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Physicochemical Properties, Antioxidant and Antimicrobial Activities of Fennel (*Foeniculum vulgare* Mill) Seed and Leaf Oils

Zekeria Yusuf[1]*, Abdi Mustefa[1], Mulugeta Desta[1], Jemal Teneshu[1], Sultan Seyida[1], Sewnet Mengistu[1], Desta Dugasa[2]

[1]*School of Biological Sciences and Biotechnology, Haramaya University, Oromia, Ethiopia.*
[2]*Department of Food Sciences and Postharvest Technology, Haramaya University, Oromia, Ethiopia.*

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Abstract

Foeniculum vulgare Mill. Commonly known as fennel has been used in traditional medicinal plant belonging to Apiaceae. The aim of this study was to examine quality and biological activities of fennel seed and leaf oils. The oil extraction was done in Soxhlet apparatus using hexane as a solvent. The result for physicochemical properties presented significantly higher oil yield (4.39%) and peroxide value (3.90) was observed for seed oil. Significantly higher antioxidant activities with respect to DPPH (24.45±3.74) and hydrogen peroxide (62.70±0.28) free radical scavenging activities for leaf oil. However, ascorbic acid was found to be significantly higher for seed oil (82.44±4.63). The strongest antibacterial activity with maximum zone of inhibition (14.25mm), minimum inhibitory concentration (MIC, 0.25µl/ml) and corresponding minimum bactericidal concentration (MBC, 0.50 µl/ml) was recorded for leaf oil extract against *Staphylococcus aureus*. On the other hand, the strongest antifungal activity with maximum zone of inhibition (13.50mm), MIC (0.38µl/ml, the least value) and minimum fungicidal concentration (MFC, 0.75µl/ml) was recorded for leaf oil against *Aspergillus Niger*. It can be observed from the result in this study that leaf oil extract has demonstrated more effective biological activities including both antioxidant and antimicrobial potentials.

1. Introduction

Foeniculum vulgare commonly known as fennel and its local name is *khamona* in Oromo. It is a biennial medicinal herb belonging to the family Apiaceae. Herbal drugs and essential oils of fennel have hepatoprotective effects, as well as antispasmodic effects **(Reynolds, 1982).** They are also known for their diuretic, anti-inflammatory, analgesic and antioxidant activities and anticancer activities **(Choi and Hwang, 2004; Anand *et al.*, 2008).** Medicinal herbs are good alternative to chemical drugs as they have low side effect compared to chemical drugs **(Abe and Ohtani, 2013).** Fennel has been used in traditional medicine for treatment of various disorders associated with digestive, endocrine, reproductive, and respiratory systems **(Badgujar*et al.*, 2014).** The dried, aromatic fruits are vastly used in culinary preparations for flavoring bread and pastry, in candies, and in alcoholic liqueurs, also used in cosmetic and medicinal preparations **(Farrell, 1985).** The oil yield (2.5 - 5%) varies according to origin and variety and the highest concentration of fennel oil is found in seeds ranging between 2 - 7%, fennel volatile oil is a mixture of different chemicals and the main ingredients are: trans- anethole (40 - 70%), fenchone (1 - 20%) and estragole (2 - 9%), other compounds (α-pinene, chavicole, dipentene, α-limenene etc.) are present in concentration usually less than 1% **(Cosge *et al.*, 2008).**

Fennel essential oil possesses valuable antioxidant, and has antibacterial, anticancer and antifungal activity **(El-Alwadi and Esmat, 2010).** Mature fennel fruits are used as flavoring agents in food products such as pickles, bread, pastries and cheese **(Zoubiri *et al.*, 2014).** Fennel fruits are used in diseases like cholera, nervous disorders, constipation, dysentery and colic pain, and also contains minerals and vitamins like calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin and vitamin C **(Deswal *et al.*, 2017).** Characterization should provide a standardized record of readily assessable plant **characters (Gimhavanekar *et al.*,2020).**

Even though fennel seeds and areal parts have long been used in ethno medicine, little work has been done on fennel varieties growing in Ethiopia. Considering the importance of fennel as medicinal plants and its potential industrial application in especially pharmaceutical and nutraceutical industries, this study is designed to assess fennel oil extracts including physicochemical properties, antioxidant and antimicrobial activities of fennel seed and leaf oil extracts will be examined, Thereby allowing the use of a readily available and affordable resources provided, prevent product loss and damage and ultimately lead to improved health and safety of the food.

