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Antioxidant, antimicrobial, and preliminary phytochemical constituents of fermented seeds of *Trigonella foenum-graecum*

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Abstract

Trigonella foenum-graecum* (Fenugreek) is among the earliest plants used as spices and medicine worldwide. This study determined the antioxidant activities of the fermented seeds of *T. foenum-graecum* by methods, ABTS 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl). The phytochemical compounds, pH, and *in vitro* agar well (antimicrobial) assay of the sample was tested at three concentrations (100%, 50%, and 25%) against the selected pathogens (*Staphylococcus aureus, Salmonella typhi,* and *Escherichia coli*) by agar well diffusion method. There was a pH decline from 5.6 (on day 1) to 3.53 (on day 7) and finally 3.38 (on day 14). The antioxidant property of the 14th-day fermented *T. foenum-graecum* revealed a free radical scavenging potential in correspondence with the tested concentrations. Flavonoids, phenol, phytosterols, terpenoids, alkaloids, and tannins were detected in the samples fermented for 7 and 14 days, respectively. The zones of inhibition (in diameter) were in tune with the tested concentrations, and this ranged between 6mm – 15mm on day 7 and 6mm-17mm on day 14 against the test pathogens. Hence, this study validates the antimicrobial, and antioxidant activity of fermented seeds of *T. foenum-graecum* and supports its use as an herbal therapeutic agent against diseases associated with the test pathogen.

Keywords: Antimicrobial; antioxidant; Fermentation; Phytochemical; *Trigonella foenum-graecum*
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Introduction

The effectiveness of many commonly used antibiotics is being compromised globally due to the emergence of multi-drug-resistant bacteria (Falcone & Paterson, 2016; Van Duin & Doi, 2017). Thus, a surge in the need to search for new antimicrobial agents to combat bacterial resistance. Plants with medicinal values constitute reliable sources of potent medicines. Going by worldwide records, around 258,650 species of plants, and more than 10% of such plants are used for the development of drugs against different ailments (Uniyal *et al.*, 2006; Shinwari, 2010; Walter *et al.,* 2011).

Fenugreek (*Trigonella foenum-graecum*), of the family Fabaceae, is an ancient medicinal plant from central Asia of origin (Altuntas *et al.,* 2006). It is widely grown and frequently exported from India, China, Africa, Algeria, Saudi Arabia, Pakistan, Egypt, Turkey, Ukraine, Spain, and Italy (Salehi, 2008). In major Nigerian dialects, it is called *Eru* among the Yorubas, *Kimba/Hulba* among the Hausas, *Mkpuru oka oyibo* among the Igbos.

Owing to its numerous medicinal values and dietary fiber content, it is one of the oldest medicinal plants used in Rome and Egypt as "Hilba" tea to ease childbirth and increase milk flow, alleviate menstrual pains, and stomach-related problems (Mortel and Mehta, 2013). Its seeds have been reported to possess some medicinal properties such as lactation aid, antidiabetic, hepatoprotective effects, and anticancer potentials (Srinivasan, 2006). There are extensive scientific reports on the hepatoprotective potentials of leaves and seeds of *T. foenum-graecum* against cancer (breast and colon) and their curative potentials for diabetes and hypercholesterolemia (Al-Oqail *et al*., 2013)

Materials and methods

Plant Material

Dried *T. foenum-graecum* seeds were obtained from vendors in Kano, Northern region of Nigeria. The seeds were stored after identification and authentication (Assigned voucher number: UILH/004/1497/2022) at the Herbarium unit, Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Test Microorganisms

Three bacterial pathogens used in the study, Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Salmonella typhi*) were gotten from the Microbiology Laboratory of Al-Hikmah University, Ilorin, Nigeria, and maintained on Nutrient agar. They were further tested for viability at 37°C for 24 and maintained on agar slant at 4°C till needed for further use.

