Antitumor and Antibacterial Activities of [1-(2-Ethyl, 6-Heptyl) Phenol] from Cuminum Cuminum Seeds

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Abstract: 1-(2-Ethyl, 6-Heptyl) Phenol (EHP), a biologically active compound formerly extracted by benzene from Cuminum cuminum (cumin) Egyptian seeds and of activity against a number of fungal pathogens, exhibited antitumor activity against six types of tumor cell lines (HEPG2, HELA, HCT116, MCF7, HEP2, CACO2). EHP showed no cytotoxicity when its activity was investigated on the normal fibroblast cell line (BHK). MCF7 was the most sensitive tumor cell line where only 33% of the cells survived followed by HEPG2 (41% of the cells survived) and HEP2 (56% of the cells survived) at an EHP concentration of 10 μg/ml. The percentage of tumor cell survival of CACO2 and HCT116 was 72% and 76% respectively exhibiting much less activity, however EHP activity against HELA was negligible. EHP activity was also investigated against eight bacterial human pathogens (four Gram-positive and four Gram-negative ones). Higher activity was observed against Gram-positive bacteria than Gram-negative ones where Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis and Bacillus thuringiensis were more sensitive than Salmonella typhi, Escherichia coli, Serratia marcescens and Pseudomonas aeruginosa. This study directs the attention to the antifungal, antibacterial and antitumor benefits of the cheap, safe, available and tasteful cumin.

Key words: antitumor agents, SRB, Cuminum cuminum, ELISA, BHK, antibacterial agents

INTRODUCTION

Cancer is a class of diseases or disorders characterized by uncontrolled division of cells and the ability of these cells to invade other tissue, either by direct growth into adjacent tissue through invasion or by implementation into distant sites by metastasis. Metastasis is defined as the stage in which cancer cells are transported through the blood stream or lymphatic system [15].

The unregulated growth that characterizes cancer is caused by damage to DNA, resulting in mutations to genes that encode for proteins controlling cell division [17].

The effect of plant extracts as antitumor agents were widely studied due to their low toxicity and side effects. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early, cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components [18].

Cuminum cuminum seeds are stomachic, diuretic, carminative, stimulant, astringent, emmenagogic and antispasmodic, hypoglycemic, contraceptive in treatment of sores, burn and slow continuous fever. It is valuable in dyspepsia, diarrhoea and hoarseness, antimalarial, laxative, and may relieve flatulence and colic [20].

In the West, it is used mainly in veterinary medicine, as a carminative, but it remains a traditional herbal remedy in the East. It is supposed to increase lactation and reduce nausea in pregnancy. It has been shown to be effective in treating carpal tunnel syndrome, as well as diarrhoea, indigestion and morning sickness [28]. Cumin also shows promise as a natural way to increase breast size. Used in a poultice, it relieves swelling of the breast or the testicles. Cumin stimulates the appetite. The leaf extracts were found to inhibit the growth of leukemic cells hence it contains active ingredients against tumor cells [31].

Scientific strategies for the in vitro evaluation of natural products with biological activity have changed in the past few years. One recent development is the highly automated bioassay screening based on colorimetric methods that quantify the proliferation of cell cultures [24,10]. These techniques which are considered quick and inexpensive for the evaluation of antitumor [5,32] and antiviral activity [41] of a large number of natural product extracts, have also easily permitted to guide the isolation and purification of their biologically active principles [7].
The effect of plant extracts on cancer cell was studied due to their low toxicity and side effects. The inhibition of ascites tumor cells by garlic extracts was investigated. Soybean seed extracts showed antitumor activity due to the presence of trypsin inhibitor [11].

Interest in a large number of traditional natural products has increased [21,40,39]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral and antitumor agents [6, 40]. Furthermore, the selection of crude plant extracts for screening programs has the potential of being more successful in its initial steps than the screening of pure compounds isolated from natural products [22, 4].

