Antioxidant activity, total phenolic and flavonoid content, and antibacterial effects of Stachys lavandulifolia Vahl. flowering shoots gathered from Isfahan

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ABSTRACT

Plants are a rich source of phenolic compounds and one of the most important nature-based antioxidants. The compounds derived from plant-based extracts are an important pharmaceutical resource. This study was conducted to investigate the antioxidant activity and total phenolic and flavonoid content, and to investigate the antibacterial effects of Stachys lavandulifolia flowering shoots extract. In this study, S. lavandulifolia was gathered from Isfahan and extracted by maceration using ethanol 70%. Total phenol content was measured by Folin-Ciocalteu reagent and gallic acid standard, and antioxidant activity was investigated by DPPH with reference to butylated hydroxytoluene (BHT). Antibacterial effects were investigated by Broth Microdilution and minimum inhibitory concentration and minimum bactericidal concentration were determined. The effects of different concentrations of S. lavandulifolia were investigated on Staphylococcus aureus and Enterococcus faecalis by disk diffusion with reference to vancomycin and nitrofurantoin. The findings demonstrated that the inhibition of DPPH free radicals was greater by hydroalcoholic S. aureus extract than BHT, and therefore the IC50 of this extract was lower than BHT. Total phenolic content was obtained 18.61 (mg gallic acid) and the flavonol and flavonoid content was obtained 2.42 and 8.93 mg/g, respectively. In this study, investigating the effects of different concentrations of hydroalcoholic S. lavandulifolia extract on pathogenic bacteria by disk diffusion and Broth Microdilution demonstrated that this extract exerted great inhibitory effects on both bacteria. S. aureus was more susceptible to S. lavandulifolia extract than E. faecalis.

KEY WORDS: Antioxidant activity, total phenolic and flavonoid content, antibacterial effects, Stachys lavandulifolia Vahl.

1. INTRODUCTION

The plants from family Laminaceae have long been used conventionally to treat gastrointestinal infections and bloat. Extract is one of the most important products derived from these plants, which is frequently used in traditional medicine, food and pharmaceutical industries, and perfumery (Tabatabaie yazdi, 2016). Stachys lavandulifolia Vahl. is a plant from family Lamiaceae that occurs in many regions of Iran, Turkey, and Iraq. This plant spontaneously occurs in several regions of Iran including Isfahan, Chaharmahal va Bakhtiari, Fars, and Lorestan provinces (Mohammadpour-Kanzaq, 2015). S. lavandulifolia is a perennial, short, hairy plant and has multiple stems more or less green or grayish, flowers are in a florescence and are pink, purple, and rarely white or yellowish (Mozaffarian, 2011). lavandulifolia is called variously in different provinces of Iran. Myrcene, alphapinene, gama-murolene, and ogneol are some of the compounds found in S. lavandulifolia essential oil (Aghaei Noroozloo, 2015). S. lavandulifolia is a tonic agent for stomach and is used to treat infections, asthma, and rheumatic diseases. Moreover, this plant can exert anti-anxiety effects and is effective in treating genital tumors, cancer-induced wounds, and inflammation (Taghkhania, 2012). Boiled S. lavandulifolia is used to treat certain diseases such as headache, diarrhea, gastrointestinal diseases, wound, cough, common cold, neuralgia, urinary and bile ducts stones, dyspepsia, bloat, skin infections, and fever (Mohammadpour-Kanzaq, 2015). Recently, medicinal plants have been investigated for antioxidant and antibacterial properties. Some studies have indicated that plants are rich resources of antioxidant and antibacterial compounds and contain large amounts of secondary metabolites including phenolic compounds, flavonols, flavonoids, glycosides, and alkaloids (Zarali, 2016). Flavonoids are the most well-known phenolic compounds with strong antioxidant properties. The protective effects of flavonoids in biological systems have been attributed to their antioxidant capacity, disposing free radicals, activating antioxidant enzymes, and reducing alpha-tocopherol radicals (Rafiee, 2012). Flavonoids can prevent platelets from accumulating and have anti-inflammatory, antibacterial, and antitumor properties (Dehghan, 2013). Phenolic compounds are an important group of plant-based products that are developed in response to environmental stresses. These compounds are able to neutralize free radicals and can act as donors of electron or hydrogen because of having hydroxyl groups (Nazari, 2013). Antioxidant compounds can prevent free radical reaction and decrease cell damage or death, cardiovascular diseases, and cancers (Aleebrahim-Dehkordy, 2016). Today, phytotherapy, as the use of plant-based products or herbal extracts, is a common approach worldwide. Given the side effects due to the use of chemical drugs and large
costs spent for mass production of synthetic drugs, the secondary compounds of medicinal plants can be suitable alternatives to synthetic drugs (Omidbaig, 2006). In this study, two gram-positive strains, Staphylococcus aureus and Enterococcus faecalis, were used to investigate the antibacterial effects of S. lavandulifolia. S. aureus is a gram-positive, catalase-producing bacterium which contributes significantly to the incidence of food poisoning, purulent, systemic, and nosocomial infections (Sepehri, 2015). abscesses, third-degree burn, trauma wounds, surgical incisions, bed sore, or atrophy wounds (Hamon-Navard, 2013). S. aureus anthrotoxin is heat resistant; therefore, S. aureus in foods is not removed by heat (Alizadeh Behbahani, 2014). Enterococci are the fourth leading cause of nosocomial infections and a common cause of urinary tract infections. The Enterococci with antibiotic resistance can colonize in gastrointestinal tract, and the Enterococci that are a constituent of natural flora of the body of patients with malignancy and those treated with broad-spectrum antibiotics are able to proliferate and cause disease (Borjjan-Borujeni, 2016). Plant extracts contain different organic compounds that can be used in food and pharmaceutical industries. Since the antimicrobial activities of plants are partly attributed to the presence of secondary metabolites such as flavonoids, phenols, and antioxidants, and given the increasing use and efficacy of these compounds in treating diseases, it is highly important to investigate the extracts derived from plants, especially the plants traditionally used in medicine. In addition, the studies conducted on the medicinal plants gathered from different regions have reported widely inconsistent findings. This may indicate that the plants of different regions may exert different therapeutic effects (Dehghan, 2013). This study was conducted to investigate the antioxidant activity, total phenolic and flavonoid content, and antibacterial effects of S. lavandulifolia gathered from Isfahan, central Iran.

