Pharmacological Study on a Potential Medicinal Herb- *Foeniculum vulgare*

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Abstract: This study was conducted to investigate the antiemetic, antimicrobial and anti-radical activity of methanolic extracts of *Foeniculum vulgare*. The antiemetic assay was carried out by using chick emetic model with minor modifications by calculating the mean decrease in the number of retching. The antimicrobial activity of the crude extract was performed by Disc Diffusion method. The anti-radical activity was determined by the 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) method. The anti-emetic activity of *Foeniculum vulgare* leaves on young chicks revealed that these extracts have a less anti-emetic effect. The group of chicks treated with Chlorpromazine was found to have 60.6 retches as compared to the 66 retches of the control group, thus Chlorpromazine reduced the retches by 7.93%. The chickens treated with leaves extracts inhibited the retches up to 2.03%. The minimum antimicrobial effect was found in this methanol crude extract. The extract did not appear potent in terms of both zones of inhibition and spectrum of activity. In anti-radical activity test, the extract showed moderate free radical scavenging activity with IC₅₀ value 240.39µg/ml while compared to that of the reference standard ascorbic acid. Methanolic extract of *Foeniculum vulgare* leaves have minimum anti-emetic and anti-microbial activities and moderate anti-radical properties.

Keywords: Antiemetic, Antimicrobial, Antioxidant, *Foeniculum vulgare*

1. Introduction

For thousands of years, nature has accomplished as a rich treasure trove of medicinal plants and a magnificent number of modern drugs have been separated from natural resources, in particular of plant origin [1]. Non-industrialized societies widely use Medicinal plants mostly because of their simple availability and relatively low in price than modern medicines. Various herbs are used as both flavoring agents and preservatives and added to food since ancient time [2]. In the last 20–25 years, the use of herbs has expanded dramatically as harmonious and substitute medicine [3]. Using herbal and medicinal plants to treat diseases is certainly the oldest way. The research strategy is progressively advanced using natural supplies with keeping in mind the drug resistance and the adverse effects of various chemical antibacterial drugs. Hundreds of chemical compounds are synthesized from plants for functions including defense against insects, fungi, and diseases. Numerous natural antimicrobials have already been identified and separated by diverse phytochemicals and pharmacological investigations on plants [4]. According to
World Health Organization (WHO), traditional medicines are trusted by about 80% of the World’s inhabitants for their key health care requirement [5]. Moreover, exposure to multiple drug-resistant strains of microorganisms has initiated a regenerating interest in herbal medicine due to miscellaneous use of antibiotics to handle infectious diseases [6].

Plants and their derivatives have been identified with a great number of Phytochemicals having potential medicinal properties or established biological activity and used as medicine for human health since ancient times. A large number of bioactive Phytochemicals with antioxidant or antimicrobial activity are found in plants such as phenols, flavonoids, anthraquinones, quinines, sugars, proteins, saponins and tannins [7].

The valuable health effects of many plants have been declared for inhibiting food decomposition and as antimicrobials against pathogenic microorganisms, which is used for centuries as seasoning agents in food and beverages. Antimicrobial prospective of distinct medicinal plants are widely studied all over the world [8]. The prime targets are Herbs and spices to hunt for safe antioxidants and antimicrobials [9]. They can supply a replacement to common pest control agent [10]. A worldwide crucial problem is to Control of the spread of antibiotic-resistant bacteria and treating the infections caused by them [11]. Epidemiological evidence suggests that at least one of the most commonly used drugs for treatment are resisted by approximately 70 percent of the bacteria causing infections in hospitals [12]. To inhibit the growth of pathogenic microorganisms those have built resistance against antibiotics, a number of bioactive ingredients were isolated from herbs and spices [13]. Gram-negative and gram-positive infections can be treated successfully using biologically active components of medicinal plants with the potential antimicrobial activity. Due to the exposure of multi-drug resistance in common pathogens, separation of such anti-microbial agents is potentially significant [14]. Due to the presence of various active components, Essential oils proceed through several modes with a broad spectrum of bioactivity [15].

A paradox in the metabolic process is that, though the immense majority of puzzling life demand oxygen for its existence on Earth, oxygen is an extremely reactive molecule that destroys living organisms by initiating reactive oxygen species (ROS) [16]. A chemical reaction producing free radicals guides to chain reactions and cell damage is known as Oxidation. An antioxidant or anti-radical agent is a substance which inhibits oxidation or potentially damaging oxidizing agents in living beings mostly used to resist the decomposition of stored food products. Antioxidants diminish the damaging effects of free radicals in the body [17]. They are extensively used as food additives to protect against free radicals caused by oxidative degradation [18]. Consequently, plant antioxidants are recommended as a fascinating alternative. A number of substances isolated from medicinal plants contain antioxidants like flavonoids compounds which can donate hydrogen to free radicals and therefore, destroy the chain oxidative reaction at the first initiation stage [19]. Plant-based natural antioxidants, for instance, phenolic substances like flavonoids, phenolic acids, and tocopherols, are achieving much acknowledgment as preventive and therapeutic medicines. They are supposed to show anticarcinogenic and health-promoting effects with their antioxidant attributes [20]. In spite of the demand for certain levels of antioxidants in the diet for good health, a remarkable debate is prevailing on whether foods well provided with antioxidant have anti-disease activity. Moreover, if they are indeed advantageous, it is still uncertain that which antioxidant(s) and in what amounts are needed from the diet [21].

