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Research Article

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Antimicrobial and phytochemical study of *Trigonella foenum graecum* against diarrhoeal pathogens

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ABSTRACT

Phytochemical study and antimicrobial screening of Trigonella foenum graecum against diarrhoeal pathogens were carried out in this study. Diarrhoea causing bacteria Escherichia coli, Salmonella spp., Vibrio spp., and Shigella spp., was selected as test pathogens. Aqueous methanol and acetone extracts of Trigonella foenum graecum seeds were showed antimicrobial activity. Phytochemical study showed the presence of carbohydrate, alkaloid, saponins, tannins, phenolic compounds and flavonoids in methanol fraction of Trigonella foenum graecum seeds.

Keywords: Antimicrobial screening, Phytochemical analysis, Diarrhoea, *Trigonella foenum graecum.*

INTRODUCTION

Gastrointestinal diseases are the most frequent causes of morbidity and mortality in developing countries. The gastrointestinal infections proned to enteric infection, diarrhoeal disease, dysentery and enteric fever (*Escherichia coli*, *Salmonella* sp., and *Shigella* sp.). The presence of enterobacteria in foodstuffs and water is a common cause of diarrhoea and dysentery among the infant population. *Escherichia coli* is a classic example of enteric bacteria capable of producing diseases.Majority of diarrhoeal disease is caused by bacterial pathogens in developing countries while virus and protozoa tend to cause diarrhoea in developed countries. *Escherichia coli*, *Salmonella* spp., *Shigella* sp., *Campylobacter jejuni*, *Vibrio cholerae*, *rotavirus*, *norovirus*, *Giardia lamblia*, *Cryptosporidium* sp., and *Entamoeba histolytica*[1] are the major causative agent of diarrhoea[2].

Infectious diseases accounted for 58 % of all deaths among children aged 5–4 years. About 18 % of deaths were due to diarrhoeal diseases. Nationally, in 2005 about 59,000 children aged 5–14 years died from diarrhoeal diseases, corresponding to mortality of 24.1/100,000. Mortality was nearly 50 % higher in girls than in boys for both diarrhoeal diseases[3].

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Forestis great storehouses of medicines, and most of our modern medicines originated from forest plants. Siddha medicinal science has a wide range of application in medicinal and non medicinal fields. To preserve ecological balance and retain green covers of earth, herbs play a very major role. Siddha is one of the three principal Indian systems of medicine and is most widely practiced in southern states, especially Tamil Nadu[4, 5, 6, 7,8].

Trigonella foenum graecum L., is an annual legume crop mainly grown for use as a spice in many parts of the world[9]. *T. foenum graecum* also is known as one of the oldest medicinal plants recognized in recorded history. Leaves and seeds of *T. foenum graecum* have been used extensively to prepare extracts and powders for medicinal uses[10];

T. foenum graecum is reported to have antidiabetic, anti-fertility, anticancer[11], antimicrobial[12], anti-parasitic and hypocholesterolaemic effects[13]. The seeds of the *T. foenumgraecum* herb possess toxic oils, volatile oils and

alkaloids have been shown to be toxic to bacteria, parasites and fungi[14]. The potential uses of in vitro propagated plants as sources for new drugs are still largely unexplored. Based on several investigative studies[15], a compound produced in an in vivo plant could be produced at the same or different levels or not produced at all in an in vitro grown plant[16]. Considering the importance of the above said medicinal plant, the study was aimed to test the antimicrobial potentials of *Trigonella foenum graecum* against enteric pathogens.

EXPERIMENTAL SECTION

2.1 Microbial Investigation

Retrospective study was undertaken for a period of 6 months, a total of 50 cases of suspected acute gastroenteritis admitted in Kavery Medical Centre Hospital, Tiruchirappalli, Tamil Nadu were subjected to microbial investigation. Stool samples were taken from patients and used for microbial investigation.

2.2 Diagnosis of sample

The stool samples were gross examined for the presence of adult worms of intestinal nemathelminthes by wet mount using saline and iodine. Helminthes and protozoans were identified based on their morphology.

Enrichment broth was used for the enrichment of enteric pathogens, *Escherichia coli*, *Salmonella and Shigella* are enriched with the help of gram negative broth, *Vibrio cholera* with the help of alkaline peptone water. Stool specimen was inoculated on enrichment broth and incubated for 3–5 h.

