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Phytochemical components, antioxidant and antimicrobial properties of fermented seeds of *Foeniculum vulgare*

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Abstract
Foeniculum vulgare (fennel) has been used in traditional medicine all over the world including Nigeria for a wide range of ailments. In the present study, the phytochemical analysis, antioxidant activities (by ABTS and DPPH methods), in vitro antimicrobial assays (against Staphylococcus aureus, Salmonella typhi, and Escherichia coli) using agar well diffusion method, and pH of the fermented seeds of F. vulgare were investigated. Phytochemical investigation showed the presence of tannins, terpenoids, phenols, phytosterols, alkaloids, and flavonoids. Alkaloids was absent on day 7 of the fermented seeds but was present on day 14. The antioxidant activity of the fermented sample of F. vulgare at day 14 was proportional to the concentration of the sample. The zones of inhibition (in diameter) obtained were dependent on the tested concentrations (100%, 50% and 25%) and ranged between 5mm-18mm on day 7 and 6mm- 20mm on day 14 against the test pathogens. The trend of acidity increased with the days of fermentation. Hence, with the observed antimicrobial and antioxidant activities of the fermented seeds of F. vulgare, it can be considered a relatively safe agent with a long remarkable history in traditional medicine.
Keywords: *Antibacterial, Antioxidant, Fermentation, F. vulgare, phytochemical.*

INTRODUCTION

The hackneying of antibiotics gives rise to the emergence of antimicrobial-resistant strains of microorganisms (Baym *et al*., 2016). Thus, there has been a shift of attention to the use of plants not just in diet, but also as safe medicinal alternatives to treat health conditions including skin disorders, gastrointestinal conditions, respiratory and urinary problems, asthma, and hepatic and cardiovascular disease (Tian *et al*., 2014).

F. vulgare Mill, commonly known as fennel, belonging to the family Umbelliferae/ Apiaceae (Kishore and Verma, 2022) can sometimes grow naturally and is commonly cultivated in the southern Mediterranean region specifically in North America, Europe, and Asia (Farooq, 2005; Badgujar *et al.,* 2014). Several varieties grow with a requirement for good soil to grow in mild climatic areas (Gori *et al*., 2012).

Traditionally, *F. vulgare* is a known plant with economic and medicinal values in Asian countries. It has been employed in folkloric medicine for a wide range of conditions linked with the reproductive, respiratory, digestive, and endocrine systems (Abou El-Soud *et al.,* 2011). It is also used as a lactation-inducing agent for lactating mothers (Kooti *et al*., 2015). Several scientific reports have stated the antioxidant, anticancer, antitumor, chemo-preventive, cytoprotective (Abou El-Soud *et al.,* 2011), hepatoprotective, hypoglycemic, galactagogue, oestrogenic, antistress, antibacterial, analgesic, and antifungal properties of *F. vulgare* (Abou El-Soud *et al.,* 2011; Kooti *et al*., 2015). In Nigeria and globally, fennel has been used as part of the condiments in spice production possibly due to its notable biological (antioxidant and antimicrobial) activities (Abou El-Soud *et al.,* 2011).

There can be variation in the resistance of microorganisms to different preparations of a specific spice due to the existence of different active phytocompounds, hence, this study probed the antioxidant and antimicrobial properties of the fermented samples of fennel seeds obtained from the Northern part of Nigeria.

MATERIALS AND METHODS

Collection and Constitution of the Plant Material

Dried seeds of *F. vulgare* (fennel) used in this study were purchased from a local market in Kano, Northern region of Nigeria, identified and authenticated (Voucher number: UILH/002/1495/2022) at the Department of Plant Biology (Herbarium unit), University of Ilorin, Ilorin, Nigeria. The dried seeds were picked to remove dirt and dust.