The tumor inhibitors of plant origin depend upon the type of cancer cells and plant species as well as the extract used. Extract of *Allamanda cathartica* gave significant activity against P-388 leukemia in mouse [13]. Different plant species growing in Egypt showed anticancer activity [32]. The principles separated from plants were also studied such as alkaloids, terpenes, flavonoids [27,16,11] and chlorophyll [4,33].

Cumin is being studied for its role in cancer prevention, says Pensiero. “Cumin contains limonene, a type of phytochemical that is being investigated for its role in blocking cancers, specifically prostate cancer”, it may also reduce cholesterol [29]. Cumin's distinctive flavor and strong warm aroma is due to its essential oil content. Also, cumin has strong antimicrobial activities such as, antibacterial activity [26], antifungal activity [19], and antitumor activity [43].

Cumin (*Cuminum cyminum*) is a widely used ingredient in food. It has been used for a very long time in traditional medicine in the treatment of gastric disorders. Cumin seeds showed antimicrobial activities for different microorganisms, including bacterial strains, yeasts and fungi. In India, many spices included cumin seeds have been traditionally used since ancient times, for the preservation of food products as they have been reported to have antiseptic and disinfectant properties. In this respect, a preliminary screening for antimicrobial activities of cumin has been carried out. Of the spices surveyed, the results indicate that cumin has potent antimicrobial activities against the test organisms *Bacillus subtilis, Escherichia coli* and *Saccharomyces cerevisiae* [8,9].

The essential oils extracted from the seeds of seven spices, including *Cuminum cyminum*, have been studied for antibacterial activity against eight pathogenic bacteria, causing infections in the human body. It has been found that the oil of *C. cyminum* is very effective against all tested bacteria, this oil is equally or more effective when compared with standard antibiotics, at a very low concentration [24].

Essential oils extracted from seeds of *Cuminum cyminum* was analyzed by gas chromatography (GC) and GC-mass spectrometry (MS) [26,19]. The main components of *C. cyminum* oil exhibited activity on many species from Gram-positive and Gram-negative bacterial. Antibacterial testing showed high activity of the essential *C. cyminum* oil against *Bacillus subtilis* and *Staphylococcus epidermidis* as well as, the activity was particularly high against the genera *Clavibacter, Curtobacterium, Rhodococcus, Erwinia, Xanthomonas, Ralstonia, and Agrobacterium*, which are responsible for plant or cultivated mushroom diseases worldwide. In general, a lower activity was observed against bacteria belonging to the genus *Pseudomonas*. These results suggest the potential use of the above essential oils for the control of bacterial diseases.

Previously, extract of *Cuminum cyminum* (cumin) seeds possessed antifungal activity in an *in vitro* study against ten pathogenic fungal isolates from human hair, nail and skin [23]. The current study focused on investigating the 1-(2-Ethyl, 6-Heptyl) Phenol (EHP) compound as an antitumor and antibacterial agent, its cytotoxicity against a normal cell line was studied as well.

**MATERIALS AND METHODS**

1-(2-Ethyl, 6-Heptyl) Phenol (EHP) formula structure (figure 1) was described and used in this study for testing its activity as an antitumor and its cytotoxicity [23]

![EHP compound](image)

**Fig. 1:** EHP compound

**I-Antitumor Activity:** Six tumor cell lines and one normal cell line (normal fibroblast) were provided from National Cancer Institute (NCI), Cairo University and listed in table (1).

<table>
<thead>
<tr>
<th>Table 1: Type of tumor cell lines</th>
<th>Type of Tumor Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPG2</td>
<td>Liver carcinoma cell line</td>
</tr>
<tr>
<td>HELA</td>
<td>Cervical carcinoma cell line</td>
</tr>
<tr>
<td>HCT116</td>
<td>Colon carcinoma cell line</td>
</tr>
<tr>
<td>CACO</td>
<td>Colon carcinoma cell line</td>
</tr>
<tr>
<td>MC7</td>
<td>Breast carcinoma cell line</td>
</tr>
<tr>
<td>HEP2</td>
<td>Larynx carcinoma cell line</td>
</tr>
<tr>
<td>BHK</td>
<td>Normal fibroblast cell line</td>
</tr>
</tbody>
</table>
Measurement of Potential Antitumor Activity and Cytotoxicity by SRB Assay: Potential antitumor activity and cytotoxicity of EHP compound was tested using SRB technique[36].

