Chemical Composition and Biological Potentials of Aqueous Extracts of Fennel (Foeniculum vulgare L)

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ABSTRACT

Foeniculum vulgare (fennel) has been widely used in Egyptian traditional medicine for treatment of various diseases. This study was designed to evaluate the potential of three aqueous extracts of fennel seeds with different extraction conditions (pH 3, 7 and 12) as natural antimicrobial, antiviral, antioxidant and antitumor agents from the safety point of view. The three aqueous extracts were then used for evaluation of yield, total carbohydrates, protein, flavonoid and total phenolic contents. Antioxidant activity was evaluated using the DPPH• radical scavenging. The results showed that the high anti-scavenging activity was noticed for three different aqueous extracts of fennel at 2.0 mg dose comparable to other doses. In addition, the antimicrobial activity of the aqueous extracts against tested Gram positive and negative bacteria, yeast and fungi strains was determined. The extract (concentration 1.6 mg/ ml) was effective against most of the strains tested, yet not against Candida albicans. These results show that water fennel extracts could be considered as a natural alternative to traditional food preservatives and be used to enhance food safety and shelf life. Plaque reduction infectivity assay was used to determine antiviral activity against herpes simplex virus type-1 (HSV-1) cells count reduction as a result of treatment with tested extracts. Acidic fennel and alkaline fennel extracts showed high activity with virucidal effect (83.4 and 58.2%, respectively). To evaluate in vitro the antitumor activity of fennel extracts, the cells were treated by fennel extracts at concentration 300, 600 and 900 ug/ml and the number of viable cells was determined by trypan blue test. In general, all three plant extracts showed moderate antitumor activity in the high concentration of extracts against the Ehrlich ascites carcinoma cells.

Key words: Antiviral, Antimicrobial, Antitumor, Antifungal, Antioxidant, Fennel, Aqueous extract.

Introduction

It is well known that the medicinal plants are the resources of promising drugs for many diseases. However, the biological and pharmacological properties of many plants are still unknown. Since ancient times, plants have been an exemplary source of medicine (Aboelsoud, 2010). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs (Schmincke, 2003). Through scientific research it is indicated that the bioactive components present in herbs and spices can reduce the risk of cancer through their antimicrobial, antioxidant, and antitumorogenic activities and their ability to directly suppress carcinogen bioactivation (Kaefer and Milner, 2008). Herbs and spices are amongst the important targets to be used for natural antimicrobial, antifungal and antioxidants from the safety point of view (Ito et al., 1985). Aqueous extracts of many allelopathic plants are known to exhibit antifungal properties. Allelo-chemicals reduce the germination of spores and mycellial growth of pathogenic fungi. Seed powder, oil and leaf extract of fennel are known for antifungal and anti bacterial properties (Singh et al., 2006).

Foeniculum vulgare Mill (Apiaceae family), commonly known as fennel, is biennial medicinal plant belonging to the family Apiaceae (Umbelliferae) with a characteristic aromatic odor. Fennel is one of the most important medicinal plants grown within the Mediterranean region, in Europe and in Egypt. It was known to the ancient Chinese, Indian, Egyptian and Greek civilizations and used as medicinal purposes and human consumption (Aboelsoud, 2010). The ripe fruit of the plant is still widely utilized in Arabian folk medicine systems as a stimulant, diuretic, carminative and sedative (Charles et al., 1993) and galactagogic, emmenagogic, expectorant and antispasmodic (Chiej, 1984). Fennel fruits are used to treat diseases like cholera, bile
disturbances, nervous disorder, constipation, dysentery and diarrhea (Leung and Foster, 1996). It is also used for control of diseases affecting chest, lungs, spleen, kidneys and in colic pains (Bown, 1995).