The fennel sample was rinsed with distilled water, and 200g of the clean sample was placed in a Mason jar containing 800mL brine (distilled water and 20g of sea salt) solution (1:8 w/v). The jar was tightly closed and stored at room temperature (26 ± 2 ℃) for 14 days with frequent agitation manually to ensure that the seeds were fully immersed in the brine solution to facilitate microbial multiplication during the process of fermentation (Guarner and Schaafsma, 1998). Some of the samples were strained after day 7, then at day 14, and filtered using number 1 Whatman filter paper. The pH was checked using a pH meter (Hanna pH210) and serial dilution was done to generate different concentrations (100%, 50%, and 25%) of the sample.

Test Microbial Bacteria

Bacteria of clinical importance were selected for use in this study. A Gram-positive organism (*Staphylococcus aureus*) and two Gram-negative strains (*Escherichia coli and Salmonella typhi*) were collected from the Microbiology Laboratory of Al-Hikmah University, Ilorin, Nigeria, and were sub-cultured for viability checking at 37 ℃ for 24 hours and sustained on nutrient agar slant at 4°C for further use. Standardization of each inoculum (Ochei and Kolhatkar, 2008) was achieved by picking respective colonies (3-4 colonies) of each strain from the sub-cultured agar plate using a sterile inoculating loop into the sterile normal saline in a sterilized test tube. This was standardized using a UV spectrophotometer (Lemfield Medical England) to get an absorbance value of 0.063 – 0.1 at a wavelength (600 nm) equivalent to 0.5 McFarland turbidity standard.

Antimicrobial Activity of Fermented Seed of *F. vulgare*

The *in vitro* activity (antibacterial) of the individual bacterial sample was investigated using Agar well diffusion method. Approximately 20 mL of sterile molten medium agar was transferred into sterile Petri dishes using the pour plate method. A sterile syringe was used to withdraw 0.5 mL of standardized inoculum of the isolates and it was introduced aseptically into the sterile nutrient agar. The dishes were allowed to solidify on a flat surface. A sterile cork borer (8 mm diameter) was used to make a well in the inoculated plate. The fermented sample (0.1 mL) was introduced into the well. A Triplicate plate was prepared for each tested concentration of the sample. The plates were incubated for 18- 24 hours at 37 °C and the zone of inhibition was recorded (Li *et al.,* 2009). Standard Gram-positive (25 µg/mL Chloramphenicol) and Gram-negative (25 µg/mL Ampicillin) drugs were tested against the test organisms. These tests were repeated thrice and the mean of the triplicate diameter of the inhibition zone was taken.

Qualitative Phytochemical Analysis

Phytochemical tests were done to ascertain the presence of a bioactive component in the fermented fennel samples. Constituents analyzed include alkaloids (Joshi *et al.,* 2013), tannins, glycosides, flavonoids, saponin (Banso and Adeyemo, 2006), terpenoids, phenol, anthraquinone (Joshi *et al.,* 2013), and phytosterols.

ABTS Radical Scavenging Activity

The ABTS (2,2-azinobis '3-ethylbenzthiazoline-6-sulphonic acid') cation radical scavenging activity was done (Re *et al*., 1999) using 7 mM ABTS solution which was reacted with 2.45 mM potassium persulfate solution and stored overnight in the dark to give a dark-colored solution containing ABTS radical cations. The ABTS radical cation was previously diluted with 50% methanol to generate an absorbance of about 0.70±0.02 at 745 nm and temperature control set at 30°C, thereafter, the free radical scavenging potential of the sample was determined by combining in a microcuvette, 300 µl of the test sample with 3.0 mL of the ABTS working standard. The decline in absorbance was quantified exactly one minute after mixing the solution and up to 6 minutes. The formula adopted for calculating the percentage inhibition is:

$$\text{Scavenging activity (\%)} = \frac{[(\text{absorbance of control} - \text{absorbance of sample})}{(\text{absorbance of control})]} \times 100$$

Thus, the antioxidant potential of the sample was given as EC50 (anti-radical activity), the concentration necessary for a 50% decrease in ABTS (Gülçin *et al*., 1999).

