SYNERGISTIC ACTIVITIES OF Hypericum perforatum L. AND GLABRIDIN AGAINST DRUG RESISTANT H. pylori ISOLATES

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Glabridin and Hypericum perforatum L. have many pharmacological activities such as antimicrobial activity, antiproliferative, cytotoxic, anticancerogenic, antiproliferative and antioxidant effect. The aim of this study was to determine the components and to investigate the antimicrobial activity of Hypericum perforatum L. and glabridin against drug resistant H. pylori isolates. The main essential oil components of Hypericum perforatum L. determined by GC-MS were caryophyllene (19,13 %), germacrene-D (13,22 %), α-Pinene (12,59 %) and β-helmspicapene (8,99 %). We also aim to determine the synergistic activity of these two natural products against the drug-resistant H. pylori strains. MIC values of Hypericum perforatum L. essential oils and glabridin against drug resistant H. pylori isolates were carried out by modifying the method described previously. In the experiments in which 1:1 ratio of Hypericum perforatum L. essential oils and glabridin was used, the MIC values (µg/mL) were as follows: 8 for standard strain; 4 for drug sensitive strain; 8 for metronidazole resistant strain; and 16 for clarithromycin resistant strains. In conclusion, it was found that Hypericum perforatum L. essential oils and glabridin had significant antimicrobial activity against HP strains in our study.

Keywords: Hypericum perforatum, glabridin, H. pylori, drug, resistant, strain

INTRODUCTION

Today, it is thought that about half of the world's population is colonized with H. pylori. Nearly all infected persons with H. pylori can develop gastritis and mild functional changes, peptic ulcer, ulcer complications, stomach cancer and maldoma (Makola Di Peura and Crowe, 2007).

Antibiotics such as Clarithromycin, Metronidazole, Amoxicillin and tetracycline are used for the treatment of H. pylori infections. For treatment, some of these drugs are given in combination. However, increased resistance to antibiotics has resulted in treatment failures. Therefore, treatment of drug-resistant H. pylori infections can lead to serious morbidity and mortality (Tonkic et al., 2012; Silva et al., 2012; Choi et al., 2018; We et al., 2014).

Therefore, new drug researches are being carried out intensively. Natural products such as plant extracts and essential oils are very important sources for new drug research (Silva et al., 2012). Natural products are very popular due to low cytotoxic and side effects. Among these natural products, Hypericum perforatum L. In addition, liquorice are two important medicinal plants.

Glabridin is one of the most commonly studied liquorice root flavonoids. Glabridin is a prenylized isoflavonoid derived from the root of G. glabra L. It has many pharmacological properties such as antioxidant, anti-inflammatory, antiatherogenic, estrogenic, neuroprotective, anti-inflammatory, anti-osteoporotic, skin whitening and regulating energy metabolism (Simmler et al., 2013).

In addition, glabridin has been reported to have many pharmacological activities such as cytoxic activity, antimicrobial activity, estrogenic and anti-proliferative activity against human breast cancer cells. It also affects melanogenesis, inflammation,
low-density lipoprotein oxidation and mitochondrial functions from oxidative stress (Choi, 2005).

Glabridin is known to have many pharmacological activities such as antimicrobial activity, antiproliferative effect and cytotoxic effect (Choi, 2005).

*Hypericum perforatum* L. has been widely used in folk medicine throughout history. Anticancerogenic, antimicrobial, antiproliferative and antioxidant efficacy of *Hypericum perforatum* L. has been reported in various studies (Kaçar and Özkan, 2004).

In this study, we aimed to investigate the antimicrobial activity of *Hypericum perforatum* L. and Glabridin against drug resistant *H. pylori* isolates. We also aim to determine the synergistic activity of these two natural products against the drug-resistant *H. pylori* strains.

**MATERIALS AND METHODS**

Experiments were performed by using drug sensitive and resistant bacteria. Also, HP NCTC (11637) strain was selected as the control strain. All clinical samples were isolated from the laboratory. Clarithromycin and metronidazole resistance of isolates were evaluated by E-test method (AB Biodisk, Sweden) (Fukazawa et al., 1999). In order to isolate, the samples were inoculated to Mueller Hinton agar with 5% sheep blood and HP agar. Then, the incubation of plates were performed at 37°C under microaerophilic conditions (Camy-Gen, Oxoid). The conventional techniques (gram staining, catalase, urease etc.) were used for the identification of *H. pylori* isolates (Owen, 1995). Standard HP NCTC (11637) strain was obtained from the Microbiology Department of Hacettepe University, Medical School.

