Identification of bioactive compounds in hydro-alcoholic extract of cinnamon by GC-MS analysis and evaluation of its in-vitro anti-gastric cancer potential

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ABSTRACT

Background: Despite the availability of various treatment methods for gastric cancer, herbal medicine remains a promising candidate. This study aimed to identify the bioactive compounds in the hydroalcoholic extract (CHE) of cinnamon (Cinnamomum zeylanicum) using gas chromatography-mass spectrometry (GC-MS) analysis.

Objectives: To study the phytochemical constituents from the hydroalcoholic extract (CHE) of cinnamon (Cinnamomum zeylanicum).

Methods: The CHE was tested for cytotoxicity against a normal human gastric epithelium cell line (GES-1) and a human gastric adenocarcinoma cell line (AGS) as a model of gastric cancer.

Results: The analysis revealed six main phytochemical compounds in the CHE, with cinnamaldehyde being the predominant compound. The CHE showed potent cytotoxicity against the AGS cell line at concentrations ranging from 100-600 μg/mL, significantly reducing the viability of these cancerous cells to 20.88% compared to the control group (p<0.05). In contrast, the CHE did not significantly affect the viability of GES-1 cells.

Conclusion: The findings obtained suggest a promising avenue for finding new anticancer compounds as potent chemotherapeutic agents for gastric cancer.

Keywords: anticancer; bioactive compounds; cinnamon; cytotoxicity
INTRODUCTION

Gastric cancer (GC), also known as stomach cancer, is a dangerous disease that leads to the death of numerous people worldwide. According to the Global Cancer Observatory (GCO) reports, GC is the fifth most common cancer and, following lung and colorectal cancer, it is considered the third deadliest cancer, with a 5-year survival rate of less than 25% and accounting for 1 in 12 cancer-related deaths globally [1]. Therefore, GC is a severe public health concern [2]. The main risk factors for GC worldwide include *Helicobacter pylori* infection, smoking, and high salt intake [3]. Currently, common treatments for GC include gastrostomy, chemotherapy, and radiation therapy [4]. However, these methods are limited by their side effects or toxicity in GC patients [5]. In addition, resistance to anticancer drugs following chemotherapy further restricts the success of treatment [6]. Consequently, finding more effective and less toxic therapies for managing GC has become a significant focus of recent research.

One of the main candidates for cancer therapy is the use of herbal medicine, which offers a safe, non-toxic, and readily available source of treatment [7]. According to the Functional Food Center (FFC), functional foods (FFs) are foods obtained from nature or derived from food processing that contain a suitable amount of biologically active compounds; these compounds provide health benefits and reduce the risk or symptoms of diseases [8]. Functional foods and bioactive compounds can be helpful in treating various clinical problems, including obesity [9], oxidative stress [10], inflammation [11-12], blood cholesterol [13], and so on [14-15]. Many studies have demonstrated that natural compounds in medicinal herbs can reduce the risk of GC [16-17]. The therapeutic effects of these herbs are primarily attributed to their phytochemicals [18]. Previous research has identified several mechanisms by which natural compounds in medicinal herbs can reduce the risk of GC [16-17]. The therapeutic effects of these herbs are primarily attributed to their phytochemicals [18]. Previous research has identified several mechanisms by which natural compounds in medicinal herbs can reduce the risk of GC [16-17]. The therapeutic effects of these herbs are primarily attributed to their phytochemicals [18].

Thus, it is important to focus on the phytochemicals in the present study. Among various herbs, cinnamon...
holds a special place in traditional medicine and has been used since ancient times for its health-promoting potential [22]. Ariaee-Nasab et al. (2014) evaluated the anticancer potential of a water extract of cinnamon on human cancer cell lines (AGS, HeLa, MCF-7 and MDA-MB-234), demonstrating its anti-neoplastic activity [23]. Despite its anticancer potential, limited studies focus on the interaction of cinnamon with the human gastric adenocarcinoma cell line (AGS). In the present study, we determined the phytochemical constituents from the hydroalcoholic extract (CHE) of cinnamon (Cinnamomum zeylanicum) using gas chromatography-mass spectrometry (GC-MS). The cytotoxicity of the cinnamon extraction was assayed in the normal human gastric epithelium cell line (GES-1) and human gastric adenocarcinoma cell line (AGS) as a model of gastric cancer.

**METHODS**

**Cinnamon hydro-alcoholic extraction:** The method described by Anderson et al. was used to prepare the cinnamon hydro-alcoholic extract (CHE) [24]. Cinnamon powder was obtained from cinnamon bark imported from China and sold in Iran. To prepare the CHE, 200 g of cinnamon powder was dissolved in 500 mL of 0.1 N acetic acid and boiled for 20 min. The mixture was then centrifuged, and the supernatant was mixed with absolute ethanol at a ratio of 1:4. The resulting mixture was kept overnight at 4 °C, filtered using Whatman No. 1 filter paper, and dried in an oven at 60°C.

