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# Investigation of Antioxidant Properties of *Polygonatum orientale* Desf and *Tilia dasystyla* Extracts by Different Methods and Solvents

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## Abstract

**Background:** Free radicals cause many diseases in humans. Antioxidants reduce the risk of cardiovascular disease and stroke by neutralizing free radicals and on the other hand, prevent the progression of cancer. The natural antioxidant enhances the antioxidant properties of plasma to prevent diseases such as heart disease, cancer, and stroke. Secondary metabolites derived from plants have a strong potential for antioxidant activity.

**Methods:** The purpose of this study was to investigate the antioxidant activities of two native plants species, including *Polygonatum orientale* Desf and *Tilia dasystyla* extracts, which are obtained with three different solvents (water, ethanol, and methanol) and two extraction methods (sonication and water bath) by DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Potential) methods. This study is the first research to investigate the antioxidant properties of *Polygonatum orientale* Desf and *Tilia dasystyla*.

**Results:** The results showed that methanol solvent had a higher efficiency among the other solvents in extracting of antioxidant compounds. Also, antioxidant level of *Tilia dasystyla* was higher than *Polygonatum oriental* Desf. In this regard, the highest FRAP values of *Tilia dasystyla* belonged to methanol solvent and water bath method with the amount of 2.72 $\pm$ 0.36 (mM). Whereas the highest FRAP values of *Polygonatum orientae* Desf belonged to methanol solvent and sonication method with the amount of 0.147 $\pm$ 0.01 (mM).

**Conclusions:** Both plants showed antioxidant properties but *Tilia dasystyla* had higher antioxidant activities than *Polygonatum orientale* Desf. Methanol was more effective solvent in extracting antioxidant compounds and also water bath method was generally an effective method for extraction.

*Keywords:* *Polygonatum orientale* Desf, *Tilia dasystyla*, Antioxidant, Frap, DPPH

## 1. Background

Reactive oxygen species may cause oxidative damage that may lead to various chronic diseases, such as cancer and heart disease (1, 2).

Cancer is the second reason for death worldwide and it is increasing every day. Cancer may have different causes such as environmental factors and lifestyle that lead to the production of free radicals in the human body (3, 4).

Prescription medicines are not preferred today because of the high cost and their unwanted side effects, such as many side effects of chemotherapy in cancer treatment. Patients are looking for herbal and other natural medicine for the cure and treatment of diseases (5, 6).

Today, several studies have shown the increase of using herbal medicines for the treatment of cancer (7).

Many Studies have shown the anti-oxidative activities of the components related to herbal medicine that prevent lipid peroxidation and have anti-cancer properties due to their antioxidant compounds. Antioxidants stop the formation of reactive oxygen species by inhibiting enzymes or forming the elements involved in the production of free radicals and neutralizing free radicals. Plants polyphenols are a group of antioxidants. These compounds are often abundant in plants and contain phenolic acids, flavonoids, tannins, and lignans. The antioxidant properties of the plants depend on the amount of polyphenolic compounds. In addition to phenolic compounds, other compounds such as ascorbic acid, phytic acid, tocopherol, carotenoids, and saponins also contribute to antioxidant activity (8-11).

"Shaghaghol" is a plant in the north of Iran. *Polygonatum orientale* Desf is the scientific name of Shaghaghol. This species belongs to the asparagus family (Aspara-

gaceae). Solomon seal is another common name for this plant. Its rhizome has some medical properties such as healing wounds, kidney stone treatment, gynecological disorders, anti-gout and rheumatism, aphrodisiac, and anti-diabetic (12).

*Tilia* is a genus in the family of *Tiliaceae* that has thirty species, this plant is domestic throughout the northern hemisphere. Medicinal potentials of the flowers and leaves of *Tilia* species (Linden) make them valuable plants in pharmacology researches (13, 14).

Extraction of phenolic compounds is influenced by their chemical nature, the method of extraction, particle size, solvent type, and the presence of interfering substances.