2. Material and methods

*Corresponding author: zakoyusuf@yahoo.com
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The experiment was conducted in Molecular Biology and Biotechnology Laboratory in the School of Biological Sciences and Biotechnology, Haramaya University. The fennel (*Foeniculumvulgare*) plant samples was collected from Bedeno district, East Hararghe, Ethiopia. The leaf and seed samples were manually washed with distilled water and residual moisture evaporated at room temperature. Thereafter, air dried and ground to a fine powder in a grinder for 2 min, the process will be stopped for 15sec so as to avoid heating of sample Determination of moisture (on dry basis) will be carried out **(AOAC, 1990).** The oil extraction was done in Soxhelt apparatus using hexane as a solvent. Then, physicochemical properties of the oil extracts were done based on determination of oil content, acid value, percent free fatty acid and peroxide values based on the standard procedures described by **AOAC (2000).** The antioxidant activities were investigated based on determination of ascorbic acid content, DPPH and hydrogen peroxide free radical scavenging activities as per the standard procedure described by **AOAC (2000).**

The antimicrobial experiment was arranged as 2x1x4 (2 source extracts (seed and leaf oil extracts from fennel (*Foeniculum vulgare Mill.*) at three concentration levels), 1 solvent system i.e., hexane, 4 test organisms (2 bacteria: *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive), two fungi *(Aspergillus versicolor* and *A. Niger)*) factorial design in three replications. The test pathogens were obtained from Plant Pathology Laboratory, Haramaya University. The fungal and bacterial pathogens were subcultured and maintained on Potato Dextrose Agar (PDA) and Nutrient Agar, respectively. Then, the fungal and bacterial cultures were incubated for 72 h at 27 ºC and for 18-24 h at 37 ºC, respectively.

Media Preparation and Standardization of Inoculum: nutrient Agar (NA), Potato Dextrose Agar (PDA), and Muller Hinton agar (MHA) was used for sub-culturing of bacterial test organism, fungal test organism, and determination of antimicrobial activities, respectively. These media were prepared and sterilized using an autoclave according to the manufacturers' instructions. The bacterial colonies and spores of the test fungi were harvested by washing the surface of the fungal colony using 5mL of sterile saline solution. This procedure repeated until the turbidity of each bacterial and fungal spore suspension matched the turbidity of 0.5 McFarland Standards as described by the Clinical Laboratory Standards Institute (CLSI, 2015). The resulting suspension will be used as inoculums for the test pathogen in the antimicrobial susceptibility test.

Disc diffusion Method: the discs of 6 mm diameter was prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then impregnated each of them was impregnated with 0.01 ml of the prepared test extract solution. The extract impregnated discs were placed onto MHA plates evenly inoculated with test pathogens **(Hudzicki, 2009).** Following this step, the impregnated discs were dispensed onto the surface of the inoculated agar plates using sterile forceps **(CLSI, 2015).** Discs of commercial gentamycin (1µl/disc) and fluconazole (1µl/disc) were used as positive controls for bacterial and fungal pathogens, respectively and distilled water impregnated discs were used as negative controls. Then the MHA plates were sealed with parafilm and incubated at 37°C for 24 hrs and 27°C for 72 hrs for bacterial and fungal pathogens, respectively. The diameters of the zone of inhibition around each disc were measured to the nearest millimeter along two axes (i.e., 90° to each other) using a transparent ruler and the means of the two readings were be recorded.

Determination of Minimum Inhibitory Concentration (MIC): The oil extracts of fennel seeds and leaves that showed significant antimicrobial activity in the antimicrobial activity tests were selected for determination of MIC using broth dilution method. Accordingly, two milliliters of nutrient broth and potato dextrose broth for bacteria and fungi respectively were added into all test tubes and 0. 1 ml of the prepared concentration of each oil extract were mixed with the nutrient broth and potato dextrose. Thereafter, standardized inoculums of 0.1 ml of the respective test pathogens were dispensed into the test tubes containing the suspensions of the broth and the oil extract. Then, all test tubes were properly corked and incubated at 37°C for 24 hrs for bacteria and 27°C for 72 hrs for fungi. After that, they were observed for absence or presence of visible growth. The lowest concentration at which no visible growth of organisms were regarded as the MIC.

