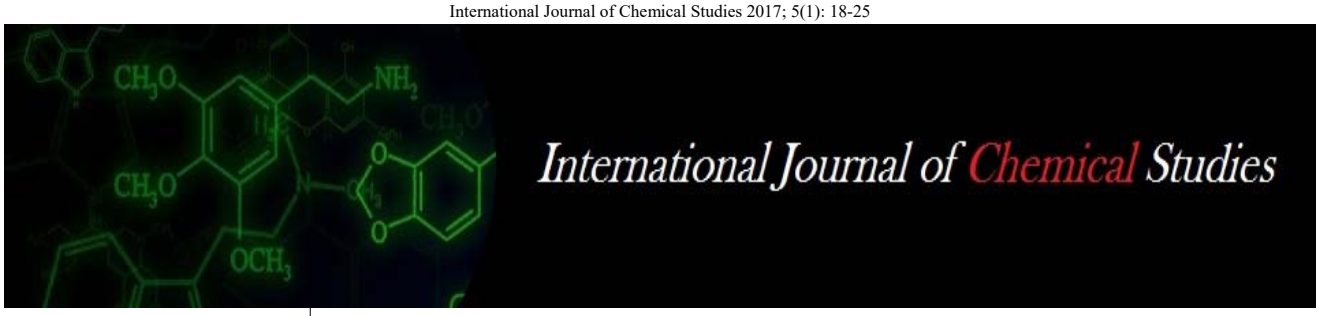

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Phytochemical screening and biological activities of leaf of *Foeniculum vulgare* (Ensilal)

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Abstract
The *Foeniculum vulgare*, known as fennel, has a long history of herbal uses as both food and medicine. Phytochemical screening, the total phenolic contents (TPC), total flavonoid contents (TFC), *in vitro* antioxidant, and antimicrobial activities of chloroform, water, methanol, and aqueous: methanol (20:80, v/v) extracts of *Foeniculum vulgare* leaf were investigated. The antioxidant activity was measured using 1, 1 Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, reducing power and total antioxidant activity by phosphomolybdate methods. TPC and TFC were evaluated using Folin–Ciocalteu and $AlCl_3$ methods respectively. *Foeniculum vulgare* extracts were investigated for *in vitro* antibacterial screening by disc diffusion method against *Bacillus megaterium, Enterococcus gallinarum, Escherichia coli* and *Pseudomonas aeruginosa.* Aqueous: methanol (20:80, v/v) extract contained the highest TPC [(24.3 ± 0.99) mg GAE/g], and TFC [(18.92 ± 0.34) µg CE/mg] and showed the highest DPPH radical scavenging activity (IC_{50} = 69.68 ± 2.28 µg/mL). The results also showed that the aqueous: methanol (20:80, v/v) extract had strongest reducing ability (0.82 ± 0.06 nm) as compared to other extracts. Chloroform extract exhibited the highest total antioxidant capacity (1.94 ± 0.76 mg AAE/g) as determined by the phosphomolybdenum method. Except for total antioxidant activity, all antioxidant activities were positively correlated TPC and TFC. Of the *Foeniculum vulgare* extracts, the highest inhibition zone was found in aqueous: methanol (20:80, v/v) extracts of the (8.74 ± 0.12 mm) against *Bacillus megaterium*. The study revealed that antioxidant and antimicrobial activities of the crude extract of *Foeniculum vulgare* extracted by different solvents indicating a high potential to be used as natural antioxidants in food preservation as well as for preventing oxidative stress mediated human disorders.

Keywords: Antibacterial activity, antioxidant activities, DPPH, *Foeniculum vulgare*, reducing power, total flavonoid and phenolic content

1. Introduction
Antioxidants are widely used as food additives to provide protection against oxidative degradation of foods by free radicals [1]. Furthermore, many synthetic antioxidant components (BHA and BHT) have shown toxic and/or mutagenic effects; therefore, plant antioxidants are suggested as an interesting alternative. Several substances from aromatic and medicinal plants have been shown to contain antioxidants like flavonoid compounds. Compounds are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step [2]. The use of plant-based natural antioxidants, such as those of phenolic substances like flavonoids and phenolic acids and tocopherols in foods, as well as preventive and therapeutic medicine, is gaining much recognition. Such natural substances are believed to exhibit anti-carcinogenic potential and offer diverse health-promoting effects because of their antioxidant attributes [3]. Some biologically active compounds isolated from spices and herbs have been in use for the inhibition of growth of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [4].

Fennel (*Foeniculum vulgare* Mill.) is a biennial medicinal and aromatic plant belonging to the family Apiaceae. *Foeniculum vulgare* is a perennial umbelliferous herb with feathery leaf. It grows to a height of up to 2.5 m with hollow stems. The leaf grows up to 40 cm long and the flowers are produced in terminal compound umbels. The fruit is a dry seed up to 10 mm in length [5]. *Foeniculum vulgare* is generally considered indigenous to the shores of Mediterranean Sea, but has become widely domesticated in many parts of the world especially on dry soils near the sea coast and on the river banks [6]. This plant has anti-inflammatory, antispasmodic, antiseptic, carminative, diuretic and analgesic effect and is effective in

gastrointestinal disorder treatment. Also with its anti-ulcer and anti-oxidant properties it is used to treat neurological disorders [7].

