Optimum Conditions for Oleuropein Extraction from Olive Leaves

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Abstract

In this study the effect of extraction solvent (type, composition, pH, and temperature), and the extraction method (maceration and soxhlet) on the amount of oleuropein extracted from olive leaves obtained from West Bank /Palestine was investigated. It was found that pure solvents (100% water, 100% methanol, and 100% ethanol) are not good solvents for oleuropein extraction, while mixtures of the solvents (methanol/water and ethanol/water) gave higher oleuropein content. It was found that 80% ethanol give the highest oleuropein content followed by 20% acetonitrile. Temperature of extraction was found to have a significant effect on the oleuropein content where higher temperature gave higher oleuropein content. It was found also that the amount of oleuropein extracted decreases with increase in pH where highest amount was obtained at pH 3. Soxhlet extraction was found to give higher oleuropein content compared to maceration method.

Keywords: Oleuropein, extraction, olive leaves, maceration, soxhlet
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1. Introduction

Olive leaves are a source of many phytochemicals like phenolics and flavonoids which possess many activities e.g. antioxidant, antibacterial, antifungal..etc. (Eberhardt, and Liu, 2000). Interest in phytochemical content and antioxidant activity of olive leaves is increasing in recent years. The main characteristic of antioxidant compounds is their ability to trap free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Prakash, Rigelhof, and MIller, 2011).

Oleuropein is the major phenolic compound in olive leaves and varies from 17% to 23% depending upon the harvesting time of the leaves (Le Toutour, and Guedon, 1992). In addition to oleuropein, different compounds present also in olive leaves e.g. hydroxytyrosol, tyrosol, caffeic acid, verbascoside, rutin, luteolin 7-O-glucoside, luteolin 4-O-glucoside, apigenin-7-O-rutinoside and apigenin 7-O-glucoside.

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Different parameters affect extraction of oleuropein from olive leaves incuding method of extraction, extraction solvent (type, composition, pH, tempearature), and time of extraction. Regarding extraction method, soxhlet, super critical fluid extraction, liquid- liquid extraction, and dynamic ultrasound-assisted extraction can be used. In the scientific literature, different studies dealing with oleuropein content of olive leaves came out of different countries (Ansari, Kazemipour, and Fathi. 2011; Tayoub et al. 2012; Ranalli et al. 2006; Aouidi et al. 2012; Ortega-García et al. 2008; Altinyay, and Altun. 2006; Amiot, Fleuriet, and Macheix. 1989; Andreadou et al. 2006; Bisignano et al. 1999; Perridis, Therios, and Samouris. 2012; Savournin et al. 2001).

Ansari et al. optimized a green and inexpensive water-based method of extraction of oleuropein from Iranian olive leaves, and found that deionised water adjusted to pH 3, at 60 °C for 4 hours had the highest amount of oleuropein extracted (Ansari, Kazemipour, and Fathi. 2011). Tayoub et al. (2012) has determined oleuropein in leaves of some Syrian olive varieties. Ranalli et al. (2006) has investigated the factors affecting the contents of oleuropein in olives from Italy. Aouidia et al. (2012) has studied quantitative determination of oleuropein in olive leaves from Tunisia using mid-infrared spectroscopy combined with chemometric analyses. However, to date, the scientific literature does not contain any report that deals with oleuropein content of olive leaves from Palestine. The aim of the current work is to determine the oleuropein content of olive leaves from Palestine, and to study the effect of different extraction parameters of oleuropein from olive leaves in Palestine. These parameters include type of extraction solvent and its composition, temperature and pH of extraction solvent, and type of extraction method (maceration and Soxhlet). Additionally, study of the oleuropein content from Palestine constitutes a valuable addition to the available literature.

2. Materials and Methods

2.1 Samples Collection

Olive leaves samples were obtained from trees localized in the sunshine area of grassland in West Bank /Palestine. The collection was directly from the trees in the middle of November 2012.

2.2 Sample Preparation

Fresh olive leaves were dried at ambient temperature, and grinded to obtain olive powder which was stored at room temperature in dark until extraction.

2.3 Chemicals

Oleuropein (40%) which used as a standard was obtained from Chengdu Biopurity Phytochemicals Ltd (China). Chromatographic grade-double distilled water, HPLC grade acetonitrile (Merck), analytical grade acetic acid, ethanol, methanol were obtained from Sigma-Aldrich company.

2.4 Extraction Procedures

2.4.1 Simple Green Extraction Method

Ten grams of olive leaves powder were macerated in 100 ml solvent for 4 hours. The solvent mixtures used for the extraction were: deionized water at various pHs (3, 5, 7, 9) (adjusted with Hydrochloric acid solution (0.1N) or sodium hydroxide (0.1N)) at 60°C and at ambient temperature. The extracts were then filtered through a Whatman No.1 filter (Whatman, UK) to separate coarse particles. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at 2-8°C until used.

