Phytochemical Investigation and Antimicrobial Evaluation of *Foeniculum vulgare* Leaves Extract Ingredient of Ethiopian Local Liquor

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Abstract: Medicinal plants are of great interest to the researcher in the field of biotechnology, as natural products, including medicinal plants, accounts 25% of prescribed drugs. Plants are sources for fragrances, drink colors and flavors in several countries including Ethiopia. All parts of *Foeniculum vulgare* were traditionally used as antispasmodic, aromatic, carminative, digestive, galactagogues, stomach and kidney ailment. *Foeniculum vulgare* leaves extract was investigated for its phytochemicals as well as antimicrobial effects. The petroleum ether, $CHCl_3$, $CHCl_3$/CH_3OH (1:1) and CH_3OH crude extract were subjected to phytochemicals screening test which revealed that it is rich in any primary and secondary metabolites such as steroids, tannins, flavonoids, cholesterol, terpenoids, saponins, phenols, cardiac glycosides, carbohydrates, and proteins. The essential oil of the plant leaves was investigated by GC-MS and was found to have (64.92%) anethole, as a major constituent followed by (30.88%) estragole and (3.21%) fenchyl acetate. The crude extracts, oil, and the isolated compound were tested against four bacterial species (Gram negative bacteria, *Escherichia coli* and *Shigella flexneri*; Gram positive bacteria: *Staphylococcus aureus* and *Streptococcus pyrogenes*) and two fungal species (*Fusarium oxysporum* and *Aspergillus niger*) using paper disc diffusion method. Tests of antimicrobial activity showed that all crude extracts and isolated pure compound were active against all the tested bacterial and fungal species. However, the hydrodistillation extract was found to have no antibacterial activity towards the tested bacterial species but active against the two fungal species and thus the present study supported the traditional claims of the plant.

Keywords: Antimicrobial activities, Disc diffusion method, Phytochemical screening, anethole, estragole, GC-MS, Essential oil, Apiaceae.

INTRODUCTION

Aromatic plants are becoming more important due to their antimicrobial activity [1]. They possess biological activities such as antioxidants [2], hypocholesterolemics [3], the stimulant effect on animals digestive systems, increase production of digestive enzymes and improve utilization of digestive products through enhanced liver functions [4]. Fennel (*Foeniculum vulgare*, Apiaceae) is one of these aromatic plants, which are containing a high percentage of the fatty acids linolenic and stearic. In addition, fennel has 16.81% *trans*- anethole plus 47.20% estragole with total sweeting components of 64.01% in essential oil. Body weight was increased and improvement feed conversion by using fennel in the diets [5].

F. vulgare has been reported to contain 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fiber and 42.3% carbohydrates. The minerals and vitamins present in *F. vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C. *F. vulgare* is well known for its essential oil. The characteristic anise odour of *F. vulgare* which is due to its essential oil makes it an excellent flavouring agent in baked goods, meat and fish dishes, ice-cream and alcoholic beverages. The major components of *F. vulgare* seed essential oil have been reported to be trans-anethole, fenchone, estragole (methyl chavicol), and α-phellandrene. The relative concentration of these compounds varies considerably depending on the phonological state, origin of the fennel, geographical location and soil type [6].

In Ethiopia, *F. vulgare* leaves are chewed with *Catha edulis* (khat) for treatment of kidney stones and gonorrhea. In addition to that, around Dessie, Ethiopia the plant is used during preparation of local liquor for best odor. Hence, the present study was aimed to investigate the phytochemicals and antimicrobial activities of leaves of *Foeniculum vulgare* as components of the plant leaves are often used for food and beverage flavoring. As the plant is a wild herb that grows in large scale it is possible to use the plant essential oil as additives in food and beverages and drugs for kidney stones and gonorrhea. Analysis of essential oil of the plant leaves has been carried out using GC-MS and antimicrobial activity tests were done using paper disc diffusion method.