The selection of the appropriate extraction method and solvent depends on the different parts of the plant and its ingredients. Also, proper extraction method and solvent can increase the concentration of effective compounds and antioxidants (15).

## 2. Objectives

In this study, the antioxidant properties of *Polygonatum orientale* Desf and *Tilia dasystyla* extracts, which is obtained with three different solvents and two extraction methods (sonication and water bath), were investigated. The antioxidant properties of these two species have not been reported to date.

## 3. Methods

### 3.1. Chemicals and Reagents

2, 4, 6-Tris (2-pyridyl) -1, 3, 5-triazine (TPTZ), hydrogen chloride, Iron (III) chloride, sodium acetate, Iron (II) sulfate, ascorbic acid, DPPH (2, 2-Diphenyl-1-Picrylhydrazyl), methanol, ethanol, and other chemicals were purchased from Merck company.

### 3.2. Plant Material

*Polygonatum orientale* Desf and *Tilia dasystyla* species were purchased from Bagh Firuze, Tehran, Iran and were approved at Al-Zahra University herbarium. The plant material (herbs) were washed with water, air-dried in the laboratory, and were grinded into powder. In this study, the leaf of *Tilia dasystyla* and rhizome of *Polygonatum orientale* Desf were used for investigation.

### 3.3. Sample Preparation

#### 3.3.1. Sonication Method

A ground dried sample of 0.1 g was weighted. The compounds of samples wereextracted with 10 mL 80% aqueous methanol and 10 mL 80% aqueous ethanol and 10 mL distilled water on an ultrasonic bath (ultrasonic cleaner set, model: WUC-A0H) for 20 min, then the extracts were centrifuged for 15 min at 3000 rpm (16).

#### 3.3.2. Water Bath Method

Another method of extraction was as follows: 0.1g of dried sample was added to 10 mL of 80% aqueous methanol and 10 mL 80% of aqueous ethanol and 10 mL distilled water, the samples were extracted by boiling in water bath for 60 min at a temperature of 70°C, then the extracts were centrifuged for 20 min at 2000 rpm (4).

### 3.4. Total Antioxidant Assay

#### 3.4.1. The Ferric Reducing Antioxidant Potential

The Ferric reducing antioxidant power (FRAP) reagent was prepared freshly by adding 10 mM of 2,4,6-Tris (2-pyridyl) -1, 3, 5-triazine (TPTZ) Dissolved with 40 mM of HCl, 20 mM of $FeCl_3$ in water, and 300 mM of acetate buffer (pH 3.6) in the ratio of 1:1:10. A blank only contained sample and solvents.

Then, 100 $\mu$L sample was added to 3 mL of FRAP reagent after incubating for 10 min at 37°C, the absorbance of the mixture was measured at 593 nm. The absorbance of the samples was compared with a FeSO4 standard curve (17).

### 3.5. DPPH Radical Scavenging Assay (DPPH)

Here, 100 $\mu$L of each extracts with different concentrations (0.25, 0.5, and 1 mg/mL) were added to 2 mL of DPPH (0.5 mM in absolute ethanol), respectively. The mixtures were shaken and kept at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. Ascorbic acid was used as the reference standard and the results compared with the standard. The percent of inhibition (I %) was calculated by this formula:

$I\% = ((\text{Abs control-Abs sample})/\text{Abs control}) \times 100$

Also, $IC_{50}$ value, which represented the concentration of the sample that caused 50% inhibition, was determined (18).

### 3.6. Statistical Analysis

Statistical analysis of variance was performed using ANOVA test by SPSS software version 24 and P value $< 0.05$ was considered significant. Data are expressed as means $\pm$ standard deviation.

## 4. Results

### 4.1. Free Radical Scavenging Capacity

#### 4.1.1. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measures the capability of a molecule to reduce the ferric 2, 4, 6-tripyridyl-striazine complex to the colored-ferrous complex (17).

