Keywords: Hypericum perforatum L.; Microwave assisted extraction; Ethanol extracts; Antioxidant activity; Folín-Ciocalteu; Flavonoids; Cardiac glycosides

Introduction
Free radicals are chemical species one or more unpaired electrons due which are extremely unstable and destroy other molecules by extraction of electrons to gain stability. The typical reactive oxygen species (ROS) are nitric oxide (NO), peroxyl radical (ROO) highly reactive hydroxyl radical (OH), hydrogen peroxide (H$_2$O$_2$), superoxide anion (O$_2^-$), and peroxyxynitrite anion (ONOO$^{-}$). Free radicals are in the body, as they are needed to supply detoxification, energy chemical signaling, and immune function [1-3]. Free radicals can trigger the bio molecules oxidation such as protein, amino acids, lipid, and DNA, causing cell damage and stimulating many illnesses. Oxidative stress can start by an imbalance between antioxidants and reactive oxygen species causing cellular damage; oxidative stress is the origin of numerous illnesses like age related diseases, cancer, cataracts, and Parkinson’s. Antioxidants reduce the oxidative stress in cells and may improve many illnesses like cancer, cardiovascular illnesses, and inflammatory diseases [4-6]. Oxidative stress can be caused by an imbalance between reactive oxygen species and antioxidants resulting in cellular damage, and oxidative stress is the major of many diseases as cataracts, cancer, age related diseases, and Parkinson’s disease. Antioxidants decrease the oxidative stress in cells and are beneficial for the improvement of many diseases such as cardiovascular diseases, cancer and inflammatory diseases. This activity occurs because of the capability of antioxidants to decrease oxidative stress through scavenging or neutralizing of reactive species via hydrogen donation [7-9]. The medicinal plants, as potential sources of drugs are rich sources of secondary metabolites including glycosides, steroids, alkaloids and flavonoids. Almost, one third of the pharmaceuticals are plant origin. Since all plants can synthesize a great value of organic molecules /phytochemicals, they are called secondary metabolites [10]. Plants derived compounds have crucial impact different clinically beneficial drugs. Phytochemicals are bioactive compounds which are found in plants that work with dietary fiber and nutrients to protect the body against diseases. They are non-nutritive compounds. These phytochemicals are the secondary metabolites which are found in small quantities in higher plants and contain flavonoids, terpenoids, alkaloids, steroids, tannins, cardiac glycosides, quinones and phlobatannins. This study may contribute to drugs development to cure different diseases.

Antioxidant Activity and Phytochemical Screening of Flowers and Leaves of Hypericum perforatum L. Ethanolic Extracts from Tonekabon-Iran
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Abstract
Medicinal plants are an important source of phytochemicals that offer traditional medicinal treatment of various ailments. This research set to assess phytochemicals in the ethanolic extracts of Hypericum perforatum L. leaves and flowers by quantitative and qualitative screening procedures. The Hypericum perforatum L. flowers and leaves were gathered, and the extract was provided from ethanol (5%) by microwave assisted extraction (MAE). The phytochemical assessment was done applying standard methods & the phytochemical evaluation by using standard methods. The total phenolic contents of ethanolic extracts were estimated by Folín-Ciocalteau method and total flavonoids contents were determined by the Aluminium Chloride Colorimetric method. Ethanolic extracts invitro antioxidant activity was assessed by via evaluating 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging activity by the standard method. Ethanolic extracts from flowers and leaves of Hypericum perforatum L. showed total phenolic contents of (15.32 ± 0.07) and (7.39 ± 0.43) mg GAE/g dry plant material respectively. Total flavonoid contents of ethanolic extracts from leaves and flowers of Hypericum perforatum L. were (1.09 ± 0.08) and (0.38 ± 0.05) mg QE/g dry plant material, respectively. The antioxidant activity of the investigated ethanolic extract of leaves and flowers of Hypericum perforatum L. were scavenging ability of DPPH radical scavenging activity and IC$_{50}$ value (89.45% to 2.15 ± 0.02) and (74.77% to 1.96 ± 0.06) mg/ml respectively. The ethanolic extract of leaves and flowers of Hypericum perforatum L. contains terpenoids, flavonoids, phenols, tannins, cardiac glycosides, quinones and phlobatannins. This study may contribute to drugs development to cure different diseases.

