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Research Article

Determination of The Concentration of Chemical Elements in The Walnut Septum Using Inductively Coupled Plasma Optical Emission Spectrometry

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Abstract

This article provides a comprehensive investigation into the chemical composition of the walnut (Juglans regia L.) fruit septum, specifically focusing on the methodologies for preparing dry ash mass from the laminae and the subsequent extraction processes from the resulting ash. The analytical study was conducted using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), which enabled the screening of 68 distinct chemical elements. The findings reveal that among the elements analysed, 35 chemical elements exhibited concentrations exceeding the Limit of Detection (<LOD). Notably, five specific macro-elements were identified in substantial quantities, demonstrating an increasing concentration gradient in the order of Na < P < Mg < K < Ca. Given that the walnut septum is exceptionally rich in these 35 macro- and micro-elements, the study provides strategic recommendations for its utilisation as an effective and promising raw material in the pharmaceutical industry, particularly for the development and production of therapeutic food supplements and nutraceuticals.

Keywords

Walnut fruit septum, micro-elements and macro-elements, ICP-OES method, limit of detection, dry ash mass, ash extract, pharmaceutics, therapeutic food supplements.

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INTRODUCTION

The walnut (Juglans regia L.) is an exceptionally widespread and renowned plant species throughout Central Asia, where it thrives indigenously within mountainous regions. Beyond its wild populations, the plant is extensively cultivated in domestic gardens and commercial orchards, maintaining a historical presence that dates back to ancient civilisations. Within the framework of modern scientific medicine, the walnut continues to hold significant pharmacological value. Its seeds are traditionally consumed in both their mature and immature states, while the timber remains a highly prized material for premium furniture manufacture. Historically, all components of the plant have been utilised for medicinal purposes, a practice that persists in contemporary phytotherapy [4].

The leaves of the walnut are characterised by a complex biochemical profile, containing iodine, α- and β-hydrojuglone, polyphenols, glycosides, flavonoids, vitamin C, carotene, vitamin B1, essential oils, and trace amounts of tannins [3, 2]. Furthermore, the foliar tissues contain various organic acids, including hydrocinnamic, caffeine, chlorogenic, ferulic, β-coumaric, and synaptic acids [9]. Advanced phytochemical analyses have also identified megastigmanic, tetralonic, phenylpropanoid, neolignan, and juglonic glycosides, along with derivatives of megastigmanic glycosides (juglanosides A–K) and tetralonic glycosides (juglanosides J–O) [15].

Phenolic compounds identified within the leaves include:

– Quercetin-3-orgamnoside;

– Quercetin-3-O-arabinoside;

– Quercetin-3-O-xyloside;

– Kaempferol-O-pentoside;

– Quercetin-3-O-galactoside;

– Kaempferol-opentoside [18].

The essential oil extracted from the leaves is predominantly composed of eugenol (27.5%), methyl salicylate (16.2%), and various sesquiterpenes such as germacrene D (21.4%) and (E)-beta-farnesene (8.2%). Additionally, monoterpenes such as α-pinene (15.1%), β-pinene (30.5%), β-caryophyllene (15.5%), and limonene (3.6%) have been documented [14, 13].

The floral components of the walnut contain a diverse array of chemical constituents, including:

– 5,6,11,12-tetrahydropyrrolo[1',2':1,2]azepino[4,5-b]indole-3-carbaldehyde;

– (±)-5,6,7,11c-tetrahydro-1H-indolizino[7,8-b]indol-3(2H)-one;

– (±)-9-hydroxy-5-oxo-2,3,4,5-tetrahydro-1H-benzo[b]azepine-2-carboxamide;

– 5-(ethoxymethyl)-1-(4-hydroxyphenethyl)-1H-pyrrole-2-carbaldehyde;

– (±)-5,8-dihydroxy-4-(1H-indol-3-yl)-3,4-dihydronaphthalen-1(2H)-one;

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– (±)-4-(6-amino-9H-purin-9-yl)-5,8-dihydroxy-3,4-dihydronaphthalen-1(2H)-one;

– (±)-4-(6-amino-9H-purin-9-yl)-5-hydroxy-3,4-dihydronaphthalen-1(2H)-one [10].