Foeniculum vulgare is a highly aromatic and flavourful herb with culinary and medicinal uses. *Foeniculum vulgare* seeds are anise like in aroma and are used as flavourings in baked goods, meat and fish dishes, ice cream, alcoholic beverages and herb mixtures [8]. *Foeniculum vulgare* (Mill.) locally called "*ensilal*" is a weed of cultivated or disturbed grounds, common in grassland areas of Ethiopia. The seeds and dried plant parts are used to flavor local drinks such as "*areke*" and "*tej*". The fresh leave is also traditionally used to treat Gonorrhoea, Gland TB (Naqarsa), Kidney disease and Nosebleed [9].To the best of my knowledge no study has been reported on total phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf *Foeniculum vulgare*. The objective of this study was to determine total phenolic contents (TPC), total flavonoid contents (TFC), *in vitro* antioxidant, and antimicrobial activities of various solvent extracts of *Foeniculum vulgare* leaf. In addition, the correlation between phenolic contents and antioxidant assays was also evaluated.

2. Materials and Methods

2.1 Chemicals

Gallic acid, butylated hydroxytoluene (BHT), Folin–Ciocalteau reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), catechin, ascorbic acid, aluminum chloride, Mueller-Hinton agar, acetic anhydride, phosphate buffer, sodium phosphate and ammonium molybdate were purchased from Sigma-Aldrich (St. Louis, USA). The other chemicals and solvents were used in this experiment were analytical grade.

2.2 Collection of plant sample

The fresh plant was collected in April 2016 from Hawassa Agricultural Research Center. Random sampling method was selected to eliminate questions of bias in selection. The plant materials were washed with distilled water and air dried in shade for ten days then ground by analytical mill using electric blender (FM100 model, China) and packed in polyethylene bags to avoid entrance of air and any other dust from surrounding material.

2.3 Preparation of plant extracts

The chloroform, water, methanol, and aqueous: methanol (20:80, v/v) extracts of all were prepared by dissolving 10 g of the leaves fine powder separately in 100 mL each solvent. The contents were kept in orbital shaker for 24 h at room temperature. Thereafter, each extract was filtered using Whatman no.1 filter paper and evaporated to dryness under vacuum at 40 °C by using a rotary evaporator (Buchi, 3000 series, Switzerland). The extraction was done in triplicate for each solvent and the resulting extracts were stored in a sealed plastic container at 4 °C until further investigation. Unless specifically mentioned, all analysis was conducted on triplicate analysis.

2.4 Determination of extraction yield

The percentage yield was obtained using the formula $W_2 – W_1/W_0$ X 100. Where W_2 is the weight of the extract and the container, W_1 is the weight of the container alone and W_0 is the weight of initial dried sample. Then the extraction yield was expressed in terms of milligram of dried extract per gram of dried material (mg/g).

2.5 Phytochemical Screening

A qualitative phytochemical test to detect the presence of alkaloids, flavonoids, saponins, steroids, tannins, and triterpenoids was carried out using standard procedures [10-12].

2.6 Determination of total phenolic content (TPC)

Total phenolic compound contents were determined by the Folin-Ciocalteau method as described in Ebrahimzadeh *et al.,* [13], with slight modification using gallic acid as standard. To 0.1 mL of the extract (1 mg/mL), 1 mL Folin-Ciocalteu reagent (diluted ten times) was added and the mixture was left for 5 min and then 1 mL (75 g/L) of sodium carbonate was added. The absorbance of the resulting blue color was measured at 765 nm with a UV- visible spectrophotometer (JENWAY, 96500, UK) after incubation for 90 min at room temperature. The total phenolic content was estimated from gallic acid calibration curve and results were expressed as milligram gallic acid equivalent/gram of dry extract (mg GAE/g). ($Y = 0.015x + 0.09$, $R^2 = 0.99$, $P < 0.0001$).

2.7 Determination of total flavonoid content (TFC)

Total flavonoid was estimated using the method of Ebrahimzadeh *et al.,* [13]. The extract (1 mL, 1 mg/mL) was diluted with 1.25 mL distilled water and 75 µL 5% $NaNO_2$ was added to the mixture. After 6 min, 150 µL 10% $AlCl_3$ was added and after another 5 min, 1 mL 1M NaOH was added to the mixture. Immediately, the absorbance of the mixture, pink in color, was determined at 510 nm versus prepared water blank. A standard curve was prepared using 5 – 120 µg/mL of catechin. Results were expressed as milligram of catechin equivalents per milligram of dry extract of the plant extract. ($Y = 0.020x + 0.011$, $R^2 = 0.991$, $P < 0.001$).