Foeniculum vulgare Mill (family- Apiaceae), generally known as fennel, is a humble genus of annual, biennial or perennial medicinal herbs cultivated for its aromatic fruits that are used as culinary spices (based on the variety), and is native to the Mediterranean area [22]. The plant is generally supposed to be indigenous to the shores of Mediterranean Sea but has become extensively habituated in numerous parts of the world especially on dry soils close by the sea coast and on the river banks [23]. It has been domesticated and initiated into various regions outside that zone; for example, Russia, India, China and Japan cultivate this herb commercially [24]. But now, it has been cultivated in almost every country [25]. The plant is usually 2.5 m high with hollow stems having up to 40 cm long leaves and the terminal compound umbels produce the flowers. The fruit is up to 10 mm long dry seed [26]. Many phytochemical studies of Foeniculum vulgare’s stem, roots, and seeds disclosed the presence of flavonoids, tannins, coumarins, saponins, sterols, essential oil and absence of anthocyanes and alkaloids [27]. The principal constituents of fennel are found to be trans-anethol and fenchone [28]. Foeniculum vulgare is an extremely aromatic and flavorful herb having medicinal and culinary uses. Mature fruit or seeds of the plant and its essential oil are anise-like in the aroma, used as a flavoring agent in food products such as baked goods, liqueurs, bread, pickles, pastries, cheese, meat and fish dishes, ice cream, alcoholic beverages and herb mixtures. Furthermore, they are applied as an ingredient in cosmetic and pharmaceutical products [29]. Fennel and its preparations are known to cure various disorders acting as carminative, digestive and diuretic agent [30]. Moreover, it has not only anti-inflammatory, antispasmodic, antiseptic, analgesic effect but also antioxidant, anti-ulcer, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic activities [31]. Numerous studies revealed its potential to control a great number of infectious disorders of bacterial, fungal, viral, mycobacterium, and protozoal origin [32]. Traditionally, the plant is employed for curing female infertility and increasing breast milk production [33]. Studies suggested that it exceptionally enhance memory and reduce stress [34].

This present study was aimed to investigate the antiemetic, antimicrobial and anti-radical activity of methanolic extracts of Foeniculum vulgare.
2. Materials and Methods

2.1. Chemicals

All of the chemicals used in this study were of analytical grade. Copper sulfate was purchased from ScharlauChem-ie S.A. Barcelona, Spain. Metoclopramide hydrochloride was purchased from Square Pharmaceuticals Ltd. Dimethyl sulfoxide (DMSO), Polyoxy-ethylene sorbitan monooleate (Tween 80) and methanol were purchased from Merck, Darmstadt, Germany.

2.2. Collection and Proper Identification of Plants Sample

For this present investigation Foeniculum vulgare was collected from Dhaka District, Bangladesh and was identified by Bangladesh National Herbarium, Mirpur (Accession no: 37667).

2.3. Drying and Grinding of Plant Materials

The collected plant parts (seeds) were sun-dried for one week. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

2.4. Extraction of Plant Materials

About 400 gm of powered material was taken in a clean, flat-bottomed glass container and submerged in 2100 ml of 100% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through cotton plug. The filtrate (methanol extract) obtained was evaporated under ceiling fan and in a water- bath until dried. It rendered a gummy concentrate of reddish black color. The gummy concentrate was nominated as crude extract of methanol.

2.5. In Vivo Antiemetic Activity

Chick emetic model [35] was used to assay the antiemetic potency of Foeniculum vulgare with minor modifications by calculating the mean decrease in the number of retching. 2-4 days young male chicks weighing from 30-32 gm were collected from a local poultry store. After 24 hrs fasting, the antiemetic activity was evaluated. All chicks were kept under laboratory conditions at room temperature with 12 h light and dark cycles. All animal experiments were carried out in accordance with the acts of the Ethical Committee of Manarat International University. After 24 hours fasting, the antiemetic activity was evaluated. The chicks were divided into three groups of five chicks each and each chick was kept in a large beaker at 25°C for 10 minutes. The extracts of Foeniculum vulgare seeds dissolved in 0.9% saline containing 5% DMSO and 1% Tween 80 and administered at a dose of 150 mg/kg orally and a volume of 10 ml/kg to the test animal on the basis of their body weights. Control group received only saline (0.9% NaCl). After 10 minutes copper sulphate was administered orally at 50 mg/kg body weight, then the number of retching was observed during next ten minutes. Chlorpromazine was used as a standard drug (150mg/kg. b. w).