2. MATERIAL AND METHODS

Flowering shoots of S. lavandulifolia were gathered from Isfahan and dried under natural conditions and aeration, after being identified with reference to botanical keys and Iranian flora, and Herbarium samples of the Medical Plants Research Center of the Shahrekord University of Medical Sciences. Extraction was done using maceration with ethanol 70% and extract concentration using rotary evaporator at 40°C. Antioxidant property was determined by DPPH using a spectrophotometer at 517 nm wavelength with reference to a synthetic antioxidant, butylated hydroxytoluene (BHT). The results were expressed as the percentage of inhibiting free radicals by the formula below, the graph plotted against the extracts concentration, and IC50 calculated.

**DPPH percentage of inhibiting:** control absorbance - sample absorbance/control absorbanceTo measure total phenolic content, Folin-Ciocalteu reagent was used. For this purpose, aluminium chloride 2% and sodium acetate 5% were used. To measure flavonoid compounds, aluminium chloride 2% and potassium acetate 5% were used. sodium carbonate was introduced and absorbance of the samples was read after 30 min at 765 nm wavelength. In this method, gallic acid was used as standard, the gallic acid standard curve plotted at 760 nm wavelength, and total concentration of the phenolic content of the extracts measured. To do microbial tests, two standard (ATCC) gram-positive bacteria, S. aureus ATCC 25923 and Enterococcus faecalis ATCC 29212, which were provided from Iranian Research Organization for Science and Technology as lyophilized were used. After culturing bacteria and isolating pure colony, we used 24 h isolates at growth phase to prepare a microbial suspension equal to 0.5 McFarland standard opacity to investigate strains susceptibility. To prepare Stok solution, different concentrations of hydroalcoholic S. lavandulifolia were prepared and DMSO 5% was used for dilution. To investigate antimicrobial effects, Broth Microdilution and Mueller-Hinton Broth base culture medium, which was prepared according to the manufacturer (Merck Co., Germany) instructions, were used. According to this method, the first well is considered as negative control and the second well as positive control. After introducing the culture medium, Stok solution, and bacteria into microplate wells and diluting them, we incubated the samples at 37°C for 24 h and the first well in which no opacity was developed was considered MIC. To determine MBC, all of the wells with no opacity were cultured on Blood agar medium separately, and the lowest concentration of the extract in which the bacteria could not grow was considered MBC. Moreover, to investigate antibacterial effects of S. lavandulifolia and compare these effects with those of vancomycin and nitrofurantoin, positive controls, disk diffusion was adopted. For this, paper sterile blank discs were kept in different prepared concentrations of S. lavandulifolia extract for 24 h to let the extract be completely absorbed by the disks. Then, the disks containing different concentrations of the extract were incubated at 37°C for one h to dry. The dried disks were placed on the plates containing cultured bacteria alongside the standard disks containing the antibiotics: vancomycin (30 g) and nitrofurantoin (300g) and then incubated at 37°C for 24 h to investigate the antibacterial property of each extract. The inhibition rate was measured by measuring the inhibition zone diameter with a ruler (in mm) and the results were compared with CLSI (2012).