3. Antioxidant activity tests

3.1 DPPH assay

The DPPH free radical scavenging activity of the extracts of leaf of *Foeniculum vulgare* was assayed according to the method of Szollosi *et al.,* [14], with slight modification. Different concentrations (50 to 1000 µg/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 mL, 0.006%, w/v) was prepared in methanol was added in each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference standards (ascorbic acid and BHT) were vortexes and left to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was then taken at 520 nm. Methanol was used as blank. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenged\ (\%) = \frac{(Ac - As)}{Ac} x100$$

Where *A*c is the absorbance of the control and *A*s is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that scavenges the DPPH radical by 50%. High IC_{50} value indicates less antioxidant capacity.

3.2 Reducing power

The reducing power of *Foeniculum vulgare* extracts was determined according to the method of Yen *et al.,* [15]. Plant extract (1 mL) solution (final concentration 100-1000 µg/mL) was mixed with 2.5 mL potassium phosphate buffer (0.1 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Then the mixture was incubated at 50 °C for 20 min. Trichloroacetic

acid (2.5 mL, 10%) was added to the mixture, which was then centrifuged at 3000 rpm (Centurion, 1000 series, UK) for 5 min. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (0.1%) and absorbance was measured at 700 nm.

3.3 Phosphomolybdenum method

The total antioxidant activities of the crude extracts were evaluated by the phosphomolybdenum method reported by Prieto *et al*., [16], with slight modification. 0.3 mL plant extract (0.5 and 1 mg/mL) was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated at 95 ^{0}C for 90 min, was cooled to room temperature and absorbance was measured at 695 nm. 3 mL of methanol was used as a blank. The total antioxidant activity was expressed as milligram ascorbic acid equivalent/gram of dried extract (mg AAE/g) ($y = 0.301x + 0.002$; $R^2 = 0.996$, $P < 0.001$) based on the calibration curve.

4. Antimicrobial Activity

4.1 Preparation of disc diffusion assay

Diffusion disc of approximately 6 mm diameter was prepared from whatman no.1 filter paper by using a paper puncher and sterilized by autoclaving and dried in oven at 120 ºC. For each test, sterile disc were soaked by aseptically 10 µL the crude extract at a concentration of 100 mg/mL of the crude extract using sterile micropipette.

4.2 Preparation of inoculums

The test bacterial strains were transferred from the stock cultures and streaked on Mueller Hinton plates and incubated for 24 hr. Well separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved Mueller Hinton that was cooled to about 45 °C in a water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petri plates, allowed to solidify and used for the biotest.

4.3 Disc diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of leaf of *Foeniculum vulgare* was carried by disc diffusion assay as described in Bauer *et al.,* [17]. Briefly, two to three sterile paper discs (6 mm as diameter) was lodged, under aseptic conditions, on Mueller Hinton agar Petri dish previously flooded with the appropriate bacterial culture which included two Gram +ve *(Bacillus megaterium and Enterococcus gallinarum)* and two Gram -ve (*Escherichia coli and Pseudomonas aeruginosa*) bacteria. The discs were aseptically impregnated with 25 µL of *Foeniculum vulgare* extract solution (100 mg/mL). These dishes were kept for 15–30 min at room temperature before incubation at 37 ºC for 24 hour. The antibacterial activity was evaluated by measuring the zone of inhibition against the tested organism. Kanamycin, and Amoxicillin was used as a positive control and blank disc impregnated with solvent was used as negative control.

4.4 Statistical Analysis

All the experiments were conducted in triplicate. The results were expressed as mean values ± standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan post-test. A probability $p <$ 0.05 value of was considered to denote a statistically significance difference. This treatment was analyzed using SPSS 16.0 software. The correlation analysis between phenolics, flavonoids and antioxidant activities were performed using origin 8 software.

5. Results and Discussion

5.1 Extraction yield

The data in Table 1 represent extraction yield (mg/g) of the four extracts of *Foeniculum vulgare* leaf. The yields of the four extracts varied from 28.1 to 246.6 mg/g of leaf extracts. The methanol extract gave the highest yield (246.6 ± 8.84 mg/g), while the chloroform extract gave the least yield (28.1 ± 1.13 mg/g).

5.2 Phytochemical analysis

The most active extract of each plant was tested for various phytochemicals. Alkaloids, saponins, terpenoids, saponins, terpenoids and flavonoids were predominantly present in active extracts (Table 2).

5.3 Total phenolic and flavonoid contents

The total phenolic contents (TPC) and total flavonoid contents (TFC) of *Foeniculum vulgare* leaf extracts are presented in Table 1. The amounts of TPC and TFC extracted from fennel leaves in different solvent systems were in the ranges 4.76 ± 0.65 – 24.3 ± 0.99 mg GAE/g and 9.64 ± 2.15 – 18.92 mg CE/g, respectively. These differences in the amount of TPC and TFC may be due to varied efficiency of the extracting solvents to dissolve endogenous compounds. TPC followed the order: aqueous: methanol (20:80, v/v) > methanol > chloroform > water extract. Aqueous: methanol (20:80, v/v) extract significantly higher ($p < 0.05$) than that of other extracts. There was no significant difference ($p > 0.05$) between methanol, water and chloroform extracts.

Similarly aqueous: methanol (20:80, v/v) extract was the richest source of TFC ($p < 0.05$) and decreased in the order of; aqueous: methanol (20:80, v/v) > methanol > water > chloroform extracts (Table 1). No flavonoids content are observed in chloroform extract. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases [18].