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Received November 09, 2017; Accepted November 30, 2017; Published December 08, 2017


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Material and Method

All chemicals and reagents had analytical grade with utmost purity. Fresh plant flowers and leaves of Hypericum perforatum L. were collected from Se hezār (N 36° 37′, E 50° 50′; at 2650 m altitude) Tonekabon, Iran, in April 2015 and the plant was identified by Fariba serpooshan in Hebarium (Islamic Azad University - Tonekabon Branch). Then leaves and flowers dried and made powder. 10 g of powders of leaves and flowers of Hypericum perforatum L. were combined with 5% ethanol exposed to microwave irradiation at 300 W; suspension was radiated in microwave oven (model GE280S) at regular intervals (10 min irradiation and 3 min off). After extracting, the extract was cooled down to room temperature to keep room temperature. Then extract was filtered use Whatman’s Filter No. 1 filter paper. The solvent was evaporated under vacuum in a rotary evaporator (model LABOROTA 4001) and finally, stored in refrigerator at 40°C till further use.

The ethanolic extracts of leaves and flowers of Hypericum perforatum L. was following subjected to various chemical experiments to detect various phytoconstituents by standard methods [33-37]. Qualitative phytochemical analysis of ethanolic extracts of leaves and flowers of Hypericum perforatum L. were done after the standard methods. Few drops of 1% NH3 solution was mixed with the extract in a test tube. Yellow coloration was seen for flavonoids. 2-3 ml of the extract was treated with 10% aqueous FeCl3, drops, and blue green color was detected.

In this experiment, 2-3 ml of extract was treated with drops of 10% aqueous FeCl3 and emergence of blue green color was observed. 1 ml of the extract and 2 ml of chloroform were mixed. Nearly 3 ml of conc. H2SO4 was transferred with care from the sides of test tube. Reddish brown coloration at interface showed terpenoids presence. For this purpose, the mixture including 0.5 ml of extract solution, 1 ml of distilled water prepared and 1-2 drops of ferric chloride solution were added and brownish green or a blue black coloration checked. 2 ml of extract and 3 drops of copper acetate solution were mixed. Emerald green solution shows the existence of Di-terpenes. 1 ml of extract was treated with 10% aqueous FeCl3 drops, and blue green color was detected.

Based on the protocol, in a test tube, 5 ml of extract was mixed with 5 ml of distilled water and heated. The stable foam formation into interface layer, manifested a deoxysugar feature of cardenolides. A violet ring may be seen below the brown ring; however, a greenish ring can be made gradually across thin layer in the acetic acid layer. To determine phlobatannins, 2 ml of extract was mixed with 2 ml of 1% HCl, and then it was boiled. A red precipitate showed the presence of phlobatannins. The phytochemicals which are present in the plant material, inducing their quantitative estimation and detecting various phytoconstituents by standard methods [33-37].

The initial phytochemical experiments may chemical constituents in the plant material, inducing their quantitative estimation and locating the origin of pharmacologically active chemical compound. Qualitative analysis of phytochemical compounds like saponins, tannins, flavonoids, terpenoids, phenols, coumarins, quinones, cardiac glycosides, quinones and phlobatannins were examined in the ethanolic extracts of flowers and leaves of Hypericum perforatum L. In present study, we have found that most of the biologically active phytochemicals were present in the ethanolic, extracts of flowers and leaves of Hypericum perforatum L. (Table 1) [44-48].

<table>
<thead>
<tr>
<th>No.</th>
<th>Phytochemical Constituents</th>
<th>Flowers</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Di-Terpenes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Coumarins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Quinones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Phlobatannins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) positive, (-) Negative, (*): Few and (ND): Not defined

Table 1: In vitro qualitative phytochemical analysis of ethanolic extract of flowers of Hypericum perforatum L.
The plants screening for medicinal value has been done using many experiments and preliminary phytochemical analyses. Phytochemical screening is very important in identification of novel sources of industrially and therapeutically valuable compounds with medicinal significance, to efficiently use natural wealth available. These phytochemicals have anti-oxidants, anti-inflammatory, anti-cancer, anti-bacterial, anti-diabetic, allergic, anti-coronary, antiseptic, analgesic, anti-arithmetic, sedative, anesthetic, hypo-cholesterolemic, and hepatoprotective activities [49-58].

Table 2: In vitro quantitative phytochemical analysis of ethanolic extract of flowers of Hypericum perforatum L.