3. RESULTS AND DISCUSSION

The inhibition rate of DPPH free radicals was compared between hydroalcoholic S. lavandulifolia and BHT (standard) (Table 1). The concentration of the extract that inhibited 50% of DPPH free radicals was obtained 98.8 mg/mL. Moreover, IC50 and inhibition rate of DPPH radicals of BHT was obtained 45.18 mg/mL (Table 1)
According to the findings, the inhibition of DPPH free radicals was obtained higher for hydroalcoholic \textit{S. lavandulifolia} extract than BHT, and therefore the IC50 of this extract was lower than BHT. Notably, \textit{S. lavandulifolia} extract is inversely associated with IC50; in other words, the more colorless the color of DPPH solution at presence of the plant, the lower the IC50 and hence the greater the antioxidant property. Table 1 shows the total amounts of flavonol, flavonoid, and phenolic compounds, antioxidant property, and BHT of \textit{Stachys lavandulifolia} Vahl. hydroalcoholic extract.

<table>
<thead>
<tr>
<th>Flavonol (mg/g)</th>
<th>Flavonoid (mg/g)</th>
<th>Total phenol (mg Gallic acid)</th>
<th>Antioxidant (IC50, %)</th>
<th>BHT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/42</td>
<td>8/93</td>
<td>18/61</td>
<td>8/98</td>
<td>18/45</td>
</tr>
</tbody>
</table>

Table 1.

Table 2. Inhibition zone diameter of \textit{Staphylococcus aureus} for different concentrations of \textit{Stachys lavandulifolia} compared to vancomycin

<table>
<thead>
<tr>
<th>Different concentrations of \textit{Stachys lavandulifolia} (µg/mL)</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>600</th>
<th>700</th>
<th>800</th>
<th>900</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition zone diameter (mL)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>17</td>
<td>18</td>
<td>20</td>
</tr>
</tbody>
</table>

Vancomycin = 16 mm (according to CLSI)

Table 3. Inhibition zone diameter of \textit{Enterococcus faecalis} for different concentrations of \textit{Stachys lavandulifolia} compared to nitrofurantoin

<table>
<thead>
<tr>
<th>Different concentrations of \textit{Stachys lavandulifolia} (µg/mL)</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>600</th>
<th>700</th>
<th>800</th>
<th>900</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition zone diameter (mL)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>15</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
</tbody>
</table>

Nitrofurantoin = 18 mm (according to CLSI)