Turbidity of the test bacterial cultures was set at 0.5 McFarland turbidity standard; a few colonies of each organism from the subcultured agar plate were standardized using a UV spectrophotometer (Lemfield Medical England, MODEL 752G) to get an absorbance reading of (0.063-0.1) at a wavelength of 600 nm (equivalent to 0.5 McFarland turbidity standard) (Ochei & Kolhatkar, 2008).

Preparation of Fermented Seeds of T. foenum-graecum

The seeds were carefully sorted for healthy ones and stones were removed, they were rinsed using flowing tap water and later distilled water. The sample was drained for 15 mins. In a sterile Mason jar, 800 mL of distilled water and 200g of the cleaned seeds were combined (8:2 v/w). The jar was covered tightly and agitated daily to combine the content and facilitate the process of fermentation (Guarner & Schaafsma, 1998). The fermentation process was carried out for 14 days at room temperature after which the sample was centrifuged for 15 min at 14,000×g. The resulting supernatant was sifted using Whatman (no. 1) filter paper and kept for further use.

Preparation of working concentration of the sample

The filtrate was diluted serially to have different concentrations (100%, 50%, and 25%). From a tube containing 100% sample, 10 mL was transferred into a clean tube containing 10% sterile distilled water to generate 50% concentration. Another 10 mL was transferred from the tube containing 50% concentrated sample into a new clean tube of 10 mL of sterile distilled water to generate 25% concentration (Al-Bakri and Afifi, 2007).

Qualitative Phytochemical Analysis

Phytochemical tests were done to ascertain the presence of some bioactive components namely flavonoids, phenol, glycosides, phytosterols, terpenoids, saponin, alkaloids, tannins, and anthraquinone (Harbone, 1998).

Antimicrobial activity of fermented seeds of T. foenum-graecum

The activity of the sample against each strain was tested by the Agar well diffusion method (Valgas *et al.,* 2007). Into approximately 20mL of sterile molten nutrient agar medium, a sterile syringe was used to introduce (by aseptic means) 0.5mL of standardized inoculum of the isolates. The impregnated dishes were allowed to solidify at room temperature on the lab flat surface, then, a sterile cork borer measuring 8mm in diameter was used to bore a well in the impregnated dish. A quantity (0.1mL) of the fermented sample was introduced into the well and the plates were incubated for 24 hours at 37 °C. The clear zone without growth was recorded after 18-24 hours. Plates were prepared in triplicates for each tested concentration of the sample.

Antioxidant testing

Antioxidant activity, involving testing for scavenging (of free radical) activity of the

fermented sample was determined using DPPH [2,2-Diphenyl-1-picrylhydrazyl] as a free radical (Bulsal & Gulcin, 2011). The extract (0.1 mL) reacted with 3.9 mL of 0.1 mM methanol DPPH solution. After 30 minutes of incubation, the absorbance of the extract was determined at 515 nm using a spectrophotometer (Lemfield Medical England, MODEL 752G). The prepared working solution (3mL) was mixed with 100 µl of the test sample at different concentrations (10 - 100 µg/mL). The mixture was shaken thoroughly and incubated at room temperature for 15 minutes in the dark. Then, the absorbance reading (at 517 nm) was taken. DPPH was calculated based on the percentage of DPPH radical scavenged using the given equation: The formula given as (%) was used for the calculation (Brand-Williams *et al.*, 1995):

Scavenging effect (%) = [(control absorbance – sample absorbance) / (control absorbance)]x 100

ABTS Radical Scavenging Activity

The antioxidant activity of the sample was assayed by the ABTS cation scavenging activity approach described by Gulcin *et al.* (2011). By this method, a 7 mM solution of ABTS was reacted with 2.45 mM of potassium persulfate solution and left in the dark overnight to give a dark-colored solution of ABTS radical cations. Before use, the resulting ABTS radical cation was thinned with methanol (50%) for the first absorbance (at 745 nm) of 0.70±0.02 at a temperature control of 30°C. Subsequently, free radical scavenging activity was estimated by mixing the test studied sample (300 µl) with 3.0 mL of working standard of ABTS in a microcuvette. The reduction in absorbance was determined every minute up to 6 min after mixing the solution. Then, the inhibition (%) was determined using the formula:

ABTS scavenging activity (%) = $(A_0 - A_1)/ A_0 \times 100$ while A_0 represents the absorbance of the control, A_1 represents the absorbance of the sample.