Tumor cells were plated in 96 – multi-well plate (104 cells/well) for 24 hrs before treatment with EHP to allow attachment of cell to the wall of the plate. Then, different concentrations of the EHP compound at (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer triplicate wells after prepared for each individual dose. Monolayer cells were incubated with the compound for 48 hrs at 37 °C and in atomaosphere of 5 % CO2.

After 48 hrs. cells were fixed, washed and stained with Sulfo – Rhodamine – B stain (SRB). Excess of stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. The color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line.

II- Antibacterial Activity:
1.Culture Medium: Nutrient agar medium was used for bacterial growth [Beef Extract, 3.0g; Bacteriological Peptone, 5.0g; Agar, 20.0g, the pH was adjusted at 6.2 ± 0.2 at 25 (±2)°C. The medium was prepared by dissolving the solid ingredients in 1 liter of cold distilled water and then heating to 60-70 °C with stirring. Medium was sterilized by autoclaving at 121°C (1.5 atm.) for 15-20 minutes.[31]

Test Organisms: Eight clinical bacterial strains employed for this investigation including four Gram-positive (Staphylococcus aureus; Streptococcus pneumoniae; Bacillus subtilis and Bacillus thuringiensis) and four Gram-negative bacteria (Escherichia coli; Salmonella typhi; Serratia marcescens and Pseudomonas aeruginosa). All strains were kindly provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), AL–Azhar University.

Antimicrobial Assays: Diffusion agar technique, antibacterial potentiality against several species was expressed as the measurement of diameter of their inhibition zone. Hole-plate diffusion method was used; 1 cm diameter of holes were made using sterile cork borer in Nutrient agar sterile plates (10x 10 cm), which had previously been seeded with tested bacterial isolates. Holes were filled with 100 µL of different concentration of EHP compound (1, 2.5, 5, and 10 µg/ml). Control holes were filled with benzene solvent. Plates were left in a cooled incubator at 4 (±2)°C for one hour and then incubated at 37 (±2) °C. Inhibition zones developed was measured after 24 hours of incubation time[26] Amoxicilli was used as a standard antibacterial agent.

RESULTS AND DISCUSSION

Biologically active compounds from plant sources have had a dramatic impact in medicine including: quinine for treatment of malaria, reserpine for controlling hypertension, cocaine as a muscle relaxant and vincristine for treating children with leukemia. The main problem in cancer therapy is represented in the sever toxicity and the side effects of the current drugs and radiotherapy. Hence, it became crucial to search for antitumor agents from the natural and safe plant resources. It should also be noted that regarding antibiotics there should always be sources for new safe ones to overcome the problems of microbial resistance, side effects and long-term therapy.

Cumin has been used as a folk medicinal plant – schools of natural medicine tout its stimulant and antimicrobial properties. Cumin does have proven carminative and antispasmodic qualities, and is therefore said to be useful in the treatment of diarrhea, stomachache, and menstrual cramps. Eastern medicine also recommends it for pregnant women to settle morning sickness and increase breast milk production [37].

Being of diverse benefits, attention has been attracted to cumin in the current study to investigate the effectiveness of EHP, a cumin seed extract, against six tumor cell lines as well as against four Gram-negative and four Gram-positive bacterial pathogens. The first step in the current study was to investigate the cytotoxic effects of EHP against normal cells where BHK was the chosen normal cell line. Results revealed negligible cytotoxic effects for EHP; increasing the concentration up to 10 µg/ml resulted in the survival of 85.6% of the normal cells (Table 2 and Figure 2).

Evaluation of the antitumor effect of the EHP compound of Cuminum cyminum seeds using six cell lines (HEPG2; HELA; HCT116; CACO2; MCF7; HEP2) was performed. It is worth noting that according to the available research, no previous work has been reported on the antitumor activity of the Egyptian seeds of Cuminum cyminum.

