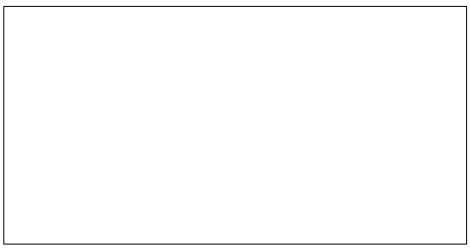

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Phytochemical assessment, elemental composition, and biological kinetics of *Foeniculum vulgare* Mill. stalks

Covenant E. Ogbonna [a], Doga Kavaz [b], Yemi A. Adekunle [c], David B. Olawade [d,e,*]

[a] Department of Bioengineering, Cyprus International University, Nicosia, Mersin-10 Turkey
[b] Biotechnology Research Center, Cyprus International University, Nicosia, Mersin-10 Turkey
[c] Department of Pharmaceutical and Medicinal Chemistry, College of Pharmacy, Afe Babalola University, Km 8.5, Afe Babalola Way, P.M.B. 5454, Ado-Ekiti, Ekiti State, Nigeria
[d] Department of Allied and Public Health, School of Health, Sport and Bioscience, University of East London, London, United Kingdom
[e] Department of Research and Innovation, Medway NHS Foundation Trust, Gillingham ME7 5NY, United Kingdom

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ABSTRACT

Introduction: The essential oil of *Foeniculum vulgare* (Fennel) has been extensively studied for phytochemical and elemental compositions, biochemical significance, and biological activities. However, the literature has a dearth of information on the Fennel stalk as a potential source of antioxidant and antibacterial agents. This study aimed to evaluate the phytochemical and elemental composition of Fennel stalks and also, to investigate the kinetic DPPH scavenging property and antibacterial activity of Fennel stalk extracts.

Methods: Fennel aqueous extract (FVAE) was prepared by mixing of powdered fennel stalks with sterile distilled water, followed by heating and filtration. Methanol (FVME) and ethanol (FVEE) extracts of *F. vulgare* were prepared by macerating the stalk with respective solvents, followed by filtration. Gas chromatography-mass spectrometry (GC–MS) analysis, Fourier Transform Infrared Spectroscopy (FTIR), determination of total phenols, and X-ray fluorescence were conducted to analyze the chemical composition of the extracts. Biological activities, including DPPH scavenging and antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*, were evaluated through spectrophotometric methods and well diffusion assay, respectively.

Results: The GCMS analysis revealed 15 compounds including anozol, myristyl iodine, flexiricin, dutadrupine, cycloheptaciloxane, neophytadiene, phthalic acid, and ribitol. Some of these compounds have not been reported from this plant before. FTIR analysis suggested hydroxyl (OH), alkane (CH), carbonyl (C = O), aromatic (C = C), and ether (CO) functional groups in the Fennel extracts. X-ray fluorescence identified elements including titanium, chromium, manganese, iron, nickel, copper, zinc, rubidium, and strontium. The ethanol extract had higher total phenolic content (72.45±0.01 mg GAE/g) than the aqueous (54.3 ± 0.01 mg GAE/g) and methanol (51.3 ± 0.01 mg GAE/g) extracts. In addition, a high% DPPH scavenging activity was observed in the ethanol extract (85.1 %) compared to that of ascorbic acid (96.2 %). Fennel's stalks methanol extract showed significant inhibition against the growth of *E. coli* 0157 and Methicillin-resistant *Staphylococcus aureus* B-4420, having zones of inhibition diameters of 16 mm and 13 mm, respectively. This is similar to what was observed for chloramphenicol and azithromycin.

Conclusion: The current study has demonstrated that Fennel stalks contain potentially useful antioxidant and antibacterial properties, in addition to the well-studied essential oil of its fruits and seeds.

1. Introduction

Plants amongst other sources for therapy have gained outstanding relevance both for their accessibility and multi-purpose [1]. Several plants such as turmeric, green tea, olives, cinnamon, ginger, garlic, and fennel amongst others have been used for their immense medicinal properties [2,3]. Despite growing worries about the safety of their use, ancient traditional medicine centered on the use of plants. The

* Corresponding author at: Department of Research and Innovation, Medway NHS Foundation Trust, Gillingham ME7 5NY, United Kingdom.
E-mail address: d.olawade@uel.ac.uk (D.B. Olawade).

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biological activities of medicinal plants are primarily because of the presence of secondary metabolites such as phenolic acids, flavonoids, triterpenes, alkaloids, tannins, and saponins [4,5].

Foeniculum vulgare Mill. (Fennel) is a medicinal and aromatic herb in *Apiaceae* family which is indigenous to the Mediterranean area but also commercially cultivated in China, India, Japan, and Russia [6]. Fennel, also known as "Xiaohuixiang" in Chinese, has been used in traditional Chinese medicine to cold pain, treat rheumatism, stomach disorders [7]. The fruit mericarp is rich in essential oils [8]. It is traditionally used to relieve stomach conditions [3], abdominal pain, cough [9], cancer, conjunctivitis, pyrexia, rheumatism [10], constipation [11], and stomachache [12]. Biological activities have confirmed the plant's antioxidant [13], antimicrobial [14–16], anti-osteoarthritic [17], anticancer [18], anti-inflammatory and analgesic [8] properties. Phytochemically, riboflavin, Fe, Na, K, Ca, niacin, and ascorbic acid are among the minerals and vitamins found in Fennel [19]. Bioactive phyto-compounds have been reported in the essential oil of Fennel seeds, and these include estragole (76.2 %), α-thujone (9.6 %), limonene (8.6 %), α-pinene (0.7 %), β-fenchol (0.6 %), α-terpinene (0.4 %), and β-myrcene (0.4 %). The liquid chromatography-mass spectrometry (LCMS) analysis of the methanol seed extract also showed protocatchuic acid, cirsiliol, apegenin, quercetin, p-coumaric acid, caffeic acid, epicatechin, rutin, chlorogenic acid, quinic acid, quercetin-3-o-rhamonoside, naringin, and 4-O-caffeoylquinic acid [20]. Other studies also reported trans-anethole (88.28 %) in the fruits [21], estragole (51.04 %), limonene (11.45 %), l-fenchone (8.19 %), and trans-anethole (3.62 %) in the essential oil [22] of Fennel.

