Phytochemical investigation and evaluation of anti-microbial and anti-oxidant activity of *Foeniculum vulgare* (fennel)

Shabana Bano, Nashrah Ahmad and A.K Sharma
Department of Zoology, University of Lucknow, Lucknow- 226007
email – shabana700@gmail.com

Abstract - In this study, methanol extract of fennel seeds was examined for its antioxidant and antimicrobial activities. Phytochemical constituents of the fennel extract were also analysed, that revealed the presence of flavonoids, terpenoids and glycosides. Total phenol content of the extract was determined by using Folin-Ciocalteu reagent and was found to be 3.48 ± 4.2 (mg GAE/g DM). The antimicrobial activity of fennel extract was evaluated against following microorganisms: *Escherichia coli, Bacillus pumilus, Staphylococcus aureus, Listeria monocytogenes and Enteropathogenic E. coli (EPEC).* Maximum antimicrobial activity of the fennel extract was found against *Staphylococcus aureus,* showing an inhibition zone of 20.00 mm. DPPH radical scavenging assay was used to evaluate the antioxidant activity of the fennel extract and its IC_{50} value was also calculated. Thus, the results of the present investigation demonstrate antimicrobial and antioxidant activities of fennel extract and also the presence of various phytochemicals.

Keywords: - Antimicrobial, Antioxidant, DPPH, Fennel, *Foeniculum vulgare,* IC_{50}

Introduction

Since ancient times, plants and herbs have been used for their medicinal properties. Plants contain a large number of phytochemicals which may have different biological activities like antioxidant activity or antimicrobial activity (Lee *et al*, 2004; Kang *et al*, 2011; Ghaima *et al*., 2013). Antioxidants are known to mitigate the harmful effects of free radicals in body (El-Hawary et al., 2012).A number of bioactive compounds like phenols, flavanoids, anthraquinones, quinines, sugars, proteins, saponins and tannins are known to be found in plants (Mandle *et al*., 2012; El-Hawary *et al*., 2012). Those bioactive compounds with antimicrobial activity have the potential to be used in the treatment of gram negative and gram positive infections. Identification of such anti-microbial agents is particularly significant due to the emergence of multi-drug resisitance in comman pathogens (Spellberg *et al*., 2003).

Foeniculum vulgare commonly called fennel, has been used in folk medicine to cure many digestive, endocrine, reproductive and respiratory disorders (Badgujar *et al*., 2014). It belongs to the Umbelliferae (Apiaceae) family, and is used by humans since olden times, due to its flavour. A number of studies showed that *F. vulgare* effectively controls many infectious disorders of bacterial, viral, fungal and protozoal origin [Manomani *et al.,* 2011; Orhan *et al*., 2012; Morales *et al*., 2012;Dua *et al*., 2013]. It has also been demonstrated that fennel possess antioxidant, hepatoprotective, antitumor and hypoglycemic activities (Oktay *et al*., 2003; Pradhan *et al*., 2008; El-Soud *et al*., 2011).

The objectives of the present investigation were to evaluate the presence of various phytochemicals in fennel extract and to elucidate its antimicrobial and antioxidant activities. Total phenol content of the extract was determined using Folin-Ciocalteu reagent. The antioxidant activities of the fennel extract was measured by using the stable radical, 2, 2-diphenyl-1-picrylhydrazylhydrate (DPPH) in comparison with standard antioxidant ascorbic acid. The antimicrobial activity of the fennel extract was determined by using well diffusion method.

Material and methods

Plant material

100 gm of seeds of *Foeniculum vulgare* were purchased from local store and identified by Prof. Y.K.Sharma, Former Head of Botany, University of Lucknow. Seeds were washed under running tap water and then dried. They were grinded with the help of grinder. Powdered form of the seeds was stored in a polythene bag for further use.

Extract preparation

100 gm powdered seeds and 500 ml methanol were subjected for extract preparation. Soxlet extracter was used for extract preparation. Whole setup was set at room temp for 48 hours; extract was filtered through Whatman No-1 filter paper and methanol was removed from the extract with the help of a distillation unit.

Phytochemical screening

Phytochemical analysis of the fennel seeds methanol extract was carried out to test for the presence of various phytochemicals like alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, and glycoside according to Khandelwal *et al*., 2001.