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MATERIALS AND METHODS

Chemicals

The chemicals used in this study were: Solvents (Petroleum ether, Chloroform (99.8%; Analytical reagents), Methanol (99.9%; HPLC-Plus-gradients) and Ethyl acetate), Anhydrous Sodium sulphate (NLT 99.5%, Analytical reagent pH (5% aqueous solution 5.0-8.0), sulfuric acid, vanillin and appropriate media for bacteria and fungi, Muller-Hinton agar (Blulux laboratories (p) ltd., India), Potato dextrose agar (Blulux laboratories (p) ltd., India), distilled water, Ammonium (Blulux laboratory ltd., India), Sodium hydroxide pellets AR 98% (Breck land scientific supplies, Thetford Norfolk, UK), 76.4% Ferric Sulfate (Baker chemical co., USA), 99% Acetic acid glacial, (Techno Pharmchem Dehi, India), Acetic anhydride (British drug house ltd., UK), ferric chloride (British drug house ltd., England), Ammonium hydroxide (Abron chemicals INSI, India) and 37% hydrochloric acid (Blulux laboratories (p) ltd., India).

Plant Materials

The leaves of *Foeniculum vulgare* were collected from Haramaya University, Haramaya in December, 2015. The botanical specimens of the plant were identified and the voucher specimen was deposited at the Herbarium, Haramaya University.

Extraction of the Plant Material

Air dried leaves of *Foeniculum vulgare* were grinded by analytical mill and packed in polyethylene bags to avoid entrance of air and any other dirt from the surrounding. A 300 g of the powdered leaves was then measured in six Soxhlet apparatus (50 g of each) and soaked with 250 ml (of each) of petroleum ether and heated with a heating mantle at 45°C for 6 h and filtered with filter paper (Whatman No. 1 Whatman Ltd, England). This procedure was repeated twice. The filtrate was collected and concentrated at 40°C using a Rotary evaporator (Buchi Rotovapour R-205, Switzerland). After petroleum ether extract the marc was dried at room temperature extracted with chloroform, methanol/chloroform (1:1) and methanol using the same procedure.

Phytochemical Screening Tests

Test for Terpenoids

5 ml of the extract was added to 2 ml of chloroform and 3 ml of conc. H_2SO_4 and formed a monomer of reddish-brown coloration of the interface a positive result for the terpenoids [7].

Test for Cholesterol

To 2 ml of the extract, 2 ml of chloroform was added to a dry test tube. Then 10 drops of acetic anhydride and 2 to 3 drops of conc. H_2SO_4 were added. A red rose color changed to a blue-green color which confirms the presence of cholesterol [8].

Test for Flavonoids

5 ml of dilute ammonia solution was added to apportion of the aqueous filtrate of plant extract followed by addition of conc. H_2SO_4. A yellow coloration was observed which confirms the presence of flavonoids which disappears on standing [7].

Test for Cardiac Glycosides

Keller Killiani Test – Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution. Concentrated sulphuric acid was added, and then the formation of two layers was observed. Lower reddish-brown layer and an upper acetic acid layer which turns bluish green would indicate a positive test for glycosides [7].

Bromine Water Test

A test solution was dissolved in bromine water and the formation of yellow precipitate showed a positive result for the presence of glycosides [7].

Test for Steroids

Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed the formation of a brown ring at the junction of two layers. Green coloration of the upper layer would indicate a positive test for steroids [9].

Test for Saponins

0.5 g of the extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins [9].

Test for Carbohydrate

To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2 ml of conc. H_2SO_4 was added on the side of the test tube. A reddish violent ring appeared at the junction of two layers immediately indicates the presence of carbohydrate [8].

Test for Proteins

To 0.5 g of extract 2 ml of protein solution 1 ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violent color indicates the presence of peptide linkage of the molecule [8].

Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed brownish green or blue-black coloration which indicated the presence of tannins [7].

Test for Phenols

1 g of extract was treated with few drops of ferric chloride solutions. Formation of bluish black color indicates the presence of phenols (Ferric Chloride Test) [9].

Antimicrobial Assay

Petroleum ether, chloroform, chloroform/methanol (1:1) and methanol extract of leaves of *F. vulgares*, isolated pure compound and volatile oil extracted by steam distillation were evaluated *in vitro* for anti-microbial assay by using the paper disc diffusion method against four bacteria (two gram positive *Staphylococcus aureus and Streptococcus pyrogenes,* and two gram negative *Escherichia coli and Shigella flexneri*) and two fungi, *Aspergillus niger (A. niger)* and *Fusarium oxysporum* [10]. The bacterial cultures were inoculated into the Muller Hinton Agar (MHA) and incubated at 37 ^{o}C. Fungal cultures were inoculated into Potato Dextrose Agar (PDA) and incubated at 27 ^{o}C.