Fennel is a soft feather-like perennial plant with the potential to attain a height of 2 m. The segments of the three to four pinnate, filiform leaves can reach a length of 1.6 inches (4 cm) [14]. Small, yellow, and arranged in broad umbels with flat tops are the blooms. Around June and July, flowers are produced and appear to bloom, growing to about 3–5 mm in length with a non-appearance of fruits, until September to October when the seeds appear and begin to mature [23]. When young, Fennel seed is a bright brown or green spice with an anise flavor; as it ages, the seed gradually turns a drab grey color. Green seeds are great for cooking. The hardy, crisp bulb can be grilled, sautéed, stewed, braised, or simply eaten raw [23].

Several *in vitro* and *in vivo* biological evaluations of Fennel showed that it has anticancer [18], antimicrobial [15,24], anti-inflammatory, analgesic [8,25], antioxidant [16] properties. Fennel is consumed as salads, as a stew component, and even as herbal teas [10]. A recent study reported that Fennel contains polyacetylene, hence the ability of Fennel seed extracts to improve vision and mitigate tumorous cell proliferation [26,27]. Some isolated compounds from the stem of *F. vulgare* include dillapional, scopoletin, dillapiol, bergapten, imperatorin, and psolaren [28].

This study aims to address a notable gap in the current literature concerning the underexplored potential of *Foeniculum vulgare* Mill. (Fennel) stalks. While Fennel has garnered significant attention for its medicinal properties, particularly in its seeds and fruits, scant attention has been paid to its stalks. Given the rich history of traditional medicine's reliance on plants like Fennel, understanding the chemical composition and potential therapeutic benefits of its stalks presents a novel avenue for exploration. With its abundance of secondary metabolites such as phenolic acids, flavonoids, triterpenes, alkaloids, and tannins, Fennel holds promise for various biological activities. Moreover, Fennel stalks, often overlooked, may possess distinct chemical profiles and bioactivities compared to other parts of the plant. Therefore, this study seeks to conduct a comprehensive phytochemical analysis and elemental composition assessment of Fennel stalks while elucidating their potential biological activities. By shedding light on the chemistry and bioactivities of Fennel stalks, this research aims to uncover new insights into the therapeutic potential of this often-neglected plant component, paving the way for its utilization in pharmaceuticals, nutraceuticals, and other health-related applications.

2. Materials and method

2.1. Chemicals and reagents

Reagents and chemicals used in the experiments were analytical grades obtained from Sigma Aldrich, (Germany).

2.2.1. Plant collection and botanical identification

Fresh Fennel stalks were collected from the Yesilirmak region in Northern Cyprus (35° 08′ 40″ N; 32° 43′ 21″ E) in February 2021. The stalks were dried on a laboratory bench surface over several weeks. The botanical identification of the dried fennel stalks was performed by a Pharmacologist in the pharmacy department. The voucher number CIU/PHARM/APIA/001 was assigned to the identified fennel stalks at the Biotechnology Research Center, Cyprus International University, Haspolat, Nicosia.

2.2.2. Preparation of Foeniculum vulgare (fennel) aqueous extract

The method proposed by Jang et al. [29] was used for the extraction process. *F. vulgare* stalk aqueous extract (10 %) (FVAE) was prepared by mixing 10 g of the powdered Fennel stalks in 100 mL of sterile distilled water in a conical flask using a magnetic stirrer at 50 °C for 30 min. Subsequently, it was placed in boiling water for 10 min. The extract was then filtered through a Whatman filter paper No. 5 (pore size 2.5 μm) and stored in a refrigerator. The extracts were further filtered with a 0.45 μm pore size syringe filter in other to remove dust particles and other bigger contaminants.

2.2.3. Preparation of methanol and ethanol extracts of F. vulgare

The method considered for the extraction was that of Kedisoet al. [30] with minor adjustments. The *F. vulgare* methanol extract (FVME) and ethanol extract (FVEE) were prepared by weighing 10 g of stalks powder into a conical flask. Next, 100 mL each of ethanol and methanol was added to the material. The mixtures were placed in an overhead shaker for 24 h at room temperature. The solution was filtered with a vacuum filter system and stored in the refrigerator at –4 °C till further use. The Extracts were further filtered with a 0.45 μm pore size syringe filter in other to remove dust particles and other bigger contaminants.

2.3. Gas chromatography-mass spectrometry (GC–MS) analysis

The various solvent extracts of *F. vulgare* were analyzed using gas chromatography–mass spectrometry (GC–MS) following a method adapted from Onyebuchi & Kavaz [31]. The GC–MS conditions were determined based on the peculiarity of the various extracts. A 30 m x 0.25 mm, (thickness of film) 0.25 μm, HP-5 fused silica capillary column was utilized. Helium (99.999 % purity) with a flow rate of 0.9 mL/min was used as carrier gas. The oven temperature of the column was programmed from 50 °C (hold 1 min) to 240 °C (hold 10 min) at a 5 °C/min rate. FVME was introduced in an Agilent 7890A GC system joined with an MS (Agilent technologies) by auto-injection. 70 eV was used to ionize the sample parts. The system was run for 26 mins. The determination of the identities of each component was done by comparing mass spectra with records present in the National Institute of Standards and Technologies (Gaithersburg, USA) and Wiley (Hoboken, New Jersey, USA) Mass Spectrometry (MS) libraries. The sample parts were ionized at 70 eV.

2.4. Elemental analysis

2.4.1. FTIR analysis

The presence of available molecular functional groups in the extract was carried out using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectrum for the extract was obtained on PerkinElmer (Model RX1) between 4000 cm^{-1} and 400 cm^{-1} wavenumber range. The resolution of the IR spectrophotometer is 8cm^{-1}.