Results

The value of ABTS antioxidant activity of *T. foenum-graecum* during the fermentation period increased along with the concentration (Figure 1).

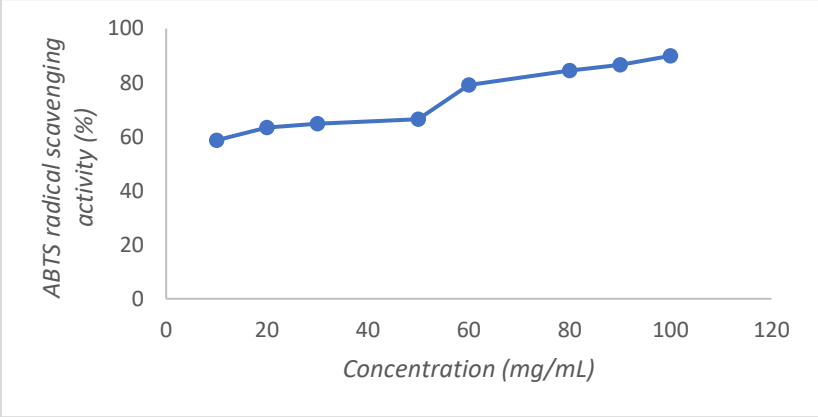

Figure 1: ABTS Radicals Scavenging Activity of Fermented Seeds of *T. foenum-graecum*

The antioxidant activity of the sample by the DPPH method increased correspondingly with the concentration tested (Figure 2).

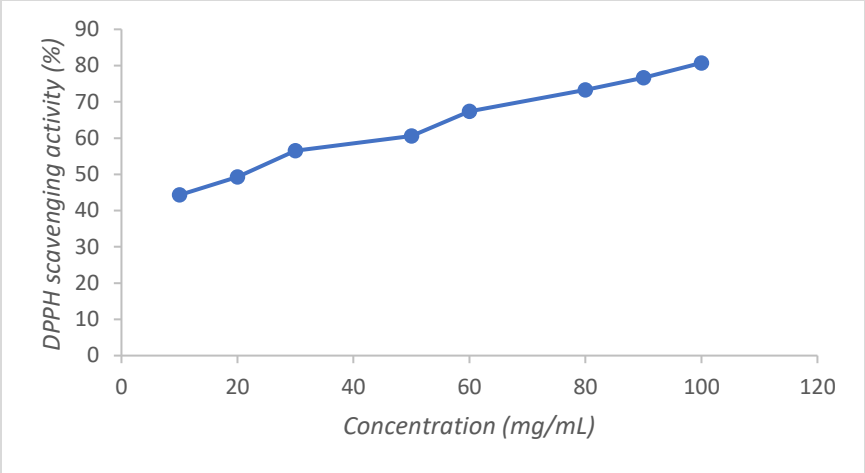

Figure 2: DPPH Radicals Scavenging Activity of Fermented Seeds of *T. foenum-graecum*

The result of the qualitative phytochemical composition of the sample showed the presence of the same constituents (flavonoids, phenol, phytosterols, terpenoids, alkaloids, and tannin) on days 7 and 14 of the fermentation process (Table 1).

Table 1: Constituents of fermented seeds of T*. foenum*-graecum

Components	**Day 7**	**Day 14**
Flavonoids	+	+
Phenol	+	+
Glycosides	-	-
Phytosterols	+	+
Terpenoids	+	+
Saponin	-	-
Alkaloids	+	+
Tannin	+	+
Anthraquinone	-	-

Key: + = positive

- = negative

The pH of fermented seeds of *T. foenum-graecum* declined during the period of fermentation from 3.45 on day 7 to 3.38 on day 14 (Figure 3).