### 4.2. DPPH Radical Scavenging Activity Assay (DPPH)

The principle of this approach is based on the free radical recovery of DPPH by antioxidants in the absence of other free radicals in the environment. The result of this reaction is to generate color in an environment that is measurable using spectroscopy.

DPPH is a stable radical having a violet color that shows the highest absorbance at 519 - 595 nm. The basis of this approach is that DPPH radicals act as an electron receiver from a donor molecule such as antioxidants. As a result, in a reaction between DPPH and antioxidant, the purple color of DPPH becomes yellow and the absorbance of this yellow complex was measured at 595 nm. The result is normally expressed as $IC_{50}$ value, defining as the concentration of antioxidant that causes a 50% decrease in the DPPH absorbance (19, 20).

The results showed that FRAP values were high in methanol extracts samples. This indicated that methanol extract was more efficient in extracting antioxidants of plant compounds compared with both ethanol and water. *Tilia dasystyla* had higher FRAP values than *Polygonatum orientale* Desf. Also, sonication method was more efficient in extraction of the antioxidant compounds of *Polygonatum orientale* Desf extract and water bath method was efficient method in extraction of *Tilia dasystyla*. According to the results that have shown in the charts (Figures 1-4), the higher FRAP value in *Tilia dasystyla* belonged to methanol extract and water bath method with the value of $2.72 \pm 0.36$ mM and for *Polygonatum orientale* Desf belonged to methanol extract and sonication method with the value $0.147 \pm 0.01$ mM. The results of DPPH method almost confirmed the results of FRAP method.

There was a significant difference between the FRAP values of methanol extract and water extract of *Tilia dasystyla* and *Polygonatum orientale* Desf ($P < 0.05$) Also, there was a significant difference between the FRAP values in methods of water bath and sonication in both *Tilia dasystyla* and *Polygonatum orientale* Desf ($P < 0.05$).

## 5. Discussion

In plants, antioxidants are very important because of their activities in the neutralizing of free radicals (19). The higher antioxidant activity is shown in a lower $IC_{50}$. All extracts were able to scavenge DPPH free radicals. The $IC_{50}$ of ascorbic acid as a standard was $0.87 \pm 0.08$ mg/mL that the $IC_{50}$ values of *Tilia dasystyla* were almost near to $IC_{50}$ value of ascorbic acid.

In another study conducted by wong et al. (2012), the $IC_{50}$ value of *polygonatum odoratum* was determined by DPPH method and the $IC_{50}$ value of ethanol extract was $2496.44 \pm 27.52$ (mg dry matter/mL) (21). The study of wang et al. (2013) showed methanol extract had a higher antioxidant activity than water extract and the study of Buřičová and Reblova showed water extract of *Tilia* cordata had a higher antioxidant activity with the value of $63.0 \pm 3.8$ (mg/g) (22, 23).

According to the study of Rice-Evans et al. (1996), there is a direct correlation between phenolic and flavonoid compounds. The amount of polyphenol compounds increase, antioxidant properties also will increase (24).

The water bath method was the most efficient in extracting of phenolic and flavonoid compounds due to the heat; the reason might be the decomposition of heavy polyphenols to a variety of phenolic compounds with less molecular weight (25).

In addition, the higher antioxidant activities of extracts, which heat was used in their extraction method, may be due to the appearance of the third structure of the proteins because of their partial denaturation in extracts by heat. In this way, the presence of amino acid residues with sulfur (cysteine and methionine) or aromatic side chains (tryptophan, tyrosine, phenylalanine) is increased and they have antioxidant properties because they easily can release protons (26).

In the extraction of 13 types of fruits and vegetables, the effect of five solvents, methanol, ethanol, acetone, and hexane was investigated; The results showed the highest efficiency of extraction belonged to water and methanol solvents (27).