Determination of minimum bactericidal (MBC) and fungicidal concentrations (MFC): for the determination of the MBC and MFC, fresh nutrient agar and potato dextrose agar plates were inoculated with one loop full of culture taken from each of the broth cultures that showed no growth in the MIC tubes. That is MBC/MFC values were determined by subculturing from respective MIC values. Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC **(CLSI, 2015).** While MBC assay plates were incubated for 48 h, MFC assay plates were incubated for 3 days. After the incubation periods, the lowest concentration of the extract that did not allow any bacterial or fungal growth on solid medium was regarded as MBC and MFC for the extract **(CLSI, 2012).** The experimental data were analyzed using SAS version 9.2. (SAS, 2011) to investigate statistical significance between the different oil quality parameters. Differences between means were considered statistically significant at $P< 0.05$.

3. Results

3.1 Assessment of physicochemical quality of oils from fennel (*Foeniculum vulgare*) seeds and leaves

The quality of *Foeniculum vulgare* seed and leaf oils was determined based on physicochemical properties including oil content, acid value, free fatty acids and peroxide values (Table 1). Significance differences between *F. vulgare* seed and leaf oils were obtained for oil content, acid value (ACV), free fatty acid. Significantly higher oil yield (4.39%) and peroxide value (3.90) was observed for seed oil. By contrast, leaf oil was recorded significantly higher acid value (2.38) and free fatty acid value (1.20).

Table 1 Physicochemical properties of *Foeniculum vulgare* seed and leaf oils

Oil extract	Oil yield	ACV	FFA	PV
Seed	4.39±0.16a	1.26±0.20b	0.63±0.10b	3.90±0.14a
Leaf	3.04±0.12b	2.38±0.19a	1.20±0.09a	3.10±0.15b

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row. ACV: acid value; FFA: free fatty acids; PV: peroxide value.

3.2 Antioxidant activities of *Foeniculum vulgare* seed and leaf oils

The antioxidant activities of oil extracted from *F. Vulgare* seed and leaf evaluated based on ascorbic acid content, DPPH and hydrogen peroxide free radical scavenging activities as in Table 2. Significantly higher antioxidant activities with respect to DPPH (24.45±3.74) and hydrogen peroxide (62.70±0.28) free radical scavenging activities, were obtained for leaf oil. However, ascorbic acid was found to be significantly higher for seed oil (82.44±4.63) than for leaf oil. The higher DPPH value (24.45) indicates higher antioxidant activities and the presence of higher essential omega-3 fatty acids in *F. Vulgare* leaf oil. The

antioxidant activities of leaf oil was found to be significantly higher than seed oil extract indicating that leaf oil might possess better biological activities, oil quality and pharmacological applications.

Table 2 Antioxidant activities of *Foeniculumvulgare* seed and leaf oils

Oil extract	DPPH	HPSA	AA
Seed	13.3±0.14b	3.65±0.07b	82.44±4.63a
Leaf	24.45±3.74a	62.70±0.28a	21.57±3.16b

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row. DPPH: 2, 2- diphenyl-1-picrylhydrazyl; HPSA: hydrogen peroxide scavenging activity; AA: ascorbic acid.

3.3 Antimicrobial Activities of *Foeniculum vulgare* seed and leaf oils

The diameter of inhibition zone, measured by paper disk diffusion method, for *F.vulgare* seed and leaf oils as in Table 3. Significance differences were recorded for both seed and leaf oil extracts at different concentration levels The mean zone of inhibition at highest concentration (3µl/ml) against bacterial test pathogens ranged from 8.00±0.50 mm to 14.25±0.25mm, while 10.50±0.50 to 13.50±0.36mm against fungal test pathogens. The strongest antibacterial activity with maximum zone of inhibition (14.25mm) at highest concentration (3µl/ml) of the oil was recorded for leaf oil extract against *S. aureus* while the weakest antibacterial activity (8.00mm) was observed for *F. vulgare* seed oil against *E. coli* indicating that *S. aureus* was more susceptible. Hence leaf oil has exhibited more antibacterial potential than seed oil in *F. vulgare.*

On the other hand, the strongest antifungal activity with maximum zone of inhibition (13.50mm) was recorded for leaf oil against *A. niger* as the weakest antifungal activity with minimum zone of inhibition (10.50mm) was recorded for seed oil against *A. versicolor* suggesting leaf oil extract might be more effective antifungal potential than seed oil extract in fennel *(F. vulgare*). Similar study was conducted by **Ahmada *et al* (2018)** who reported antibacterial activity of fennel vegetable oil cake.