DPPH Radical Scavenging Activity Assay

The 2, 2 - diphenyl-1-picrylhydrazyl (DPPH) assay was carried out using the method recounted by Brand-Williams *et al.* (1995); a stock solution was made by constituting 24 mg DPPH with 100 mL methanol and kept at 20°C for further use. The working solution was prepared by diluting the DPPH solution with methanol to have an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. An aliquot (3 mL) of the working solution was combined with the test sample (100 µl) at different concentrations (10 - 100 µg/mL). The reaction mixture was shaken thoroughly before incubation in the dark for 15 minutes at room temperature. Then, the absorbance (at 517 nm) was taken. Following the same method, a control setup was prepared without any sample. The scavenging potential was determined based on the percentage of DPPH radical scavenged using the equation:

$$\text{Scavenging activity (\%)} = \frac{[(\text{absorbance of control} - \text{absorbance of sample})}{(\text{absorbance of control})]} \times 100$$

RESULTS

The diameter of zones of inhibition (mm) of the different tested concentrations of the sample against the test pathogens showed concentration-dependent activities (Figures 1, 2, and 3) across the period of fermentation; a higher activity (18 mm at day 7 and 20 mm at day 14) was obtained against *S. typhi* at the highest tested concentration (100%) while the least activity (5 mm at day 7 against *E. coli* and 6 mm at day 14 against *S. typhi*) was obtained at the lowest tested concentration (25%). With *E. coli*, the highest activity (10 mm on day 7 and 15 mm on day 14) was obtained at the highest tested concentration (100%), while, the least activity (5 mm on day 7 and 8 mm on day 14) was obtained at the lowest tested concentration (25%). Against *S. aureus*, the highest activity (8 mm on day 7 and 10 mm on day 14) was obtained at the highest tested concentration (100%) while the least activity (6 mm on day 7 and 14, respectively) was obtained with the lowest tested concentration (25%).

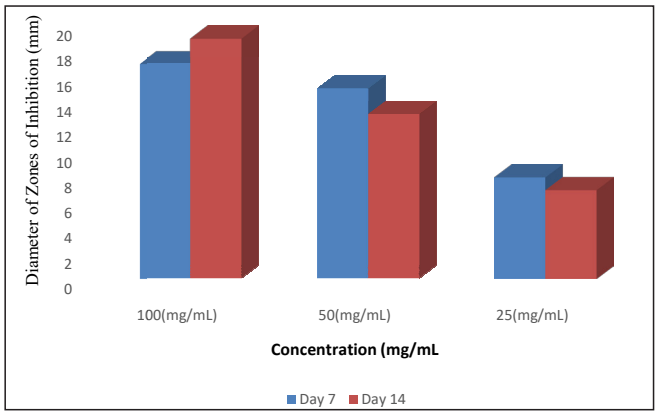

Figure 1: Diameter of zone of inhibition of the fermented seeds of *F. vulgare* against *S. typhi*

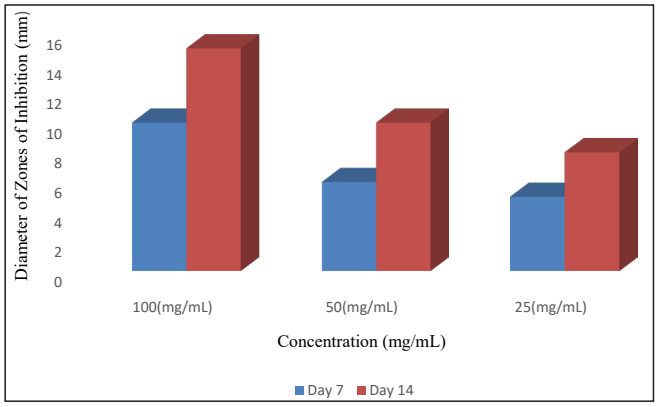

Figure 2: Diameter of zone of inhibition of the fermented Seeds of *F. vulgare* against *E. coli*