In the study of Sun and Ho, antioxidant properties of Buckwheat were investigated by different solvents (acetone, butanol, ethanol, methanol, ethyl acetate). The results of their studies showed that the highest extraction efficiency was related to methanol extract (28).

In extracting of cinnamon by the Soxhlet and cold solvent methods, the methanol solvent efficiency was higher in the extraction of antioxidant compounds than acetone solvent (29).

The extraction of antioxidant compounds from barley seeds with three acetone, ethanol, and methanol solvents showed that the maximum extraction yield belonged to methanol extract (30).

The results of the study on the Plum showed that the most suitable solvent for the extraction of phenolic and

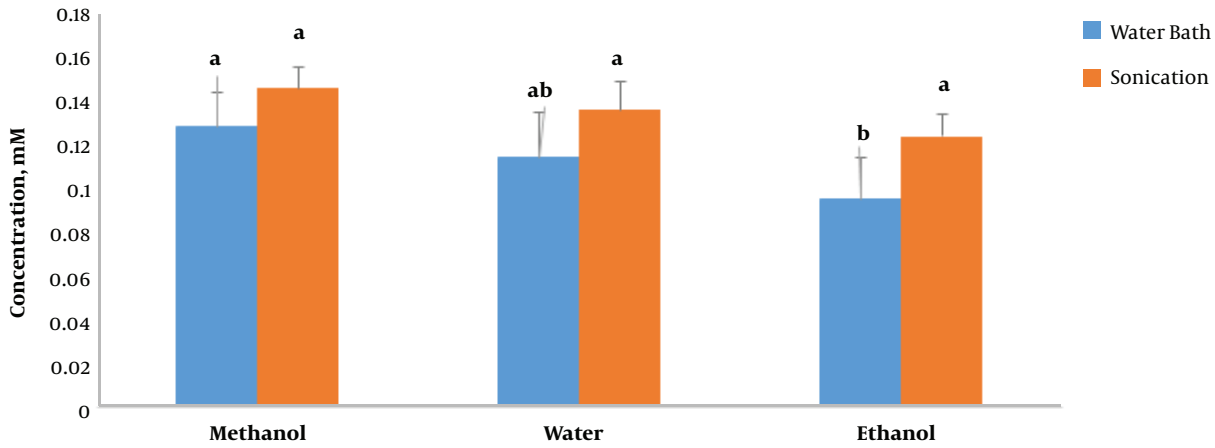


**Figure 1.** FRAP values of *Polygonatum orientale* Desf with different solvents and methods of extraction. The experiment was performed in triplicate and expressed as mean ± SD. Values in each column marked with different letters showed significant differences ($P < 0.005$).