2.4.2. Determination of total phenols

Determination of total phenols was done by a method described by [32,33] with slight modifications. About 500 μL of 15 %v/v Folin-Ciocalteu reagent was added to 100 μL of a pre-determined concentration of FVAE, FVME, and FVEE in test tubes. The mixture was agitated gently for about 5 min. Another 400 μL of 20% w/w sodium carbonate was further added to the mixture. The absorbance of the resulting solution was read at 765 nm on a UV-spectrophotometer and compared with a gallic acid standard curve. The experiment was carried out in triplicate ($n = 3$).

2.4.3. X-ray fluorescence

X-ray fluorescence was used to determine the elemental constituents in the *F. vulgare* powdered stalks. X-RAY Fluorescence ZSX Primus II was used to measure the amount of fluorescent X-ray radiation that was emitted from the plant samples when exposed to a primary light source.

2.5. Biological activity

2.5.1. Kinetic evaluation of dpph scavenging

The antioxidant potential of the plant material was carried out by spectrophotometric methods measuring its ability to neutralize the DPPH free radicals [31]. The DPPH stock solution was prepared by dissolving 4 mg of DPPH in 0.1 L of analytical-grade methanol. Various concentrations from the stock DPPH solution were prepared: 0.3, 0.6, 1.25, 2.5, 5, and 10 mg/mL. Then 1 mL of each of the dilutions was added to 3 mL of DPPH solution and incubated for over 40 mins in a dark cupboard. The absorbance of the solution was then read at 517 nm on a UV spectrophotometer [34,35]. The DPPH stock served as a positive control. A calibration curve was obtained with the standard drug ascorbic acid. Percentage DPPH inhibition was determined with the equation below [36].

$$\%\ \textbf{DPPH inhibition} = \left[\frac{\boldsymbol{Vt}}{\boldsymbol{Vc}} - 1 \times 100\right]$$

where, Vt = absorbance of test sample, Vc = absorbance of standard drug (control)

2.5.2. Antimicrobial activity by well diffusion assay

The bacteria used for this experiment were the Methicillin-resistant *Staphylococcus aureus* (MRSA B4420) (gram-positive) and *Escherichia coli* (0157) (gram-negative). Briefly, each sterile petri dish with a diameter of 9 cm was prepared with 20 mL of nutrient agar medium. A standard quantity of bacterial suspension (108 CFU/mL) was dispersed on the plates after solidification. A well-borer was used to make sterile wells of about 6 mm on the plates. The wells were filled with 20 μL of different *F. vulgare* extracts after 5 min. To accelerate extract diffusion into the agar, the plates were incubated at 4 °C for 1 h and then incubated at 37 °C overnight [37,38]. Zones of inhibition (mm) were determined after the period of incubation. Gentamycin (30 μg/disk) and chloramphenicol (30 μg/disk) were used as reference antibiotics. All experiments were carried out in duplicates.

2.6. Statistical analysis

The results were presented as mean ± standard deviation. Data were subjected to one-way ANOVA using GraphPad Prism (8.0.2) followed by Tukey's post hoc test. $P < 0.05$ were considered significantly different.

3. Results

3.1. Gas chromatography

The methanol extract of Fennel was subjected to gas chromatography-mass spectrometry (GC–MS) analysis to evaluate its phytochemical constituents (Fig. 1). Table 1 described the chemical compounds identified in the extract. A total of fifteen compounds including anozol, myristyl iodine, flexiricin, dutadrupine, and ribitol were identified (Fig. 2). This results indicated that the chemical constituents of the stalks of Fennel are very different from those found in the essential oil of its seeds [22].

3.2. FTIR analysis

FTIR spectroscopy was used to identify the functional groups of phyto-constituents present in the Fennel extract (Fig. 3). The observed peaks in the FTIR spectrum of the extract and their corresponding functional groups are represented in Table 2. The broad O–H stretching around 3325.2 cm^{-1} shows the presence of alcohol group [39]. The peak around 2928.0 cm^{-1} is absorbing for C–H group.

3.3. X-ray fluorescence

The result obtained in Fig. 4 showed several elements as its oxide including titanium (TiO_2), chromium (Cr_2O_3), manganese (MnO), iron (Fe_2O_3), nickel (NiO), copper (CuO), zinc (ZnO), rubidium (Rb_2O), and strontium (SrO). The elements were detected in varying concentrations (Fig. 4). No macronutrients such as Ca, P, K, Mg were detected [42]. Lead, cadmium, mercury, and arsenic, among other potentially harmful and poisonous elements, were not found in Fennel stalk material.

3.4. Total phenolic content

The total phenol content was determined using the calibration graph ($R^2 = 0.9962$) obtained from the reference drug gallic acid (Fig. 5). The results showed that the three extracts of Fennel have high total phenolic contents (Table 3). The FVEE had the highest total phenolic content (72.45±0.01 mg gallic acid equivalent [GAE]/g dry extract weight). Second to the ethanol extract is the aqueous extract. It was observed that the methanol extract which had the lowest total phenolic content was in close range with the aqueous extract (Table 3).

3.5. Biological activity

3.5.1. DPPH free radical scavenging activity

The extent of scavenging activity was concentration- and time-dependent. As shown in Fig. 6a, the scavenging activity of ascorbic acid reached about 96 % at a concentration of 10 mg/mL. The activity was found to decrease over time. Similarly, Figs. 6b and 6c illustrated the scavenging activity of F. vulgare extracts ranging from about 20 % to about 97 % in a concentration- and time-dependent manner. FVME and FVEE at 10 mg/mL showed the lowest activity which increased with time. However, there was no significant difference in the activities of the extracts at 30 mg/mL, 40 mg/mL, and 50 mg/mL. For FVME, the activities of these three concentrations were maintained over a period of 60 min while for FVEE, the activities were slightly reduced.