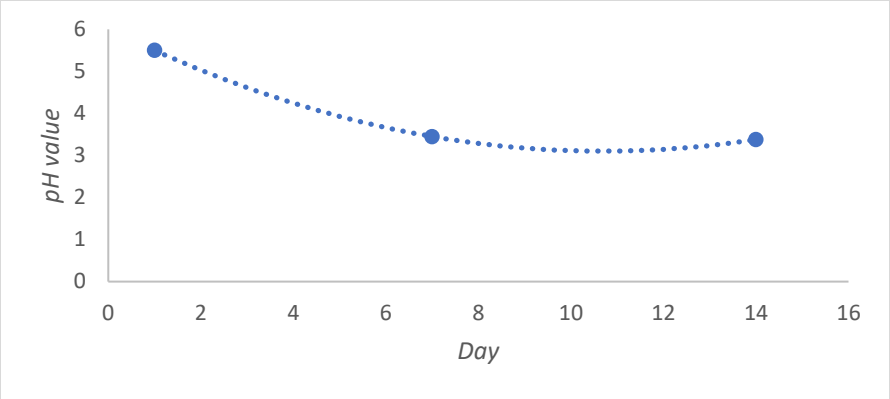

Figure 3: pH Value of fermented seeds of *T. foenum-graecum* during the period of fermentation

A varying degree of concentration-dependent activities was obtained for the different concentrations of the sample against the test pathogens (Figures 4, 5 and 6). On day 7 of the fermentation process, the highest zone of inhibition (15 mm) was obtained with the highest concentration tested (100%) against *S. aureus* while the least zone of inhibition (6mm) was obtained against the same organism at the lowest concentration (25%).

On day 14 of the process, the highest zone of inhibition (17 mm) was obtained against *S. typhi* at the highest concentration (100%) while the least zone of inhibition (6 mm) was obtained against *S. aureus* at the lowest tested concentration (25%)

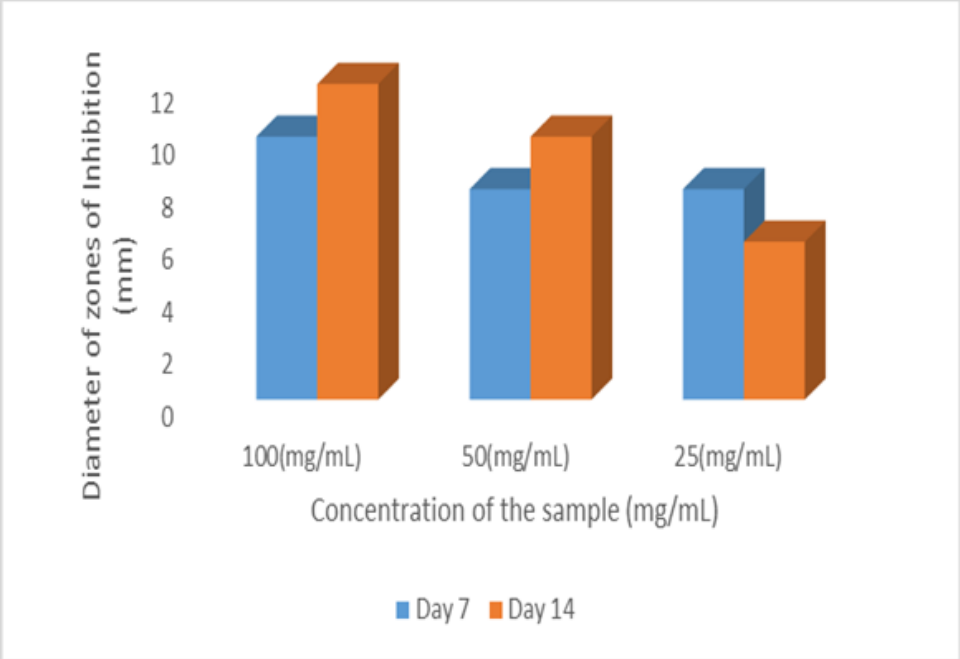

Figure 4: *In vitro* Antibacterial Activity of Fermented Seeds of *T. foenum-graecum* Against *S. aureus*