Determination of total phenolic content

Total phenolic content of the fennel seed extract was determined by using Folin–Ciocalteu reagent, according to the method by Wolfe *et al*. (2003), with slight modifications. Gallic acid was used as a standard and total phenolic content of the extract was calculated by interpolation of standard curve of gallic acid. The result of analysis of total phenolic content in the extract was reported as gallic acid equivalents. The experiments were performed in triplicates. C was calculated from the equation: C= C1 x (v/m), where C=total concentration of phenolic component in mg/gm in GAE equivalent, C1=concentration of GAE established from calibration curve (mg/ml), V=volume of extract taken (ml), W=weight of extract (gm). From the gallic acid standard curve of Follin-Ciocalteau assay, y=0.006x-0.067, where y is the absorbance value at 765 nm & C1 is x.

Determination of Antimicrobial activity

Test microorganisms and bacterial culture

The following microorganisms obtained from NCL Pune were used to test the anti-microbial activity of fennel extract: *Escherichia coli* (NCIM 2065*), Bacillus pumilus* (NCIM 9369), *Staphylococcus aureus* (NCIM 5021) and *Listeria monocytogenes* (NCIM 5279). *Enteropathogenic E. coli* E 2347(EPEC) was procured from KGMU, Lucknow. The glycerol stock cultures of these micro-organisms were maintained at -80°C. Working cultures were stored at 4°C and were regularly subcultured. To prepare the inocula of micro-organisms, a loopful of working culture was transferred into 5 ml of sterilized LB media and incubated in incubator shaker at 37°C for overnight. The turbidity of bacterial culture was compared with Mc-Farland turbidity standard and the culture which has attained 0.5 Mc-Farland units was used for the anti-microbial assay.

Well diffusion method

Well diffusion technique was used to test the antibacterial activity of fennel extract (Hood *et al.,*2003).25 ml autoclaved sterile LB Agar was poured in sterile Petri plates (90 mm) to make LB agar plates. Overnight bacterial cultures having .05 OD were swabbed over sterilised LB agar plates. A standard cork borer was used for the creation of uniform 6mm wells on the surface of LB agar plates. 3 wells were made on the LB agar plates, one for positive control, one for negative control and one for sample. 50 mg of concentrated methanol extract of fennel was dissolved in 1 ml of 0.5 % of DMSO and 40 µl of this extract was poured in one of the well. Streptomycin was used as positive control for antibacterial activity assay. 20 mg streptomycin was dissolved in 1 ml DMSO and 40 µl was poured in the other well as positive control. 40.5 % of 40 µl DMSO was poured in the last well as negative control. The experiment was performed in triplicates and the plates were placed at 37°C for overnight. The inhibition zone was calculated as mean (n=3).

Determination of antioxidant activity: DPPH radical-scavenging

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging reagent was used for the determination of antioxidant activity of fennel extract. DPPH free radical scavenging capacity of fennel extract was evaluated according to the protocol of Brand *et al*., (1995) with some minor modifications. 1mM solution of DPPH was prepared in methanol. Ascorbic acid was taken as a positive control. Following concentrations of ascorbic acid was used: 30 µg/ml, 60 µg/ml, 90 µg/ml, 120 µg/ml and 150 µg/ml. 1 ml of each sample was mixed with 1ml DPPH solution by shaking strongly for 1min by vortexing and then incubated in dark for 30 minutes. After an incubation period of 30 minutes at 37°C, absorbance of each sample (A_{sample}) at 517nm was measured using UV spectrophotometer. Corresponding methanol blanks were taken. Likewise, following concentrations of fennel extract was used: 30 µg/ml, 60 µg/ml, 90 µg/ml, 120 µg/ml and 150 µg/ml. Likewise, 1 ml of each test sample was mixed with 1ml DPPH solution by shaking strongly for 1min by vortexing and then incubated in dark for 30 minutes. After an incubation period of 30 minutes at 37°C, absorbance of each test sample (A_{sample}) at 517nm was measured using UV spectrophotometer. Corresponding methanol blanks were taken and the whole set of experiment was performed in triplicate. The negative control ($A_{control}$) was taken after adding DPPH solution to 1 ml of methanol. A low absorbance of the reaction mixture shows higher radical scavenging activity.