**Gas chromatography-mass spectrometry (GC-MS):** A gas chromatography-mass spectrometry (GC-MS) analysis of the CHE was conducted using an Agilent 6890 N system (US), following established protocols [25]. The GC-MS system was equipped with an HP 5989 mass spectrometer detector. The Agilent 5MS-HP column (US) used was 30 m in length, with an inner diameter of 0.025 mm and a particle size of 0.25 μm. Nitrogen was used as a carrier gas to transport the evaporated extract into the column. For data analysis, Agilent G1701DA MSD ChemStation software (US) was utilized.

**Cell culture:** The human gastric epithelial cell line (GES-1) and the human gastric adenocarcinoma cell line (AGS) were obtained from the National Cell Bank of Iran (Tehran, Iran). The GES-1 and AGS cells were cultured in H-DMEM (HyClone) and RPMI 1640 (Gibco) media in 96-well (TC) plates (NUNC). The media were supplemented with 10% fetal bovine serum (Invitrogen) and penicillin–streptomycin (Sigma). The cells were maintained at 37°C in a 95% humidified atmosphere enriched by 5% CO₂.

**Cell exposure and cell cytotoxicity assay:** The resulting powder, or the CHE, was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution with a concentration of 100 mg/mL. An MTT assay was used to evaluate the cytotoxic effects of CHE on GES-1 and AGS cells. Cells were seeded in 96-well plates at a density of 1 × 10⁴ cells per well and incubated for 24 h. The plates were then treated with various concentrations of CHE (0, 100, 200, 400, 600, 800, and 1000 μg/mL) and incubated for 24, 48, and 72 h. After incubation, the medium in each well was removed and replaced with 100 μL of MTT at 0.5 mg/mL concentration. The plates were incubated for an additional 4 h at 37°C in a humidified atmosphere with 5% CO₂. Following this, the MTT solution was removed, and 100 μL of DMSO solution was added to each well to dissolve the purple formazan crystals. Optical absorbance was measured using an Awareness Technologies Stat Fax 2100 ELISA reader (US) at a wavelength of 570 nm. The results were reported as the IC₅₀ values and cell viability percentages [26].
**Statistical analysis:** The statistical analysis was performed using OriginPro 2018 9.5 version Software. The results were analyzed using one-way ANOVA and Tukey's multiple-range test to compare one factor among multiple groups. Data are presented as the mean ± SD at a 95% confidence level. The probit analysis method was used to transform the growth inhibition data, and the IC50 values were derived by linear regression from plots of probit units against the correspondent log concentrations.

**RESULTS:**

**Cell cytotoxicity:** Treatment with different concentrations of CHE did not exhibit high cytotoxicity in GES-1 cells, except at concentrations of 800 and 1000 μg/mL, which led to a significant decrease in the percentage of viable cells (p<0.05) (Figure 2).

In contrast, the survival of AGS cells showed no significant change after treatment with 100 and 200 μg/mL of CHE (p>0.05) (Figure 2). However, treatment with CHE at concentrations ranging from 400-1000 μg/mL resulted in a significant decrease in cell viability compared to the control group (p<0.05) (Figure 2). IC50 values for AGS cells at 24, 48, and 72 h are presented in Table 1, with the lowest IC50 value observed after 72 h of CHE treatment.

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**Figure 1.** GC/MS spectrogram for cinnamon hydro-alcoholic extraction.

**Table 1.** Phytocomponents identified in Cinnamon hydro-alcoholic extraction.

<table>
<thead>
<tr>
<th>Pike number</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Peak Area %</th>
<th>RF</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.053</td>
<td>2-Propenal, 3-phenyl, 3-phenylpropenal cinnamal</td>
<td>21.47</td>
<td>39272</td>
<td>000104-55-2</td>
</tr>
<tr>
<td>1</td>
<td>21.053</td>
<td>2-Propenal, 3-phenyl-(CAS), cinnamaldehyde 3-phenylpropenal cinnamal</td>
<td>21.7</td>
<td>39276</td>
<td>2-55-000104</td>
</tr>
<tr>
<td>1</td>
<td>21.053</td>
<td>2-Propenal, 3-phenyl-cinnamaldehyde acrolein, 3-phenyl-5S cinnamal</td>
<td>21.7</td>
<td>39274</td>
<td>2-55-000104</td>
</tr>
<tr>
<td>7</td>
<td>26.523</td>
<td>1S, cis-calamenene, 6-dimethyl-1,2,3,4-tetrahydronaphthalene</td>
<td>8.64</td>
<td>86283</td>
<td>2-77-000483</td>
</tr>
<tr>
<td>17</td>
<td>48.169</td>
<td>Megastigma-5(11),7,9-trien-1H-purin-2-amine, 6-methoxy-N-meth 1,2-dihydro-4-(2-propenylamino)</td>
<td>7.53</td>
<td>159760</td>
<td>072523569</td>
</tr>
<tr>
<td>6</td>
<td>25.808</td>
<td>alpha-Muurolene</td>
<td>6.11</td>
<td>191751</td>
<td>9-22031983</td>
</tr>
</tbody>
</table>
Figure 2. Effect of cinnamon hydro-alcoholic extract with various concentration on cell viability of GES-1 (a) and AGS (b) cell line at 24, 48, and 72 h. Different letters in each group indicate significant differences, P < 0.05. (n = 3, Mean ± SD).