All the microbial were obtained from Plant Pathology Laboratory of the School of Plant Science, Haramaya University and Chloramphenicol was used as standard drug against bacteria whereas Tilt was used against fungi. All solvents including, petroleum ether, chloroform, methanol/chloroform and methanol were also used as a negative control. From inhibition zone data, correlations of structures with antimicrobial activities compounds were critically examined by comparing the mean inhibition diameters at various concentrations and relating them to the control. The crude extracts, isolated pure compound and oil were subjected to antimicrobial assay by using the disk diffusion technique. The procedures are described below.

Preparation of Inoculums

The test bacterial species, two Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyrogenes* (*S. aureus* and *S. pyrogenes*) and two Gram-negative bacteria *Escherichia coli and Shigella flexneri* (*E. coli* and *Sh. flexneri*) were transferred from the stock cultures and streaked on Muller Hinton plates and incubated for 24 h at 37 ^{o}C. Well separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved Muller Hinton that was cooled to about 45 ^{o}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 bio-test [11].

For test fungi, mycelial plugs from stock cultures were transferred to PDA plates and incubated for 5-7 days. Then spores of *A. niger were* harvested by washing the surface of the colony using 10 ml sterile distilled water and transferred to 250 ml autoclaved PDA cooled to about 45 ^{o}C. in a water bath. Likewise, mycelium of *Fusarium oxysporum* were washed with 10 ml sterile distilled water, macerated in a blender and the mycelia suspension, which were be transfer to 250 ml autoclaved PDA cooled to about 45 ^{o}C in a water bath. The medium containing spore or mycelia suspension was poured to sterile plates allowed to solidify and used for the disc diffusion bioassay [11].

Preparation of Test Solutions

Each of the crude extracts (petroleum ether, chloroform, methanol/chloroform and methanol) was dissolved in the respective solvents, at a concentration of 0.2 g/ml, the isolated compound was dissolved in petroleum ether /ethyl acetate (9:1) at a concentration of 0.023 g/ml and the volatile oil of 100% was used to test both antibacterial and antifungal activities.

Evaluation of Antifungal Activity

Filter paper discs of about 6 mm diameter placed in a beaker were sterilized in an oven at 180°C for 1 h. The crude extract and each of the concentrations of the fractions or oil were then pipetted to the sterile paper discs. 10 µL and 20 µL of the extracts were pipetted to the discs in three replications. The paper discs impregnated with the extract solutions were then transferred using sterile forceps to PDA seeded with spore or mycelia suspension of test fungi as described under inoculums preparation above. The Petri dishes were incubated at 24°C for 3 days. The antifungal activity was evaluated by measuring the zone of inhibition against the tested organism.

Evaluation of Antibacterial Activity

Similar procedures to that of the antifungal test were followed. Sterilized paper discs were transferred to

Mueller Hinton agar plates seeded with bacteria and incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the zone of inhibition against the tested organism.

RESULTS AND DISCUSSION

Percentage Yield of the Solvent Extract

The air-dried powdered leaves of *Foeniculum vulgare* (300 g) was pulverized and extracted with petroleum ether at 45°C for 6 h by Soxhlet apparatus to yield dark green extract (22.3 g, 7.5 %). After petroleum ether extract mass of marc was weighed as 277.7 g and extracted with chloroform at 45°C for 6 h by the same apparatus with petroleum ether to yield green extract (14.8 g, 5.2 %). Again, after chloroform extract mass of marc was 262.9 g and extracted with chloroform/methanol (1:1) ratio at 45°C with Soxhlet apparatus for 6 h to yield bright green extract (30.6 g, 11.6 %). Finally, after chloroform/methanol (1:1) ratio extract mass of marc was 232.3 g and extracted with methanol at 48°C for 6 h. with the same procedure of above it yields blue-black color (53.0 g, 22.8 %).