Recently, essential oils of the fruits of three organically grown cultivars of Egyptian fennel (Foeniculum vulgare var. azoricum, Foeniculum vulgare var. dulce and Foeniculum vulgare var. vulgare) showed dramatically high antimicrobial and antioxidant activities (Shahat et al, 2011). In addition, the vascular effect of aqueous extract of fennel leaves was tested using pentobarbital-anaesthetised rats. The aqueous extract of fennel inhibited the hypotensive effect in a dose-related manner (Abdulghani and Amin, 1988) but there is no information about in vitro antioxidant activity of water extract of fennel seeds. Anand et al (2008) reported that Anethole, the principal active component of the spice fennel, has shown anticancer activity. In 1995, Al-Harbi et al. studied the antitumor activity of anethole against Ehrlich ascites tumor (EAT) induced in a tumor model in mice. The study revealed that anethole increased survival time, reduced tumor weight, and reduced the volume and body weight of the EAT-bearing mice.

To our knowledge, little information is available on antitumor, antiviral, antioxidant and antimicrobial activities of the aqueous extracts of fennel seeds. Therefore, the purpose of current study was designed to evaluate the total phenolic and total flavonoid contents and to assess and compare between antimicrobial, antitumor and antioxidation properties in aqueous extracts of fennel seeds by different extraction methods.

Materials And Methods

2.1. Collection of plant material:

Fennel seeds (Foeniculum vulgare L) - Apiaceae) were purchased and identified by Central Administration of Horticulture and Agricultural crops, Ministry of Agriculture and Land Reclamation (Egypt) and stored in deep freeze at -20 °C until analysis.

2.2. Preparation of aqueous extracts:

Fennel sample was dried for 48 h in a hot air-drier at 50 °C. After drying, 100 g of dried sample were extracted with 2000 mL of deionized water at different pH conditions either with HCl (pH 3), water (pH 7), or NaOH (pH 12) at 80 °C for 3hr. After cooling to the room temperature and then filtering (Whatman No 1), the extracts were neutralized and dialyzed against distilled water for 48hr., dried under vacuum and weight. The extracts were completely dried under freeze-drier and stored at -20 °C until further uses.

2.3. Microorganisms and media:

The antimicrobial and antifungal activities of aqueous extracts were individually tested against a panel of microorganisms, including one Gram-positive bacteria (Staphylococcus aureus), Gram-negative bacteria (Escherichia coli), one yeast (Candida albicans) and two molds. All the strains were grown on Mueller Hinton agar (MHA) for the bacteria and Saboureaud Dextrose Agar (SDA) with chloramphenicol for yeasts and moulds.

2.4. Preparation of the inoculums:

Culture media: Bacteria were grown on Mueller Hinton Agar (MHA, g/L): beef infusion 2.0; acid casein peptone 17.5; starch 1.5; bacteriological agar 17.0.

Sabouraud Dextrose Agar (SDA, g/l): peptone, 10.0; glucose, 20.0; agar-agar, 17.0 with chloramphenicol 0.5 g was used for yeasts and moulds. The inoculum used for all the assays reached the microbial density of the order of $10^8$–$10^9$ CFU/ml for the bacteria and yeasts and $10^5$–$10^6$ spores/ml for the moulds were used.

Bacteria: The strains preserved in the nutrient agar at 8°C, were revived in nutrient solution and incubated at 37 °C for 24 h.

Yeasts: The strains preserved at 8°C in the Sabouraud agar supplemented with chloramphenicol were revived in nutrient solution and incubated at 30°C for 48 h., 0.1 ml of each culture was added to 10 ml sterile saline.

Moulds: The inoculum was presented in the form of spore suspension in sterile saline with 0.1% of Tween 80 (Tantaoui-Elaraki et al, 1992).