Table 3 Antimicrobial Activities oil extracts from fennel leaf and seed as mean diameter of zone of inhibition against test pathogenic microbes

Test Pathogens	Oil extract	Concentrations of the oil extract (v/v)			amoxicillin (2µl/ml)
		2µl/ml	2.5µl/ml	3µl/ml	
E. coli	Leaf	0.00±0.00bD	9.50±0.50bC	12.13±0.32bB	18.33±0.50aA
	Seed	0.00±0.00bD	6.43±0.40cC	8.00±0.50cB	18.50±0.29aA
S. aureus	Seed	12.00±0.50aB	13.10±0.36aB	14.25±0.25aB	18.50±0.50aA
	Leaf	11.33±0.76aB	12.77±0.25aB	13.75±0.25aB	18.67±0.29aA
					ketokonazole (2µl/ml)
A. niger	Seed	6.00±0.50bD	8.00±0.40bC	11.50±0.60bB	17.67±0.29abA
	Leaf	7.77±0.25aD	9.50±0.50aC	13.50±0.36aB	17.17±0.29bA
A. versicolor	Seed	0.00±0.00cD	7.00±0.53cC	10.50±0.50cB	17.83±0.29aA
	Leaf	5.97±0.55bD	8.50±0.36bC	12.00±0.51bB	17.33±0.29abA

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row. *E. coli*: *Escherichia coli; S.aureus*: *Staphylococcus aureus.*

3.4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) fennelleaf and seedoils

The effectiveness of fennel seed and leaf oils against pathogenic microbes was evaluated by MIC, MBC and MFC as indicated in Table 4. The fennel leaf oil extracts has exhibited strongest bactericidal activity with MIC (0.25µl/ml) and corresponding MBC (0.50 µl/ml) against *S. aureus* while the weakest bactericidal activity with MIC (0.50 µl/ml, the largest value) and corresponding MBC (1.00 µl/ml) was recorded for seed oil against *E. coli* indicating that *S.aureus* is more susceptible to the oil extract than *E. coli,* and also suggesting leaf oil possesses stronger antibacterial potential.

Table 4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) fennel leaf and seed oils

Pathogens	Oil extract	MIC(µl/ml)	MBC/MFC (µl/ml)
Escherichia coli	Seed	0.50	1.00
	Leaf	0.38	0.75
Stapylococcus aureus	Seed	0.38	0.75
	Leaf	0.25	0.50
Aspergillusversicolor	Seed	1.75	2.75
	Leaf	1.38	2.25
A. niger	Seed	0.75	1.25
	Leaf	0.38	0.75

For antifungal activity, fennel leaf oil extract has demonstrated strongest antifungal activity with MIC (0.38µl/ml, the least value) and MFC (0.75µl/ml) against *A. niger* whereas the weakest antifungal activity with MIC (1.75µl/ml) and the corresponding MFC (2.75µl/ml) was observed for the seed oil extract against *A.versiclor* showing that *A. niger* was more susceptible to the oil extract than *A. versicolor,* and the leaf oil was more effective antifungal potential than seed oil in funnel.

4. Discussions

The result for the quality of *F. vulgare* seed and leaf oils indicated significance differences between *F. vulgare* seed and leaf oils were obtained for oil content, acid value (ACV), free fatty acid. Significantly higher oil yield and peroxide value was observed for seed oil. This finding was in accordance with **Saini et al (2014)** who reported physicochemical parameters of the essential oil of fennel fruit**. Wahba *et al* (2018)** compared the volatile oils isolated from green herb and dry seeds with the oil extracted from the fennel waste, so as to benefit from waste as a new source of oil. In most cases there was a great similarity between fennel oil constituents isolated from the dry waste after harvest with oil extracted either from green herb or dry seeds.

