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Antimicrobial and Antioxidant Assessment of *Trigonella foenum-graecum*

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ABSTRACT

Background: *Trigonella foenum-graecum* (fenugreek) is an important medicinal herb of the family Fabaceae. It has wide utility in culinary and ethnomedicinal formulations. Globally, the different folkloric formulations use this plant as an immunity booster. Unfortunately, the lack of proper studies imposes a major obstacle to the successful and fruitful realization of its full pharmacological potential. In the current study, pharmacological testing was performed on Trigonella seed oil.

Methods: The seed oil was extracted using the Soxhlet apparatus. The antimicrobial study of fenugreek seed oil was done using the disc-diffusion and broth dilution method against different bacterial and fungal strains. The antioxidant assessment was done by DPPH and ABTS assay.

Result: The yield of essential oil obtained from Trigonella seeds was 4.35%. GC-MS analysis has confirmed the presence of diverse phytocompounds in Trigonella seed oil. Dibutyl. phthalate was recognized as the major phytocompound of Trigonella seed oil. The oil had significant antifungal and antioxidant activity. In conclusion, oil is a good pharmacological agent and can be explored further for therapeutic purposes.

Key words: Essential oil, Fenugreek, Pharmacology, *Trigonella foenum-graecum*.

INTRODUCTION

Trigonella foenum-graecum is an imperative medicinal herb of Leguminosae family (Meena *et al*., 2021). Trigonella is the largest genera in the family Fabaceae (Ahlawat *et al*., 2023). It is one of the renowned Indian spices used in different cuisines and is commonly known as fenugreek (Qadir *et al*., 2022). The plant has a global distribution. India is the largest producer of fenugreek, followed by China, Pakistan, Iran, Afghanistan, Russia, Australia, Canada, USA and Europe (Singh *et al*., 2022). Fenugreek is the third largest seed spice after coriander and cumin (Shekhawat *et al*., 2023). *T. foenum-graecum* is a well-known anthelmintic, expectorant, hepatoprotective and immunity booster herb. Traditionally, it is used to cure gastric problems, joint pain, anxiety, pneumonia, bronchitis, fever, cold and cough. The plant is highly recommended for curing digestive problems, hair loss and reproductive health. The folkloric use of this medicinal herb is either inherited from elders or acquired through trials. These traditional uses also endorse multiple pharmacological efficacies of fenugreek (Sulieman *et al*., 2008).

Many studies have also documented the anticancer, anti-diabetic and anti-inflammatory role of fenugreek.The plant is effectively used in treating digestive problems, reproductive health problems and hypocholesterolemic issues (Syed *et al*., 2020). These pharmacological effects result from their diverse biologically active phytoconstituents, such as alkaloids, flavonoids, phenols, vitamins, minerals, amino acids, fibers and bioactive metabolites. These phytocompounds are mainly concentrated in their essential oil and bestow them numerous pharmacological effects. As a result, the essential oil help in curing many maladies in the case of human beings and animals (Salman and Qadeer, 2021). Moreover, EOs and their derivatives are increasingly used in the pharmaceutical and food industries. Numerous studies have highlighted the high efficacy of EOs against pathogenic microbes.

The EOs derived from spices are frequently used as flavouring, antimicrobial and antioxidant agents in food processing and pharmaceutical industries. The demand for herbal food preservatives and therapeutic agents is increasing daily. Hence, the present study was planned to evaluate the antimicrobial and antioxidant potential of *T. foenum-graecum.*

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MATERIALS AND METHODS

The work was carried out at Maharshi Dayanand University, Rohtak, Haryana (124001) from 2021-2023. The analytical-grade chemicals were procured from Sigma-Aldrich and Himedia. In the present study, we worked on the trigonella seeds. The seeds were purchased from the local market

of Rohtak (Haryana), India. The different plant parts of *T. foenum-graecum* are presented in Fig 1.

Extraction of trigonella seeds

The extraction was done using the Soxhlet apparatus (Fig 2). The thimble was prepared with 30 gm of seed powder. The extraction was performed in petroleum ether at 40-60°C for 4-5 hours. The extract was left to evaporate the solvent and to obtain the pure oil. The water droplets were removed using anhydrous sodium sulfate (Gungor *et al*., 2022).