Regarding its antitumor activity, the SRB assay of benzene extract of Cuminum cyminum treated cells showed that 1 µg/ml concentration of the EHP compound had activity against MCF7 cell line followed by HEPG2 where 73% and 84% of surviving cell lines was obtained respectively. While, the rest of the tumor cell lines exhibited great resistance to the cumin compound at the same concentration. An EHP concentration of 2.5 µg/ml exhibited activity only
against the MCF7 cell line (only 45% of the cells survived). While, a negligible effect was observed regarding the other ones (Table 2 and Figure 2).

Table (2) and figure (2) reveal that a concentration of 5µg/ml of the EHP cumin compound introduced good activities toward two of the investigated tumor cell lines (MCF7 and HEp2); 38% of MCF7 tumor cells survived and 58% of HEp2. However mild activities were detected in case of CACO2 (72% of cells survived) and HEpg2 (77% of cells survived). Negligible effects were observed for the rest of cell lines.

In case of 10 µg/ml EHP concentration, MCF7 cell line was the most affected (33% of cells was survived) followed by HEpg2 and then HEp2 cell lines where 41% and 56% of the cells survived respectively. The effect of EHP on the rest of the investigated cell lines was mild to negligible (72% of CACO2 cells survived while for HT116 and HELA cell lines only 24% and 15% of cells was affected, respectively).

Antitumor activity has been reported from other plant extracts; acetone and ethyl acetate extracts of *Stevia rebaudiana* showed cytotoxic activity on HEp2 cells. Acetone extracts showed the highest cytotoxic activity followed by ethyl acetate and chloroform extracts [20]. Also, the ethanol extract from *Annona* sp. exhibited activity on MDBK and HEp2 cells where CC50 values were 34.5 and 55 mg/ml at 24 hr respectively. Furthermore the value for the same extract on HEp2 cells at 72 hr was 49.6x10^-9 mg/ml. The cytotoxic activity of the *Annona, maricata, Annona, cherimolia* and *Ruagea membranacea* species has been extensively proven [14,19,35].

Moreover, *Jacaranda copaia* and *Tapirira guianensis* extracts had cytotoxic effect on eight human tumor cell lines (representing lung, breast, colon, and pancreas) [30]. While, great reduction of tumor cell lines was exhibited in mice when injected with 10 µg/ml of root extract of *Gossampinus malabarica* [42].

The compound of interest was characterized by possessing aromatic ring and terminal hydroxyl group and this type of compounds was believed to possess activity against cancer. This conclusion was confirmed by many reports that these compounds interact with DNA by intercalation and act as topoisomerase inhibitors [2,20].

Further in vivo studies will be conducted using experimental mice suffering carcinoma they will be fed cumin to investigate the effect of cumin ingestion on cancer.

The antibacterial activity of EHP against four Gram-negative and four Gram-positive bacterial pathogens is tabulated in table (3) and illustrated in figure (3). The most sensitive bacterial strain was *Staphylococcus aureus* where an inhibition zone of 0.8, 1.6, 2.8, and 3.5 cm was obtained when applying EHP concentrations of 1, 2.5, 5.0, and 1.0 µg/ml respectively. *Staphylococcus aureus* was followed by *Bacillus subtilis, Streptococcus pneumoniae* and *Bacillus thuringiensis*.

In the present study, the activity of EHP against Gram-negative bacteria was less than that against Gram-positive ones where the largest zone of inhibition in Gram-negative bacteria was observed against *E. coli*; 1.5 and 1.8 cm inhibition diameters were reported at 5 and 10 µg/ml EHP concentration respectively. It should also be noted that *E. coli* was the only Gram-negative bacteria affected by the lower EHP concentration of 2.5 µg/ml (0.6 cm diameter of inhibition zone). It has been reported that alcoholic and aqueous extracts of eight herbs were prepared after proper drying and grinding. The in vitro activities of the extracts in two concentrations were evaluated against *E. coli*. Among the all spices tested in vitro, the highest degree of anti-*E. coli* activity was exhibited by alcoholic and aqueous extracts of *Cuminum cyminum* at a concentration of 500 mg/ml [25].

