, HAQLE: Hot Aqueous Leaf Extract

Table 2. Preliminary Phytochemical Screening of Leaf Extracts

Leaf extracts	Alkaloids	Flavonoids	Saponins	Tannin	Glycosides
Cold Methanol	-	+	+++	++++	+++
Hot Methanol	-	+	+++	++++	+++
Cold Acetone	-	+	+++	++++	+++
Hot Acetone	-	+	+++	++++	+++
Cold Chloroform	-	-	+++	-	+
Hot Chloroform	-	-	+++	-	+
Cold aqueous	-	+	+	+	+
Hot aqueous	-	+	+	+	+

++++ = abundantly present, +++ = moderately present, + = present in limited amount, - = absent

## DISCUSSION

Plants are one of the most powerful alternative chemotherapeutic agents to control diarrheal infections in animals. Plants and plant products has gained much importance for treatment of various infections (Anibijuwon and Udeze, 2009). However, very limited work has been carried out in field of animal health. Chukwuka *et al.* (2011) reported that a very limited work has been carried out in field of medicinal plants for curing diseases allied to animal health. Bakari *et al.* (2012) reported antibacterial usefulness of plant extract of *Commiphora swynnertonii* against *E. coli* and other organisms associated with infections in animals. In the present study most of the extracts of *Acacia arabica* showed effective inhibitory response against four different diarrheagenic serogroups of *E. coli* associated with diarrheal infections in ruminants. The antibacterial effectiveness of extracts was due to presence of large amount of tannins, saponins and glycosides. Hot methanolic extract was found to be more effective detected on the basis of minimum inhibitory concentration. Antibacterial effectiveness of extracts is always due to presence of bioactive compounds. Acharyya *et al.* (2009) reported that presence of polyphenols and flavonoids in extracts are related to bactericidal activity.

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