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Research Article

ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF *TRIGONELLA FOENUM-GRAECUM* LINN

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ABSTRACT
In the present study the ethanolic extract of the seeds of *Trigonella foenum graecum* was prepared and evaluated for antimicrobial activity against eight bacterial strains by determining minimum inhibitory concentration and zone of inhibition. Minimum inhibitory concentration values were compared with control and zone of inhibition values were compared with standard tetracycline in concentration (20 µg/ml and 100 µg/ml). The result revealed that the ethanolic extract is potent in inhibiting bacterial growth of both gram-positive and gram-negative bacteria.

KEYWORDS: Antimicrobial activity, bacteria, Cup Plate Diffusion method.

INTRODUCTION

Trigonella foenum-graecum (Leguminosae) is an annual herb [1]. Plants are hardy to about -15^{0}C[2]. They are cultivated in India, Africa, Egypt, Morocco, occasionally in England [3]. Various medicinal properties like anticholestrolemic, anti-inflammatory, antitumor, cardio tonic, carminative, demulcent, diuretic, emollient, expectorant, febrifuge, galactogogue, hypoglycemic, hypotensive and laxative have been attributed to this plant in the traditional system of Indian medicine. Fenugreek is much used in herbal medicine, especially in North Africa, the Middle-East and India. It has a wide range of medicinal applications. The seeds are given to convalescents. Research has shown that the seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an antidiabetic effect [2].

MATERIAL AND METHODS

The present investigation was undertaken to find out the antibacterial potentiality of the ethanol extract of the seeds against gram-positive and gram- negative bacteria. The seeds of *Trigonella foenum-graecum* were collected from Asansol, West Bengal and identified at Botanical Survey of India, Kolkata (Ref no.BSI/CNH/AD/Tech. /2008 dated19/06/2008).The seeds of *Trigonella foenum-graecum* were shade dried, then it was powdered in a mechanical grinder. The powders were then used for extraction with suitable solvents.

The dried powder of the seeds (100 g) was subjected to extraction with 1000 ml ethanol for 48 h. The ethanol extract was collected, filtered and concentrated in vacuum under reduced pressure and dried in desiccators. The yield was about 10.11 % (w/w). The ethanol extract obtained was tested for the antimicrobial activity against eight bacterial strains. These strains were collected from the Department of Pharmaceutical technology, Jadavpur University, Kolkata. All sub cultured microbes used were pure cultured preserved as slant agar culture at 4^{o}C.

The molten nutrient agar medium containing various concentrations of the extract (0.0mg/ml, 50mg/ml, 100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml) were poured and solidified onto sterile 100 mm Petri dishes to give sterile nutrient agar plates with varying dilutions of the extract. Then these plates were kept in a refrigerator (4^0) for 24 h for uniform diffusion of the extract into the nutrient agar media. The plates were then dried at 37^0 for 2 h before spot inoculation [4]. One loopful (diameter 3mm) of an overnight growth peptone water culture of each test organism was placed in a Petri dish marked by checker board technique [4]. The spot inoculated plates were incubated at 37^0 for 24 h and the MIC values were obtained.
Tetracycline was taken as a standard compound against *Staphylococcus aureus* for comparing the results obtained with. Two sets of two dilutions (400mg/ml and 2000mg/ml) each of *Trigonella foenum-graecum* seeds extract and tetracycline (solvent: sterile distilled water) were prepared in sterile McCartney bottles. Sterile nutrient agar plates were prepared and incubated at 37^0 for 24 h to check for any sort of combination. Then four cylinders were placed in appropriate position of the Petri dish marked as quadrants at the back of the Petri dishes. Two different dilutions of the extract were given to each cylinder of each Petri dish. The Petri dishes were incubated at 37^0 for 24 h and diameter of zones of inhibition use measured in mm. A similar procedure was adopted for the pure tetracycline. Corresponding zone diameters were compared accordingly and % potency was estimated according to Two-level factorial assay for Cylinder plate method [5,6].

RESULTS

The observations of the MIC study has been tabulated in Table 1 and it was found that the minimum inhibitory concentration of the ethanol extract was found to be 400mg/ml , with respect to most of the test bacteria like *Escherichia coli* (ATCC25922), *Salmonella typhi* (NCTC 74), *Vibrio cholerae* (ATCC 14033), *Staphylococcus aureus* (NCTC 8530), *M.lutea*, *Bacillus subtilis*, *L.bacillus*. But in case of *Shigella sonnei* 2, it was not showing any antimicrobial activity on that concentration. The result of ZOI of the extract and its comparison with standard antibiotic tetracycline (20 µg/ml and 100 µg/ml) was recorded in Table 2. The antimicrobial potency of ethanolic extract was found to be 69.54%.against *S.aureus*. From the result of MIC and zone of inhibition value and by the comparison with standard tetracycline, it is evident that the ethanol extract possessed antimicrobial activity [7].

DISCUSSIONS

The compounds responsible for this antimicrobial activity have not been investigated. However, preliminary phytochemical analysis and literature review of the ethanol extract revealed the presence of flavonoids, saponins, alkaloids and glycosides. The antimicrobial properties of the plant may be attributed to the individual or combined effect of the above mentioned chemical groups. The findings of the present investigation offer a scientific support to the ethno medicinal use of the plant by the traditional healers.

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Table 1: MIC of ethanol Extract of *Trigonella foenum-graecum* against different bacteria

Name of bacteria	Growth in nutrient agar containing different concentrations of extract (mg/ml)					
	0	50	100	200	300	400
Escherichia coli ATCC25922	+	+	+	+	+	_
Salmonella typhi NCTC 74	+	+	+	+	+	_
Vibrio cholerae ATCC 14033	+	+	+	+	+	_
Shigella sonnei 2	+	+	+	+	+	+
Staphylococcus aureus NCTC 8530	+	+	+	+	+	_
M.lutea	+	+	+	+	+	_
Bacillus subtilis	+	+	+	+	+	_
L.bacillus	+	+	+	+	+	_

All determinations were done in triplicates '0' Control(without extract), '+' Growth; '-' No growth

Table 2: Zones of inhibition produced by the ethanol extract and tetracycline

Name of bacteria	Ethanol extract(mg/ml)		Tetracycline(mg/ml)	
	400	2000	0.1	0.2
Staphylococcus aureus NCTC 8530	16.8	13.1	21.5	16.5

Zone of inhibition, including the diameter of the cylinder; mean value of the three independent experiment; tetracycline was used as positive control.

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