In the current study, *E. coli* was followed by *Salmonella typhi* where 0.6 and 1.2 inhibition zone diameters were recorded at 5 and 10 µg/ml EHP concentration respectively. For *Serratia marcescens*, an inhibition zone diameter of 1cm was detected at both 5 and 10 µg/ml. *Pseudomonas aeruginosa* was the least affected; 0.5 mm inhibitory zone was recorded at high EHP concentrations of 5 and 10 µg/ml.

The current antibacterial cumin results agree with other studies reporting more effect against Gram-positive than Gram-negative bacteria. For Gram-negative bacteria, the antibacterial activity of cumin by disk diffusion was studied [14,19,35] where Cumin essential oil showed complete zone of inhibition against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus* and *Bacillus subtilis* at 2 and 6 µL levels. Regarding Gram-negative bacteria, earlier studies reported reduction in bacterial growth due to the incorporation of cumin seed ingredients in the medium with *Cuminum cyminum* essential oil showing little effect against *E. coli, P. aeruginosa* and *Salmonella* sp. [38]. Alternatively, Rajan and Nagaraj [31] studied the antimicrobial activity of *Cuminum cyminum* against *E. coli* and *S. aureus*, its seeds had the least effect against *Staphylococcus aureus*, while *E. coli* was susceptible to all the spices extracts.

Conclusively, the uses of natural products as drugs are crucial instead of the synthetic compounds which possess severe toxicity and side effects besides being very expensive. Hence, the use of natural products represents a valuable solution. This study conveys the use of cumin as a helper in the therapy or the control of the cancer of liver, breast and larynx directing the attention to a cheap treasure called cumin. Moreover, it can be used in the treatment of various pathogenic bacterial diseases. The antiviral activity of EHP will be investigated in a further study.
Fig. 2: Cytotoxicity activity of 1-(2-Ethyl, 6-Heptyl) Phenol extracted from the seeds of Cuminum cyminum

Fig. 3: Antibacterial activity of 1-(2-Ethyl, 6-Heptyl) Phenol extracted from the seeds of cumin

Table 2: antitumor activity and cytotoxicity of 1-(2-ethyl, 6-heptyl) phenol compound

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>MCF7</th>
<th>HEPG2</th>
<th>HEP2</th>
<th>CACO2</th>
<th>HCT116</th>
<th>HELA</th>
<th>BHK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0.73</td>
<td>0.846</td>
<td>0.965</td>
<td>0.96715</td>
<td>0.9826</td>
<td>0.99599</td>
<td>0.98702</td>
</tr>
<tr>
<td>2.5</td>
<td>0.45</td>
<td>0.83</td>
<td>0.8385</td>
<td>0.8958</td>
<td>0.9319</td>
<td>0.93654</td>
<td>0.95053</td>
</tr>
<tr>
<td>5</td>
<td>0.38</td>
<td>0.774</td>
<td>0.5854</td>
<td>0.72485</td>
<td>0.9</td>
<td>0.8557</td>
<td>0.90542</td>
</tr>
<tr>
<td>10</td>
<td>0.33</td>
<td>0.416</td>
<td>0.5657</td>
<td>0.72011</td>
<td>0.768</td>
<td>0.85982</td>
<td>0.85644</td>
</tr>
<tr>
<td>IC50 (µg)</td>
<td>2.21</td>
<td>8.93</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

IC50: The dose of compound which reduced 50% of survival tumor cell lines
Table 3: *In vitro* antibacterial susceptibility of pathogenic bacterial strains to EHP.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Concentration (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.8</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>0.5</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>1.2</td>
</tr>
<tr>
<td><em>Bacillus thuringiensis</em></td>
<td>0.5</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>0</td>
</tr>
</tbody>
</table>

Data are expressed as mean diameter of inhibition zone (cm)

REFERENCES


had been stored for up to 36 years. International Journal of Food Science & Technology, 40(3): 305-310.