Table 2. The value of test solution IC\textsubscript{50} on gastric cancer cell AGS using Probit analysis. (IC\textsubscript{50} ± SD).

<table>
<thead>
<tr>
<th>IC\textsubscript{50} value in AGS cells (μg/mL)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>940 ± 50</td>
<td>24 hours</td>
</tr>
<tr>
<td>740 ± 35</td>
<td>48 hours</td>
</tr>
<tr>
<td>490 ± 22</td>
<td>72 hours</td>
</tr>
</tbody>
</table>

DISCUSSION

The use of natural products as potential anticancer agents holds great promise in current therapeutic strategies. Moreover, structural modifications of natural compounds can help generate novel clinical candidates targeting cancer cells [27]. Cinnamon, known for its antitumor properties demonstrated in previous studies [28], stands out among similar herbs. Hence, this study selected cinnamon as the focal plant for investigation. The present study used the hydro-alcoholic method to extract cinnamon. This method offers several advantages, notably its environmental friendliness, safety, and efficiency. Moreover, this method can be used to extract polar compounds, such as cinnamaldehyde [29]. A previous study showed the effectiveness of the hydro-alcoholic method in reducing the proliferation and growth of cancer cells, as observed...
with the plant *Capparis spinosa*, attributed to the presence of flavonoids, including cinnamaldehyde [30].

In this study, results showed that the main compound in CHE was cinnamaldehyde, a finding consistent with previous research. Uma et al. similarly reported cinnamaldehyde as the dominant compound in the alcoholic extract of *Cinnamomum zeylanicum* [31]. Previous studies have revealed that aromatic aldehydes, like cinnamaldehyde, have unique biological and pharmacological properties, including antioxidant, antimitogenic, and anti-inflammatory activities [32-33]. Recently, cinnamaldehyde has gained considerable attention as a powerful anticancer agent [33]. It shows efficacy in treating various conditions, such as diabetes, atherosclerosis, cancer, inflammation, and cardiovascular disease. Its anticancer potential originates from its ability to induce apoptosis and cell cycle arrest. Moreover, it also has an inhibitory role in angiogenesis, oxidative stress, inflammation, cellular invasion, and metastasis [34].

In the present study, cytotoxicity investigations revealed that CHE treatment did not cause significant changes in viability and proliferation of GES-1 cells, while it exerted a strong inhibitory effect on the viability and proliferation of gastric cancer cells (AGS cell line). In one study, cinnamaldehyde extracted from cinnamon in hepatoma cells (HepG2 and Hep3B) inhibited cell proliferation [35]. In another study, polyphenols containing cinnamaldehyde inhibited cell proliferation and induced cell death in human ovarian cancer cells via cell cycle arrest [36]. Furthermore, low molar concentrations of cinnamon extract have been found to inhibit cell proliferation in the melanoma A375 cell line by increasing intracellular reactive oxygen species (ROS) [27]. Consistent with the observations of the present study, previous research has reported that cinnamaldehyde derived from cinnamon extract exhibits a potent cytotoxic effect on cancer cells, while demonstrating minimal cytotoxicity to normal cells [32-37].

The present study also observed that treatment with CHE at concentrations of 800 and 1000 μg/mL did not induce significant changes in the viability and proliferation of GES-1 normal cells. However, CHE had a strong inhibitory effect on the proliferation and viability of gastric cancer cells. In a past study, it was demonstrated that trans-cinnamaldehyde, not CHE, at a concentration of 2000 μg/mL reduced the development of AGS cells [38]. Trans-cinnamaldehyde was found to induce apoptosis in the AGS cell line by increasing the levels of cleaved caspase-9, cleaved PARP, p53, and Bax proteins [34]

Although studies on the effect of cinnamon extract on gastric cancer and its underlying molecular mechanisms are still in their infancy, present evidence suggests that cinnamaldehyde, a prominent component of cinnamon, may suppress gastric cancer cell growth via apoptotic agents, such as caspase-3 cleavage and Bcl-2 reduction, along with autophagic markers.

**CONCLUSION**

The results of the present study supported the idea that the hydroalcoholic extract of cinnamon, which contains cinnamaldehyde, showed high anticancer properties in gastric cancer cells, while demonstrating no toxic effect on healthy gastric cells. These findings warrant further investigation to explore the anti-gastric cancer effects of HCE in vivo and to evaluate its therapeutic potential against gastric cancer in clinical trials.

**List of Abbreviations:** CHE, cinnamon hydroalcoholic extract; GES-1, human gastric epithelium cell line; AGS, human gastric adenocarcinoma cell line; GC, gastric cancer; GCO, Global Cancer Observatory; DMSO, dissolved in dimethyl sulfoxide.
**Authors’ Contributions:** M. O. designed the study and wrote the manuscript, A. A. G. edited the manuscript, A. R. performed the experiments, and M. T. performed the statistical analysis.

**Competing Interests:** The authors have no conflict of interest.

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