2.5. Chemicals, solvents and reagents:

2.2-Diphenyl-1-picrylhydrazyl (DPPH*), trypan blue, gallic acid, quercetin and Folin–Ciocalteu reagents were obtained from Sigma Chemical Co. All other solvents and chemicals were of analytical grade.
2.6. Animal and tumor:

Female Swiss albino mice (8-10 weeks) weighing 22-25g were used. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of 25 °C, humidity of 45–65% fed with a balanced commercial diet and water. EAT cells, derived from a spontaneous murine mammary adenocarcinoma were maintained in the ascites form by peritoneal transplantation of 2.10^6 cells. EAT cell counts were done in a hemocytometer slide. The cells were found to be more than 99% viable by the Trypan blue dye exclusion method.

2.7. Cell culture and virus:

Herpes simplex virus type 1 (HSV-1 local isolate) was used as a model of DNA virus for antiviral screening. The virus was isolated, propagated and identified by Prof. Mohamed Ali, Virology Laboratory of the Department of Water Pollution, National Research Center. African green monkey cells (Vero) were used as virus host. cells grew in minimum essential medium with Hank’s buffer (HMEM) supplemented by 1% antibiotic–antimycotic mixture (GIBCO-BRL), 8% fetal bovine serum and the pH adjusted to 7.2–7.4 by 7.5% sodium bicarbonate solution. Cells grew as monolayer sheets dissociated by trypsin–versine solution (0.15% trypsin and 0.04% ethylene diamine tetracetic acid, EDTA 2Na). The dissociated cells sub-cultured in a 96-well plate to measure the cytotoxicity of the extract by incubation with the cells at 37°C/24 h. Cytotoxicity was measured microscopically and by viable cell counting.

The optimal concentration of extract was estimated; compared with acyclovir as standard (>95%-4669) Sigma. Control virus and cells were treated identically without the extract or standard. Virus plaques were counted and the percentage reduction was calculated (Papageorgiou, et al, 2000). The virucidal activity was determined by plaque reduction method.

2.8. Chemical characterization of crude aqueous extracts:

The carbohydrate content was analyzed by the phenol–H_2SO_4 method (Dubois et al, 1956) without previous hydrolysis of the polysaccharide. Total protein was estimated by the method of Lowry et al. (1951). The sugar composition was determined after complete hydrolysis with H_2SO_4 (2 mol/l) at 100°C for 8 h, neutralized with BaCO_3, then centrifuged, filtered, neutralized with Dowex 50 resin (H^+ form) and concentrated. The hydrolyzed products were spotted in Whatmann no.1 paper and subjected to chromatography (Wilson, 1959) in butanol: acetone: water (4:5:1 v/v) for 24 h. The chromatogram was visualized by spraying with aniline phthalate (Partridge, 1949).

2.9. Determination of total phenolics:

The total phenols of aqueous extracts of powdered fennel seeds were estimated according to the method described by Makkar et al. (1997). One ml of the extract were taken in a test tube, then 0.5 ml of Folin Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentilly in each tube. After one hour of incubation at room temperature, the absorbance was measured at 725 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents (mg gallic acid equivalent/ g extract) and the values are presented as means of triplicate.

2.10. Determination of total flavonoids:

Total flavonoids content was determined spectrophotometrically using the method of Ordonez et al. (2006) based on the formation of a complex flavonoid-aluminum. An aliquot (0.5 ml) of aqueous extract was mixed with AlCl_3 solution (2%, 0.5 ml). Then the mixture was properly mixed and allowed to stand for 30 minutes at room temperature. The intensity of colour was measured at 420 nm after filtration if it is necessary. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve and the values are presented as means of triplets analyses.

2.11. Antioxidant activity (DPPH assay):

The free radical scavenging activity using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) reagent was determined according to Brand-Williams et al. (1995). The samples (10 mg) were extracted with 80% aqueous methanol. To 0.75 ml of the extract sample 1.5 ml of freshly prepared DPPH solution (prepared with 20 mg of DPPH/L of methanol) was added and stirred. The decolorizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control. The percentage of DPPH scavenging activity is expressed by the
following formula, Antioxidant activity% = [(control absorbance – sample absorbance /control absorbance x 100)]. The values are presented as means of triplets analyses.