Hydro-Distillation of the Leaves

The air-dried powdered leaves (150 g) was crushed to fine particles and hydro-distilled for 3 h with Clevenger's apparatus to yield an aromatic pale yellow oil (1.3 g, 0.9 %).

Phytochemical Screening Test Result

The phytochemical screening test was carried out on the petroleum ether, chloroform, chloroform/ methanol and methanol extract of leaves of *Foeniculum vulgare* using standard procedures and identified the presence and absence of the different secondary metabolites present in the plant (Table **1**).

GC-MS Analysis of Essential Oil of Leaves of *Foeniculum vulgare*

The oil of *F. vulgare* leaves was analyzed by combined gas chromatography and mass spectrometry. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The identification of the chemical constituents of the oil was determined by their GC retention times and interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) [12].

The chemical constituents of essential oil from *Foeniculum vulgare* are listed in Table **2**. Four components were identified by using GC-MS of the oil. The main constituents of essential oil were identified as: *L*-fenchone (1, 3, 3-trimethyl bicyclo [2, 2, 1] heptan-2-one) (**1**) (0.98%), estragole (1-allyl-4-methoxybenzene) (**2**) (30.88%), fenchyl acetate (1, 3, 3-trimethyl bicyclo [2, 2, 1] heptan-2-yl-acetate (**3**) (3.21%), and (Z)-anethole (Benzene, 1-methoxy-4-(1-propenyl) (**4**) (64.92%). (Z)-Anethole was found as a major component in the oil. Estragole is an isomer of (Z)-anethole differing with respect to the location of the double bond. Fenchyl acetate is ester derivative of L-fenchone. The constituents of essential oil in the plant leaves were identified by GC-MS representing 99.99 % (Tabe **2**).

Anethole is an organic compound that is widely used as a flavoring substance. It is distinctly sweet,

Table 1: Phytochemical Screening Test Results of Petroleum Ether, Chloroform, Chloroform / Methanol and Methanol Extract of Leaves of *Foeniculum vulgare*

No	Constituents	Petroleum ether extract	Chloroform extract	$CHCl_3$/MeOH	MeOH
1	Flavonoids	+	+	-	+
2	Cholesterols	+	+	+	+
3	Terpenoids	+	+	+	+
4	Steroids	+	+	+	-
5	Carbohydrates	-	+	+	+
6	Cardiac Glycosides	+	-	+	-
7	Proteins	-	-	-	+
8	Saponins	+	-	-	-
9	Tannins	+	-	+	-
10	Phenol	+	_	+	+

Table 2: Major and Minor Constituents Identified from Fennel (*Foeniculum vulgare*) Essential Oil

Compound	Retention time	Area %	Name of compound
1	12.142	0.98	L-Fenchone
2	16.971	30.88	Estragole
3	18.454	3.21	Fenchyl acetate
4	20.866	64.92	*z*-Anethole
Total		99.99	