3.5.2. Antibacterial activity

In this study, the methanol extract of Fennel's stalks showed significant inhibition against the growth of *E. coli* 0157 and Methicillin-resistant *S. aureus* B-4420 (MRSA) (Table 4). The extract appeared to be more active against *E. coli* than MRSA with zones of inhibition diameters of 16 mm and 13 mm, respectively. A similar pattern was observed for the reference drug chloramphenicol while azithromycin had equal effects on the two microorganisms (Table 4). No activity was observed for the aqueous and ethanolic extracts of Fennel against the two bacteria.

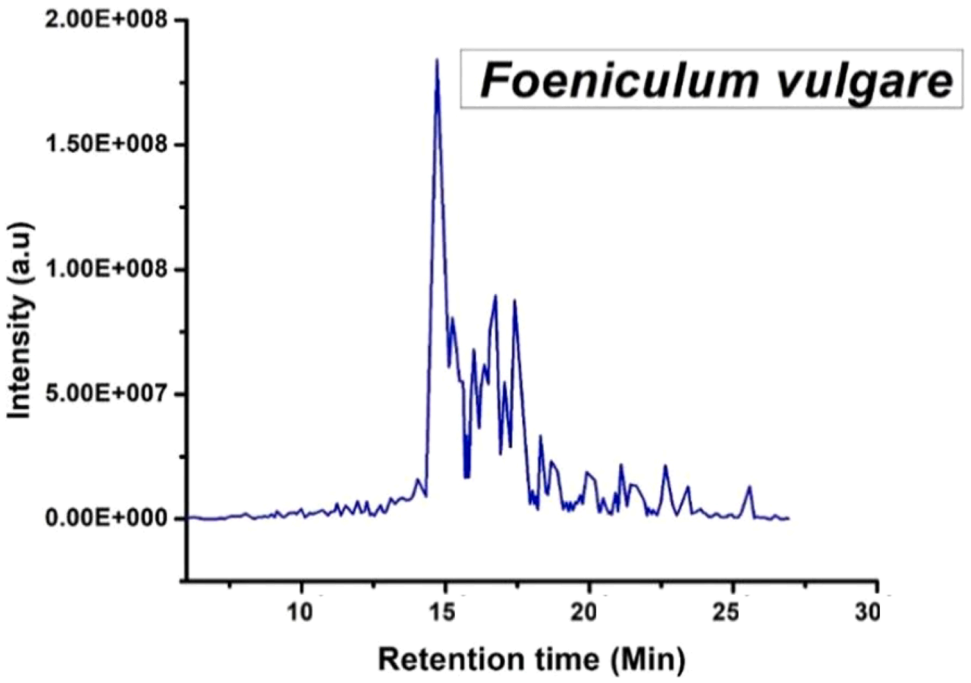

Fig. 1. Retention time (min) and peak intensity of *Foeniculum vulgare* extract.

Table 1
Phytochemical compounds from the gas chromatogram (GC–MS).

Peak Number	Retention time (mins)	Compound name	Molecular formula	Peak area (%)	Molecular weight (g/mol)
1	14.705	Anozol	$C_{12}H_{14}O_4$	–	222.24
2	15.240	Phthalol	$C_8H_4O_4$	–	
3	17.05	Diethyl ether	$(C_2H_5)_2O$	–	74.12
4	17.230	Myristyl iodide	$C_{14}H_{29}I$	–	324.28
5	18.303	Cycloheptaciloxane	$C_{14}H_{42}O_7Si_7$	0.52	519.08
6	19.083	Cyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	0.20	352.68
7	20.2	Neophytadiene	$C_{20}H_{38}$	0.74	278.5
8	13.76	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl-hexasiloxane	$C_{12}H_{38}O_5Si_6$	0.37	430.94
9	23.5	Indolizinol	C_8H_7N	0.11	161.16
10	25.52	Cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	0.63	667.39
11	21.101	Cyclododecasiloxane	$C_{24}H_{27}O_{12}Si_{12}$	0.24	889.8
12	21.3	Phthalic acid	$C_8H_6O_4$	0.24	166.14
13	15.5	Flexricin P-4	$C_{21}H_{38}O_4$	–	400
14	16.7	Dutadrupine	$C_{17}H_{15}NO_3$	–	281.3
15	19.0	Ribitol	$C_{15}H_{22}O$	0.20	152.146

4. Discussion

4.1. Chemical and elemental compositions of Fennel stalks

Gas chromatography/mass spectrometry (GC–MS) is an hyphenated analytical technique used to identify, quantify or separate mixtures of volatile and semi-volatile compounds of organic or biochemical sources [43]. Some of the compounds identified in Fennel extract included anozol, myristyl iodine, flexiricin, dutadrupine, and ribitol. Ribitol is a constituent of riboflavin (vitamin B2) that helps in the prevention of tooth decay. A recent study also showed that in breast cancer cells, ribitol significantly enhances the expression of matriglycan [44]. During a six-month of daily ribitol treatment, skeletal, respiratory, and cardiac muscle functions were significantly improved in dose-dependent way in limb girdle muscular dystrophy 2I mouse model [45]. Dutadrupine is an active anticancer ingredient in some green herbs [46,47]. Twenty-five compounds including trans-anethole, myristicin, eucalyptol, α-pinene, d-limonene, l-fenchone, estragole were identified in the essential oil of dry seeds of Fennel [48]. Chen et al. [21] also reported about 88.28 % trans-anethole in the Fennel fruits extracted by double-condensed microwave-assisted hydrodistillation method. Ahmed et al. [22] found estragole (51.04 %), limonene (11.45 %), l-fenchone (8.19 %) and trans-anethole (3.62 %) as the major components of Egyptian fennel essential oil while trans-anethole (54.26 %), estragole (20.25 %), l-fenchone (7.36 %) and limonene (2.41 %) were found as the major constituents of Chinese Fennel essential oil. Another study showed twenty-eight compounds by GC–MS analysis of seed's essential oil with trans-anethole (68.53 %) and estragole (10.42 %) as the major components [49]. It is interesting to note that the essential oil of the fruits and seeds of Fennel contain trans-anethole and other compounds as the major chemical constituents. However, the stalk extract as demonstrated in this study did not indicate the presence of trans-anethole.