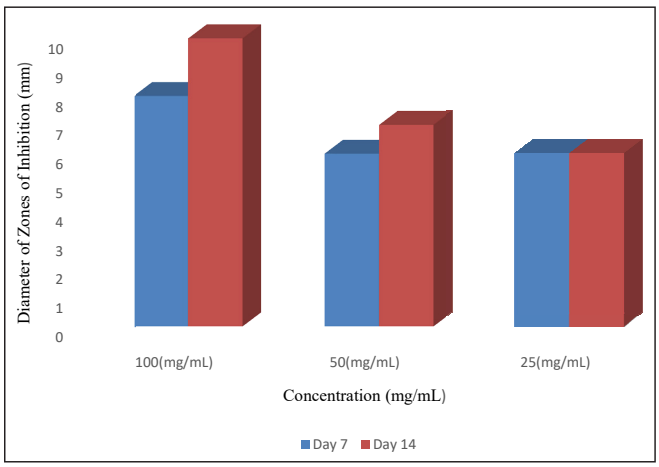

Figure 3: Diameter of zone of inhibition of the fermented seeds of *F. vulgare* against *S. aureus*

The result of the qualitative phytochemical analysis revealed the presence of five constituents on day 7 and six constituents on day 14 of the fermentation (Table 1).

Table 1: Qualitative phytochemical composition of the fermented seeds of *F. vulgare*

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

+ = Present; - = Absent

The tested sample showed high scavenging activity which increased as the tested concentrations (10-100 mg/mL) of the sample increased (Figure 4).

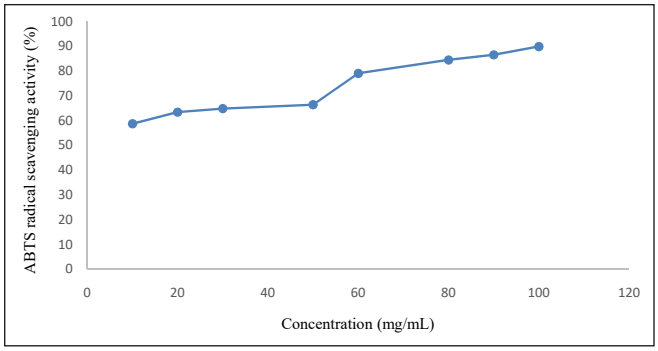

Figure 4: ABTS radical scavenging activity of the fermented seeds of *F. vulgare*

The tested sample showed inhibition of the DPPH radical by the antioxidant increased along with the tested concentrations (10-100 mg/mL) of the sample (Figure 5).

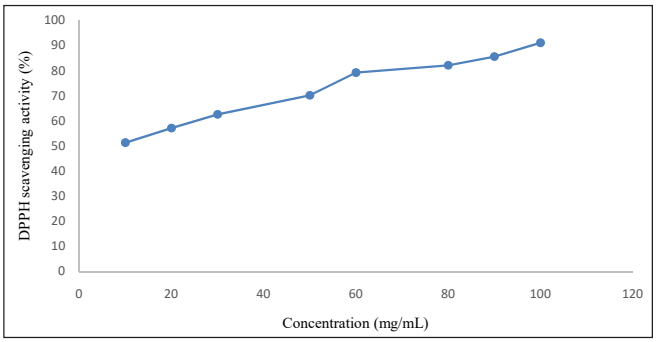

Figure 5: DPPH radical scavenging activity of the fermented seeds of *F. vulgare*

The pH values of *F. vulgare* sample were observed to decline during the period of fermentation from 3.52 on day 7 to 3.47 on day 4 (Figure 6)