2.12. Antimicrobial screening:

Paper disc diffusion technique (Belaiche, 1979) was used to test the microbial activity of aqueous extracts of fennel seeds as follows: The agar plate containing the appropriate medium was spread with the inoculum containing $10^{10}$ CFU/ml. The filter paper disc (5 mm in diameter, whatman No 3) was absorbed with 0.025 ml of extract (1.6 mg/ml) and then placed onto agar plates. After incubation at 37°C for 24 h for bacteria, at 30°C for 48 h for yeasts and 7 days at 28°C for moulds, the diameters of inhibition zones were measured in mm. Positive activity was defined as an inhibition zone of over 7 mm surrounding a disc (Irkin and Korukluoglu, 2009). Streptomycin (15 µg/disc) was used as positive control for bacteria, yeast and Greseofulvin (20 µg/disc) for moulds, respectively.

2.13. Plaque reduction infectivity assay:

On a six-well plate, cell culture (10⁷ cell/ml) was cultivated and incubated for 2 days at 37°C. HSV-1 and mixed with the tested extract and cyclovir was used as a positive control at concentration of 20 µg/10⁷ cell/mL and 20 µg/10⁴ cell/mL, respectively, and incubated overnight at 4°C. Growth medium was removed from the multiwell plate, and virus-extract mixture was inoculated (100 µl/well). After 1 h contact time, the inoculum was aspirated and 3mL of HMEM with 1% agarose was overlaid with cell sheets. The plates were left to solidify and incubated at 37°C until the development of virus plaques. Cell sheets were fixed in 10% formaline solution for 2 h and stained with crystal violet stain. Control virus and cells were treated identically without any chemical compound. Virus plaques were counted, and the percentage of reduction was calculated (Zeid, et al, 1999).

2.14. Trypan Blue Exclusion Test:

To detect the cell viability trypan blue exclusion test, the suspension of the tumor cells was attained from peritoneal cavities of tumor-bearing mice and then diluted with phosphate buffered saline (PBS) (pH 7) so that the final preparation comprised 2.5*10⁷ cells/0.1 ml. Briefly, in a set of sterile test tubes, aliquots (0.1 ml/tube) of the cell preparation were distributed followed by addition of aliquots (0.8 ml/tube) of (PBS). The investigated samples (Dissolved in phosphate-buffered saline) were then applied to the tubes in aliquots (0.1 ml/tube) at different concentration of the dry samples. The tubes were incubated at 37°C for 2h under 5% CO₂, then the tubes were centrifuged at 1000 rpm for 5 min and separated cells were suspended in saline. For each examined tube and control, a new clean, dry small test tube was used and 0.1 ml of cells suspension, 0.8 ml saline and 0.1 ml trypan blue were added and mixed, and then the number of living cells was calculated using a hemocytometer slide. Viable cells appeared as unstained bodies while non-viable cells stained blue (El-Merzabani et al, 1979).

2.15. Statistical analysis:

Data were statistically analyzed using SPSS, version 10.00 for windows SPSS (Inc., Chicago, IL, USA). Data was presented as mean ± standard deviation.

Results And Discussion

3.1. Chemical composition of extracts:

The individual extraction with hot water under different pH conditions (pH3, pH7 and pH12) at constant temperature (80°C) was performed in order to obtain extracts with high molecular weight compounds, such as polysaccharides and low molecular weight compounds, such as phenolic and flavonoid compounds. Both kinds of high and low molecular weight compounds play important roles in medicinal functions of fennel seeds.