FTIR analysis was used to identify the functional groups present in the Fennel's chemical constituents as seen in Fig. 3. The spectrum of *F. vulgare* revealed absorption signals for hydroxyl group (O—H stretch), aliphatic hydrocarbons (C—H stretch), aliphatic ether (C—O stretch), and unsaturated system (C = C stretch). X-ray fluorescence (XRF) was used to determine the percentage mass of elements in the extract of Fennel as shown in Fig. 4. The result showed that the extract contained several elements including titanium, chromium, manganese, iron,

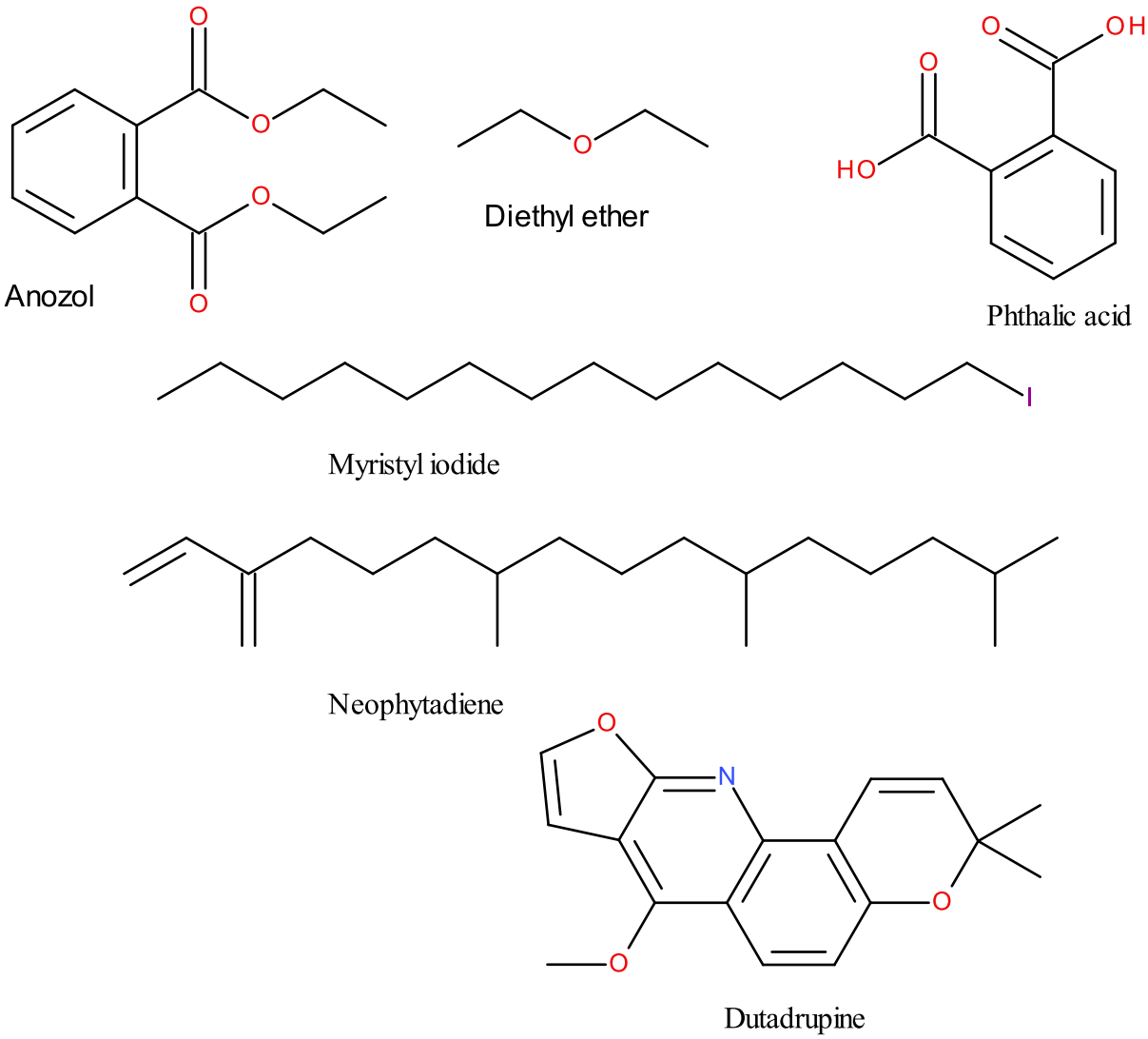

Fig. 2. Structures of some of the compounds identified in *Foeniculum vulgare* extract.

nickel, copper, zinc, rubidium, and strontium. Ca, P, K, and Mg were not detected [42]. Heavy metals such as lead, mercury, and arsenic were not found in Fennel stalk material. A study on the elemental compositions of herbal infusions and tea samples (which include Fennel) found that Mn, Fe, Ni, Cu, Cl, P, Zn, and Rb are present in Fennel fruits [3]. Another study reported essential macro-elements (P, S, K, Mg, Ca), micro and trace elements (Cr, Mn, Fe, Co, Ni, Cu, Zn), halogens (Cl, Br, I), and non-essential or toxic elements (Al, As, Rb, Sr, Cd, Sn, Hg, Pb) from the seeds of Fennel [2]. Contamination by heavy metals is a widely acknowledged environmental problem that poses a major hazard to human life. In addition to impairing plant growth, toxic elements in the environment that plants absorb can also negatively impact human health by way of the food chain [50]. X-ray fluorescence (XRF) is a non-destructive analytical technique for the analysis or determination of elements compositions of a sample [42].