Table 1: Extraction yield (mg/g), total phenolic content (TPC) (mg GAE/ g dried extract) and total flavonoid content (TFC) (µg CE/mg dried extract) of leaf of *Foeniculum vulgare.*

Extraction solvents	Extraction yield (mg/g)	TPC (mg GAE/g)	TFC (mg CE/g)
Chloroform	28.1 ± 1.13^a	6.83 ± 3.8^a	ND
Water	158.8 ± 8.28^b	4.76 ± 0.65^a	9.64 ± 2.15^a
Methanol	246.6 ± 8.84^c	9.19 ± 1.89^a	11.78 ± 0.58^a
aqueous: methanol(20:80, v/v)	160.8 ± 5.87^b	24.3 ± 0.99^b	18.92 ± 0.34^b

All values are means ± standard deviations (n=3). In each column, different superscript letters (a - c) are significantly different at $p < 0.05$) (one way ANOVA followed by Duncan post-test). ND = not detected.

Table 2: Phytochemical constituents of the extracts of *Foeniculum vulgare* leaf.

Components	Chloroform extract	Water extract	Methanol extract	Methanol 80% extract
Alkaloids	-	+	+	+
Tannis	-	+	+	+
Saponins	-	+	+	+
Terpenoids	+	+	+	+
Flavonoids	-	+	+	+
Steroids	-	-	+	+

6. Antioxidant activity

6.1 DPPH scavenging

DPPH is a stable free radical with characteristic absorption at 520 nm and antioxidants react with DPPH radical and convert it to diamagnetic 2, 2- diphenyl-1-picrylhydrazine molecule. The degree of discolouration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating ability [19]. Figure 1 shows the dose–response curve of DPPH radical scavenging activity of the *Foeniculum vulgare* leaf of chloroform, water, methanol, and aqueous: methanol (20:80, v/v) extracts compared with BHT and L- ascorbic acid. As the concentration of sample increased, the percent inhibition of DPPH radical also increased (Figure 1). At a concentration of 1000 µg/mL, the scavenging effect of L-ascorbic acid, BHT, and *Foeniculum vulgare* leaf extracts, on the DPPH radical scavenging decreased in the order of L- ascorbic acid > BHT > methanol > aqueous: methanol (20:80, v/v) > water > chloroform, which were 96.98 ± 0.04%, 95.56 ± 0.04%, 95.46 ± 0.02%, 92.20 ± 0.50%, 75.34 ± 6.33%, and 45.74 ± 3.07%, respectively.

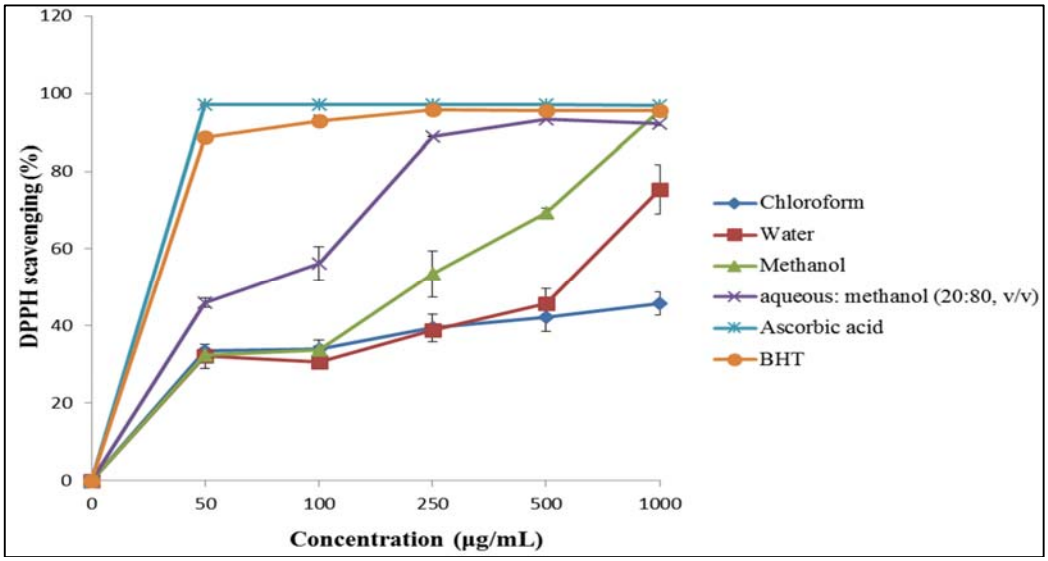


Fig 1: DPPH radical scavenging activity (%) of chloroform, water, methanol, and aqueous: methanol (20:80, v/v) extracts from dried leaf *Foeniculum vulgare* and controls (L- ascorbic acid and BHT). Each value is expressed as mean ± standard deviation (n=3).

The parameter IC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity. Effectiveness of antioxidant properties is inversely correlated with IC_{50} values. The IC_{50} values of all the extracts were calculated from plotted graph of percentage scavenging activity against concentration of the extracts (Table 3). Aqueous: methanol (20:80, v/v) of *Foeniculum vulgare* had the highest scavenging activity as compared to the other extracts due to its least IC_{50} value (Table 3). The extracts with the weakest scavenging potency were chloroform extract (IC_{50} > 1000 µg/mL) which had significantly lower activity.