**GC/MS Analysis**

Analysis of essential oil was performed using the Thermo Scientific Focusgas chromatograph equipped with a DSQ II single quadrupole mass spectrometer, Triplus autosampler and fused-silica capillary columnTR-5MS (5% phenyl-polysilphenylene-siloxane, 30 m×0.25 mm inner diameter, film thickness 0.25 μm). The injection volume was 2 μL. The samples were injected with a split ratio of 250:1 by using helium (99.99 %) as carrier gas, at a flow rate of 1 mL/min; ionization energy was 70 eV. The transfer line temperature of the mass spectrometer was 220°C, while the temperature of orifice injection was of 220°C. The temperature of oven was programmed in the range 50–220°C at a rate of 3°C/min. Data acquisition was made in the scanning mode. Identification was done on full scan mode in the m/z range of 50–650 a.m.u.

**Preparation of Bacterial Suspension**

Glabridin was purchased from Sigma (Sigma, USA). In order to solve glabridin, DMSO was selected as the solvent. MIC values of *Hypericum perforatum* L. essential oils and glabridin against drug resistant *H. pylori* isolates were carried out by modifying the method described by Imamura et al. For this purpose, the microdilution broth method was used. Clinical isolates and standard *H. pylori* strain were suspended in Brucella Broth (BBL 4311086) with 5% fetal calf serum (FCS). *Hypericum perforatum, Glabridin* and antibiotics were diluted from 0.5 to 256 μg/mL by 2-fold serial dilution. All bacterial strains were incubated in Brucella Broth agar containing 5% FCS at 37°C under microaerophilic conditions. The bacterial cell concentration in the experiments

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was adjusted to 1x10^8 cells/mL. MIC assays were evaluated at the end of 5 days of incubation (Hirschl and Makristathis, 2007).

**Determination of Minimum Bactericidal Concentration (MBC)**

Bacterial strains were diluted from 1024 to 0.5 µg/mL (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 µg/mL). MBC assay was studied according to method described by O’Mahnoy et al 2005. Briefly, 900 µL of solution containing different concentrations of *Hypericum perforatum* L. essential oils and Glabridin was added to 100 µg/ml of bacterial suspension (1x10^8 cells/mL) and incubated at the 37°C under microaerophilic conditions for 72 hours. At the end of incubation, 100 µL of each dilution was inoculated to *H. pylori* agar for growth. As positive controls, metronidazole (100 µg/mL), clarithromycin (10 µg/mL), amoxicillin (10 µg/mL), levofloxacin (20 µg/mL) and tetracycline (40 µg/mL) were selected. DMSO (1%; w/v) was used as negative control. All experiments were performed in triplicate.

**Antimicrobial Sensitivity Tests**

Resistance against clarithromycin and metronidazole in clinical isolates were determined by the E test method as described by Farshad et al. In the present study, the following values were accepted as resistance threshold: ≥8 µg/mL for metronidazole and ≥1 µg/mL for clarithromycin. HP NCTC 11637 was used as control strain.

The resistance threshold in this study was as follows: 8 µg/ml for metronidazole and 1 µg/ml for clarithromycin. HP NCTC 11637 was used as standard strain.

**Statistical Analysis**

Chi-square and Student's t-test were used to analyze of data. p≤0.05 was considered as statistically significant. All statistical analyses were performed by using SPSS for Windows Version 17.0.

**RESULTS**

Forty-four essential oil components, representing 98.6 %, were detected in the essential oil of *Hypericum perforatum* L. (Figure 1, Table 1). The main essential oil components were caryophyllene (19,13 %), germacrene-D (13,22 %),α-Pinene (12,59 %) and β-helmiscapene (8,99 %).

![GC/MS chromatogram of Hypericum perforatum L.](https://doi.org/10.24264/icams-2018.1.12)
Synergistic Activities of *Hypericum perforatum* L. and Glabridin against Drug Resistant *H. pylori* Isolates

Table 1. The main essential oil components of *Hypericum perforatum* L.

