

Screening of antimicrobial activity of fenugreek seeds

Abstract

The pharmaceutical industry has produced a large number of new antibiotics over the last three decades, but resistance to these antibiotics by microorganisms has increased. Statistically, WHO estimates that medicinal plants are the primary medicine for 2/3 of the world's population. Recent studies focus on plant research all over the world to extract appropriate and efficient antimicrobial drugs. Fenugreek (*Trigonella foenum-graecum Linn.*) is an annual herb which has widely been consumed throughout the world as a food, a food additive and in the traditional remedies science civilizations. This study was aimed preliminary to investigate *in vitro* antimicrobial activity of fenugreek seeds against Gram-negative and Gram-positive bacteria and other microorganisms such as *Escherichia coli* (*E. coli*), *Proteus vulgaris* (*P. vulgaris*), *Staphylococcus aureus* (*S. aureus*), *Candida albicans* (*C. albicans*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus saprophyticus* (*S. saprophyticus*) using two different solvents: aqueous extractions (cold, hot & boiling) and methanol extractions. The antimicrobial activities were evaluated using two different methods: agar disc diffusion and agar-well diffusion method. The results indicated that only the boiling water extract contains the antimicrobial active ingredients of fenugreek seeds, while both cold water extract and methanol extract are not suitable for such purposes.

Keywords: fenugreek, seeds, antibiotics, solvent extraction, antimicrobial, staphylococcus aureus.

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Introduction

Plants have formed the basis for traditional medicine systems in most societies and have been used for thousands of years in countries like China and India. Plant-based systems still play a vital role in health care, and WHO has estimated about 80% of the world's inhabitants rely mainly on traditional medicine for their primary health care. Various plants (whole or some parts) are known to be useful use for human benefit and welfare. Of these benefits, is the capacity of some plant seeds, fruits, leaves and other parts exerting pharmacological activities such as, analgesics, diuretics, antispasmodics, antimicrobial activity.. etc. Fenugreek (*Trigonella foenum - graecum Linn.*) is an annual herb which has widely been consumed throughout the world as a food, a food additive and in the traditional remedies science civilizations. Fenugreek, being rich in photochemicals, has traditionally been used as a food, forage and medicinal plant.¹ The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components.² Fenugreek seeds contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects.³ The steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) are thought to inhibit cholesterol absorption and synthesis and hence its potential role in arteriosclerosis.

Locally, fenugreek seeds have traditionally and commonly been used to treat diabetes, coughs, congestion, bronchitis, fever, high blood pressure, headache, migraines, diarrhea, flatulence, anemia, irregular menstrual cycles and arthritis, to ease labor pains and

menstruation pain, and as an appetite stimulant.⁴ Fenugreek has also been used as an external poultice to control inflammation and dandruff. Modern medicine is beginning to provide confirmation of many of the traditional medicinal applications of fenugreek seeds.^{5,6} To the best of our knowledge, no previous studies have been reported for antimicrobial activity of fenugreek. Thus, this study was aimed to evaluate the antimicrobial activity of fenugreek seeds in order to be used in some infectious diseases.

Materials and methods

Fenugreek was procured from the local market. Sixty gram of fenugreek (seeds and powdered seeds) was extracted by maceration in 200ml of cold, hot and boiling water. Different concentrations of fenugreek powder (20, 40, and 100gm) were extracted in methanol as organic solvent. *In vitro*, antimicrobial activities of the extractions of different concentrations were tested for the antimicrobial activity using both Gram positive and Gram negative bacteria (six microorganisms including *E. coli*, *C. albicans*, *P. vulgaris*, *S. aureus*, *S. epidermidis* and *S. saprophyticus*). Antimicrobial activity evaluations were performed using two different methods: agar disc diffusion and agar-well diffusion method.^{7,8} Gentamicin (10 μ g) was used as a positive control,^{9,10} pure methanol and distilled water were used as a negative control in both methods. In addition, the extraction of fenugreek seeds in boiling water was used to test its effect on some staphylococcus species (*S. epidermidis*, *S. saprophyticus* and *S. aureus*) using disc diffusion method. Samples were incubated at 37°C. The diameter of clear zone of inhibition surrounding each disc was measured in millimeters. The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates \pm SD of two replicates.

Results

Tables 1 and Table 2 show that the extraction of fenugreek seeds by boiling water has some inhibitory effect on the growth of *St. Aureus* bacteria in disc diffusion method and agar well diffusion method with a zone inhibition of diameters of 22mm and 10mm respectively. To verify this finding; other species of *staphylococcus* were used to test this effect. Table 3 indicates that fenugreek has some antimicrobial

effect on all the species of *staphylococcus* and it also shows that the inhibition is directly proportion to the extract concentration. The other microorganisms (*E. coli*, *C. albicans*, and *P. vulgaris*) were found to be resistance to all aqueous extractions including the boiling water. The effects of different methanol fenugreek powder extract concentrations by disc diffusion method and by agar well diffusion method did not show any inhibitory effect on the growth of all the microorganisms used (concentrations: 20, 40 and 100gm per 200ml, data not shown).