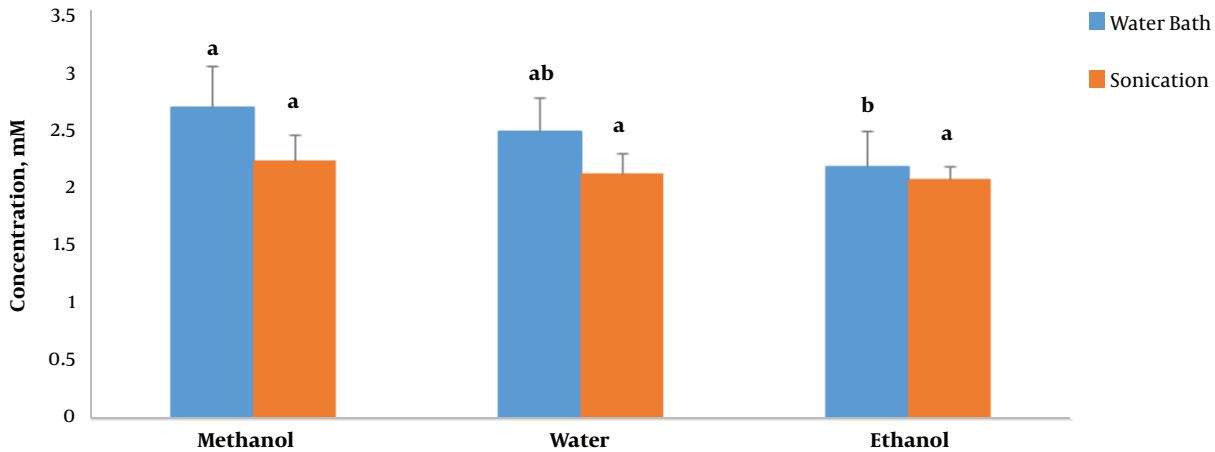


**Figure 2.** FRAP values of *Tilia dasystyla* with different solvents and methods of extraction. The experiment was performed in triplicate and expressed as mean ± SD. Values in each column marked with different letters showed significant differences ($P < 0.05$).

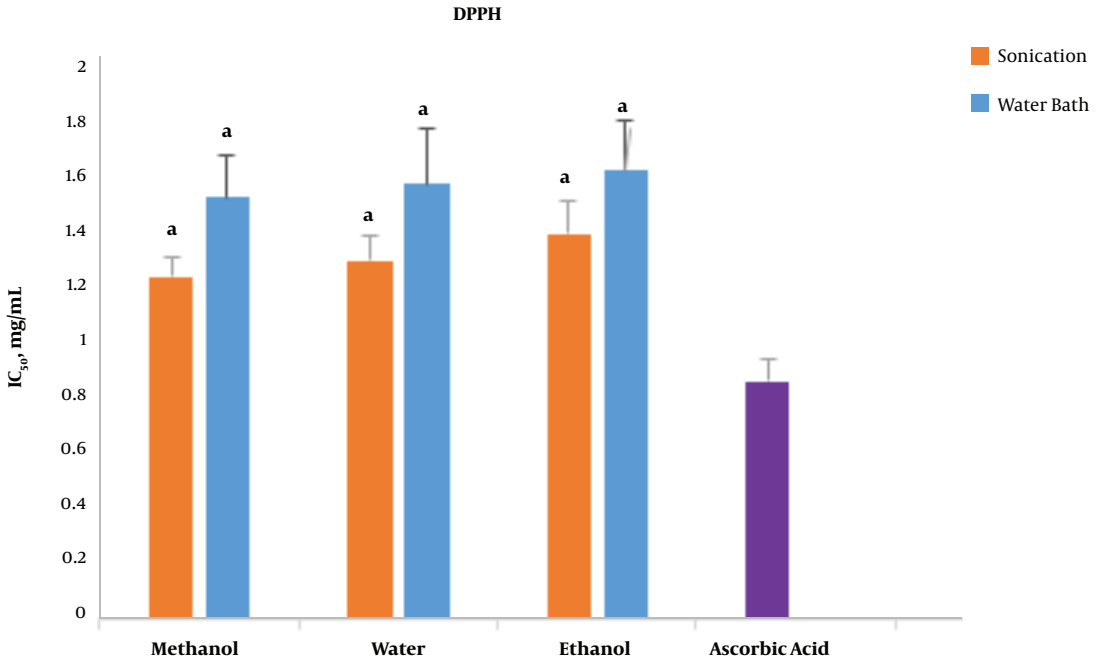


**Figure 3.** DPPH values of *Polygonatum orientale* Desf with different solvents and methods of extraction. The experiment was performed in triplicate and expressed as mean ± SD. Values in each column marked with different letters showed significant differences ($P < 0.05$).