<table>
<thead>
<tr>
<th>RT</th>
<th>Compound Name</th>
<th>SI</th>
<th>RSI</th>
<th>Cas #</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,68</td>
<td>α-Pinene</td>
<td>991</td>
<td>992</td>
<td>80-56-8</td>
<td>12,59</td>
</tr>
<tr>
<td>21,45</td>
<td>trans-Caryophyllene</td>
<td>979</td>
<td>984</td>
<td>87-44-5</td>
<td>19,13</td>
</tr>
<tr>
<td>25,59</td>
<td>Germacrene D</td>
<td>968</td>
<td>974</td>
<td>23986-74-5</td>
<td>13,22</td>
</tr>
<tr>
<td>25,91</td>
<td>β-helmscapene</td>
<td>963</td>
<td>981</td>
<td>17066-67-0</td>
<td>8,99</td>
</tr>
<tr>
<td>26,12</td>
<td>α-helmscapene</td>
<td>952</td>
<td>960</td>
<td>473-13-2</td>
<td>5,47</td>
</tr>
<tr>
<td>34,89</td>
<td>Caryophyllene oxide</td>
<td>950</td>
<td>960</td>
<td>1139-30-6</td>
<td>5,26</td>
</tr>
</tbody>
</table>

Figure 2 presents results for *Hypericum perforatum* L. essential oils in *H. pylori* NCTC 11637 strain and clinical *H. pylori* strains found to be sensitive to antimicrobial agents. It was found that the essential oils showed significant bactericidal activity at 8 µg/mL for *H. pylori* NCTC 11637 strain, while MIC value was 16 µg/mL for the drug-sensitive clinical isolates. It was found that MIC value for drug-sensitive clinical isolates was significantly higher when compared to standard *H. pylori* strain (p<0.05).

Similarly, as shown Figure 2, when the efficacy of Glabridin against *H. pylori* is evaluated; the MIC value for the standard *H. pylori* strains (16 µg/ml) was lower than for drug-sensitive strains (32 µg/ml). A statistically significant difference was found between the MIC values obtained against the standard strain and the drug-sensitive strains (p<0.05).

It was found that *Hypericum perforatum* L. essential oils and glabridin had higher MIC values when compared to standard antibiotics (metronidazole, clarithromycin, amoxicillin, levofloxacin and tetracycline) used in HP treatment (Figure 3).

In our study, MIC value was found to be 8 µg/mL for metronidazole, 4 µg/mL for clarithromycin, 2 µg/mL for amoxicillin, 1 µg/mL for levofloxacin and 8 µg/mL for tetracycline whereas MIC value of glabridin was 64 µg/mL in metronidazole-resistant HP isolates. MIC value was found to be 4 µg/mL for metronidazole, 4 µg/mL for clarithromycin, 2 µg/mL for amoxicillin, 2 µg/mL for levofloxacin and 4 µg/mL for tetracycline whereas 32 µg/mL for glabridin in clarithromycin-resistant *H. pylori* isolates (Figure 3). Also, MIC value of *Hypericum perforatum* L. essential oils against *H. pylori* was 32 µg/mL in both metronidazole-resistant and clarithromycin-resistant HP isolates.

In the experiments in which 1:1 ratio of *Hypericum perforatum* L. essential oils and Glabridin was used, the MIC values were as follows: 8 for standard strain; 4 for drug sensitive strain; 8 for metronidazole resistant strain; and 16 for clarithromycin resistant strains (Figure 4).

![ MIC Values of standard drugs, *Hypericum perforatum* L. essential oils and Glabridin against the *H. pylori* isolates](https://doi.org/10.24264/icams-2018.1.12)
Today, treatment of individuals infected with drug-resistant *H. pylori* strains is extremely difficult. Our results show that both *Hypericum perforatum* L. essential oils and glabridin were quite effective against *H. pylori* colonizing nearly half of the world's population. In our study, MIC values of both *Hypericum perforatum* L. essential oils and glabridin against drug-sensitive *H. pylori* isolates of were found to be lower than drug-resistant strains.

Another important finding of our study was that the combined use of *Hypericum perforatum* L. essential oils and Glabridin was more potent against drug-resistant *H. pylori* strains. Further studies *in-vivo* are needed in this regard.

It should not be forgotten that the combined use of natural products which have antimicrobial effectiveness in combating drug-resistant strains may exhibit additive or synergistic effects.

In conclusion, it was found that *Hypericum perforatum* L. essential oils and Glabridin had significant antimicrobial activity against *H. pylori* strains in our study.
REFERENCES


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