The free radical scavenging effect (%) was calculated by using the following formula:

$$\text{Scavenging effect (\%)} = [1 - (A_{sample}/A_{control})] \times 100$$

The percentage of scavenging effect (%) was plotted against the respective concentrations of ascorbic acid and fennel extract to calculate the IC_{50} values. IC_{50} value is the concentration that is required for scavenging 50% of the DPPH. The concentration of fennel extract providing 50% inhibition (IC_{50}%) was calculated from the graph plotting inhibition percentage against the concentration of extract (Chatterjee *et al*., 2012).

Results

Extraction Yield

The percentage of extraction yield was calculated by the following formula:-

$$\text{Percentage of Yield (\%)} = \frac{\text{Amount of extract (g)}}{\text{Amount of dried part used (g)}} \times 100$$

Table-1 Extraction Yield of fennel methanol extract

Sample	Amount of dried part used(g)	Amount of extract yield	Percentage of yield (%)
Fennel	100	27.47	27.47

Phytochemical analysis

Phytochemical analysis of the fennel methanol extract demonstrated the presence of flavonoids, glycosides, phenol and terpenoids (Table- 2).

Table-2 Phytochemical analysis of fennel methanol extract

Sample	Alkaloids	Flavonoids	Phenol	Glycosides	Saponins	Tannins	Terpenoids
Fennel	-	+	+	+	-	-	+

Antibacterial activity

A preliminary antibacterial study of the fennel extract is summarized in Table-3. The fennel extract showed various degree of sensitivity against test microorganisms. The study demonstrated *Staphylococcus aureus* (20.00 mm) as the most sensitive organism for the fennel extract followed by *Enteropathogenic E. Coli (EPEC)* (19.33 mm) and *Listeria monocytogenes* (17.66 mm). *Escherichia coli* and *Bacillus pumilus* were not sensitive to the fennel extract (12 mm and 11.33 mm inhibition zone respectively).

Table-3. Antibacterial activity of fennel extract as measured by Well Diffusion Assay

Bacteria strain name	Diameter of zone of inhibition for fennel methanol extract (mm)	Diameter of zone of inhibition for positive control i.e. Streptomycin (mm)	Diameter of zone of inhibition for negative control i.e. DMSO (mm)
Listeria monocytogenes	17.66	26.3	No zone of inhibition
Enteropathogenic E. Coli (EPEC)	19.33	22.33	No zone of inhibition
Staphylococcus aureus	20.00	24.33	No zone of inhibition
Escherichia coli	12.00	19.66	No zone of inhibition
Bacillus pumilus	11.33	24.33	No zone of inhibition

Antioxidant activity

The results of the DPPH radical scavenging activity of the fennel extract along with the reference standard ascorbic acid are shown in Table- 4. Figure 1 illustrates the dose dependent curve of DPPH radical scavenging activity of fennel extract. Fennel extract concentration necessary to decrease initial concentration of DPPH free radicals by 50% (IC50 value) was calculated from the graph in figure 2 and was found to be 31 µg/ml. The lower the IC50 value, the greater is the antioxidant power (Kandhasamy *et al*., 2010).

Table-4 % of radical scavenging activity of fennel extract as measured by DPPH assay

	Concentration (µg/ml)				
Sample	30 µg/ml	60 µg/ml	90 µg/ml	120 µg/ml	150 µg/ml
% of inhibition by Ascorbic Acid (Positive control)	88.7	90.1	92.3	98.6	99.03
% of inhibition by Fennel extract	50.2	65.4	69.0	85 .1	88.9