2.3 Isolation of diarrhoea causing bacteria

A loopful of culture from Gram negative broth was inoculated on Hektoen enteric agar for isolation of *Escherichia coli*. Xylose-Lysine Deoxycholate (XLD) and Salmonella Shigella (SS) agar was streaked with the loopful of culture from Gram negative broth and observed for specific colony morphology after 24 h. Rajhans medium was used for differentiation of *Salmonella* from *Shigella*, *Salmonella* spp., was also identified using Bismuth sulphite agar for the differentiation of *Salmonella enteriridis*. Colonies obtained was further subjected to biochemical tests and identified. Specimen was inoculated into alkaline peptone water before inoculation into the Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar for enrichment. TCBS agar was incubated at 37 °C for 24 h. under aerobic condition. Colonies obtained was further subjected to biochemical tests and identified.

Aeromonas was enriched with selenite F broth and inoculated with ampicillin blood agar and TCBS agar. Colonies obtained was further subjected to biochemical tests and identified. Specimens were inoculated on Campy blood agar plates and incubated under microaerophilic condition for 2 days for Campylobacter spp. Colonies obtained was further subjected to biochemical tests and identified. To isolate *Yersinia enterocolitica* bacteria faecal specimen was directly streaked on Yersinia selective medium and observed for dark pink colonies after overnight incubation.

2.4 Collection of sample

The plant *Trigonella foenum graecum* seeds were collected from in around Tiruchirappalli. The freshly collected seeds were washed and dried in shade at room temperature for 10–15 days. The dried seeds were used for powdering by using mortar and pestle. The larger plant debris was removed and powdered seeds were used for extraction for antimicrobial compounds

2.5 Extraction of crude compounds

***Aqueous extract*:** One gram of seeds were taken and washed with sterile distilled water. The seeds were crushed by using mortar and pestle. The seeds paste was mixed with 20 ml of sterile distilled water in 50 ml beaker. The aqueous seeds mixture was covered with aluminium foil and kept at room temperature for 24 h.

***Solvent extract*:** Five different solvents such as methanol, chloroform, ethyl acetate, dichloromethane and acetone were used for the extraction of antimicrobial compounds from seed powder. One gram of dried plant seed powder was taken in a 50 ml beaker and 20 ml of methanol was added into it. This content was mixed well and the beaker was covered with aluminium foil and kept for extraction at room temperature for 24 h. The procedure was adopted for remaining solvents.

2.6 Antibacterial activity of crude extracts

***Aqueous extracts*:** The antimicrobial activity of aqueous extracts was studied by well diffusion method using Muller Hinton agar (MHA) plates. About 18 h old bacterial culture was prepared and inoculated into MHA plates. Five millimeterin diameter well was cut on plates. Each 10 µl of aqueous plant extracts were added in wells using micropipette. Ten micro liter sterile distilled water was used as a control well. All the plates were incubated at 37 °C for 24 h and plates were observed for zone of inhibition.

***Solvent extracts*:** The antimicrobial activity of solvent extracts was studied by disc diffusion method using MHA plates. About 18 h old bacterial cultures were inoculated into MHA plates. 0.25mg of crude extracts were added into sterile filter paper disc (5 mm diameter) and allowed to dry at room temperature for few minutes. Crude plant extract impregnated discs were placed on MHA plates inoculated with test bacterial strains. Sterile empty disc was used as a control. All the plates were incubated at 37°C for 24 h. After incubation the plates were observed for zone of inhibition.

2.7 Phytochemical study

Methanol extract was refluxed with 2N HCL in methanol. This concentration was saponified with 5 % KOH in ethanol. Solvent was removed by evaporation under reduced pressure and diluted with water. Then the mixture was extracted several times with chloroform. The extracted compounds were dissolved in chloroform and analyzed for the presence of flavanoids, alkaloids, terpenoids, saponins, tannins, amino acids, anthraquinone, steroids, glycosides and reducing sugar.

RESULTS AND DISCUSSION

Microscopic examination and gross examination of stool specimen revealed, absence of adult worms in all the 50 samples and 14% of the sample showed positive to Entamoeba cyst. The prevalence of diarrhoea with reference to age showed that bacterial incidence was higher than any other microbial etiology. Incident rate of diarrhoea is higher among children who are 2–5 years of age (Table 1).