Quantification of trigonella oil

The extracted oil yield was calculated using the standard equation given by Akbari *et al*. (2019). The Eq. was as follows:

$$\% \text{ yield of obtained EO} = \frac{\text{Mass of oil obtained (gm)}}{\text{Weight of the sample taken for extraction (gm)}} \times 100 \quad \text{...Eq. 1}$$

GC-MS analysis

GC-MS was carried out to identify the phytocompound composition of Trigonella seed oil. The analysis was done through394143602-GC Single Quadruple Mass Spectrometer SCION 436 GC; Fill Scan SIM, with CP-739651-CP8410 Liquid Auto Sample (Caputo *et al*., 2022).

Antimicrobial assessment

Microbial strains

The antimicrobial activity was carried out against four bacterial and two fungal strains as shown in Fig 3.

Disc-diffusion assay

The primary culture was obtained by inoculation and incubation (37°C overnight) of freshly prepared autoclaved nutrient broth media. The secondary culture was prepared by inoculating the freshly prepared autoclaved nutrient broth media from the primary culture and incubating for 3-4 hours to achieve the microbial concentration of 8 $\times 10^8$ cells/mL at 600 nm. The freshly prepared agar plates were inoculated with 100-150 µL of microbial suspension, followed by infusion of sterile discs with 30 µL of different concentrations of serially diluted Trigonella seed oil (stock solution of 10 mg/mL prepared in DMSO). The standard used for antibacterial and antifungal was Ciprofloxacin and fluconazole discs, respectively. In the case of fungal strains, the media was potato dextrose broth and incubation were done at 30±5°C for 48 hours (Errouane *et al*., 2020).

Broth dilution assay

For minimum inhibitory concentration, 20 µL aliquot (prepared in 0.85% of NaCl) was added to test tubes containing different concentrations of test samples, followed by a standard incubation period. The test tube without turbidity (MIC) was further tested for minimum bactericidal concentration. For MBC, 10 µL of non-turbid culture was again incubated at optimum temperature and duration. The microbial growth was observed visually (Errouane *et al*., 2020).

Antioxidant activity

DPPH assay

The assay was performed according to Akhlaghi and Najafpour-Darzi (2021). The 0.1 mM DPPH was prepared in methanol. One mL of methanolic DPPH was mixed with 1 mL of different concentrations of essential oils. The solution was left in the dark for 30 minutes. The solution was stirred vigorously and then poured into quartz cuvettes. The fluctuations in the absorbance were monitored at 517 nm. Pure methanol was used to zero down the spectrophotometer. Ascorbic acid was used as standard. The assay was done in triplicates. The radical inhibition by DPPH assay was calculated as follows Eq 2:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad \text{...Eq. 2}$$

Where,

$A_{control}$ = Absorbance of the control reagents excluding test sample.

A_{sample} = Absorbance of the test sample.

ABTS Assay

ABTS radical cation was prepared by mixing an aqueous ABTS solution (7 mM) with an aqueous solution of $K_2S_2O_8$ (140 mM). The solution was kept for 16 hours in the dark followed by dilution in methanol up to an absorbance of 0.8 at 734 nm. The one mL ABTS solution was dissolved with one mL concentrations of different essential oils. After vigorous stirring, the solution was poured into quartz cuvettes. The spectrophotometer was zeroed down with pure methanol. Ascorbic acid was used as the positive control. The decreased absorbance for each sample was registered at 734 nm. The readings were taken thrice to deduce the mean value (Kaviarasan *et al*., 2007).

RESULTS AND DISCUSSION

Yield of oil

The oil extracted from Trigonella seed was yellow in colour with a viscous texture. The oil had a characteristic aroma of fenugreek seeds. The yield of trigonella seed oil was calculated using Eq. 1.

Trigonella oil specific density = 0.98 g/mL.

The volume of the obtained oil = 20 mL.