2.4.2 Simple Extraction (Maceration) Method with Organic Solvents

Ten grams of olive leaves powder were macerated in 100 mL of different solvents for 4 hours. The solvents used for the extraction were: 100% methanol, 80% methanol, 50% ethanol, 80% ethanol, 100% ethanol, and 20% acetoniltrile at ambient temperature. The extracts were then filtered through a Whatman No.1 filter (Whatman, UK). The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum, and the concentrated extracts were stored in a refrigerator at 2-8°C until used.

2.4.3 Soxhlet Extraction

15 grams of olive leaves sample were placed in the thimble of Soxhlet apparatus and extracted with 300 ml of different solvents for 4 hours. The solvents used for the extraction are: 80 % ethanol, 20% acetonitrile at 60 ◦C. Extract were cooled to room temperature, and filtered.

The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at 2-8°C until used.

2.5 Determination of Oleuropein in Olive Leaf Extracts by HPLC

For determination of oleuropein from olive leaf extract, reversed phase HPLC method was used with silca-based C_{18} bonded phase column (C_{18}, 250mm × 4.6 ID) with mobile phase consisting of a mixture of water and acetonitrile (80/20 volume ratio) containing 1% acetic acid at a flow rate of 1.0 mL/min. UV detector at 240 nm was used for oleuropein determination.

The injection volume used is 20.0 µl for both standard and sample solutions. Identification of oleuropein in olive leaves extracts was based on retention times in comparison with standard of oleuropein. The quantitation was carried out using external standard method. The concentration of oleuropein was calculated using peak area and the calibration curves obtained from oleuropein standard solution. The amount of oleuropein was expressed as milligram per gram of olive leaf powder.

2.6 Statistical Analysis

Three samples of olive leaves extract of each treatment were independently analyzed in each sampling, and all of the determinations were carried out in triplicate. The results are expressed as means ± standard deviations. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of solvent type, pH, temperature, and extraction method were carried out using the GLM procedure, treating main factors (solvent type, pH, temperature, and extraction method) separately using one-way analysis of variance (ANOVA). The Bonferroni procedure was employed with multiple t-tests in order to maintain an experiment-wise of 5%.

3. Results and Discussion

3.1 Effect of Extraction Solvent on Oleuropein Content

The effect of extraction solvent on oleuropein content was investigated by extracting of a fixed quantity of olive leaves in different solvents (types) and using different composition (volume fraction). To this end, seven extraction procedures were performed (each time using 10 gram of olive leaves) using 100 ml of seven extraction solvents (water, 80%methanol, 100% methanol, 50% ethanol, 80% ethanol,100% ethanol, and 20% acetonitrile) for 4 hours. Results (Figure 1) showed that the highest oleuropein content was obtained when the olive leaves are extracted with 80% ethanol (13 mg/g) followed by 20% acetonitrile (10.0 mg/g), and 80% methanol (5.31 mg/g), and 50% ethanol (2.57 mg/g). Regarding pure water, pure methanol and pure ethanol, the amount of oleuropein extracted using these solvents is low (0.16, 0.10, and 0.02 mg/g for water, methanol and ethanol respectively). Statistically there is a significant difference in the oleuropein content with change of solvent (type and composition) which is indicated by different small letters (a, b, c, d, e, f, and g).

These results show that mixture of an organic solvent with water is needed to effectively extract oleuropein from olive leaves as very low amounts of oleuropein was extracted using pure solvents compared to solvent mixtures. These results showed that using water as co-solvent with organic solvents increase the amount of oleuropein extracted from olive leaves. Additionally, the solvent mixtures are better as they deactivate the enzymes which are responsible for conversion of olueropein into other compounds which have high protein-denaturing, and protein-cross linking activities (Fransisca et.al., 2008).

3.2 Effect of Temperature on Oleuropein Content

The effect of temperature of extraction solvent was studied by extracting of a fixed quantity of olive leaves in 100 ml of water at different temperatures (25, 40, and 60^{o}C) for 4 hours. Table 1 shows that oleuropein content increase with increasing temperature of extraction solvent where there is 18 fold increase in oleuropein content as temperature increased from 25^{o}C to 40^{o}C, and 43 fold increase as temperature increased from 25^{o}C to 60^{o}C. This can be attributed to increase in solubility of oleuropein with increasing temperature. Statistically there is a significant difference in the oleuropein content with increase of temperature (indicated by different small letters a, b, c).

