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Comparative analysis of phytochemical and antibacterial activity of ginger (*Zingiber officinale,* Rosc.) and fenugreek (*Trigonella foenum-graecum,* L.)

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Abstract
Spices like *Zingiber officinale*, Rosc.; (ginger) and *Trigonella foenum-graecum*, L.; (fenugreek) are indispensable for the preparation of our daily food and are reported to possess phytochemicals, which have varied beneficial biological effects. This study evaluates the comparative analysis of phytochemical screening and antibacterial activity of ginger and fenugreek. Ethanol and methanol are the two solvents used for making the extracts from both spices. The comparative analysis of phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, saponins, terpenoids and cardiac glycosides. On comparing the presence of phytochemicals in both solvent extracts, methanol showed the presence of more phytochemicals than ethanolic extract. Antibacterial activity of *Zingiber officinale*, Rosc.; and *Trigonella foenum-graecum*, L.; against two common pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* was carried out. Both the spices showed antibacterial activity in which *Zingiber officinale*, Rosc.; has the largest inhibition zone. The present study reveals the quality of *Zingiber officinale*, Rosc.; and *Trigonella foenum-graecum*, L.; as a raw material for drug manufacturing and an inevitable nutrient diet in our daily food.

Keywords: *Zingiber officinale*, *Trigonella foenum-graecum*, phytochemical, antibacterial activity

Introduction
Herbs and spices have been used during the Middle Ages for flavoring, food preservation, and medicinal purposes. They stimulate appetite by increasing the flow of gastric juice (Nwinuka *et al*., 2005) [12]. Spices, which include leaves (coriander, mint), buds (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stem (cinnamon), rhizomes (ginger) and other plant parts, have been defined as plant substances from indigenous origin. The beneficial health effects of spices have been well documented (Mensah *et al*., 2009; Bhattacharjee and Sengupta, 2009) [7, 2]. Many spices have been reported to have antimicrobial properties, cholesterol lowering effects, anti-diabetic and anti inflammatory properties. Currently there is an increasing interest both in the industry and scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties which may be due to the presence of vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens and minerals. These compounds render spices to act as preservative agents in food.

Ginger (*Zingiber officnale*, Rosc.;) is one of the most commonly consumed dietary condiments in the world. The crop is cultivated both as spice and a medicine in almost all tropical and sub-tropical parts of India, particularly in Kerala, Karnataka, Tamil Nadu and West Bengal. Ginger is primarily used to treat nausea, but it is also used as an anti-inflammatory, a pain remedy and a cholesterol-lowering herb. The main active phytochemicals present in ginger are gingerols, shogaols and paradols which have strong antioxidant and chemopreventive properties (Gloria *et al*., 2010) [4].

Fenugreek (*Trigonella foenum-graecum*, L.;) is an annual herb indigenous to the countries bordering on the eastern shores of the Mediterranean and largely cultivated in India, Egypt, and Morocco. In Indian and Ethiopian medicine it is used as a carminative and tonic for gastric troubles. When soaked in water the seeds swell and produce soothing mucilage to aid digestion. Fenugreek extracts are found in soaps and cosmetics. Fenugreek is used as a herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts and micro greens). Sotolon is the chemical responsible for fenugreek's distinctive sweet smell (Srinivasan *et al*., 2007) [17]. Seeds of fenugreek are cuboid-shaped and yellow- to amber-coloured which are encountered in the cuisines of Indian subcontinent. Studies with type 2 diabetics have shown a blood glucose normalizing effect and decreased insulin resistance (Helambe *et al*., 2012) [5].

Phytochemicals are plant derived chemicals, which protect human from numerous diseases. These chemicals are naturally occurring in medicinal plant leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. The present study is aimed to assess the phytochemical constituents in ethanol and methanol extracts of *Zingiber officinale*, Rosc.; and *Trigonella foenum-graecum*, L.

This work evaluated the antibacterial activity of the spices, ginger *(Zingiber officnale, Rosc.;)* and fenugreek (*Trigonella foenum-graecum,* Linn.;*)* against two bacteria *Staphylococcus aureus* and *Escherichia coli.* Through ages spices have served humans in many areas such as food, flavors and drugs. The present work ascertained whether these spices could affect growth inhibition on the test organisms *in vitro*. Furthermore, the research was geared towards finding out if the effects on growth inhibition were dependent on the solvents of extraction of the plant chemicals.

Materials and Method

Collection and Identification of Plant Materials

Ginger rhizomes bought from a vegetable market and Fenugreek seeds were bought from a local market in Coimbatore.

Extraction of Plant materials

The plant materials were washed with clean water and allowed to air dry in order to reduce the microbes present in the plant material due to handling and transportation. The outer covering of ginger is manually peeled off and the materials were sliced into small pieces. The materials were placed in a hot air oven for drying. And the dried material is powdered and weighed. Fenugreek seeds were directly air dried in hot air oven and powdered. All powders were stored at 4^0C when not in use.