Mass = Density × volume; 0.98 g/mL × 19.6 mL= 19.6 gm

$$\% \text{ yield of Trigonella oil} = \frac{19.6 \text{ gm}}{450 \text{ gm}} \times 100 = 4.35\%$$

The yield of oil obtained from Trigonella seeds was 4.35%.

GC-MS results

GC-MS analysis revealed the presence of numerous phytocompounds in Trigonella seed oil. The different retention times represented the presence of different

phytocompounds. Dibutyl.pthalate was recognized as the major phytocompound of Trigonella seed oil. Other compounds namely Benzaldehyde 4-(1-methylethyl), Cinnamaldehyde, *n*-Pentadecanol, Phenol, 2,4-bis (1,1-dimethyl ethyl), Aspidospermidin-17-ol, 1-acethyl-19,21-epoxy-15,16-dimethoxy, Murolan-3,9 (11) diene-10-peroxy, Pthalic acid butyl hexyl ester and i-Proxy 7,10,13,16,19-docosapent have also been identified. The physicochemical properties of different phytocompounds of Trigonella seed oil are shown in Fig 4.

Antimicrobial activity

The maximum antibacterial activity was observed against *E. coli*. In fungal strains, the oil had shown a zone of inhibition against *F. oxysporum* only. In the case of MIC, the oil had shown good MIC except for*S. aureus*. Though the oil had no bactericidal potential but it has shown fungicidal potential against *A. oryzae*. The antimicrobial results are shown in Table 1 and 2.

Antioxidant activity

The compounds with lesser absorbance are found to have higher free radical scavenging activity and antioxidant potential. In the present study, Trigonella oil was also reported to have significant antioxidant potential (Fig 5). ABTS assay has given more pronounced antioxidant results than the DPPH assay.

Medicinal plants have always remained integral to treating routine maladies and maintaining healthcare. They are an affluent reservoir of various phytocompounds (Singh *et al*., 2022). These phytocompounds endow diverse therapeutic attributes to the plants. The phytocompounds are mainly concentrated in the plant essential oil. Essential oils are used as food additives. They not only enhance the food taste but also preserve the food items. In developing countries, the fenugreek plant can be used as a nutrient supplement to avoid fungal infections, oxidative stress and associated health issues (Gurjar *et al*., 2016). Considering

Fig 1: Different plant parts of *T. foenum-graecum*.

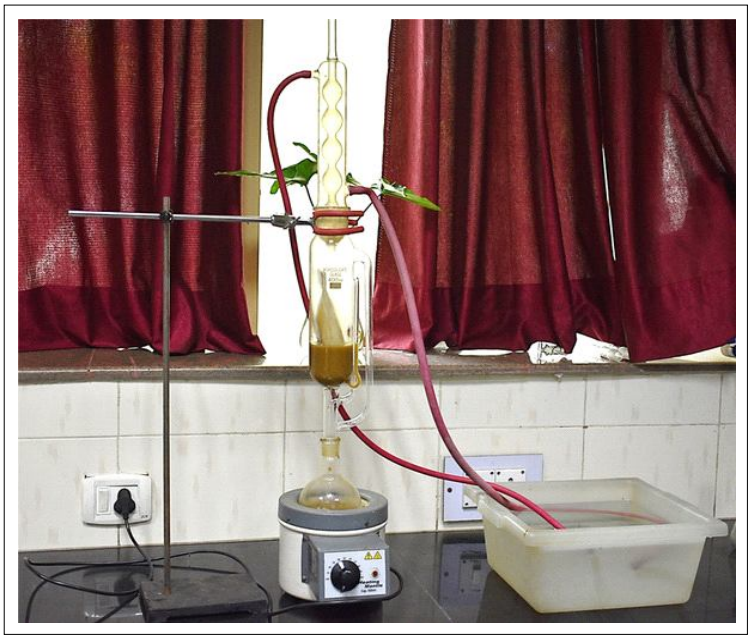


Fig 2: Soxhlet apparatus.