The results of the extract yields, total phenolic and flavonoid values obtained for the three extracts are shown in Table 1. Significant differences were observed in the extraction yield of three different extracts with the highest (14.3%) and lowest (3.8%) values recorded for Alk.Fen and Ac.Fen extracts, respectively. However, no high differences were found for total phenolic contents (25.04-33.18 mg GA/g) and total flavonoid (2.05-2.65 mg QE/g). On the other hand, Kim et al (2011) had reported that water extraction yields, non volatile total phenolics, flavonoids of fennel were 11.38%, 9.36 and 44.76%, respectively. Similarly, this study is in agreement with of Hinneburg et al (2006) and Kim et al (2011).
The results of total carbohydrates and water soluble proteins, for the three extracts were shown in (Table 1). The Neut.Fen extract showed the highest total carbohydrates and protein concentrations (47% and 44.6%, respectively). While, lowest concentration of total carbohydrates and water-soluble protein were recorded in Ac.Fen (38.6 and 32.9 %) and Alk.Fen (39.9 and 30.4%), respectively.

Table 1: Yield, total phenolics, flavonoids, Carbohydrate and protein contents of aqueous fennel seeds extracts.

<table>
<thead>
<tr>
<th>Extract*</th>
<th>Extraction yield (%)</th>
<th>Total phenolic content (mg GA/g)</th>
<th>Total flavonoid content (mg QE/g)</th>
<th>Total Carbohydrates (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac.Fen</td>
<td>3.8±0.89</td>
<td>33.18±1.54</td>
<td>2.65±0.06</td>
<td>38.6</td>
<td>32.9</td>
</tr>
<tr>
<td>Neut.Fen</td>
<td>5.6±1.32</td>
<td>31.94±0.27</td>
<td>2.46±0.11</td>
<td>47</td>
<td>44.6</td>
</tr>
<tr>
<td>Alk.Fen</td>
<td>14.3±1.78</td>
<td>25.04±0.29</td>
<td>2.05±0.07</td>
<td>39.9</td>
<td>30.4</td>
</tr>
</tbody>
</table>

3.2. Monosaccharide constituents of extracts:

In relation to the sugar composition (Fig 1) of aqueous extracts, the Ac.Fen and Alk.Fen extracts yielded galactose as the main sugar (87.7% and 66.5%, respectively). The highest values of glucose, rhamnose, xylose and uronic acids (41.4; 12; 8.7 and 5.8%) were found in Neut.Fen extract. In contrast, xylose and rhamnose were not detected in sugar composition of Ac.Fen extract.

Fig. 1: Monosaccharide constituents of acid hydrolyzates of aqueous fennel seeds extracts UA (Uronic acid), Gal. (Galactose), Glu. (Glucose), Xyl. (Xylose), Rham. (Rhamnose) and Tr. (Traces) (<1%)

3.3. Free-radical scavenging activity:

The free-radical scavenging activity of F. vulgare extracts evaluated using the DPPH method is presented in Fig. 2. The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreased as a result of a color change from purple to yellow as the radical was scavenged by antioxidants through donation of hydrogen or electron to DPPH radical to become a stable diamagnetic molecule (Soares, et al., 1997). The antioxidant activity of water extracts of Fennel seeds increased with increasing concentration.

Figure (2) illustrates a significant decrease the concentration of DPPH radical due to the scavenging ability of soluble solids in the three extracts of fennel seeds. The scavenging effect of water extracts at 0.5, 1.0, 1.5 and 2.0 mg doses on the DPPH radical decreased with increasing the concentration. However, the scavenging effect of three aqueous extracts on the DPPH radical decreased in the order of Alk.Fen>Neut.Fen>Ac.Fen water extracts were 35.0%, 33.6% and 31.7% at the dose of 2.0 mg, respectively. These results indicated that all the three extracts have a noticeable effect on scavenging free radical. As have been noticed that free radical scavenging activity also increased with increasing concentration. The good correlation between the results from total phenolics analysis and the antioxidative assays has been previously reported (Zheng & Wang, 2001). Moreover, Liu et al (2008) had reported that the clove extract was significantly higher in the total phenolic content and DPPH radical scavenging activity than other Chinese herbal plants. Further studies are needed to explore the potential
phenolics compound, and flavonoids from these extracts and in vivo studies are needed for better understanding their mechanism of action.