Furthermore, researchers have isolated 4,5,8-trihydroxy-alpha-tetralone-5-O-beta-D-glucopyranoside, 4,5-dihydroxy-alpha-tetralone-4-O-beta-D-glucopyranoside, 5-hydroxy-4-methoxytetralone, 5-hydroxy-1,4-naphthoquinone, rutin, vanillin, and the 2,3-dihydroxypropyl ester of tetrasanoic acid [12].

The green husk (pericarp) of the walnut is a rich source of pentacyclic triterpenes, sesquiterpenes, tetralones, naphthoquinones, phenolic acids, diarylheptanoids, neolignans, flavonoids, phenylethanoids, and tannins [16]. It also contains:

– Salidroside;

– (6S, 9S)-roseoside and (6S, 9R)-roseoside;

– Blumenol C glucoside;

– Byzantionoside B;

– 5-hydroxy-2-methoxy-1,4-naphthoquinone;

– Gallic acid;

– Various glycerol derivatives, including 1-(9Z-octadecenoate)-2-(9Z, 12Z-octadecadienoate)-3-(9Z, 12Z, 15Z-octadecatrienoate) [11].

Notably, the vitamin C content in the walnut pericarp exceeds that found in rosehips [4]. The walnut kernels themselves contain up to 75% lipids, alongside amino acids reaching concentrations of 6000 mg%, vitamin E, □-carotene, vitamin C, and essential mineral salts of K, Ca, Mg, S, and P [1, 8]. The primary fatty acid in walnut oil is linoleic acid (omega-6), a polyunsaturated fatty acid accounting for 54.64% of the total acid content, followed by oleic acid (28.72%), which is a monounsaturated fatty acid [6].

The kernels also contain the characteristic pigment juglone and a significant mineral profile:

– Iron: 5.1 ± 1.3 mg / 100 g;

– Zinc: 3.2 ± 0.9 mg / 100 g;

– Copper: 1.0 ± 0.26 mg / 100 g;

– Manganese: 3.9 ± 1.1 mg / 100 g;

– Nickel: 0.21 ± 0.08 mg / 100 g;

– Cobalt: 7.5 ± 2.5 µg / 100 g;

– Chromium: 7.0 ± 0.88 µg / 100 g [5].

The plant is also notably rich in fluoride salts. Within the walnut fruit septa (internal partitions), researchers have identified traces of iodine, alkaloids, glycosides, tannins, and various organic substances [7]. Specific compounds isolated from the walnut septum include (+)-dehydrovomifoliol, (6R,9R)-9-hydroxymegastigman-4-en-3-on, dihydrophaseic acid, blumenol B, and (4S)-4-hydroxy-1-tetralon [17]. It is also significant to note that immature fruits can contain vitamin C concentrations as high as 2500 mg% [4].

Experimental section

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Preparation of the working solution from walnut septum samples. The sample preparation was conducted using the dry ashing method. Initially, the walnut septum samples were pre-dried and finely ground. A 1 g portion of the prepared sample was precisely weighed using an analytical balance (Navigatortm, OHAUS®) with an accuracy of 0.001 g. The sample was then placed in a porcelain crucible and subjected to thermal decomposition in a muffle furnace (Nabertherm, Germany). The ashing protocol involved a stepwise heating procedure:

– Heating to 95°C for 30 minutes;

– Incremental heating to 120°C for 60 minutes;

– Progressive heating to 300°C for 120 minutes;

– Final heating to 550°C for 60 minutes.

The samples were maintained at a constant temperature of 550°C for a duration of 5 hours to ensure complete combustion. To the resulting ash, 3 ml of 70% HNO3 (ICP-MS grade, Sigma Aldrich, USA) and 2 ml of 60% H2O2 were added. The mixture was heated on a hot plate within a fume cupboard until the emission of white smoke ceased. Once cooled, the solution was quantitatively transferred into a 100 ml polypropylene volumetric flask and diluted to the graduation mark using ultra-pure water. The final working solution was filtered through a 0.45 µm syringe filter prior to instrumental analysis.