the various traditional medicinal uses of *Trigonella foenum-graecum*, we extracted essential oil from Trigonella seeds in the present study. The yield of oil obtained in the current study (4.35 %) was compared to a previous where they reported a 5.55±0.05% yield (Akbari *et al*., 2019). The difference in yield may be due to the difference in solvent

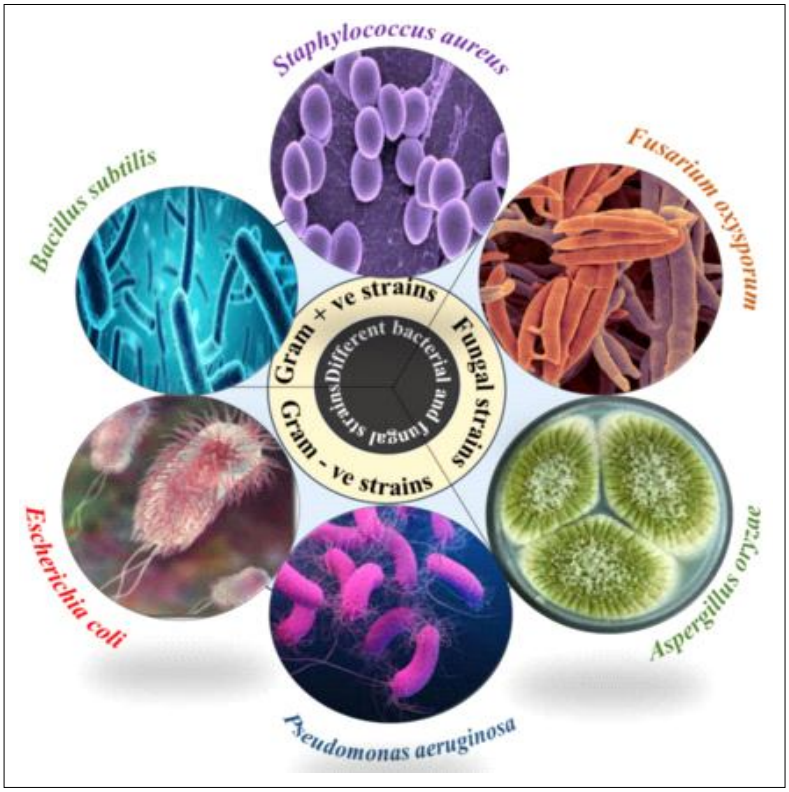


Fig 3: Selected bacterial and fungal strains.