The strongest scavenging activity (lower IC_{50} value) was recorded for aqueous: methanol (20:80, v/v) extract which appeared more than eight times stronger than that of water extract and three times stronger than that of the methanol extract (Table 3). When compared with the synthetic antioxidant (L- ascorbic acid and BHT), all extracts offered lower antioxidant activity. The results indicated the proton donating ability of the extractives which could serve as free radical inhibitors or scavengers and can also be used as primary antioxidants.

All values are means ± standard deviations (n=3). The IC_{50} values are presented with their respective 95% confidence limits. Different lowercase letters within each column is significantly different at $p < 0.05$ (one way ANOVA followed by Duncan post-test)

Table 3: IC_{50} values of DPPH scavenging activities of leaf of *Foeniculum vulgare*

Extraction solvents	IC_{50} (µg/mL)
Chloroform	>1000
Water	576.45 ± 76.77[a]
Methanol	228.94 ± 37.24[b]
aqueous: methanol(20:80, v/v)	69.68 ± 2.28[c]
L- ascorbic acid	26.91 ± 1.78
BHT	30.58 ± 3.41

6.2 Reducing power

Figure 2 shows the reductive capabilities of samples *Foeniculum vulgare* extracts compared to BHT. The reducing power of *Foeniculum vulgare* leaf extracts decreased in the order of: aqueous: methanol (20:80, v/v) > chloroform > methanol > water with values (0.819 ± 0.062), (0.349 ± 0.114), (0.338 ± 0.039), and (0.237 ± 0.053) respectively (Figure 2). It was suggested that the electron donating capacity, reflecting the reducing power of bioactive compounds, is associated with antioxidant activity. The greater the intensity of the colour, the greater was the absorption; consequently, the greater was the antioxidant activity.

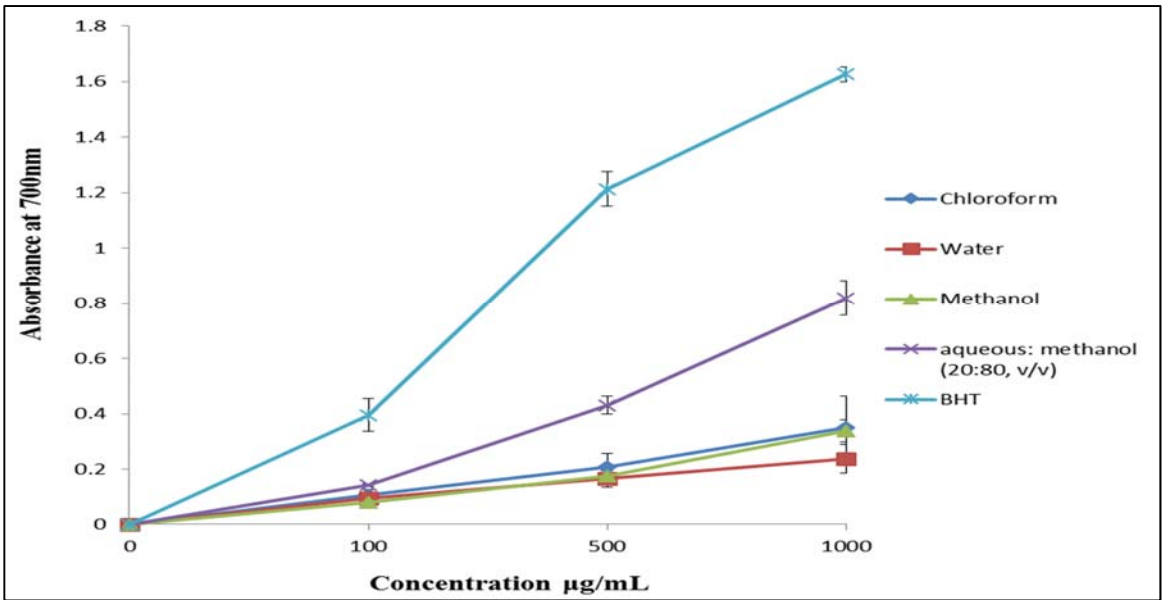


Fig 2: Ferric reducing power of chloroform, water, methanol, and aqueous: methanol (20:80, v/v) extracts from dried leaf *Foeniculum vulgare* and controls (BHT). Each value is expressed as mean ± standard deviation (n=3).

6.3 Total Antioxidant Capacity (TAC) by Phosphomolybdenum Method

TAC of the different extracts of *Foeniculum vulgare* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The TAC of *Foeniculum vulgare* leaves extracts was found to decrease in the order: chloroform > aqueous: methanol (20:80, v/v) > methanol > water; values at 1000 µg/mL was found (1.94 ± 0.76 µg/mL), (0.91 ± 0.03 µg/mL), (0.80 ± 0.07 µg/mL), and (0.43 ± 0.06 µg/mL) respectively (Figure 3). Chloroform extract showed the highest TAC and significantly different from other extracts whereas there was no significant difference ($P > 0.05$) between water and methanol extracts. The difference in the amount of antioxidant of extracts may be attributed to the differences in the amount and type of existing antioxidant compounds. This result is inconsistent with the lowest antiradical effect of chloroform extract determined by the DPPH assay.