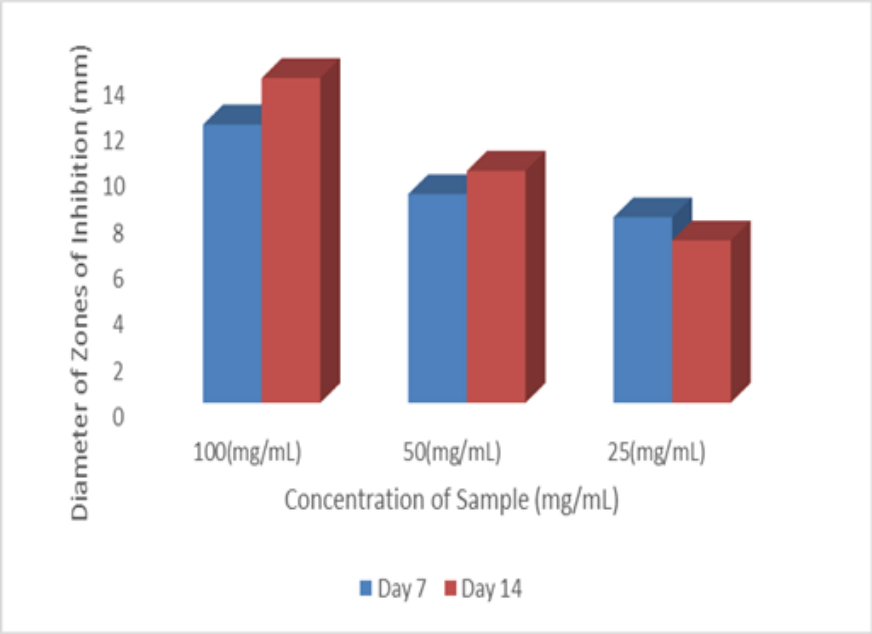

Figure 5: *In vitro* antibacterial activity of fermented seeds of *T. foenum-graecum* against *E. coli*

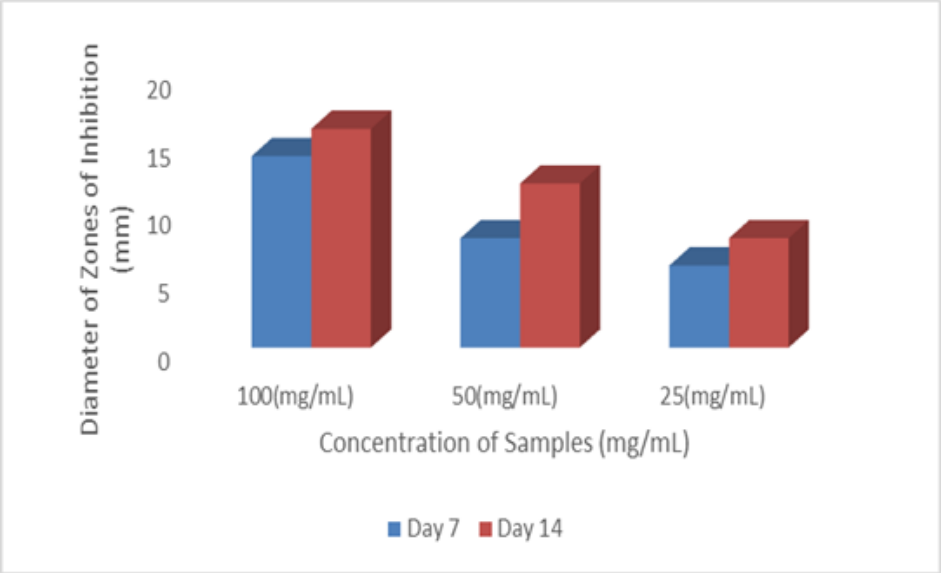

Figure 6: *In vitro* antibacterial activity of fermented seeds of *T. foenum-graecum* against *S. typhi*