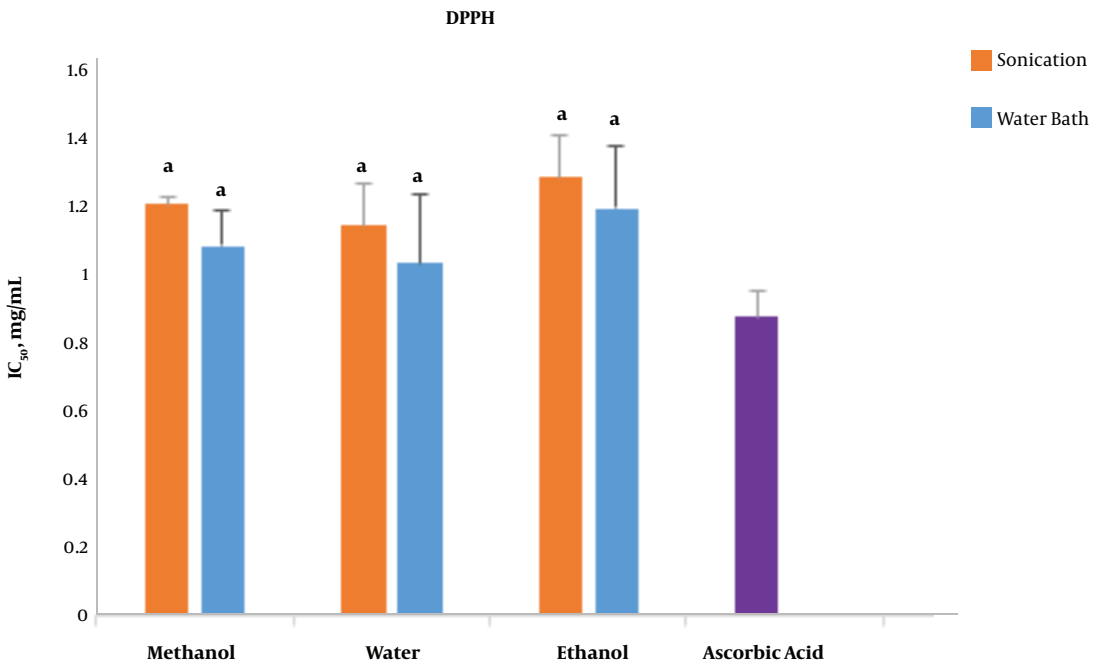


**Figure 4.** DPPH values of *Tilia dasystyla* with different solvents and methods of extraction. The experiment was performed in triplicate and expressed as mean ± SD. Values in each column marked with different letters showed significant differences ($P < 0.05$).

flavonoids from this plant is methanol solvent (31).

The results of this study showed that solvents with different chemical compositions and polarities have the ability to extract various plant compounds that affect its antioxidant properties. Extracting phenolic compounds using polar solvents is significantly more efficient.

Since the phenols have a polar structure, they are further solved with polar solvents. Methanol and water solvents are more effective in extracting low molecular weight phenols (15).

In this study, aqueous methanol solvent was more effective in the extraction of antioxidant compounds.

### *5.1. Conclusions*

Both plants showed antioxidant properties. The higher antioxidant activities belonged to *Tilia dasystyla* extract with methanol solvent and water bath extraction method. The results indicated that methanol solvent was more effective in extracting antioxidant compounds and also water bath method was generally an effective method for extraction. It is suggested that the effect of other solvents and extraction methods on the amount of antioxidant compounds can be further investigated in these two plants. In this study, aqueous methanol was more efficient than other solvents in the extraction of antioxidant compounds.

## Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

## Footnotes

**Authors' Contribution:** Study concept and design: Parichehr Hanachi and Roshanak Zarringhalami. Analysis and interpretation of data: Parichehr Hanachi and Roshanak Zarringhalami. Drafting of the manuscript: Roshanak Zarringhalami and Parichehr Hanachi. Critical revision of the manuscript for important intellectual content: Parichehr Hanachi, Roshanak Zarringhalami, and Reihaneh Ramezani Tamijani. Statistical analysis: Roshanak Zarringhalami.

**Conflict of Interests:** The authors have no conflict of interests.

**Ethical Considerations:** This study does not need any ethical considerations.

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