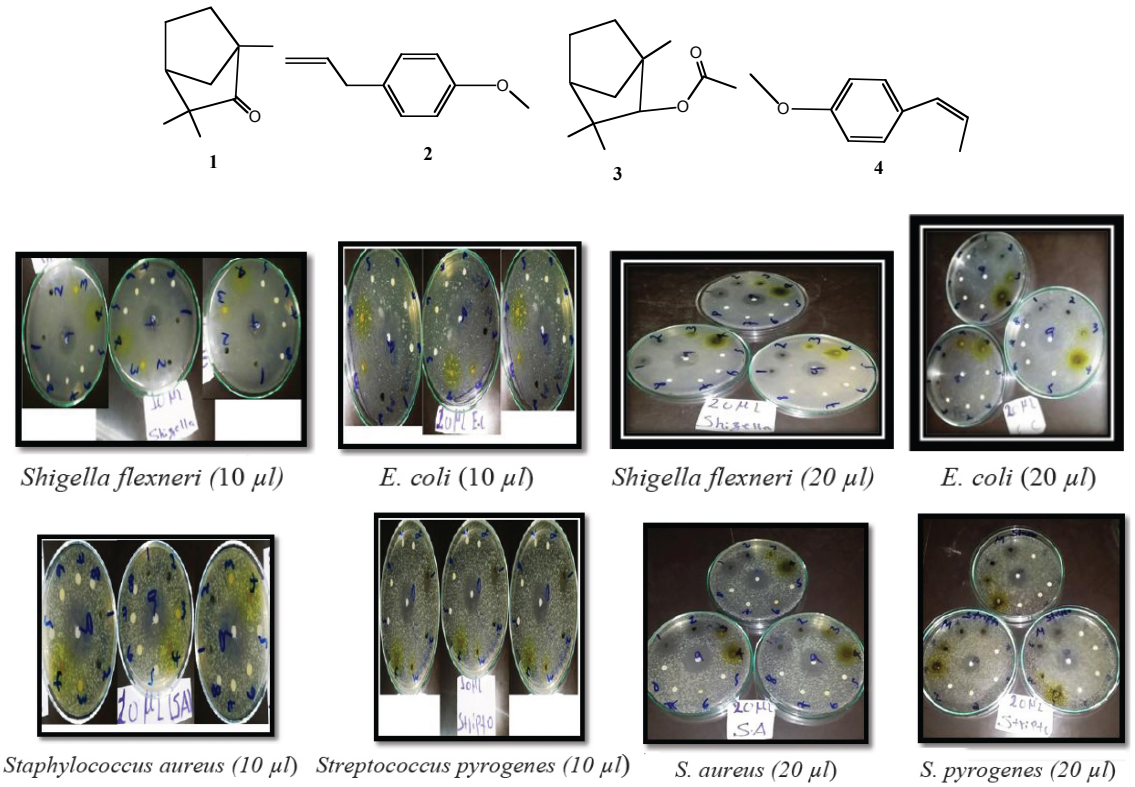

Figure 1: Gram-negative and Gram-positive bacteria test of for volatile oil, crude extracts and isolated pure compound from leaves of *Foeniculum vulgare* (10 µl and 20µl).

measuring 13 times sweeter than sugar. It is perceived as being pleasant to the taste even at higher concentrations. It is used in alcoholic drinks such as ouzo. It is also used in seasoning and confectionery applications, oral hygiene products, and in small quantities in natural berry flavors [13] (https://en.wikipedia.org/wiki/Anethole). Estragole is also used in perfumes and as a food additive for flavor. Upon treatment with potassium hydroxide, estragole converts to anethole [14, 15]. This information supports the use of *Foeniculum vulgare* as an ingredient of local liquor in Ethiopia.

Anethole has potent antimicrobial properties, against bacteria, yeast, and fungi [16]. Anethole is also a promising insecticide. Several essential oils consisting mostly of anethole have an insecticidal action against larvae of the mosquitoes *Ochlerotatus caspius* [17] and *Aedes aegypti* [18, 19]. Thus *Foeniculum vulgare* might be considered as one of a potential candidate for control of mosquitoes and other harmful insects. Fennel volatile oil is a mixture of at least a dozen of different chemicals and the main ingredients are *Z*-anethole (40-70%), fenchone (1-20%) and estragole (20-30% [20, 21, 22]. Therefore this study is consistent with those reported in the literature.

Antimicrobial Effects

Infectious diseases are the leading cause of death worldwide, accounting for nearly one-half of all deaths in tropical countries which are also becoming a

significant problem in all countries. Microbial infections are the great challenge to human health concern and it is even exacerbated by the growing resistance to the conventional drugs [23]. Thus, researchers have resort to find a remedy from plants for infectious diseases.

The antimicrobial activities of *Foeniculum vulgare* leaves petroleum ether, chloroform, methanol/ chloroform (1:1) and, methanol extracts against the microorganisms were examined in the present study, and their potency was assessed by the presence or absence of inhibition zones and zone diameter compared with some antibiotics. The *in vitro* antibacterial activity of the *F. vulgares* leaves extracts against four (two Gram negative and two Gram positive) bacterial strain was assessed by using the disk diffusion method. *In vitro* antimicrobial activity was screened using MHA. The MHA plates were prepared by pouring 41.7 ml of molten media into sterile Petri dish. The plates were allowed to solidify for 5 min and 0.1 % inoculums suspension of tested organisms were swabbed uniformly and the inoculums were allowed to dry for 5 min. 10 µl and 20 µl of the crude extract and isolated compound were used to the study.