Table 1: Incidence of diarrhoea with reference to age

Age (year)	No. of samples	Microbial etiology			
		Bacteria	Protozoans	Nematodes	Combination
0–1	13	13	Nil	Nil	Nil
2–5	17	14	3	Nil	Nil
6–10	10	5	2	2	1
11–15	10	4	3	2	1

3.1 Isolation of pathogens

Totally three genus were isolated from stool samples. All the test pathogens were identified by cultural characteristics and biochemical analysis and confirmed by Bergey'sManual of Systematic Bacteriology.

Selective and differential culturing results showed that 50 % of the infection was due to *E.coli* followed by *Shigella* 24 % and *Salmonella* 26 %. This result correlates with study[17] in which 38 % of the diarrhoeal episode is caused by *E.coli*. The isolated *E. coli, Salmonella* spp., and *Shigella* spp., used as test pathogens.

3.2 Antibiotic sensitivity test

Antibiotic sensitivity pattern of enteric isolates shows an alarming rate of increasing resistant properties of the isolates. This result highly correlates with previous studies[18]. The sensitivity pattern of isolated test pathogens was represented in Table 2.

Table 2: Antibiotic sensitivity assay of enteric isolates

S.No.	Antibiotics	Quantity(mcg)	Percentage of resistance		
			E.coli	***Salmonella* spp.**	***Shigella* spp.**
1	Amikacin	30	S	S	S
3	Azithromycin	30	I	S	S
3	Vancomycin	30	S	S	S
4	Chloramphenicol	30	R	R	R
5	Amoxyclav	30	R	I	S
6	Kanamycin	30	R	R	R
7	Erythromycin	30	R	I	S
8	Tetracycline	30	R	R	R
9	Streptomycin	30	S	R	R
10	Ampicillin	30	I	I	R

R- Resistant; I-Intermediate; S-Sensitive

3.3 Antimicrobial activity of crude extract

Antimicrobial activity of plant seed *Trigonella foenum graecum* aqueous extract showed activity against test pathogens (*E. coli, Salmonella* spp., *Shigella* spp.) (Table 3).

In this study, solvents such as methanol, chloroform, ethyl acetate, dichloromethane and acetone were tested for extraction of crude compound. Among the various solvents tested, the crude compounds were extracted only in methanol and acetone but not in other solvents. Solvent extracts of *Trigonella foenum graecuma* showed antibacterial activity with average zone of inhibition from 12 to 16 mm (Table 3). *Trigonella foenum graecum* a grown as a tree has shown to have the antimicrobial activity due to the seed oil as their component.

Table 3: Antibacterial activity of *Trigonella foenum graecum* a crude extract

S.No	Extract	Zone of inhibition in mm		
		E. coli	*Salmonella* spp.	*Shigella* spp.
1	Aqueous	12	10	–
2	Hexane	–	–	–
4	Chloroform	–	–	–
5	Ethyl acetate	–	–	–
6	Methanol	14	16	12
7	Acetone	13	–	–

Trigonella foenum graecum showed positive result for the presence of carbohydrate, alkaloid, phenolic compounds, saponins, tannins and flavonoids in methanol fraction and presence of some of these compounds in acetone, aqueous fraction water and absence in other extracts (Table 4). Tannins, phenolics and flavonoids contributed for the antibacterial property of the plant. This is similar to the effect observed by Randir et al.[19]

Table 4: Phytochemical analysis of *Trigonella foenum graecum*

Extracts	Steroid	Triterpenoids	Reducing sugar	Carbohydrates	Alkaloids	Phenol	Saponins	Xanthoproteins	Tannins	Flavanoids
Hexane	–	–	–	–	–	–	–	–	–	–
Benzene	–	–	–	–	–	–	–	–	–	–
Chloroform	–	–	–	–	–	–	–	–	–	–
Ethyl acetate	–	–	–	–	–	–	–	–	–	–
Methanol	–	–	–	+	–	+	+	–	+	+
Acetone	–	–	–	+	–	+	+	–	+	+
Aqueous	–	–	–	+	–	+	+	–	+	+

+ ——> Present; – ——> Absent

CONCLUSION

Among the three isolates from clinical samples (*E.coli*, *Salmonella* and *Shigella*) *E. coli* shows highest predominance (50 %). *Trigonella foenum graecums* are traditional medicinal plants used for the treatment of diarhoea. Methanol extract, Acetone extract and aqueous extractshowed activity against diarrhoeal pathogens. Phytochemical study of *Trigonella foenum graecum* reveal that to avoid the side effects of allopathic medicine this type of study will provide information about the effect of medicinal plant and will encourage their usage.

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