3.3 Effect of pH on the Extraction of Oleuropein

The effect of pH of extraction solvent on oleuropein content was studied by extracting fixed amount of olive leaves (10 gram) in 100 ml distilled water (at room temperature for 4 hours) adjusted to different pH's (3, 5, 7, and 9, pH adjusted with 0.1N hydrochloric acid or 0.1N sodium hydroxide). Results showed that the highest amount of oleuropein was got when olive leaves are extracted with water at acidic medium, pH 3 (6.85 ± 0.17 mg/g). With increase of pH to 5, oleuropein content was sharply reduced (0.26 ± 0.02mg/g) which is only 3.8% of oluropein amount extracted at pH 3, and with further increase of pH to 7 and 9, oleuropein content further decreased sharply (0.16 ± 0.01, and 0.09 ± 0.01 mg/g, respectively). This decrease in oleuropein content with pH increase can be attributed to the ionization of the hydroxyl groups of oleuropein with pH increase which results in poor oleuropein recovery from the leaves. Statistically there is a significant difference in the amount of oleuropein extracted in water with different pH's indicated by different small letters a, b, c).

3.4 Effect of Type of Extraction on the Oleuropein Content

The effect of two extraction methods: maceration and soxhlet on the oleuropein content in olive leaves was studied by extraction of fixed amount of olive leaves (15 grams) using Soxhlet and maceration methods using two types of solvents (80% ethanol and 20% acetonitrile). Results showed that soxhlet method gave higher amount of oleuropein compared to maceration using the two solvents studied (Table 2). Statistically there is a significant difference between the oleuropein content extracted with the two extraction methods which is indicated by different small letters (a, b) within the same row. Soxhlet is more effective compared to maceration since olive leaves are exposed more to the solvent resulting in high amounts of oleuropein extracted.

Conclusion

Oleuropein is one of the most important phenolic compounds found in olive leaves. The extraction solvent, temperature, pH and type of extraction method are important parameters in the recovery of oleuropein from olive leaves. Mixtures of organic solvents (water/ethanol, water/acetonitrile, and water/methanol) give higher oleuropein content compared to pure solvents e.g. water, methanol, ethanol. Temperature and pH also affect significantly the content of oleuropein extracted from olive leaves where higher temperature and acidic pH is required condition of extraction procedure to get high amount of oleuropein from olive leaves. Soxhlet is more efficient method for oleuropein recovery from olive leaves and gives significant higher amounts of oleuropein compared to conventional maceration method.

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Table 1: Oleuropein Content in Olive Leaves Extracted with Water at Different Temperatures. Different Small Letters within Each Column Indicate Significant Difference ($P < 0.05$, $N = 3$)

Temperature (°C)	Oleuropein Content (mg/g)	Percentage
25°C	0.16 ± 0.13 c	0.016%
40°C	2.84 ± 0.24 b	0.28%
60°C	6.84 ± 0.31 a	0.68%

Table 2: Oleuropein Content in Olive Leaves Extracted by Maceration and Soxhlet Methods

Solvent mixtures	Oleuropein content (mg/g)	
	Soxhlet method	Maceration method
80%ethanol	19.0 ± 0.66 a	13.0 ± 0.52 b
20%acetonitrile	15.6 ± 0.32 a	10.0 ± 0.69 b

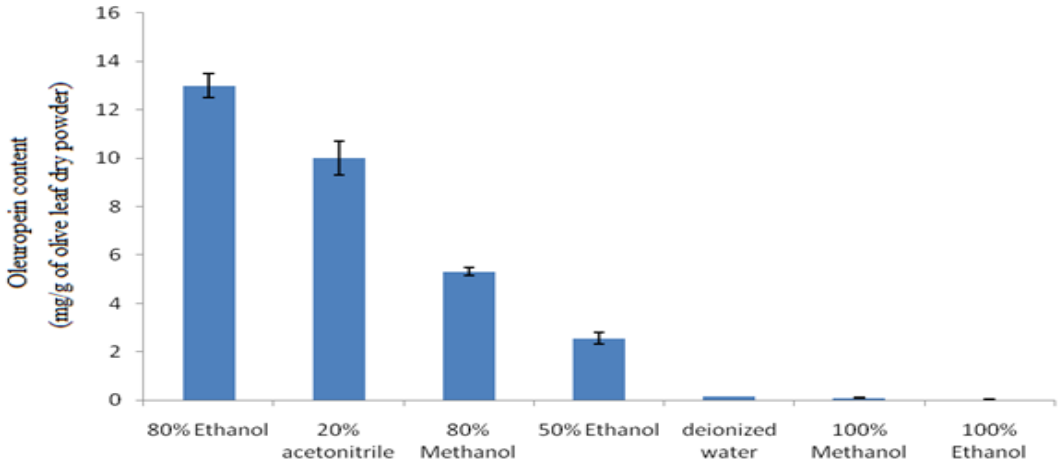

Figure1: Oleuropein content in olive leaves extracted by using different solvents.