The leaves extracts exhibited moderate inhibitory effect against the tested bacterial pathogens at a concentration of 10 µl and 20 µl (Table **3**). Among the tested bacterial strains, *Shigella flexneri*, which is the Gram-negative bacteria, was found to be the most susceptible to the methanol crude extract of *Foeniculum vulgare* leaves, with a zone of inhibition of 24.5 ±0.19 mm. Also, pure compound isolated from the plant leaves showed the least antibacterial activity against the Gram-positive bacteria, *S. aureus*, with a zone of inhibition of 7.2±0.17 mm (Figure **1** and Table **3**).

All crude extracts have higher inhibition effect against the tested bacteria in dose 20 µl compared with another dose 10 µl of the leaves of *Foeniculum vulgare*. Isolated pure compounds have lower inhibition effect against the tested bacteria in dose 20 µl compared with other all crude extract, which might be due to naturally occurring combinations of these components, might have synergistic effects [24]. Inhibition zones of the pure compound were to a little extent higher in *Escherichia coli* in dose 20 µl than in *Staphylococcus aureus* and *Shigella flexneri*, hence, the pure compound has a stronger antibacterial activity towards *Escherichia coli* than *Staphylococcus aureus*. The commercial standard drug (Chloramphenicol) showed the greatest inhibition effect against both tested bacteria in both doses (10 µl and 20 µl)

Table 3: Zone of Bacterial Growth Inhibition (mm) for Volatile Oil, Crude Extracts and Isolated Pure Compound from Leaves of *Foeniculum vulgare*

Sample	Dose (*µl*)	Types of bacteria with mean inhibition diameter (mm)			
		Gram negative bacteria		Gram positive bacteria	
		E. coli	*Sh.flexneri*	*S. pyrogenes*	*S. aureus*
Petroleum ether crude extract	10	-	14±0.05	8.5 ±0.08	-
	20	19±0.14	18.17±1.09	11.8 ±0.09	18.7 ±1.11
Chloroform crude extract	10	-	-	-	-
	20	9 ±0.06	13.8±0.10	11±0.09	14.8±0.08
Chloroform/methanol (1:1) crude extract	10	-	5.0±0.16	-	-
	20	12.3±0.17	13±0.13	8.8±0.08	14 ±0.09
Methanol crude extract	10	10.5±0.11	-	-	5±0.06
	20	23 ±0.33	24.5 ±0.19	17.5±0.10	22±0.18
Essential oil	10	-	-	-	-
	20	-	-	-	-
Pure compound	10	-	-	-	-
	20	19 ±1.07	12.5±0.10	-	7.2±0.17
DMF	10	-	-	-	-
	20	-	-	-	-
Chloramphenicol	10	29.1 ±0.18	-	-	18.4±1.10
	20	34±1.20	25.8±0.12	23.7±0.18	32.5 ±0.19

Value represents mean of three replication, ±SD, - Stands for no inhibition.

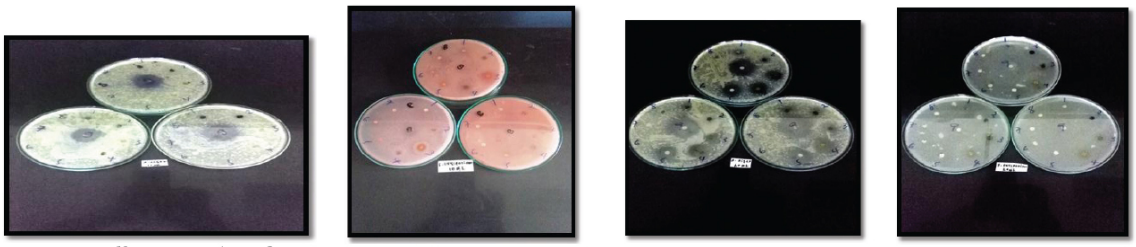

Figure 2: Anti fungi test of for volatile oil, crude extracts and isolated pure compound from leaves of *Foeniculum vulgare* (10 µl and 20µl).

compared with the tested samples and the control (solvent). The crude extract and pure components are known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Therefore, leaves of *Foeniculum vulgare* extracts are valuable and could be a future target for replacing synthetic antibacterial agents. As revealed from the results presented in Table **3**, the antibacterial activities of the tested leave extract of the plant were more pronounced on the Gram-negative bacterium (*E. coli* and *Sh. flexneri*) than the Gram-positive bacterium (*S. aureus* and *S. pyrogenes*).