The two solvents used for extraction are ethanol and methanol. 80% ethanol was prepared by adding 80ml of 100% ethanol to 20ml distilled water. 0.5g of ginger powder was mixed with ethanol. The same was repeated for fenugreek powder. The mixture was vigorously stirred with a sterile glass rod. After 24 h, with interval stirring, the mixture was filtered, using Whatmann No 1 filter paper. One part of the filtrate is tested for phytochemical analysis. The precipitate was discarded and the supernatant was collected for evaporation and tested for antibacterial activity. The same procedure was followed to make methanolic extract.

Phytochemical Analysis

The extracts of the ginger and fenugreek were tested for carbohydrates, proteins, starch, aminoacids, steroids, glycosides, flavonoids, alkaloids, tannins, saponins, terpenoids, gums, phlobotannins and cardiac glycosides. This phytochemical screening of the extracts are carried out by standard methods (Raman, 2006; Karpagam *et al*., 2008; Kokate *et al*; 2001).

Screening the extracts for antibacterial activitty

Antibacterial activity is determined by the Agar diffusion method (Threlfall *et al*., 1999, Walker 2000) [18, 19]. Two bacterial cultures were used namely *Staphylococcus aureus* and *Escherichia coli*. The methanolic and ethanolic extracts of both the plants are tested against the two strains of bacteria.

Procedure

10 mg/ml of sample is prepared. The concentrations in which the bacteria screened are 1000, 500, 250, 100, 50, 25 µg. Ciprofloxacin is used as the standard antibiotic.

Media Used are Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different volumes. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted.

Results and discussion

The present study was carried out to find out the phytochemical screening and antibacterial activity of *Zingiber officinale*, Rosc.; and *Trigonella foenum-graecum*, L.;

Phytochemical analysis

Zingiber officinale, Rosc.; revealed the presence of carbohydrates, glycosides, flavonoids, alkaloids, saponins, terpenoids, phlobotannins and cardiac glycosides. Carbohydrate is present in ethanolic extract and methanolic extract of ginger. Phytochemical constituents which are absent in both the extracts of ginger are proteins, starch, amino acids, steroids, tannins and gums. Ethanolic extract exhibited the absence of flavonoid whereas the same is present in methanolic extract. Glycosides, alkaloids, saponins, terpenoids, phlobotannins and cardiac glycosides are present in both the solvent extractions of ginger. The phytochemical studies conducted on ginger showed that it contains alkaloids, tannins cardiac glycosides and flavonoids (Sarafa Akeem *et al*., 2016) [14]. In the present study flavonoids and tannins are absent in ethanol and methanol extract of ginger. Steroids are absent in both ethanol and methanol extract of ginger. Terpenoids are one of the main course phytochemical present in ginger (Humayun Riaz *et al*., 2015) [6] which is confirmed in the present study. Shamsuddeen *et al*., (2009) [15] confirmed the absence of tannins whereas the present study proves the presence of tannins.

In *Trigonella foenum-graecum*, L.; phytochemical screening exhibited the presence of carbohydrates, steroids, flavonoids, alkaloids, tannins, saponins, terpenoids and cardiac glycosides. Proteins, starch, aminoacids, glycosides, gums and phlobotannins are absent in both the solvent extracts of fenugreek. Glycosides are absent in both the ethanol and methanol extract in the present study although it is said to be present in the ethanol extract of fenugreek (Sita *et al*., 2016) [16]. Carbohydrates are present along with alkaloids and steroids whereas flavonoids are absent in ethanol extract of fenugreek (Mowl *et al*., 2009) [9]. In present study flavonoids were found in moderate amount. In general, the presence of these phytochemicals could account for medicinal properties of these spices in various disease conditions such as atherosclerosis, arthritis, nausea, asthma, worm expeller, bacterial infections and cancer (Nandagopal *et al*., 2012) [10].

Zingiber officinale, Rosc.; and *Trigonella foenum-graecum*, L.; contain many phytochemical constituents which are responsible for several health effects. In both the plants, methanolic extract answers for more positive result. The phytochemicals which are equally present in ginger and fenugreek are carbohydrate, alkaloid, flavonoid, saponin, terpenoid and cardiac glycosides. Cardiac glycosides are a class of important organic compound that increase the output force of heart and increase its rate of contraction. These compounds have a diverse range of biochemical effects and also use in cancer treatments. Glycosides and phlobotannins are present in ginger but absent in fenugreek. Steroids, flavonoids and tannin are present only in fenugreek extract and absent in ginger. On comparing the presence of phytochemical constituents in ginger and fenugreek, maximum compounds are present in fenugreek.