4.2. DPPH free radical scavenging and antibacterial activities of Fennel stalks

A calibration curve obtained using various concentrations of gallic acid was used to determine the total phenol contents of Fennel extracts. The results showed that all the tested extracts of Fennel possessed high total phenolic contents with the ethanolic extract having the highest total phenolic content (72.45±0.01 mg gallic acid equivalent [GAE]/g dry extract weight). The methanol and aqueous extracts also showed high total phenol contents. The Egyptian and Chinese Fennel seed extracts had 42.24 and 30.94 mgPE/g, respectively, total phenolic contents [22]. The total phenolic contents analysis of methanol extracts of sixteen wild Fennel populations growing in Tunisia showed values that ranged from 6.00±0.57 to 29.86±5.69 GAE/g dry extract weight [51]. This indicated that the ethanolic stalk extract of Fennel has more total phenol content than the seed extracts. The consumption of vegetables, fruits, and herbs containing high phenol and flavonoid contents has been associated with reduction is the incidences of inflammatory and oxidative stress-linked diseases [52]. Medicinal plants are good source of antioxidants. With their redox characteristics, phenolic compounds are significant phytochemicals that exhibit antioxidant property [53].

The scavenging activity of F. vulgare extracts was determined using DPPH inhibitory assay. The DPPH free radical scavenging activity ranged from about 20 % to about 97 %. The activity was found to be both concentration- and time-dependent. Free radicals play significant roles in the pathogenesis of some non-communicable diseases such as cancer and cardiovascular disorders [16]. Several studies have echoed the fact that green plants tend to have significant antioxidant activity against the reactive oxygen species (ROS), hence their ability to resist oxidative stress [54]. The phytochemicals and functional groups present within the plant may be responsible for the high scavenging activity levels observed across its concentrations. Additionally, the lower

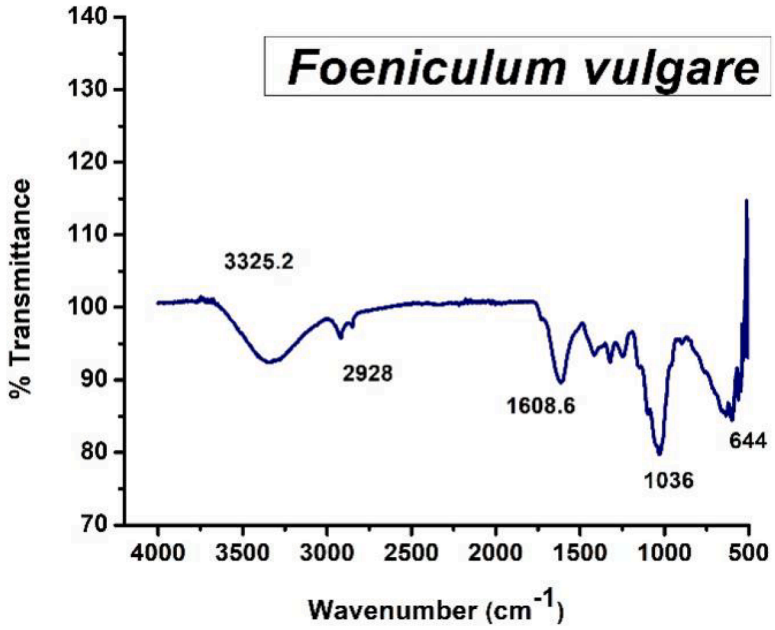

Fig. 3. The FTIR spectrum of *F. vulgare* extract.

Table 2
FTIR band values and functional groups of *F. vulgare* extract.

S/N	Peak/band values (cm^{-1})	Chemical bond	Functional groups	References
1	3325.2	O–H	O–H stretching/alcohol group	[39]
2	2928.0	C–H	C–H stretching/alkane group	[40]
3	1608.6	C = C	C = C stretching/ unsaturated hydrocarbon	[39]
4	1036.0	C–O	C–O stretching/aliphatic ether	[41]

activity of the F. vulgare extract relative to the ascorbic acid standard may stem from interference by the unbound elements alongside the free radicals [25]. Using the double-condensed microwave-assisted hydrodistillation technique, the IC_{50} value for DPPH free radical scavenging activity of the essential oil of Fennel fruits was determined to be 14.05 µL/mL [21]. Haematological and biochemical toxicity induced by carbendazim in male mice was attenuated by Fennel extract. This was attributed to its antioxidant capacity [55].

The extracts were also investigated for their antibacterial activity using the agar diffusion method. The methanol extract showed significant inhibition against *E. coli* 0157 and Methicillin-resistant *S. aureus* B-4420 (Table 4). More activity was observed against *E. coli* than MRSA. However, no antibacterial activity was observed for the aqueous and ethanolic extracts. Several studies have demonstrated the antimicrobial potential of the extracts and the essential oil of Fennel's seeds [21,49, 14–16]. The essential oil of Fennel's dry seeds had been shown to possess pronounced activity against bacterial strains of *Acinetobacter baumannii, Staphylococcus aureus* and *Staphylococcus epidermidis* [48].