Discussion

The concentration-dependent manner of antioxidant activity reported in this study indicated the free radicals scavenging property of the *T. foenum-graecum* Previous studies have shown that plants harbor some compounds with anti-inflammation and antioxidant activities (Arora & Pandey-rai, 2014; Benzidia *et al.,* 2018). Similar antioxidant ability of fenugreek has also been reported by Naidu *et al.* (2011)

Phytochemical compounds are natural biologically active compounds occurring in plants, which confer enormous medicinal and nutritional benefits on humans (Hasler & Blumberg, 1999). Fenugreek harbors secondary metabolites such as flavonoids, alkaloids, and tannins (Taylor *et al.,* 2006) which in abundance, may predominantly have deleterious effects on its nutrients. However, microorganisms associated with fermentation can alter the food sample by causing the structural breakdown of the walls of

the sample, leading to the synthesis and release of new bioactive compounds (Liu *et al*., 2017; Ricci *et al.,* 2019). Hence, in this study, the phytochemical screening of *T. foenum-graecum* conveyed the presence of flavonoids, alkaloids, phenol, phytosterols, terpenoids, and tannin which may individually or collectively, attributed to the antibacterial properties of fenugreek seeds as previously reported (Khursheed *et al.,* 2012). Polyphenol compounds, including rhaponticin and isovitexin, remain the major bioactive compounds in fenugreek seeds (He *et al.,* 2015).

Flavonoids are ever-present polyphenolic compounds occurring in numerous derivatives such as glucosides, aglycones, and methylated (Narayana *et al.,* 2001; Mahmomoodally *et al.,* 2005). They have numerous biochemical properties with the best, being their antioxidant potential (Kelly *et al*., 2002). Terpenoid shows immunobiologically properties and may have therapeutic importance on various infections. Tannins have well-known antimicrobial and astringent activity (Chung *et al*., 1998).

The decline in the pH value of the fermented sample means an increase in the acidity of the sample which may be due to the production of organic acid-producing organisms e.g., Lactic acid bacteria (Broadbent *et al.,* 2010). which creates stressful conditions for spoilage-causing microorganisms and outcompete them in foods by reducing the pH (Meyer *et al.,* 2009).

The concentration-related zone of inhibition obtained by different concentrations of the sample against the test organisms (*S. aureus*, *E. coli,* and *S. typhi*) agrees with the work of Ritu *et al*. (2010) which reported the activity of fenugreek seed extracts against *E. coli, L. acidophilus, B. cereus,* and *Pneumococcus*. The wider zone of inhibition recorded against *S. typhi* than *E. coli* authenticates the work of other researchers where a broad spectrum of antagonistic activity was reported against Gram-negative pathogens (Liasi *et al.,* 2009; Grosu-Tudor *et al.,* 2014; Setyawardani *et al.,* 2014; Jose *et al*., 2015). However, the work of Kloos (1998) is in disagreement with previous reports that Staphylococci (a Gram-positive organism) develops resistance swiftly to antibiotics. Anas *et al.* (2008) contradict this as a higher degree of antagonistic activity was reported against Gram-positive organisms than Gram-negative organisms.

Conclusion

From this study, fermented seeds of *T. foenum-graecum* harbor phytochemical constituents with several health importance. It has also shown antioxidant and antimicrobial activity against selected microorganisms and thus, can be exploited for the development of safe antimicrobial agents.

Acknowledgements

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Conflict of interest

The authors declare that there are no conflicts of interest.

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