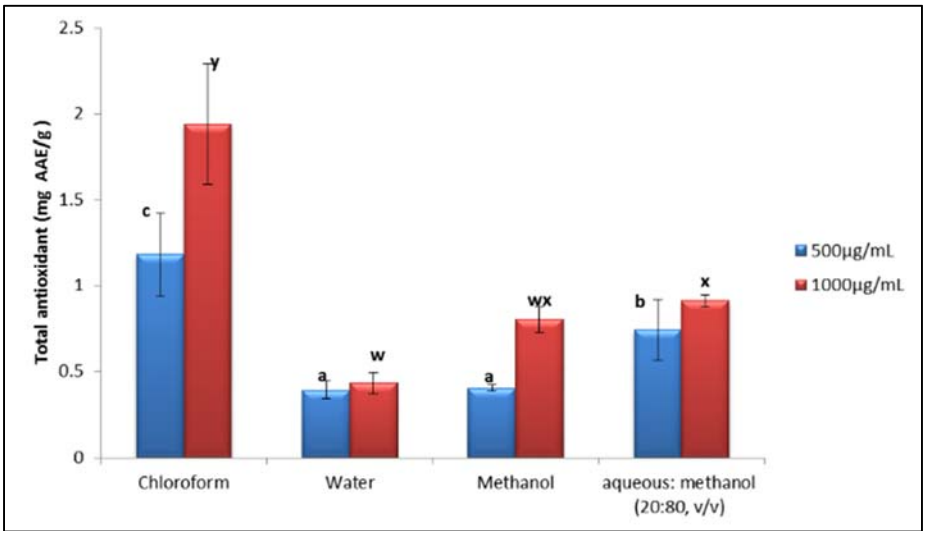


Fig 3: Total antioxidant capacity (mg AAE/g) of chloroform, water, methanol, and aqueous: methanol (20:80, v/v) extracts from dried leaf of *Foeniculum vulgare* at different concentrations (µg/mL). Values are average of triplicate measurements (mean ± SD). Values within the same concentration with different letters in the histogram bar are significantly different at $p < 0.05$.

6.4 Correlation analysis

A relationship between phenolic content and antioxidant activity was extensively investigated, and both positive and negative correlations were reported. Engeda D., [20], Boulanouar *et al*., [21], Terpinc *et al*., [22] and many other research groups stated that there was a positive correlation. In the present study, the dependence of antioxidant activity, obtained by different assays, in relation to the TPC (Figure 4) and TFC (Figure 5), was evaluated. TPC was strongly correlated with DPPH scavenging (IC_{50}) (R = -0.71, $p > 0.05$), and ferric reducing power (R = 0.99, $p < 0.01$). However, there was no correlation TPC with total antioxidant activity (R = -0.07, $p > 0.05$). The results suggested that the phenolic compounds contributed significantly to the antioxidant activities of the *Foeniculum vulgare* extracts. The strong correlations observed between antioxidant activities and

phenolic content indicate phenolic compounds were a major contributor to the antioxidant activities in the *Foeniculum vulgare* extracts. This is in agreement with other studies that found strong correlations between high phenol content and high antioxidant activities in and herbs and medicinal plants [23].

