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Anti-microbial property of *Trigonella foenum-graecum* L. Methanol Extract [TFGME] on Pathogenic organisms

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ABSTRACT

Methanol extract of *Trigonella foenum-graecum* L. and its role on pathogenic micro-organisms is the scope of this study. Initially, *Trigonella foenum-graecum* L. was treated with methanol and the obtained extract was termed as *Trigonella foenum-graecum* L. Methanol Extract [TFGME]. TFGME shows the presence of Polyphenols, glycosides, phytosterol, saponins and etc., when it subjected to initial preliminary screening. HPLC analysis of TFGME suggests that the presence of several organic molecules as it elutes 9 major peaks after the solvent peak in reverse-phase HPLC at 216nm. In addition, TFGME exhibit anti-microbial property by exhibiting zone of inhibition when it was incubated with pathogenic organisms such as *E. coli, S. aureua, Pseudomonas, Salmonela and shigella.* The obtained results were recorded in MIC (Minimum Inhibition Concentration) of TFGME values. Furthermore, when TFGME was subjected to analysis of minerals content by using ICP-OES instrument, it was confirmed that TFGME contains aluminium, copper, iron, manganese and zinc in the extract.

Keywords: TFGME; RP-HPLC; *E. coli*; *S. aureua*; Pseudomonas; Salmonela; Shigella; Minimum Inhibition Concentration; ICP-OES.

1. Introduction

Antimicrobial property is nothing but the inhibition of microbial growth in the parasite cell or organisms [1]. The established drug or a biomolecule agent which bare the capacity to lysis the pathogenic organism cell membrane to degrade the biomolecules of the cell or organism leads to become a good antimicrobial agent [2]. In the other hand the chemical agent or a biomolecule which possess the capability to suppress the growth of pathogenic organism by degrading the genetic material or by inhibiting the replication of microbes also leads to be an excellent anti-microbial agents/drug in the medicinal field [3].

Identifying the novel anti-microbial agents from herbal source or plant seeds is the big task for the emerging scientists. Even though at present modernized world there is a lot of effective synthetic anti-microbial drugs are available in the market, due to its several side effects explode the herbal medicines without any side effects play a pivotal role in the treatment of several pathogenic infections [4]. In the contrast Fenugreek seeds which is scientifically termed as *Trigonella foenum-graecum L.* belongs to Fabaceae family is an annual herb [5]. *Trigonella foenum-graecum L.* Seed contains 369 calories, 7.8% moisture, 28.2g proteins, 5.9g fat, 54.5g carbohydrates, 8g fiber, 3.6g ash and less than 1% minerals [6]. It contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, galactomannans, diosgenin, tigogenin, neotigogenin, triterpenoids, trigonelline, choline, amino acid 4-hydroxyisoleucine, flavonoids, nicotinic acid, sapogenins phytic acid, scopoletin and trigonelle which has therapeutic effects [7].

Majorly, it was cultivated as spice crop in many part of the world. *Trigonella foenum-graecum L.* is rich in anti-oxidants and phytochemicals, thus it has been traditionally used as food, forage and as a medicinal herb. Medicinally, it was used in the treatment of wounds, arthritis, bronchitis, heart disease and digestive problems [8].

1.1. Study Objectives

The general objective of this study was to evaluate the role of TFGME on pathogenic organisms.

The specific objectives were: (i) to study the preliminary assay of TFGME; (ii) to assess the chemical characterization of TFGME in HPLC; (iii) to study the minerals composition of TFGME in ICP-OES; (iv) to evaluate the anti-microbial property of TFGME; and (v) to determine the non-toxic property of TFGME.

2. Materials and Methods

All the chemicals used were of analytical grade. Pathogenic cultures were purchased from MTCC.

2.1. Preparation of TFGME

Trigonella foenum-graecum L. seeds were purchased from local market. It was subjected for Soxhlet extraction method using methanol to obtain the extract. The finally obtained extract was termed as *Trigonella foenum-graecum L.* Methanol Extract (TFGME) and it utilized for further assays.

2.2. Preliminary phytochemical screening of TFGME

TFGME was screened for terpenoids, phytosterol, tannin, phenolic, glycoside, saponins, flavonoids, carbohydrates, proteins, steroids and lipids [9].

2.3. Reverse Phase High Performance Liquid Chromatography analysis of TFGME

TFGME was subjected to RP-HPLC using C_{18} column (150mm×3mm, particle size 2.7µm) with VWD detector in Agilent 1260-infinity II. The column was pre-equilibrated with HPLC water and Acetonitrile and sample was eluted at the flow rate of 1ml/min in linear gradient mode [10].

2.4. Antimicrobial assay of TFGME

The bacterial cultures (*E. coli, Salmonella*, *Pseudomonas*, *Shigella* and *S. aureus*) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient agar petri plates were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates evenly with the help of a glass spreader. Wells were made on the solidified agar using a cork borer. The test solution was made by dissolving 50mg TFGME in 1.0mL of water to get 50mg/mL concentration followed by sonication for 2min. The 10µL of this test solution containing 0.5mg of TFGMA was added into the respective wells by varying the concentration (0-5mg). The standard antibiotic drug Amoxycillin was kept as positive control and tested against all the pathogens. These plates were incubated at 37°C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [11]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported.