Many studies recently have been conducted on the effects of different plant species on bacteria (Rafieian-Kopaei, 2013; Ghasemi-pirbalouti, 2015). These studies confirmed the inhibitory effects of the studied plants on bacteria. In this study, the effects of hydroalcoholic \textit{S. lavandulifolia} extract were investigated on \textit{S. aureus} and \textit{E. faecalis}. Antimicrobial properties of \textit{S. lavandulifolia} essential oil and extract have already been investigated by...
Various methods (Shahnama, 2015) Taheri et al studied the antibacterial effects of aqueous, ethanolic, and methanolic (80%) S. lavandulifolia extract on S. aureus, Escherichia coli, and P. aeruginosa using disk diffusion and determination of MIC and MBC, and observed no antibacterial effects for aqueous extract, but ethanolic and methanolic (80%) extracts exerted anti-inhibitory effects on growth (Taheri, 2013). Some investigations have indicated that another species of Stachys genus, inflate, exerts antimicrobial effects on E. coli, S. aureus, P. aeruginosa, and Salmonella (Meshkibaf, 2010). An important explanation of medicinal plants inhibiting bacteria is the presence of phenolic compounds in their essential oils and extracts, which enable them play an effective role, thanks to their hydrophobicity, in decomposing cell and mitochondrial membrane lipids and changing membrane permeability and hence bacterial cell death through bonding with amino groups and hydroxylamine proteins (Tavassoli, 2011). Many plants contain phenolic compounds. As well, the phenolic content was investigated in S. lavandulifolia in this study. Phenolic compounds have antibacterial effects. Moreover, the number of hydroxyl groups is directly associated with their toxicity on microorganisms. The structures of flavonoids and flavonols are phenolic with antibacterial effects. In addition to exerting antimicrobial activities, these compounds are highly useful because of having antioxidant properties (Sharafati-Chaleshtori, 2010). It can be inferred that most of the antibacterial compounds identified in medicinal plants are aromatic or saturated organic compounds with greater solvency in methanolic solvents such as methanol and ethanol. However, difference in antibacterial effects may be related to different compounds identified in different medicinal plants (Hamon-Navard, 2013). Regarding MBC and MIC, hydroalcoholic S. lavandulifolia extract exerted acceptable inhibitory and bactericidal effects on S. aureus and E. faecalis of which S. aureus was more susceptible to S. lavandulifolia extract (Table 4).

Table 4. MIC and MBC (μg/mL) of hydroalcoholic Stachys lavandulifolia extract gathered from Isfahan

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>MIC</th>
<th>MBC</th>
</tr>
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<tbody>
<tr>
<td>Staphylococcus aureus (ATCC 25923)</td>
<td>300</td>
<td>600</td>
</tr>
<tr>
<td>Enterococcus faecalis (ATCC 29212)</td>
<td>400</td>
<td>800</td>
</tr>
</tbody>
</table>

The difference in the effects due to S. lavandulifolia extract between S. aureus and E. faecalis can be related to difference in structures of these two bacteria, the type of culture medium used, and extraction method, and the compounds identified in S. lavandulifolia (Tajkarim, 2010). Moreover, this work confirmed that the antibacterial activity of S. lavandulifolia was associated with phenolic, flavonolic, and flavonoid compounds.

4. CONCLUSION

Overall, hydroalcoholic S. lavandulifolia extract has a high antioxidant property and large amounts of phenolic, flavonolic, and flavonoid compounds, and can inhibit DPPH free radicals. Because antioxidant compounds of plants can exert protective effects on the body’s cells, S. lavandulifolia extract can be considered an effective factor for human beings’ health and used as a nature-based antioxidant to prevent and treat diseases. Besides that, S. lavandulifolia extract can be replaced partly with synthetic antioxidants to reduce the risk of liver damage and development of cancer using nature-based antioxidants. Besides that, this study demonstrated that hydroalcoholic S. lavandulifolia extract had antibacterial effects on S. aureus and E. faecalis strains. This finding requires further investigations so that other effective concentration of S. lavandulifolia extract can be studied on these two bacteria and clinical strains. In the light of climatic diversity and occurrence of plants in different regions in Iran, a great deal of attention should be paid to identification of the best species in terms of the amounts of effective substances, because climatic conditions, the type of soil and gathered plant in each province can influence the amounts of effective compounds and antibacterial properties of the plants. Regarding the findings of this work, we can argue that hydroalcoholic extract of S. lavandulifolia gathered from Isfahan is a rich source of phenolic, flavonolic, flavonoid, and antioxidant compounds which can be used in food, pharmaceutical, and cosmetic industries, as an inhibitory agent of bacterial growth, and a suitable alternative to antibiotics and synthetic drugs.

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