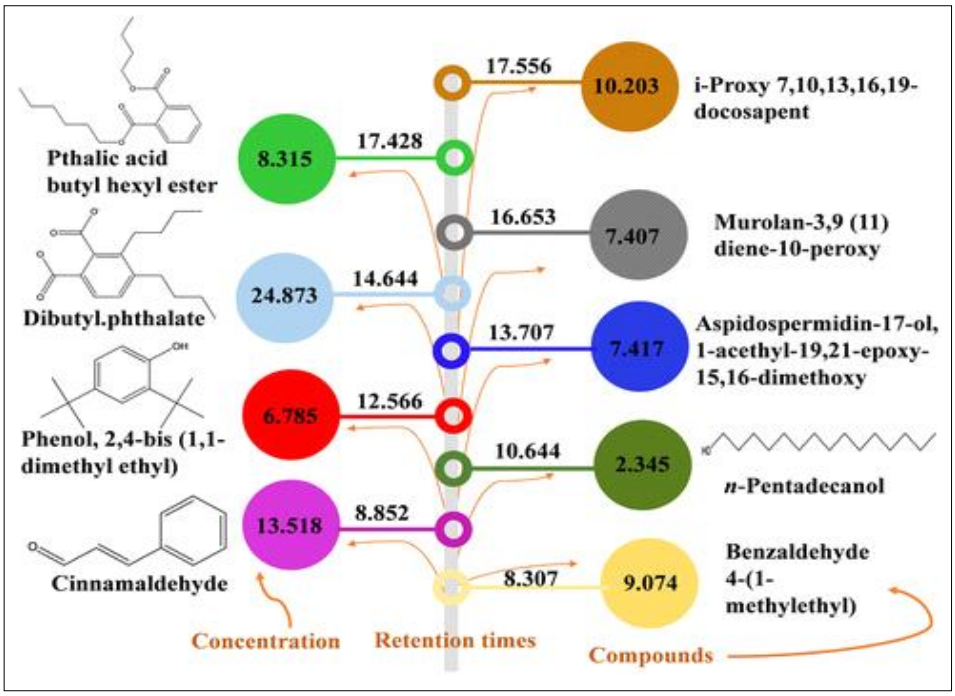
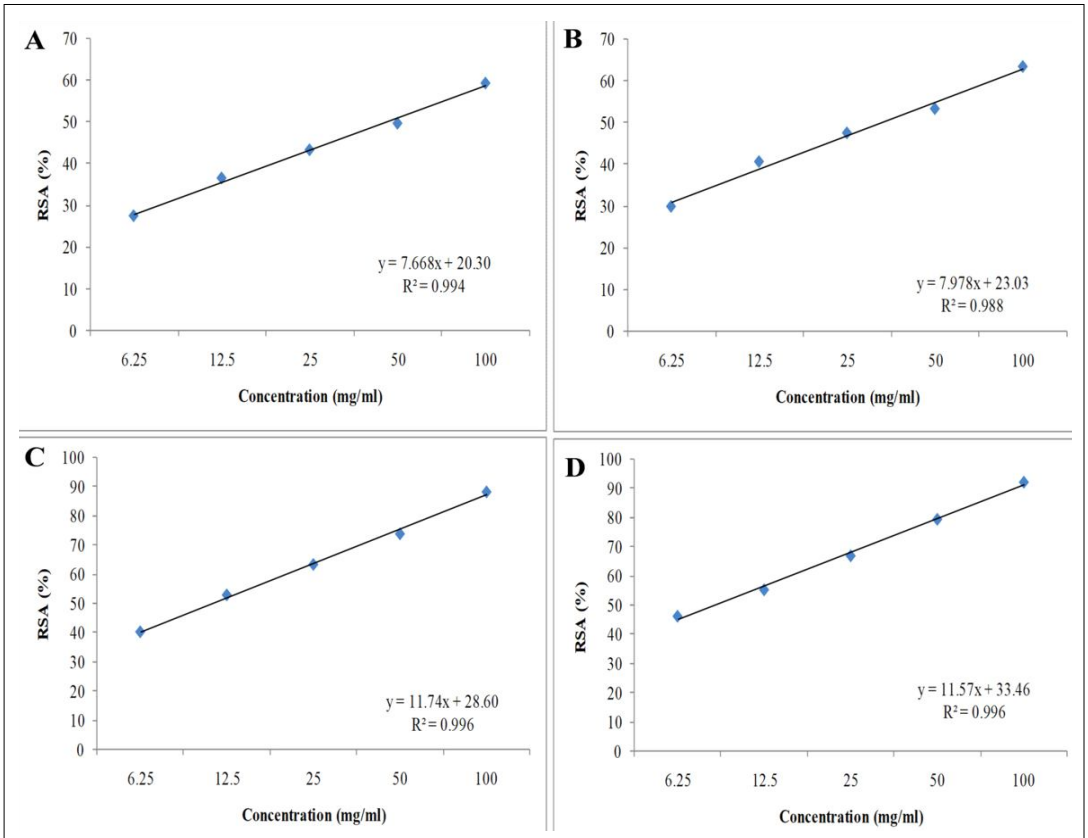


Fig 4: Different phytoconstituents of trigonella seed oil.



(A) DPPH; (B) ABTS; (C) Ascorbic acid (DPPH); (D) Ascorbic acid (ABTS).

Fig 5: Antioxidant potential of trigonella seed oil.

Table 1: Zone of Inhibition (mm) of trigonella seed oil.