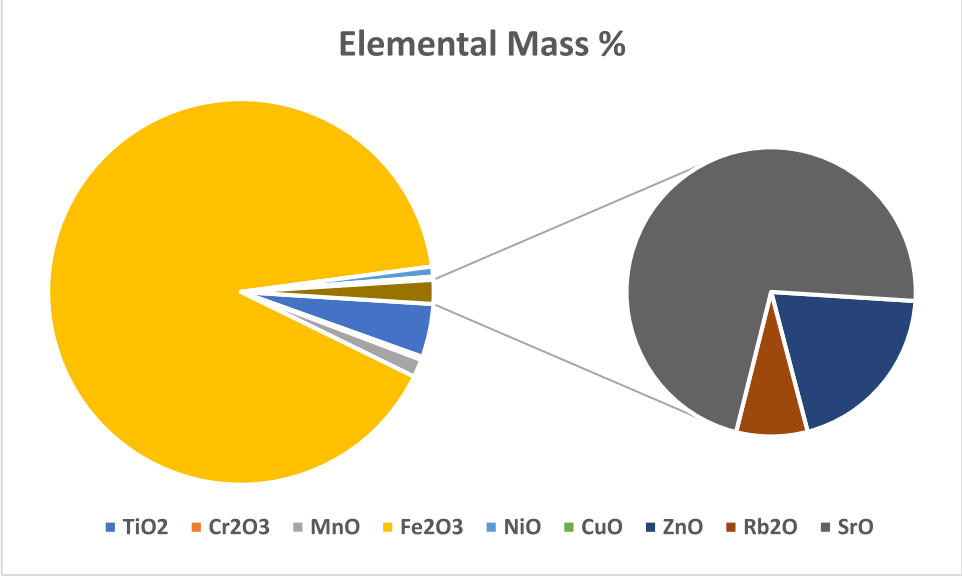

Fig. 4. X-ray fluorescence showing the percentage mass of elements within the Fennel stalk.

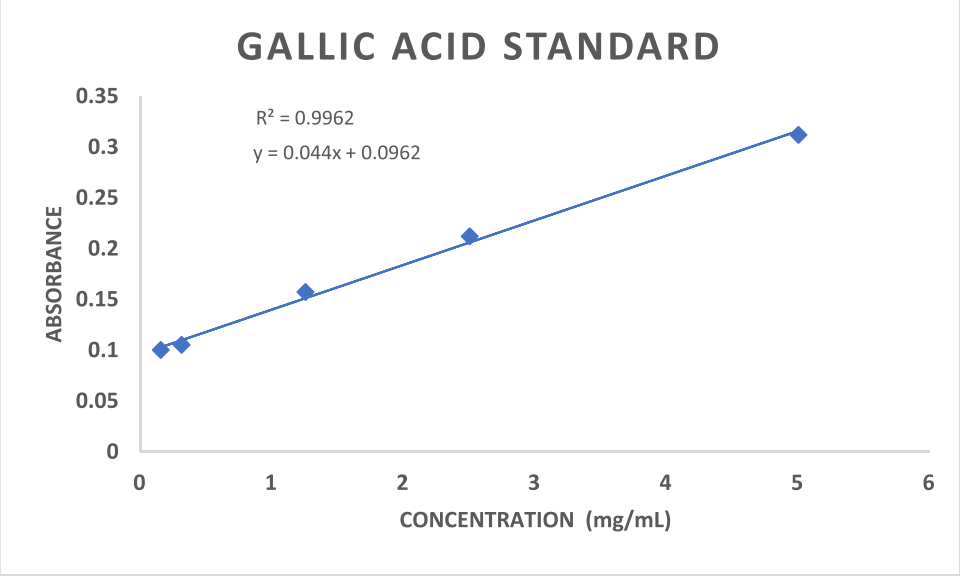

Fig. 5. Gallic acid calibration curve for total phenol content determination.

Table 3
Total phenol content of *Foeniculum vulgare* extracts.

Extracts	Total Phenol content (mg GAE/g dry extract wt
FVEE	72.45±0.01
FVME	51.3 ± 0.01
FVAE	54.3 ± 0.01

FVEE: Foeniculum vulgare ethanol extract; FVME: Foeniculum vulgare methanol extract; FVAE: Foeniculum vulgare aqueous extract. Results are expressed as Mean ± SD of duplicates ($n = 3$).

5. Limitations and strengths of the study

This study has some limitations that should be acknowledged as stated below:

i. **Variability in Extraction Process:** Although efforts were made to standardize the extraction process, variations in factors such as temperature, time, and solvent concentration during extraction could lead to differences in the chemical composition of the extracts. This variability may affect the reproducibility and comparability of results between experiments.
ii. **Limitations of GC–MS Analysis:** While gas chromatography-mass spectrometry (GC–MS) is a powerful tool for identifying phytochemical compounds, it may not capture all constituents present in the extracts. Minor compounds with significant biological activities could be overlooked, impacting the comprehensiveness of the chemical profile obtained.
iii. ***In vitro* Biological Assays:** The biological assays conducted to evaluate antioxidant and antimicrobial activities were performed *in vitro*, which may not fully replicate the complexity of biological systems *in vivo*. Biological responses observed *in vitro* may not translate directly to therapeutic effects in living organisms, necessitating further *in vivo* studies for validation.
iv. **Focus on Stalks Only:** The study focused solely on the stalks of Foeniculum vulgare Mill., neglecting other parts of the plant that may possess distinct chemical profiles and bioactivities. A more comprehensive approach considering all plant parts could provide a more holistic understanding of the medicinal potential of Fennel.

Despite these limitations, our study offers several strengths as identified below:

i. **Addressing a Gap in Literature:** This study fills a notable gap in the current literature by exploring the phytochemical composition and biological activities of Fennel stalks, an underexplored component of the plant. By focusing on this neglected aspect, the study contributes to a more comprehensive understanding of Fennel's therapeutic potential.
ii. **Standardized Protocols and Analytical Techniques:** The use of standardized protocols and advanced analytical techniques, including GC–MS, FTIR, and X-ray fluorescence, ensures the reliability and validity of the study findings. These methods provide precise and detailed characterization of the chemical composition and elemental profile of the Fennel stalk extracts
iii. **Comprehensive Assessment of Total Phenolic Content:** The study includes a comprehensive assessment of the total phenolic content of Fennel stalk extracts, providing valuable insights into their antioxidant potential. This quantitative analysis enhances the understanding of the health-promoting properties of Fennel stalks.
iv. **Significant Antimicrobial Activity:** The observed inhibition of pathogenic bacteria by Fennel stalk extracts highlights their potential antimicrobial properties. This finding suggests that Fennel stalks may have therapeutic applications in combating bacterial infections, contributing to the development of novel antimicrobial agents.