The antiemetic effect was assessed as the decrease in the number of retches in the treated group in contrast to the control. The inhibition (%) was calculated as follows:

\[ \text{Inhibition (\%) = \left[ \frac{(A-B)}{A} \right] \times 100} \]

Where A is the frequency of retching of control group and B is the frequency of retching of the treated group.

2.6. Antimicrobial Screening

The crude extracts of Foeniculum vulgare were tested for antimicrobial activity by disc diffusion method [6]. The bacterial and fungal strains used for the experiment were collected as pure cultures from the Microbiology research laboratory, University of Dhaka, Bangladesh. Both gram positive and gram-negative organisms were taken for the test. Staphylococcus aureus was taken as a gram-positive organism and Escherichia coli, Pseudomonas aeruginosa, Enterobacter and Vibrio cholera were taken as gram-negative organisms. Nutrient agar medium (DIFCO) was used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

2.7. Preparation of the Medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it. To make a clear solution the contents were heated in a water bath and the pH was adjusted at 7.2-7.6 using NaOH or HCl at room temperature. To prepare plates and slants 10 ml and 5 ml of the medium was then transferred in screw cap test tubes respectively. Capped test tubes were sterilized by autoclaving at 15-lbs pressure at 121°C for 20 minutes. The slants were used for making fresh culture of bacteria and fungi that were in turn used for sensitivity study.

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop. The inoculated strains were then incubated for 24 hours at 37°C for their optimum growth. The test organisms were then transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms and the bacterial and fungal suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media. Three types of discs were used for antimicrobial screening. Standard Discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the
test sample. In this investigation, Ciprofloxacin 5mg standard was used as the reference. Blank Discs were used as negative controls which ensure that the residual solvents and the filter paper were not active themselves.

6mg methanolic extract of the seeds of Foeniculum vulgare was dissolved in methanol to obtain the desired concentrations (400 (g/disc) in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petridish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. After keeping in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium, the plates were then inverted and kept in an incubator at 37°C for 24 hours.

The antimicrobial potency of the test agents was determined by their activity to prevent the growth of the microorganisms surrounding the discs by measuring the diameter of the zones of inhibition in millimeter with a transparent scale. In order to avoid any type of cross-contamination by the test organisms, the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. Petri dishes and other glassware, Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

2.8. Anti-Radical Activity

The free radical scavenging activities (antioxidant aptitude) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand- Williams [36]. Being a stable free radical, DPPH has delocalized spare electron over the molecule offering a deep violet color. If a substance with the potential to donate a proton is mixed with DPPH solution, the violet color vanishes from sight representing a reduced form of DPPH [37]. At first 0.3mg DPPH (0.004%w/v) is weighted accurately and dissolved in 15ml methanol to make the concentration 20µg/ml.100 mg of dried sample extract was dissolved in 10 ml of methanol for the concentration of sample solution 10µg/ml.21 test tubes were taken and each of this labeled for 5ml, in which 10 test tubes for different conc. of sample solution, 10 test tubes for each conc. of standard solution and 1 test tube for blank which was filled with 3ml DPPH solution and 2ml of methanol. In each test tube (Except blank test tube) 3ml of DPPH solution (20µg/ml) was taken and then mixed at different concentration (500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml, 16.625µg/ml, 7.813µg/ml, 3.906µg/ml, 1.953µg/ml and 0.997µg/ml) of 2ml sample solution (10µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was taken at 517 nm against methanol as blank by UV spectrophotometer. Control sample was prepared by using same conc. of ascorbic acid instead of sample solution (plant extract). The absorbance was recorded and percentage scavenging (IC$_{50%}$) was determined using the following equation and was compared with ascorbic acid which was used as a standard.

\[
\text{IC}_{50\%} = \frac{1 - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100
\]

Where, absorbance of the control means blank absorbance (containing all reagents except the test material).

Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration.

2.9. Statistical Analysis

All numerical data are expressed as the mean ± SEM (standard error of the mean) and Statistical analysis was carried out using t-test and differences between means were considered to be significant when p < 0.05.

3. Result

3.1. In Vivo Antiemetic Activity

Number of retches recorded for crude methanolic extracts of Foeniculum vulgare and standard drug are given in table below.