2.5. ICP-OES analysis of TFGME

TFGME was analyzed in Agilent Make ICP-OES instrument, model number 5110. To evaluate the content of minerals in the extract, the samples were aspirated at 12RPM pump speed, 25sec sample uptake time, 30sec of rinse time, 5sec, read time, 1.2KW RF power, 15sec stabilization time, Axial viewing mode, 8mm viewing height, 0.7L/Min nebulizer flow, 12L/Min plasma flow, 0.75L/Min Aux flow.

3. Results and Discussion

3.1. Chemical Characterization of TFGME

The TFGME was found to be withholding of terpenoids, phytosterol, phenolic-compounds, glycosides, saponins, flavonoids, alkaloids, steroids and lipids as per the preliminary screening (Table 1).

Table 1. Chemical Characterization of TFGME

SL NO	Phytochemical Analysis	Results
01	Terpenoid	Present
02	Phytosterol	Present
03	Tannin	Absent
04	Phenolic	Present
05	Glycoside	Present
06	Saponin	Present
07	Flavonoid	Present
08	Carbohydrates	Present
09	Proteins	Absent
10	Alkaloid	Present
11	Steroids	Present
12	Lipids	Present

3.2. HPLC analysis of TFGME

TFGME found to presence of 09 different set of compounds, as per HPLC analysis it found to elute 09 peaks at the retention time of 8.2, 8.9, 9.7, 10.3, 11.8, 15.2, 17.6, 18.5 and 19.2 respectively (Figure 1). In addition, TFGME withholds several minerals such as Aluminium, Copper, Iron, Manganese, Zinc and Strontium (Table 2).

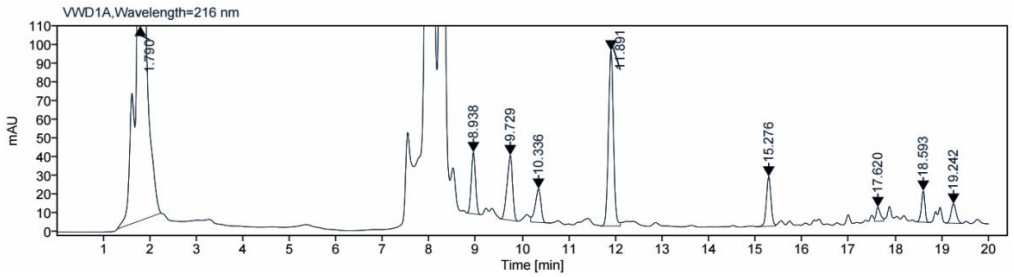

Figure 1. HPLC Chromatogram of TFGME

Table 2. HPLC analysis of TFGME

SL NO	Name of the Metal	TFGME In ppm
01	Aluminum	0.01
02	Boron	0.00
03	Cadmium	0.00
04	Copper	0.02
05	Iron	0.13
06	Manganese	0.02
07	Molybdenum	0.00
08	Nickel	0.00
09	Lead	0.00
10	Zinc	0.07
11	Chromium	0.00
12	Strontium	0.01

3.3. Antimicrobial property of TFGME

TFGME antimicrobial property was performed with pathogenic bacterial strains namely *E. coli, S. aureus, Salmonella, Pseudomonas* and *Shigella*. Interestingly, TFGME exhibits antibacterial property by inhibiting all bacterial strains used for antimicrobial assay. Minimum Inhibitory Concentration value of TFGME was demonstrated individually (Figure 2).

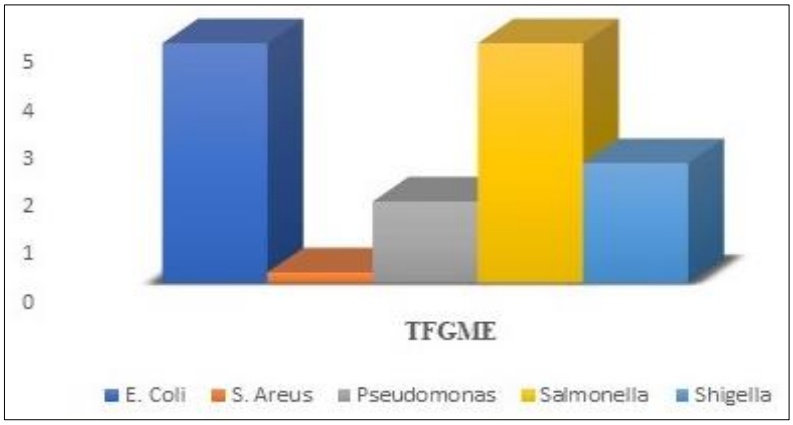

Figure 2. Anti-microbial property of TFGME

4. Conclusion

In conclusion, this study reveals the preliminary characterization of TFGME and its anti-microbial property.

Future study focus on: (i) isolate the purified compound from TFGME; (ii) characterize the purified compound isolated from TFGME; (iii) evaluate the molecular weight of purified compound isolated from TFGME; (iv) study the anti-microbial property of purified compound isolated from TFGME; and (v) determine the non-toxic property of purified compound isolated from TFGME.

Declarations

Source of Funding

This study did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis and manuscript writing equally.

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