Table 1 Effect of different aqueous extracts of fenugreek seeds on microorganisms by disc diffusion method.

Microorganism	Zone of inhibition in mm								
	Seed (60 gm/200 ml)			Powder (60 gm/200 ml)			Control		
	Hot	Cold	Boiling	Hot	Cold	Boiling	H ₂ O	Gentamicin	
<i>E. coli</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	19	
<i>St. aureus</i>	-ve	-ve	22±2.0	-ve	-ve	-ve	-ve	27	
<i>C. albicans</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	/	
<i>P. vulgaris</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	16	

-ve: Negative (no zone of inhibition is formed); Data of Mean±SD.

Table 2 Effect of different aqueous extracts of fenugreek seeds on microorganisms by agar well diffusion method.

Microorganism	Zone of Inhibition in mm								
	Seed (60 gm/200 ml)			Powder (60 gm/200 ml)			Control		
	Hot	Cold	Boiling	Hot	Cold	Boiling	H ₂ O	Gentamicin	
<i>E. coli</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	18	
<i>St. aureus</i>	-ve	-ve	10±0.8	-ve	-ve	-ve	-ve	30	
<i>C. albicans</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	/	
<i>P. vulgaris</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	30	

-ve: Negative (no zone of inhibition is formed); Data of Mean±SD.

Table 3 Antibacterial activity of aqueous boiling extraction of fenugreek seeds on *staphylococcus* species by disc diffusion method

Species	Zone of Inhibition 60 gm/200ml	Gentamicin 10 μ g
<i>St. epidermidis</i> (60gm/200ml)	09±1.0 mm	32 mm
<i>St. saprophyticus</i> (60gm/200ml)	6.5±0.7 mm	19 mm
<i>St. aureus</i> (60gm/200ml)	22±2.0 mm	18 mm
<i>St. aureus</i> (100gm /200ml)	24±3.0 mm	22 mm

Data of Mean±SD.

Discussion

The potential for developing antibacterial into medicine appears rewarding from the perspective of drug development and phytomedicine. There are several studies of antibiotics resistance of

human pathogens to available antibiotics.^{11,12} Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens. In North Africa, fenugreek is one of the medicinal plants that are traditionally used for therapy. In the present study, crude extracts of the plant materials (seeds and powder) obtained in aqueous and alcoholic solvents were tested against six different microorganisms including *E. coli*, *C. albicans*, *P. vulgaris*, *St. aureus*, *St. epidermidis* and *St. saprophyticus*. The results show that only the boiling water extraction of fenugreek seeds has an inhibitory effect on the growth of *St. aureus* by the two different testing methods, agar disc diffusion and agar-well diffusion (Tables 1) (Table 2). This may be explained by that only the boiling water extract contains the antimicrobial active ingredients of fenugreek that are responsible for the antimicrobial effect, while both cold water extract and methanol are not suitable for such purposes. This antimicrobial effect is confirmed by testing the boiling water extract of fenugreek seeds on other three different species of *staphylococcus* (*St. epidermidis*, *St. aureus*, and *St. saprophyticus*). All the species show sensitivity to this extraction type (Table 3). The antimicrobial activity of the boiling water extraction of fenugreek seeds is affected by its concentration, as changing the seed concentrations from 60 gm per 200 ml of water to 100 gm of

seed per 200ml of water results to about 2-fold in zone of growth inhibition from 7mm to 12mm for *St. aureus* (Table 3). The finding of this preliminary screening of antimicrobial activity of fenugreek seeds suggest that the minimum inhibitory concentration (MIC, MIC50) is determined in future studies when a quantitative sensitivity result is required. Furthermore, the two antimicrobials (antimicrobial-like effect of fenugreek seeds and gentamicin) interactions are suggested to be studied to identify the nature of interaction (additive/synergistic using summed fractional inhibitory concentration). However, before such studies carried out, mode of action and active ingredients of the fenugreek seeds are needed to be identified (see below).

All these preliminary indications dedicate further studies for the effect of fenugreek seed extraction on staphylococcus growth since infections caused by *St. aureus* are difficult to treat with conventional antibiotics.¹³ *In vitro* inactivity of other extractions on staphylococcus and other microorganisms may not necessary translate to their *in vivo* inactivity. The determination of the exact compound which is responsible for this effect may pave the way for new potential drugs. Previously, it has also been shown that scopoletin, a coumarin derivative of coumaric acid, responsible for the antimicrobial activity of fenugreek against prokaryotes.¹⁴

Conclusion

This study suggests that extracts of fenugreek may have antibacterial activity against some human pathogens. Therefore, it paves the way for further research to identify the active compounds responsible for the plant biological activity and its mechanism.

Conflict of interest

The author declares no conflict of interest.

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