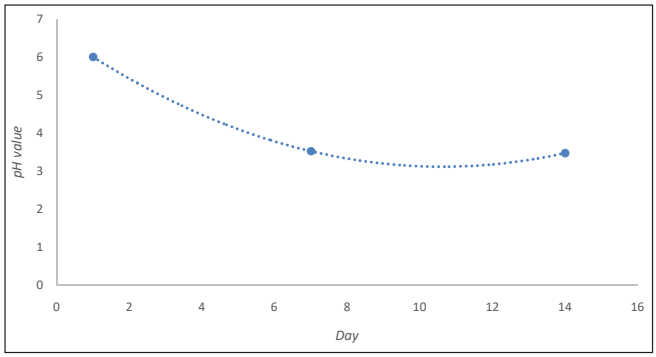

Figure 6: pH value of fermented seeds of *F. vulgare* during the period of fermentation

Discussion

Phytochemicals play vital roles in the bioactivity of medicinal plants of medicinal value (Ekwenye and Elegalam, 2005; Cao *et al*., 2022). The antimicrobial activities of some herbs against different bacteria strains might be attributed to the existence of active phytocompounds such as alkaloids phenolics, and terpenoids, with proven antimicrobial and antioxidant effects (Hoult and Payá, 1996; Rios and Recio, 2005). In humans, flavonoids are known to exhibit various biological roles such as anti-oxidative, anti-inflammatory, antitumor, antiviral, and antibacterial (Cushnie and Lamb, 2011). Flavonoids also play a protective role against coronary diseases and help in vascular activity (Cazarolli, 2006).

The present study showed antioxidant potential by ABTS and DPPH assay techniques. The assayed sample exhibited effective concentration-dependent free radical-scavenging ability. This may be due to the phytochemical constituents present in the sample. Fennel seeds are rich in free flavonoids or occurring as glycosides, and are known to exhibit antioxidant effects against free radicals. Anwar *et al.* (2009) also reported substantial contents of total phenolic, flavonoids, and DPPH radical scavenging activity with peroxidation inhibiting potential by 45–70 % level.

The decreasing antimicrobial activity demonstrated by the sample during the period of fermentation might also be attributed to the organic acids and other compounds (such as hydrogen peroxide, lactic acid, and bacteriocins) with antibacterial properties in the fermented sample (Agriopoulou *et al*., 2020). Similarly, organic acids (e.g., acetic and lactic acids) and alcohol produced, are known to play a physiological role during the fermentation process of Kefir (2011). A wider inhibition zone recorded against *S. typhi* and *E. coli* than *S. aureus* is in disagreement with the work of Anas *et al*. (2008) which reported a higher degree of antagonistic activity by strains of Lactic acid bacteria against Gram-positive pathogens than Gram-negative counterparts. However, de Almedia Junior *et al*. (2015) reported no relationship between the degree of Latic acid bacteria antagonistic activity and the gram status of pathogens tested.

Microorganisms are affected by the hydrogen ion concentration in their environment. Acidic medium prevents the growth of pathogens and only promotes the growth of acid-producing microorganisms. Thus, the decline in pH values seen in this study might be linked to the presence of acid-producing microorganisms that are associated with fermented foods (Falana *et al*., 2012). Such acids include lactic acid being produced by lactic acid bacteria or acetic acid being produced by acetobacter (Broadbent *et al.,* 2012; Papagianni, 2012) from sugars in the fermentation medium to inhibit the growth of pathogens. Organisms associated with fermentation, such as Lactic acid bacteria, have been reported to contribute to beneficial effects in fermented foods (Mokoena, 2017) and enhance the bioavailability of some nutrients (Rollán, 2019).

CONCLUSION

The seeds of *F. vulgare* are used in herbal medicine since old times. This study further shows that based on the antioxidant and antimicrobial potential of the seeds, they can be regarded as a valuable source of natural antioxidants that can resist the harmful effects of many health conditions such as inflammation, cancer, hypertension, diabetes, and diseases associated with the tested pathogens.

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CONFLICT OF INTEREST
The authors declare no conflict of interest

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