Fig. 2: DPPH radical scavenging activities of water extracts of fennel seeds at different concentrations.

3.4. Antimicrobial activities:

Antimicrobial activity of the three aqueous extracts obtained from fennel seeds at concentration of 1.6 mg/ml saline are shown in (Table 2). Streptomycin (15 μg/disc) was used as a reference material or positive control for the antibacterial activity and Greseofulvin (20 μg/disc) was used as a reference or positive control for the antifungal activity. The results indicated that all extracts have antibacterial activity against examined Gram negative and Gram positive bacteria.

The recorded highest antibacterial activity of water extracts against Gram negative bacteria which are less effective than streptomycin by 75% for Ac.Fen and Neut.Fen and 60% for Alk.Fen in the E. coli bioassay, respectively. Also, the most effective of Ac-Fen extract against Gram positive bacteria was represented 75% inhibition zone of ampicillin, Neut.Fen and Alk.Fen represented 60% in the Staph. aureus, respectively. These data coincide with those of Okoli and Iroegbu (2004), who reported that water and methanolic extracts of some plants displayed a significant antimicrobial activities. Similarly, Basri and Fan (2005) had reported that the aqueous and acetone extracts of galls of Quercus infectoria (Oak) displayed similarities in antmicrobial activity on the bacterial species and as such, it is the potentially source of antimicrobials.

Interestingly, water extracts of fennel seeds (Table 2) have effective antifungal activity as compared to reference commercial fungicial Greseofulvin. Ac.Fen, Neut.Fen and Alk.Fen. The effective extracts against Asp. niger were less effective than Greseofulvin by 55% for all three extracts and 63% for Ac.Fen and 75% for Neut.Fen and Alk.Fen in P. chrysogenum bioassay, respectively. The results also showed low activity against yeast (C. albicans) for all three extracts. These results were in agreement with those of Zahid et al (2012) who had reported that the aqueous extract of fennel (Foeniculum vulgare Mill) had potential antifungal activity against three soil borne fungi namely: Macrophomina phaseoli, Rhizocotina solani and Fusarium moniliforme.

Table 2: Antimicrobial activity of aqueous Fennel seeds extracts using disc diffusion assay

<table>
<thead>
<tr>
<th>Products</th>
<th>Inhibition zone diameter (mm*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested Microorganisms</td>
</tr>
<tr>
<td></td>
<td>E.Coli</td>
</tr>
<tr>
<td>Ac.Fen</td>
<td>15</td>
</tr>
<tr>
<td>Neut.Fen</td>
<td>15</td>
</tr>
<tr>
<td>Alk.Fen</td>
<td>12</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>20</td>
</tr>
<tr>
<td>Greseofulvin</td>
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</tbody>
</table>

*The diameter (mm) of the inhibition zone is the mean of three independent experiments including the diameter of the paper disc (5 mm).

3.5. Antiviral Activity:

As shown in Table (3), antiviral activity against herpes simplex virus 1 (HSV-1) of Ac.Fen, Neut.Fen and Alk.Fen extracts was tested using the plaque reduction infectivity assay in Vero cell line. For comparison between Acyclovir as control and the tested, the antiviral screening was performed at concentration of 20 µg for
tested and positive control. The acidic extract (Ac.Fen) and alkaline extract (Alk.Fen) showed high activity with virocidal inhibition effect (83.4 and 58.2%, respectively). In contrast, the inhibitory activity of Neut.Fen extract was 7.5%. Interestingly, at concentration 20 µg, Ac.Fen and Alk.Fen extracts were found to be more effective than Acyclovir (54.3%). The inhibitory effect of these two extracts might be due to the presence of galactose unit of sugar moiety. Supporting this outcome, anti-HSV-1 activity was observed in both crude arabinogalactan and homogeneous arabinogalactan (Oliveira et al., 2012). Another pectin-type polysaccharide isolated from Prassica oleracea L., which was composed of galactose as predominant unit showed potential anti-HSV-2 activity (Dong et al., 2010). On the other hand, aqueous extracts with phenolics in them, were already known to be potent inhibitors of plant viruses (Joshi & Prakash, 1981). Further work on the isolation of active antiviral compound of the aqueous extracts is in progress. These natural products will be useful as potent native anti-HSV-1. Nevertheless, the use of these crude aqueous extracts at a commercial level must await the results of large-scale trials and the examination of application modalities.