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>Total Phenol Contents (mg GAE/g)</th>
<th>Total Flavonoid Contents (mg QE/g)</th>
<th>Radical Scavenging Activity (%)</th>
<th>IC₅₀ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers</td>
<td>7.39 ± 0.43</td>
<td>0.38 ± 0.05</td>
<td>74.77</td>
<td>1.96 ± 0.06</td>
</tr>
<tr>
<td>Leaves</td>
<td>15.32 ± 0.07</td>
<td>1.09 ± 0.08</td>
<td>89.45</td>
<td>2.15 ± 0.02</td>
</tr>
</tbody>
</table>

The radical scavenging activity (%) and IC₅₀, ethanolic extracts of flowers of Hypericum perforatum L. for DPPH radical scavenging activity were 74.77%, 1.96 ± 0.06 mg/ml. But, the radical scavenging activity (%) and IC₅₀, ethanolic extracts of leaves of Hypericum perforatum L. for DPPH radical scavenging activity were 89.45% and 2.15 ± 0.02 mg/ml, while that of ascorbic acid used as the reference control was (0.0116) mg/ml (Table 2).

Discussion

Medicinal plants have become very popular because they have very few side effects as compared to synthetic drugs. Phytochemicals compounds are widely studied because they are highly abundance in nature and often used as parts of defense mechanisms in plants. The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study. The initial phytochemical experiments may chemical constituents in the plant material, inducing their quantitative estimation and locating the origin of pharmacologically active chemical compound [59-61]. Terpenoids have been implicated in antibacterial and anti-neoepileptic functions hence the use of the plant to treat burns, skin diseases and insect stings [62-64]. The initial screening experiments can be useful beneficial in detecting the bioactive compounds and can bring about the drug development and discovery (phytoestrogens related to relief of menopausal signs, reducing osteoporosis, improving blood cholesterol levels, and reducing the risk of some hormone-related cancer and coronary heart illnesses. Also, several flavonoids and cardiac glycosides possess hypoglycemic activities [65]. Our results showed Hypericum perforatum L. from Tonekabon has high level of flavonoids which can be used for herb based therapy. In addition, Polyphenols can treat stomach and kidney ailments and protect and prevent against numerous degenerative illnesses and pathological processes like coronary heart disease, ageing degenerative illnesses, neurodegenerative disorders, Alzheimer’s disease, and atherosclerosis cataracts [66]. Tannins may have potential value such as cytotoxic, anti-cancer agents and hasten the healing of wound and inflamed mucous membranes [67,68]. Coumarins raise the flow of blood in the veins and reduces capillary permeability and has anti-apoptotic feature [69]. Saponins can precipitate and coagulate red blood cells; they also possess cholesterol binding features. Formation of foams in aqueous solutions, hemolytic activity [70] and traditionally saponins are widely employed as molluscides and detergents, in addition to their industrial usages as surface active agents and foaming, and they also have useful health impacts [71]. Saponins are applied in hyperglycemia, anti-oxidant, anti-inflammatory, anti-cancer, and hyper-cholesterolemia weight loss etc. [72,73]. Cardiac glycosides have severe toxicity since they may influence the heart and atrial fibrillation [74]. Among many quinone compounds such as natural compounds; the naphthoquinone derivatives in particular, possess a broad variety of bioactivities. Potency as anti-inflammatory agent, anti-asthma medicine, anti-allergic agent, medicine, anti-dragon goe medicine, bronchodilator, thrombus prevention, and hypotension are seen in their derivatives [75]. Also, plants generate numerous secondary metabolites with antioxidant capacity. Antioxidants obstruct the function of free radicals implicated in the aging process and the pathogenesis of several illnesses. A crucial role is played by free radicals in monitoring different biological processes essential for the body. They can implicate cell-signaling mechanism in the body. Revealing the free radicals are essential, but at the same time, they are detrimental harmful for the body. Thus, it has several mechanisms to reduce free radical stimulated injury. The injury was repaired by many enzymes such as superoxide dismutase, catalase and etc. [76-78]. Additionally, antioxidants are critical for these defense mechanisms; some compounds containing vitamin A, vitamin E, vitamin C, and polyphenols basically show these tasks.

Conclusion

The findings revealed that the ethanolic extracts of leaves and flowers of Hypericum perforatum L. have medicinally crucial bioactive agents, justifying its usage in the traditional medication for treating various illnesses.

Acknowledgement

We gratefully acknowledge the financial support from the research council of Tonekabon Branch Islamic Azad University.

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