6. Conclusions

Fennel is an edible and aromatic vegetable plant that is widely cultivated for its essential oil and its traditional importance. Although several studies have been conducted on seeds, fruits and essential oil of Fennel, literature has scanty information on Fennel stalks. This study has investigated the phytochemical analysis using GCMS, elemental composition using X-ray fluorescence, total phenol content, DPPH scavenging activity, and antibacterial activities of various extracts of *F. vulgare* (Fennel) stalks. FTIR analysis suggested functional groups of Fennel's phytochemicals including OH, CH, C = O, C = C, and CO. GCMS analysis showed 15 compounds. X-ray fluorescence quantified elements including titanium, chromium, manganese, iron, nickel, copper, zinc, rubidium, and strontium. The ethanol extract indicated a high total phenolic content as well as a high% DPPH scavenging activity. Fennel's

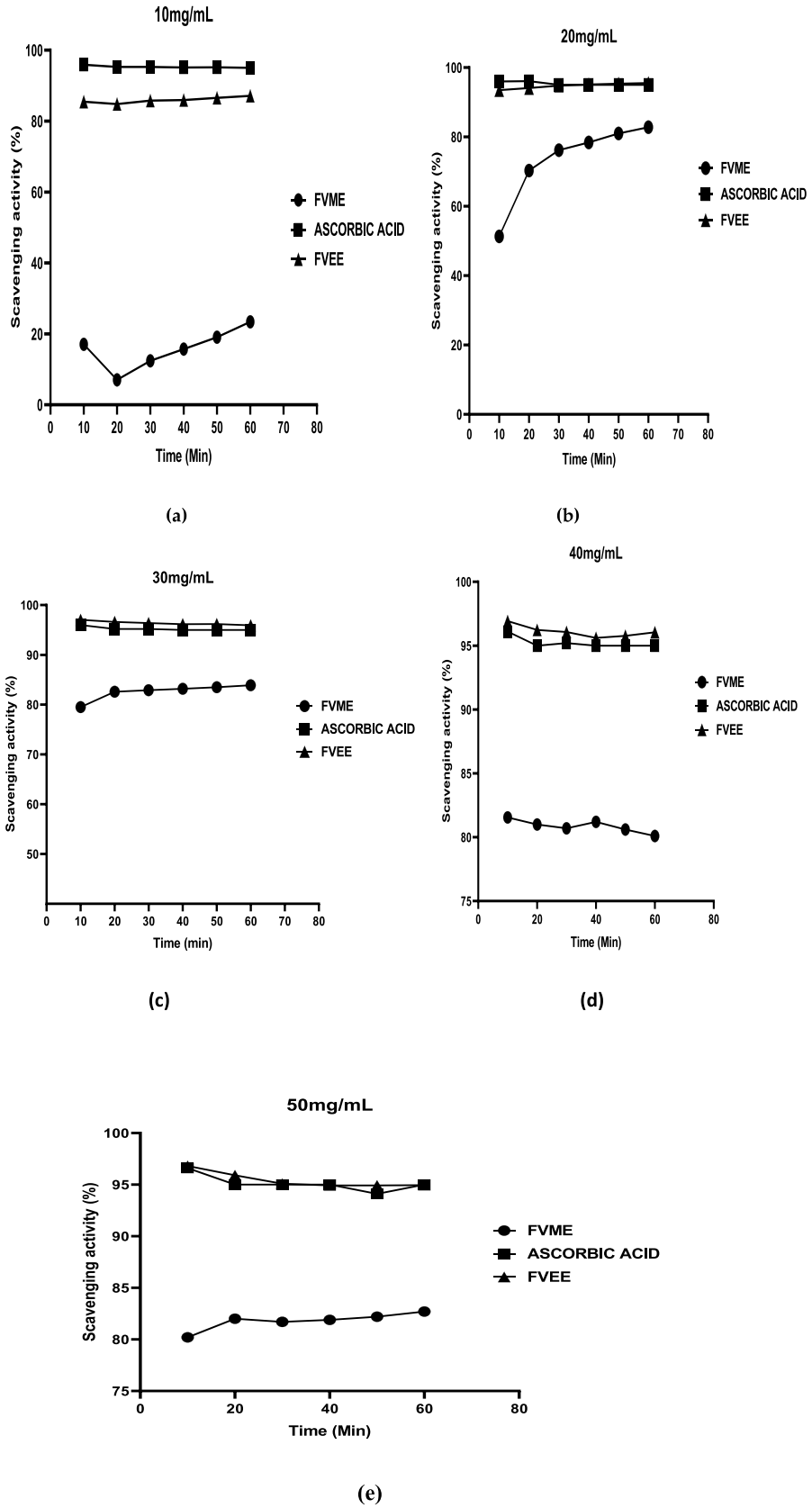

Fig. 6. The time-dependent kinetic DPPH scavenging activity of Fennel extracts at various concentrations.

Table 4
Showing the zone of inhibition for each pathogenic organism.

	Zone of inhibition (mm)	
	E. coli (HZ 0105)	Methicillin-resistant *S. aureus* (B-4420)
FVAE	–	–
FVEE	–	–
FVME	16	13
Chloramphenicol	16	13
Azithromycin	15	15
DMSO	–	–

Key: –: No growth. DMSO: negative control.

stalks methanol extract showed significant inhibition against the growth of *E. coli* 0157 and Methicillin-resistant *S. aureus* B-4420. This study has demonstrated that aside from the well-researched Fennel oil, the plant's stalks is also a potential source of antioxidant and antibacterial agents.

List of Abbreviations

FVAE	Fennel aqueous extract
FVME	Methanol Fennel extract
FVEE	Ethanol Fennel extract
GC–MS	Gas chromatography-mass spectrometry
FTIR	Fourier Transform Infrared Spectroscopy
MRSA	Methicillin-resistant Staphylococcus aureus

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

Covenant E. Ogbonna: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Doga Kavaz:** Writing – original draft, Supervision, Methodology, Conceptualization. **Yemi A. Adekunle:** Writing – review & editing, Formal analysis, Data curation. **David B. Olawade:** Writing – review & editing, Writing – original draft, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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