Table 3: Antiviral activity of the aqueous Fennel seeds extracts.

<table>
<thead>
<tr>
<th>Fennel extracts</th>
<th>Initial virus count (PFU/ml)</th>
<th>Virus count (PFU/ml)</th>
<th>Virucidal effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac.Fen</td>
<td>5.36*10^7</td>
<td>0.89*10^7</td>
<td>83.40</td>
</tr>
<tr>
<td>Neut.Fen</td>
<td>5.36*10^7</td>
<td>4.96*10^7</td>
<td>7.46</td>
</tr>
<tr>
<td>Alk.Fen</td>
<td>5.36*10^7</td>
<td>2.24*10^7</td>
<td>58.21</td>
</tr>
<tr>
<td>Acyclovir*St</td>
<td>4.16*10^7</td>
<td>1.90*10^7</td>
<td>54.33</td>
</tr>
</tbody>
</table>

3.6. In vitro antitumor activity:

The viable and dead cell counting after incubation with different concentrations of extracts was summarized in (Table 4). By counting viable cells through trypan blue exclusions test, it was noticed that the three aqueous extracts at concentration (300-900 μg/tube) didn’t show a reasonable effect on the viable cell population. In higher concentrations the viable cells were decreased which it demonstrated tumoricidal effect with 22.1, 22.6 and 25.3% of Ac.Fen, Neut.Fen and Alk.Fen extracts, respectively on Ehrlich ascites carcinoma cells. The most effective concentration, in which the number of viable cells was decreased, was 900 μg/tube of Alk.Fen extract. In general, the results of the antitumor activity were rather disappointing. All three plant extracts showed in the highest concentration tested moderate antitumor activity against the Ehrlich ascites carcinoma cells. As has been previously reported (Pradhan, et al., 2008), the in-vitro antitumor activity of methanolic extract of F. vulgare against B16F10 melanoma cell line by Trypan blue exclusion assay for cell viability. Melanoma cell culture treated with 70% aqueous methanolic extract of F. vulgare showed good antitumor activity at the concentration of 200μg/ml.

In summary, the new aqueous plant seed extracts had shown impressive antimicrobial, antiviral, antioxidant and antitumor activities. The water extraction process was simple and safe, compared with those extraction procedures with organic solvents, so that will be easily put into industrial application. Furthermore, it has the advantages of lower cost without the undesirable residual organic solvents. Therefore, the water extraction process has great potential to open the possibility of using the spice water extracts as alternative natural medicinal drugs.

Table 4: Effect of Fennel extracts on the viability of Ehrlich ascites carcinoma cells (EACC) at different concentrations

<table>
<thead>
<tr>
<th>Samples</th>
<th>Conc (ug/mL)</th>
<th>Tumoricidal effect% Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac.Fen</td>
<td>300</td>
<td>20.8±0.52</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>19.2±0.46</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>22.1±0.26</td>
</tr>
<tr>
<td>Neut.Fen</td>
<td>300</td>
<td>17.7±1.66</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>19.0±0.58</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>22.6±0.44</td>
</tr>
<tr>
<td>Alk.Fen</td>
<td>300</td>
<td>19.6±0.81</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>20.9±0.21</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>25.3±0.63</td>
</tr>
</tbody>
</table>

Acknowledgment

Authors are grateful to National Research Centre (NRC), Cairo, Egypt for financial support and Prof. Mohamed A. Ali, Virology Laboratory, NRC for antiviral evaluation.
References