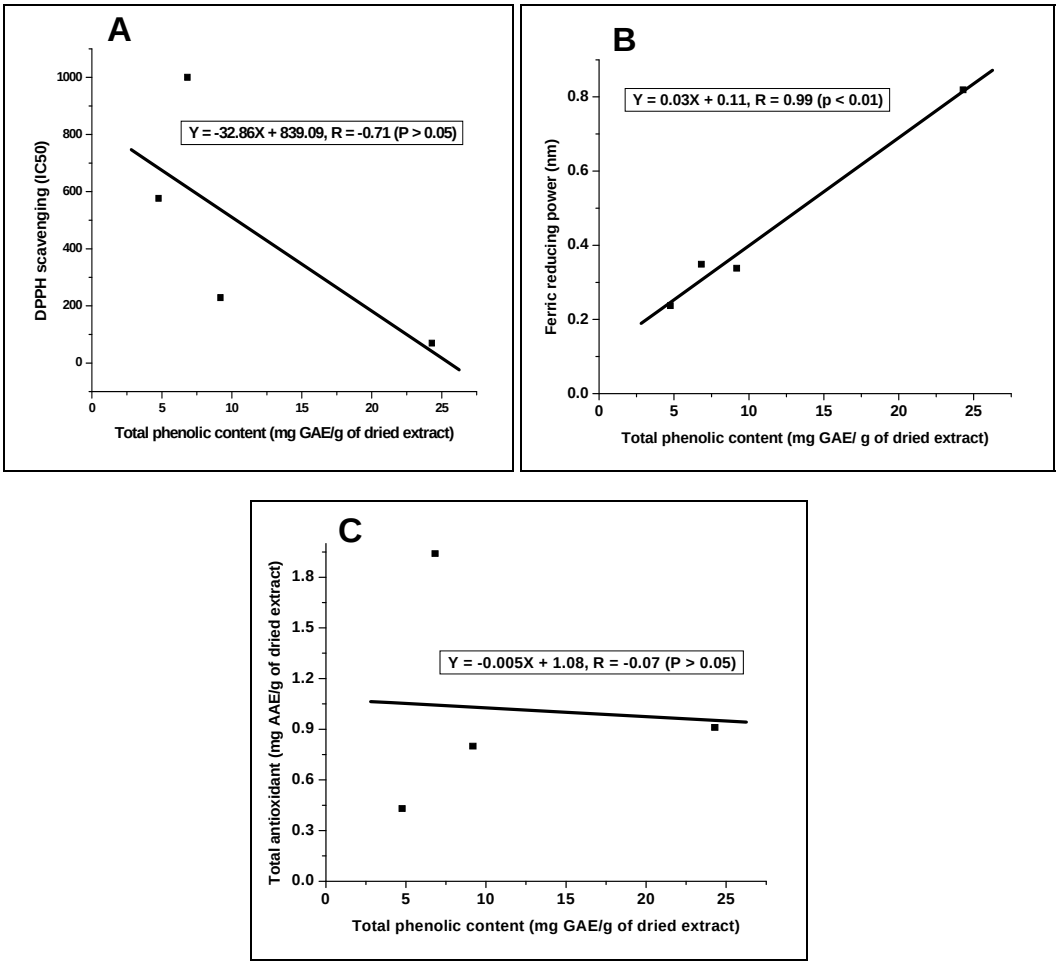
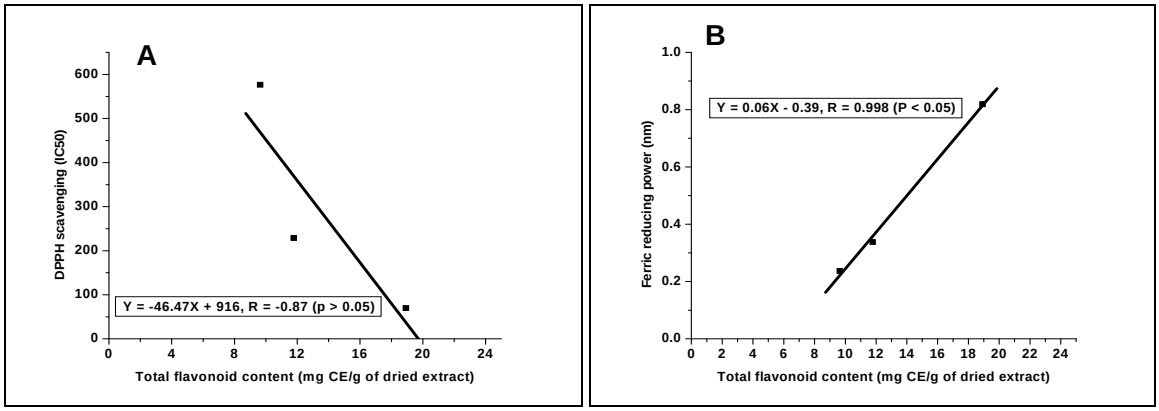


Fig 4: Correlation between total phenolic contents expressed as mg GAE/g of dried extracts and various measurements of antioxidant activity; DPPH scavenging (IC_{50}) (A), ferric reducing power at 700 nm (B), and total antioxidant expressed as mg AAE/g of dried extract(C).

Similarly, a strong positive correlation was also found between DPPH scavenging (IC_{50}) (R = -0.87, $p > 0.05$), ferric reducing power (R = 0.998, $p < 0.05$), total antioxidant activity (R = 0.822, $p < 0.05$) and TFC in *Foeniculum vulgare* extracts. These results may be due to hydroxyl groups existing in the chemical structure of flavonoids compounds from *Foeniculum vulgare* extracts that can provide the necessary component as a radical scavenger.



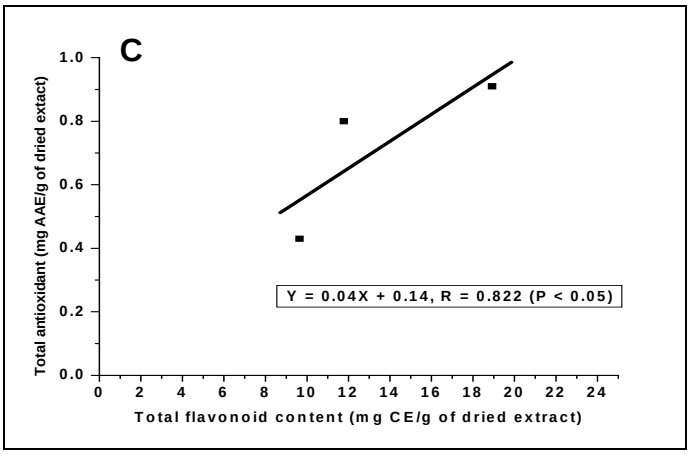


Fig 5: Correlation between total flavonoids contents expressed as mg CE/g of dried extracts and various measurements of antioxidant activity; DPPH scavenging (IC_{50}) (A), ferric reducing power at 700 nm (B), and total antioxidant expressed as mg AAE/g of dried extract(C).