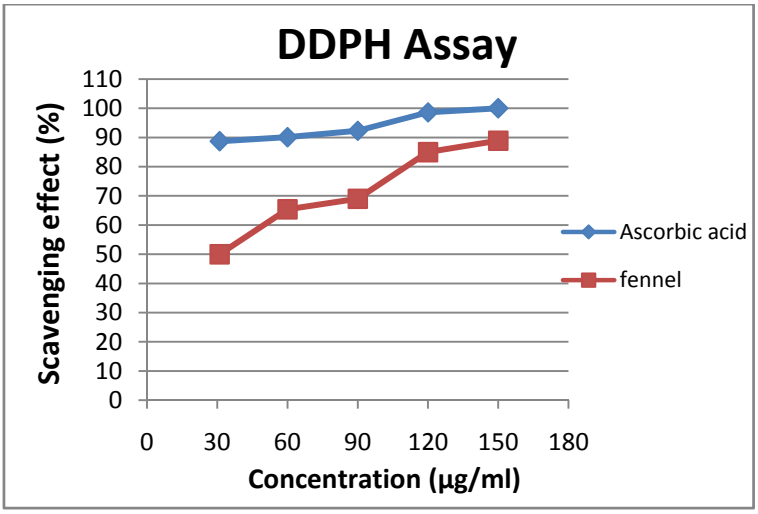

Figure-1. % Inhibition of ascorbic acid and fennel extract by DPPH assay

Total phenolic content

The concentration of total phenolic component in the fennel seed extract was determined spectrometrically and calculated as gallic acid equivalents; mgs of gallic acid per grams of dried extract (mg GAE/g DM). The total phenolic contents of the methanol extract of fennel was 3.48 ± 4.2 (mg GAE/g DM). These results are almost similar to those reported by Hernandez-Hernandez et al., (2009), Roby *et al*., 2012.

Discussion

In this study, various concentrations of methanol extract of fennel were tested for their antioxidant activity using the DPPH radical scavenging assay. Free radicals formed due to oxidation are known to cause a number of harmful effects on our body. Antioxidants are the molecules that fight with the free radicals and thus provide protection from the harmful effects of free radicals (Umamaheswari *et al*,. 2008). DPPH assay is a commonly used method to determine the free radical scavenging capability of plant extracts. It is based on the reduction of DPPH solution in the presence of antioxidant that results in the synthesis of non radical DPPH-H. In this study, the fennel seed extract demonstrated different values of antioxidant activities depending on the concentration tested. Phytochemical screening and evaluation of anti microbial activity of fennel extract were also carried out in our study. Among all the micro-organisms tested, the most significant anti-microbial activity of fennel extract was observed against *Staphylococcus aureus*. Roby *et al.*, 2012 and Bagdassarian *et al.*, 2013 have also demonstrated the anti-microbial activity of fennel extract but against different microbes. Here, we have evaluated the anti-microbial activity of fennel methanolic extract against *Enteropathogenic E. coli (EPEC), Staphylococcus aureus*, *Listeria monocytogenes, Bacillus pumilus and Escherichia coli.* Very little information is available about the polyphenolic compounds associated with fennel. In this study we have determined the total phenolic content in fennel which was found to be 3.48 ± 4.2 (mg GAE/g DM. Hence, the current study is focused towards phytochemical screening and study of anti-microbial and anti-antioxidant activity of fennel extract which can be used for the development of relevant drugs in future. Compounds isolated from fennel having anti-microbial and anti-oxidant activity will possess minimal toxicity and will be cost effective as compared to compounds that are chemically synthesized.

Acknowledgement

The authors are grateful to Prof. Madhu Tripathi, Head, Department of Zoology,University of Lucknow for providing the necessary facilities for research. We are also thankful to Prof. A.M Saxena and Prof. Mohammad Serajuddin from Department of Zoology, University of Lucknow for their guidance and help.

References

[1] Badgujar SB, Patel V and Bandivdekar AH: A Review of Foeniculum vulgare and Its Botany, Phytochemistry, Pharmacology, Contemporary Application, and Toxicology. BioMed Research International 2014; 842674

[2] Bagdassarian Christova VL , Bagdassarian KS:Phenolic profile, antioxidant and antimicrobial activities from the Apiaceae family (dry seeds) Mintag. Journal of Pharmaceutical&Medical Sciences2013.

[3] Brand WW, Cuvelier ME, Berset C: Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie1995; 28: 25-30.

[4] Chatterjee S, Goswami N and Bhatnagar P:Estimation of Phenolic Components and in vitro Antioxidant Activity of Fennel (Foeniculum vulgare) and Ajwain (Trachyspermum ammi) seeds.Advances in Bioresearch2012; 3 [2]: 109 – 118.