Table 1: Comparative Analysis of Phytochemical Screening Done In Ethanol and Methanol Extract of *Zingiber officinale*, Rosc.; and *Trigonella foenum-graecum*, L.;

S. No	Phytochemicals	*Zingiber officinale*, Rosc.;		*Trigonella foenum-graecum*, L.;	
		Ethanolic extract	Methanolic extract	Ethanolic extract	Methanolic extract
1	Carbohydrates	+	+	+	+
2	Proteins	-	-	-	-
3	Starch	-	-	-	-
4	Amino acids	-	-	-	-
5	Steroids	-	-	+	+
6	Glycosides	++	+++	-	-
7	Flavanoids	-	-	++	++
8	Alkaloids	++	+++	++	+++
9	Tannins	-	-	++	++
10	Saponins	+	++	++	+++
11	Terpenoids	+++	+++	+++	+++
12	Gums	-	-	-	-
13	Phlobotannins	+	+	-	-
14	Cardiac glycosides	+++	+++	+++	+++

+++ Appreciable amount, ++ Moderate amount, + Trace amount, -Absent

Antibacterial activity

Antibacterial activity is tested for ginger and fenugreek extracts by Agar diffusion method. Methanolic and ethanolic extract of ginger exhibited antibacterial activity against *Escherichia coli* and *staphylococcus aureus*.

Maximum inhibition zone is observed in ethanol extract of ginger in concentration of 1000 µg against *Escherichia coli* bacteria. Inhibition zones were observed in other concentrations (500, 250, 100, 50, 25µg) were slightly lesser when compared to concentration of 1000µg. Ethanolic extract showed inhibition zones in all the concentrations except in 25µg, the inhibition zone is very less. Aqueous and ethanolic extract of ginger showed no inhibition activity for *Escherichia coli* and *Staphylococcus aureus* (Onyeagba *et al*., 2004) [13] whereas a mixture of spices exhibited inhibition zone for the same bacteria. Diameter of inhibition zone in 1000µg concentration obtained was 9mm (Ekwenye *et al*., 2005) [3]. In the present study 12 mm is obtained in a concentration of 1000 µg which is an increased inhibition zone compared to previous works carried out in the same species. An inhibition zone of 6 mm was observed against *S. aureus* (Akoachere *et al*., 2002) [1] which is considered as less active.

Fenugreek exhibited very less antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* when compared to *Zingiber officinale*. An inhibition zone of 5 mm is observed in ethanol extract of fenugreek in concentration of 1000µg. Methanolic extract showed more inhibition zone than ethanolic extract. The antibacterial activity shown by methanolic extract of fenugreek is 7 mm in 1000 µg and 6mm in 500µg concentrations respectively.

The ethanolic extract of ginger inhibited the growth of staphylococcus aureus. Maximum diameter of inhibition is shown in 1000 µg concentration which is 14 mm and 10mm in the same concentration by methanolic extract. Inhibition zone is observed only in 1000 and 500 µg concentrations of both ethanolic and methanolic extracts of ginger. Extract of fenugreek exhibited an inhibition zone measuring 6mm in 1000µg concentration. As like *Escherichia coli* methanolic extract of fenugreek exhibited a large zone of inhibition compared to ethanol extract. The inhibition zones measured 7mm in 1000 µg and 5mm in 500µg concentrations.

In the present study fenugreek exhibited antibacterial activity against two bacteria. Range of inhibition zone generally observed for the extracts of fenugreek seeds (1.0 mm for *Escherichia coli* and 1.1 mm for *S. aureus*) is very less (Sita Kumari *et al*., 2016) [16]. In present study a larger inhibition zone of 7 mm was obtained in 1000µg which is comparatively a good result compared to previous studies. Inhibition zone of 4.8 mm in ethanolic extract and 3.4 mm in methanolic extract against *Escherichia coli* was observed (Norziah *et al*., 2015) [11] which is comparatively similar to present study.

Table 2: Antibacterial activity of ginger and fenugreek against *Escherichia coli*

Sample	1000 µg	500 µg	250 µg	100 µg	50 µg	25 µg
Ethanol extract of ginger	12	9	7	5	3	1
Methanol extract of ginger	10	7	4	3	2	1
Ethanol extract of fenugreek	5	2	3	2	2	2
Methanol extract of fenugreek	7	6	1	3	1	2
Standard antibiotic	31	28	26	23	20	18

Table 3: Antibacterial activity of *Zingiber officinale*, Rosc.; and *Trigonella foenum-graecum*, L.; against *Staphylococcus aureus*

Sample	1000 µg	500 µg	250 µg	100 µg	50 µg	25 µg
Ethanol extract of ginger	14	6	1	4	2	4
Methanol extract of ginger	10	3	1	6	4	3
Ethanol extract of fenugreek	6	3	4	2	3	3
Methanol extract of fenugreek	7	5	3	2	1	1
Standard antibiotic	13	18	21	25	27	34

Conclusion

Using spices to add flavor and variety to food may help motivate dietary change and reduced nutrition related disease risk. Spices such as ginger and fenugreek have a wide variety of bio-functions and their additive or synergistic actions are likely to protect the human body against a variety of diseases. The present study leads to a conclusion that spices are not only a dietary source of food but also a potential medicine for several hectic diseases. As well as its antibacterial property reveals the capability of spices to act against pathogenic bacteria leads to another possibility of utilization of spices in drug manufacturing.

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