6.5 Antimicrobial Activity

The antibacterial activity of *Foeniculum vulgare* extracts were observed by measuring the diameter of the growth inhibition zone. The results are shown in Table 4 at concentration of 100 mg/mL. The results of antibacterial activity of *Foeniculum vulgare* by disc diffusion method with the aqueous: methanol (20:80, v/v) extract showed high inhibition against *Bacillus megaterium* with the inhibition zone 8.74 ± 0.12 mm. Whereas the methanolic extract against *Bacillus megaterium* (7.39 ± 0.06 mm) and aqueous: methanol (20:80, v/v) extract against *Enterococcus gallinarum* (8.02 ± 0.43 mm), showed moderate inhibition zone. All chloroform and water extracts had no remarkable effect on all studied microbial strains. The diameter of the inhibition zone for each sample against each micro organism was found to be less than that of the standard antibiotics.

Table 4: Average zone inhibition (in mm) of different extracts against test microorganisms at concentration of 100 mg/mL.

Extraction solvents	Diameter of inhibition zone in mm, Including Disc Diameter of 6 mm			
	Gram +ve		Gram -ve	
	Entrococcus	*Bacillus*	*E. coli*	*Pseodominas*
Chloroform	-	-	-	-
Water	-	-	-	-
Methanol	-	7.39 ± 0.06^{ab}	6.86 ± 0.31^{a}	-
Aqueous: methanol (20:80, v/v)	8.02 ± 0.43^{b}	8.74 ± 0.12^{b}	6.93 ± 0.23^{a}	-
Kan	19.98	17.99	12.8	20.19
AMX2	18.75	14.89	-	19.36

Zone of inhibition is expressed as mean ± standard deviation, (n = 3) -: no inhibition, Kan: Kanamycin, AMX2: Amoxicillin. Data was analyzed by One-way ANOVA followed by Duncan post test. Different letters represent significant difference between samples ($P < 0.05$).

Most studies concerning the antimicrobial activity of *Foeniculum vulgare* showed that on gram-negative bacteria are generally less susceptible than gram-positive bacteria. The outer membrane of gram-negative bacteria contains hydrophilic lipopolysaccharides (LPS), which create a barrier toward macromolecules and hydrophobic compounds, providing gram-negative bacteria with higher tolerance toward hydrophobic antimicrobial compounds like those found in crude extracts [24]. Main components of *Foeniculum vulgare* extracts, such as anethole, estragole (methyl chavicol), fenchone and α-pinene have been previously reported to have antibacterial activity [25].

Similar findings and conclusions were drawn by various authors in their experiment they found out the aqueous extract was more effective inhibitor of bacterial growth than the other extracts [26]. Earlier reports also support the view that antibacterial activity was due to different chemical constituents including flavanoids, alkaloids, terpenoids and other compounds which are classified as active antimicrobial compounds [27]. Based on the above comparison of the result with other studies, the presence of secondary metabolite and antibacterial activities of the same plant species from different location can vary. This antimicrobial potential could be utilized in the preparation of phytomedicines in compacting diseases caused by microorganisms in plants and human diseases.

7. Conclusions

Plants and their products have been used for many years for human health. The current study showed that *Foeniculum vulgare* leaf extracts contained secondary metabolites which act as antioxidant agents. The phytochemical screening test of crude extract confirmed the presence of different phytochemicals like; alkaloids, saponins, tannins, triterpenoids, flavonoids, and steroids in aqueous: methanol (20:80, v/v), methanol and water extracts.

The study showed that TPC and TFC of *Foeniculum vulgare* were highest in aqueous: methanol (20:80, v/v) extract. According to the results obtained, the amounts of phenolic contents in extracts are significant for their antioxidant activities. The highest DPPH scavenging was observed in aqueous: methanol (20:80, v/v) extract. Similarly, in reducing power, the maximum absorbance was recorded for aqueous: methanol (20:80, v/v), which was greater the antioxidant activity. On the other hand, chloroform extract was the best antioxidant as demonstrated by the highest value of TAC compared to other extracts. The antioxidant activity of the

extracts strongly correlated with the TPC and TFC and indicated that phenolic compounds are dominant contributors to the antioxidant activity of the extracts.

Foeniculum vulgare extract could inhibit the growth of gram positive bacteria and gram negative bacteria. The results of the present study demonstrated that the highest inhibition zone was found in aqueous: methanol (20:80, v/v) extract against *Bacillus megaterium.* This antimicrobial potential could be utilized in the preparation of phytomedicines in combating diseases caused by micro organisms in plants and human beings. *Foeniculum vulgare* extract can play dual role as antioxidant and antibacterial agent, and has good potential to be applied in the food and pharmaceutical industry. In addition, *Foeniculum vulgare* showing antioxidant activity might be explored for functional food and nutraceutical applications, besides its traditional uses.

8. References

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