Microbes	Zone of inhibition (mm)				
	100%	50%	25%	12.5%	6.25%
S. aureus	7.5±0.17	6.2±0.13	6.1±0.21	NA	NA
B. subtilis	6.9±0.23	6.5±0.18	6.0±0.17	NA	NA
E. coli	8.1±0.22	7.3±0.11	7.1±0.26	6.3±0.19	NA
P. aeruginosa	7.8±0.14	7.2±0.26	6.7±0.15	6.0±0.24	NA
A. oryzae	8.3±0.25	7.1±0.22	6.9±0.14	6.3±0.11	6.0±0.27
F. oxysporum	9.1±0.16	8.0±0.24	7.7±0.20	7.4±0.12	6.6±0.14

[a]Measurement in triplicate, NA= No activity.

Table 2: MIC/MBC and MFC of trigonella seed oil.

Microbes	MIC (mg/mL)	MBC/MFC (mg/mL)
S. aureus	50	100
B. subtilis	25	100
E. coli	25	100
P. aeruginosa	25	100
A. oryzae	25	25
F. oxysporum	25	100

[a]Measurement in triplicate.

used. The seed oil was characterized by GC-MS. They also analyzed the chemical composition of Trigonella seeds oil and reported approximately 23 compounds (Akbari *et al*., 2019). The Trigonella essential oil composition was also evaluated by Qadir *et al*. (2022) and the findings were in concordance with the present study's findings. Though we also analyzed the chemical composition of Trigonella oil but the phytochemical composition obtained in the present study was different from the previously documented results. In the present study, Dibutyl. phthalate was obtained as the major

phytocompound. In other studies, linoleic acid was reported as the major phytocompound (Ali *et al*., 2012; Ciftci *et al*., 2011). The difference may be due to one or more of the following reasons: (a) different variety types, (b) growth stages, (c) climatic conditions, (d) harvesting time and others (Behbahani *et al*., 2019; Lal *et al*., 2017).

Spice essential oils are good antimicrobial agents and are effective against many bacterial and fungal strains. Therefore, in the present study, we screened Trigonella essential oil against a panel of bacterial and fungal strains. The oil had shown significant antifungal activity but almost negligible antibacterial activity. Sulieman *et al*. (2008) also studied the antimicrobial efficacy of Trigonella seed oil. They reported that the oil possesses higher antifungal activity than antibacterial activity (Sulieman *et al*., 2008). Yousefipour *et al*. (2022) also reported the antimicrobial activity of Trigonella aqueous extract.

The antioxidant potential of essential oils improves the immune system of human beings. They act as good free radical scavengers, avoiding oxidative stress and associated disorders. In the present study, we carried out DPPH and ABTS assays to assess the antioxidant potential of Trigonella seed oil. Priya *et al*. (2011) also reported the antioxidant potential of hydroalcoholic extract of trigonella seeds. Though both the assays in the present study have shown significant results but the maximum activity was obtained with the ABTS assay. It may be primarily because ABTS radicals are more reactive than DPPH assay.

CONCLUSION

Trigonella foenum-graecum is an important dietary spice with plenty of phytocompounds. The presence of different phytocompounds was confirmed by GC-MS. GC-MS has shown Dibutyl.pthalate as the major phytocompound of trigonella seed oil along with Benzaldehyde 4-(1-methylethyl), Cinnamaldehyde, *n*-Pentadecanol, Phenol, 2,4-bis (1,1-dimethyl ethyl), Aspidospermidin-17-ol, 1-acethyl-19,21-epoxy-15,16-dimethoxy, Murolan-3,9 (11) diene-10-peroxy, Pthalic acid butyl hexyl ester and i-Proxy 7,10,13,16,19-docosapent. These phytocompounds are backed scientifically by various therapeutic potentials. Though the oil has shown less antibacterial activity but has shown significant antifungal activity. The antioxidant assays also confirmed the radical scavenging activity of Trigonella seed oil. The maximum antioxidant activity was obtained with ABTS assay. Further *in-vivo* and clinical trials are needed to explore the full efficacy of Trigonella essential oil.

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Author contribution

Neetu Singh designed the paper, wrote the paper draft and performed the experiments; Surender Singh Yadav and Balasubramanian Narasihman supervised the study, read the draft and gave valuable suggestions.

Ethical approval

Not required.

Conflict of interest

There is no conflict of interest.

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