The antioxidant activities of oil extracted from *F. vulgare* seed and leaf showed significantly higher antioxidant activities with respect to DPPH and hydrogen peroxide free radical scavenging activities, were obtained for leaf oil. However, ascorbic acid was found to be significantly higher for seed oil than for leaf oil. The higher DPPH value indicates higher antioxidant activities and the presence of higher essential omega-3 fatty acids in *F. vulgare* leaf oil. The antioxidant activities of leaf oil was found to be significantly higher than seed oil extract indicating that leaf oil might possess better biological activities, oil quality and pharmacological applications. A strong antioxidant activity exhibited by fennel seeds was reported by several previous studies **(Esmaeilzadeh Kenari et al., 2014; Patel and Jasrai, 2015)**. Such differential scavenging activities can be due to the presence of different types of bioactive compounds especially phenolics in the extracts, to the extraction method but also to the type of used solvent. Gallic acid, caffeic acid, ellagic acid, quercetin and kaempferol are the main phenolic compounds identified in fennel seed extracts **(Li *et al.,* 2015; Dua *et al.,* 2013). Khan and Mushara (2014)** reported that the fennel has antioxidant property. Due to the high content of polyphenolsand flavonoids, this plant can stop free radicals. Phenolic compounds in this herb such as caffeoylquinic acid, rosmarinic acid, eriodictyol-7-orutinoside, quercetin- 3-O-galactoside, and kaempferol-3-O-glucoside showed antioxidant activity. Fennel volatile oil also has strong antioxidant activity.

The fennel leaf oil in the present study has presented stronger antimicrobial potential than seed oil. In studies conducted by various authors **(Singh and Singh, 2000; Manonmani and Khadir, 2011; Ahmada *et al*., 2018)** who reported that aqueous extract of fennel seed was the most active among other extracts. This differential antibacterial activity can be related firstly to the characteristics of each bacterial strain, and secondly to the presence of different phytochemicals such as phenols, flavonoids, tannins, alkaloids and others in the seeds residues **(Sudhira *et al.,* 2015).** Fennel essential oil antifungal properties stronger than potassium sorbate as a preservative in the food industry. So, this plant can be used in food industry as a preservative (especially in baking products) (**Khosravi *et al.,* 2014).**

As MIC is the lowest concentration of antimicrobial agent that has inhibitory effects on the growth of a particular microorganism, this means that the microorganism is present but cannot reproduce. Reduce the number of microorganisms in the circumstances, but not essential because lethal effects caused by microorganisms reach the dying phase, and the other does not multiply the number decreases. It should be noted that the use of plant essential oils in food storage does not create slightest problem in terms of health for the consumer **(Aussalah *et al.,* 2007; Siger *et al.,* 2007).**

5. Conclusion

The result of this study demonstrated the antioxidant and antimicrobial potential of fennel oil extracts. Leaf oil found to have better biological activities than seed oil extract in F. vulgare. Therefore, leaf oil should be exploited further for application as food preservatives, pharmaceuticals, alternative medicine and natural-based therapies. Fennel (Foeniculum vulgare) oils and extracts could be a promising bio-resource with significant interest as a rich source of both vegetable oil and essential oil, as well as nutraceuticals.

LIST OF ACRONYMS/ABBREVIATIONS

AA	Ascorbic acid
ACV	Acid value
AOAC	Association of Analytical Chemists
CLSI	Clinical and Laboratory Standards
DPPH	2, 2- diphenyl-1-picrylhydrazyl
FFA	Free fatty acids
HPSA	Hydrogen peroxide scavenging activity
LSD	Least Significance Difference
MBC	Minimum bacterial inhibitory concentration
MFC	Minimum fungal inhibitory concentration
MHA	Muller Hinton Agar
MIC	Minimum inhibitory concentration
PDA	Potato Dextrose Agar
PV	Peroxide value

Declaration of interests

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. Zekeria Yusuf reports financial support was provided by Haramaya University. Zekeria Yusuf reports a relationship with Haramaya University that includes: employment. Zekeria has patent pending to Haramaya University. The authors declare no conflict of interest of any kind.

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Availability of data and materials

The data materials will be available on request.

Ethics approval and consent to participate

The ethical approval is not applicable for this manuscript since it has no animal experiment according to Haramaya University's ethical committee.

Conflict of Interest

The authors declare no conflict of interest. All authors have agreed for submission of this manuscript. The authors declare that they have no competing interests.

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