Gram-positive cell walls consist of many layers of peptidoglycan and do not possess a lipid outer membrane. Gram-negative cell walls on the other hand, have only one or a few layers of peptidoglycan but posses an outer membrane consisting of various lipid complexes. In addition to that Gram-positive spores develop as a new structure inside the protective interior of an existing cell wall, Gram-negative cells morph directly into spores while maintaining their cell wall structural integrity [25]. The differences between the Gram-positive and Gram-negative organisms only establish that the passage of certain small molecules (inorganic phosphate, nucleo-tides, etc) across the wall or wall-membrane of Gram-positive bacteria is impeded when they are suspended in high concentrations of sample, whereas many of the Gram-negative bacteria were affected to a lesser degree [26]. For all samples the inhibition effect against the tested fungi increases with doses (10 µl) to dose (20 µl) (Figure **2** and Table **4**). The commercial standard drug (Tilt) shows higher inhibition zone against antifungal activity compared

Table 4: Zone of Fungal Growth Inhibition (mm) for Volatile Oil, Crude Extracts and Isolated Pure Compound from Leaves of *Foeniculum vulgare*

Sample	Dose (*µl*)	Types of fungi with mea inhibition diameter (mm)	
		Aspergillus niger	***Fusarium oxysporum***
Petroleum ether crude extract	10	2.3±0.01	1.6±0.05
	20	3.6±0.08	1.8±0.16
Chloroform crude extract	10	1.5±0.03	-
	20	1.7±0.15	0.6±0.07
Chloroform/methanol(1:1) crude extract	10	-	-
	20	3.4±0.09	3.0±0.10
Methanol crude extract	10	-	2.1±0.04
	20	4.4±0.12	2.9±0.18
Essential oil	10	-	1.9±0.02
	20	4.4±0.16	2.1±0.13
Pure compound	10	-	-
	20	-	2.3±0.06
DMF	10	-	-
	20	-	-
Tilt	10	6.9±0.05	3.1±0.09
	20	6.9±0.11	6.4±0.20

with all the tested samples. The antifungal activity of these extracts was found to be selective. The largest inhibition zones were recorded with methanol crude extract and essential oil against *A. Niger* respectively (4.4±0.12 mm and 4.4±0.16 mm). The antifungal activities of other crude extracts were followed by petroleum ether, $CHCl_3$/MeOH, and $CHCl_3$ respectively. The lowest activity was determined in a chloroform crude extract. As can be seen from Table **4**, the entire test samples showed considerable antifungal activity against the test organisms. As a whole, methanol crude extract and essential oil showed maximum activity followed by petroleum ether, $CHCl_3$/MeOH, and $CHCl_3$ in respective order.

CONCLUSION

Foeniculum vulgare leaves were extracted by different organic solvents such as petroleum ether, chloroform, methanol/chloroform and methanol. The petroleum ether crude extracted was fractionated and purified using preparative thin layer chromatography (PTLC).

Essential oil was also extracted by the method of hydro-distillation and the components of the essential oil were identified by GC-MS. Four compounds namely, L-fenchone, (*Z*)-anethole, estragole and Fenchyl acetate were identified from the hydro distilled oil. The crude extract, pure fraction and the hydro distilled oil were tested for their antimicrobial activities against two Gram-negative bacteria (*Escherichia coli* and *Shigella flexneri*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyrogenes*) and two fungi (*Fusarium oxysporum* and *Aspergillus niger*). All crude extracts and pure compound were active towards the four bacterial and two fungal strains. Lack of antibacterial activity of the essential oil may be due to the low concentration of the samples used. The study has showed that the observed antimicrobial effect of *Foeniculum vulgare* leaves crude extracts on the bacterial and fungal isolate, though *in vitro* appear interesting and promising. Besides the present antimicrobial studies on the six microbes this investigation would be best if the work is extended on many other microbes. So the same work should be carried out on large variety of microbial strains so as to have a clear picture of the spectrum of antimicrobial activities of the herb.

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