<table>
<thead>
<tr>
<th>Drug/dose</th>
<th>No. of retches</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10 ml/kg)</td>
<td>66±3.67</td>
<td>---</td>
</tr>
<tr>
<td>Chlorpromazine (150 mg/kg)</td>
<td>60.6±0.4</td>
<td>7.93%</td>
</tr>
<tr>
<td>Foeniculum vulgar Seeds (150 mg/kg)</td>
<td>64.8±6.16</td>
<td>2.03%</td>
</tr>
</tbody>
</table>

3.2. Antimicrobial Screening

The methanolic crude extract didn’t inhibit the growth of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera, Enterobacter having zone size 3.33mm, 2.15mm, 1.33mm, 4.33mm and 5.00mm respectively. Out of all the samples, methanolic crude extract was not appeared potent in terms of both zone of inhibition and spectrum of activity.

<table>
<thead>
<tr>
<th>Test microorganisms</th>
<th>Diameter of zone of inhibition (mm)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCE</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>Gram positive Bacteria</td>
<td>3.33±0.33</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2.15±0.57</td>
</tr>
<tr>
<td>Gram negative Bacteria</td>
<td>1.33±0.33</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4.33±0.33</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5.00±0.57</td>
</tr>
<tr>
<td>Vibrio</td>
<td>*MCE: Methanolic crude extract of the seeds (400g/disc)</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>*MCE: Methanolic crude extract of the seeds (400g/disc)</td>
</tr>
</tbody>
</table>

3.3. Anti-Radical Screening

The methanol extract of plant of Foeniculum vulgare were tested for Free radical scavenging activity. Absorbance of different concentrated of methanol extract of plant seeds of Foeniculum vulgare is given below.
Table 3. IC$_{50}$ value of methanol soluble fraction of Foeniculum vulgare and ascorbic acid.

<table>
<thead>
<tr>
<th>Serial</th>
<th>Sample</th>
<th>IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol soluble fraction</td>
<td>240.39</td>
</tr>
<tr>
<td>2.</td>
<td>Ascorbic acid</td>
<td>27.23</td>
</tr>
</tbody>
</table>

4. Discussion

The extracts of Foeniculum vulgare (seeds) were tested for determination of secondary metabolites and various phytochemicals which revealed the presence of alkaloids, carbohydrates, steroids, tannins and the chemical composition of essential oils of F. vulgare and identified a total of 76 volatile components [38]. Another study revealed the presence of 28 components of essential oil that accounted for 98% of the total oil [39].

The extracts of Foeniculum vulgare seeds revealed less anti-emetic effect on young chicks after administration of a dose of 150mg/kg Chlorpromazine and the extracts of seeds, reducing the number of retches. The group of chicks treated with Chlorpromazine was found to have 60.6 retches as compared to the 66 retches of control group, accounting for 7.93% reduction of the retches while Foeniculum vulgare seeds extracts inhibited the retches up to 2.03% signifying less anti-emetic potential.

A study revealed significant antimicrobial activity of the fennel extract against staphylococcus aureus (with inhibition zone of 20.00mm), Enteropathogenic E. Coli (EPEC) (19.33mm) and Listeria monocytogenes (17.66mm) [40]. Another study indicated potential inhibitory effects of essential oil of F. vulgare on E. coli (10mm) and S. aureus [41]. Earlier studies on aqueous and organic extracts of Foeniculum vulgare disclosed its antibacterial activity against some bacterial strains. However, antimicrobial effect of Chloroform, Water or Methanol extracts of fennel was not observed remarkable in the investigation [42] which was also similar in the present study. The methanolic crude extract didn't inhibit the growth of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera, Enterobacter having zone size 3 mm, 2 mm, 1 mm, 4 mm and 4 mm respectively.

Natural antioxidants are generally used against oxidative stress damage in a view to protect human being. The IC50 value is used as an indicator to measure the toxicity exhibited by the plant crude extracts. It is the concentration of drug required to restrain the growth and proliferation of the cells by 50% [41]. Study revealed potent antioxidant activity of the fennel extract having IC50 value of 31µg/ml [42]. Another investigation revealed the strongest scavenging activity (lower IC50 value) for aqueous: methanol (20:80, v/v) extract (IC50 = 69.68 ± 2.28µg/ml) which was eight times stronger than that of water extract (IC50=576.45 ± 76.77) and three times stronger than that of the methanol extract (IC50=228.94 ± 37.24). However, in the present investigation the methanol extract of plant of Foeniculum vulgare were tested for Free radical scavenging activity. The IC50 value was observed 240.39 µg/ml which was not appeared as potent antioxidant to inhibit cell proliferation.

5. Conclusion

In the context of the above discussion, it can be revealed that the crude extract of Foeniculum vulgare possess minimum anti-emetic and anti-microbial activities and moderate anti-radical properties. However, further studies on this plant extract require to find out the bioactive compounds which are mainly responsible for these pharmacological activities.

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Conflict of Interest

Authors have no conflict of interest.

References