[5] Dua AG. Garg P, and Mahajan R:Polyphenols, flavonoids and antimicrobial properties of methanol extract of fennel (Foeniculum vulgare Miller). European Journal of Experimental Biology2013; vol. 3, no. 4, pp. 203–208.

[6] El-Hawary S, Sokkar NM, Ali ZY, Yehia MM :A profile of bioactive compounds of Rumex vesicarius L. J. Food Sci2012;76: 1195-1202.

[7] El-Soud NA, El-Laithy N, El-Saeed G:Antidiabetic activities of Foeniculum vulgare mill. Essential oil in streptozotocin-induced diabetic rats, Macedonian Journal of Medical Sciences2011; vol. 4, no. 2, pp. 139–146.
[8] Ghaima KK, Hashim NM, Ali SA:Antibacterial and antioxidant activities of ethyl acetate extract of nettle (Urtica dioica) and dandelion (Taraxacum officinale). J App Pharm Sci2013; 3 (05): 096-099.
[9] Hood JR., Wilkinson JM and Cavanagh HMA:Evaluation of common antibacterial screening methods utilized in essential oil research. J Essential oil Res2003 ; 15:6.
[10] Kandhasamy S, Jince MJ, Dharmar RK, Sellamuthu M:In vitro antioxidant characteristics of different parts of Melothria maderaspatana (l.) Cogn. Int J Pharmacy and Pharm Sci2010Vol 2, Issue 3, 117-123.
[11] Kang NS, Lee JH:Characterisation of phenolic phytochemicals and quality changes related to the harvest times from the leaves of Korean purple perilla (Perilla frutescens). Food Chem2011;124:556-562.
[12] Khandelwal KR:Preliminary phytochemicals screening,Practical Pharmacognosy Techniques and Experiments2001;8th edn. pp-149-156.
[13] Lee S, San D, Ryu J, Lee YS, Jung SH, Kang J:Anti-oxidant activities of Acanthopanax senticosus stems and their lignin components., Arch Pharm Res 2004; 27:106.
[14] Mandle VS, Salunke SD, Gaikwad SM, Dande KG, Patil MM :Study of nutritional value of some unique leafy vegetable grown in Latur district. J.Animal Sci. Adv2012; 2 (3.1): 296-298.
[15] Manonmani R and, Abdul Khadir VM:Antibacterial screening on Foeniculum vulgare Mill, International Journal of Pharma and Bio Sciences2011; vol. 2, no. 4, pp. 390–394.
[16] Morales P, Carvalho AM, Sanchez-Mata MC:Tocopherol composition and antioxidant activity of Spanish wild vegetables, Genetic Resources and Crop Evolution2012; vol. 59, no. 5, pp. 851–863.
[17] Oktay M, Gulc I. and Ufrevioglu OIK :Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts, LWT-Food Science and Technology2003; vol. 36, no. 2, pp. 263–271.
[18] Pradhan M, Sribhuwaneswari S, Karthikeyan D:In-vitro cytoprotection activity of Foeniculum vulgare and Helicteresisora in cultured human blood lymphocytes and antitumour activity against B16F10 melanoma cell line, Research, Journal of Pharmacy and Technology2008;vol. 1, no. 4, pp. 450–452.
[19] Orhan IE., B. Ozlik B, Kartal M, and Kan Y:Antimicrobial and antiviral effects of essential oils from selected Umbelliferae and Labiatae plants and individual essential oil components, Turkish Journal of Biology2012; vol. 36, no. 3, pp. 239–246.
[20] Roby, MHH:Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare L.) and chamomile (Matricaria chamomilla L.). Ind. Crops Prod2012; 44:437-445.
[21] Spellberg B, Powers JH, Brass EP and Edwards JET:The trends in antimicrobial drug development: implications for the future. Clin Infect Dis2003; 38:1279-1286.
[22] Umamaheswari M, Chatterjee TK:In vitro antioxidant activities of the fractions of Coccinia Grandis L. leaf extract. Afr J Trad Comp Alter Med2008; 5:61–73.
[23] Wolfe KX, Liu Rh: Antioxidant activity of apple peels. J. Agric. Food Chem2003; 51, 609–614.