Preparation of standard solutions

To ensure analytical accuracy, a series of standard working solutions for 69 chemical elements were prepared in a 2% HNO3 matrix. The following certified reference materials were utilised:

– A multi-element standard solution containing 68 elements at a concentration of 10 mg/l in 2% HNO3 (High-purity standards, USA);

– A standard solution containing 4 elements at 1000 mg/l in 2% HNO3 (CPAchem, Sweden);

– A multi-element standard containing 20 elements at 1000 mg/l in 5% HNO3 (CPAchem, Sweden).

Using these concentrated standards and 70% HNO3 (Sigma AccuStandard, USA), six distinct levels of standard working solutions were prepared through serial dilution. A 2% HNO3 solution was employed as the blank sample. Calibration curves were constructed for all 69 elements, and the analysis proceeded only when the coefficient of determination (R2) for each calibration curve exceeded 0.995.

Analytical procedure

The elemental analysis was performed using an iCAP PRO X Duo Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) manufactured by Thermo Fisher Scientific (USA). Method development, system control, and data processing were managed via the Qtegra ISDS software platform. The operational parameters for the analytical method are detailed in Table 1 below.

Table 1. Operational Parameters of the Analytical Method

Parameter	Settings

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Pump tubing	Tygon® yellow/white (for sample)	Tygon® white/white (for drainage)
Pump speed	45 rpm	
Spray chamber	Glass cyclonic	
Nebuliser	Glass concentric	
Nebuliser gas flow	0.6 L·min^{-1}	
Coolant gas flow	12.5 L·min^{-1}	
Auxiliary gas flow	0.5 L·min^{-1}	
Centre tube diameter	2 mm	
RF power	1150 W	
Replicates	5 times	
Integration time	Axial	Radial
	15 sec	15 sec

Results and Discussion

Taking into consideration the research led by V.A. Litvinskiy and other Russian scientists, it is noted that the regulatory documents of the Russian Federation necessitate the determination of copper content through both dry and wet ashing for atomic emission spectrophotometric analysis. Consequently, they proposed an integrated approach involving sample preparation followed by analysis using modern, high-precision microwave-assisted inductively coupled plasma atomic emission spectroscopy [19].

Determination of micro- and macro-element concentrations in sample powder via icp-oes. In this study, we successfully determined the concentration of micro- and macro-elements within the ash derived from the combustion of walnut fruit septa grown under the environmental conditions of the Andijan region using the ICP-OES method. To achieve this, an extract prepared from 1 g of the walnut septum ash was analysed, and the subsequent elemental concentrations were calculated per 100 g of the original sample. During the experimental procedures, the presence of 68 chemical elements was screened based on the prepared multi-element standard solutions. Table 2 below presents only those chemical elements whose concentrations were found to be above the Limit of Detection (<LOD).

Table 2. Results of the determination of chemical elements in the walnut septum using the ICP-OES method, µg/100 g.

Al	B	Ba	Ca	Cr	Cs	Cu	Fe
106.22±9	3285.84 ±32	818.45 ±7	928876.31 ±5862	16.76±1	1623.58± 282	253.54 ±0	1418.97 ±16

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Ga	Hf	In	Ir	K	La	Li	Mg
14.75±26	3.87±1	35.99±10	7.28±4	536686.62±3721	0.7±1	356.75±2	183113.94±2442
Mn	Na	Nb	Nd	P	Pb	Pd	Pt
1221.3±5	13540.49±86	4.45±2	49.48±10	34884.09±23	0.45±7	2.48±7	15.89±7
Rb	Re	Si	Sn	Sr	Th	Ti	Tl
343.54±302	157.15±6	403.52±8	458.72±6	6723.36±23	30.04±7	15.46±2	1.49±4
U	V	Zn					
22.54±31	64.37±6	862.66±2					

Based on the data presented in the table, it can be established that the largest proportions of chemical elements per 100 g of the sample are comprised of the following: 13,540.49 ± 86 µg of Na, 34,884.09 ± 23 µg of P, 183,113.94 ± 2,442 µg of Mg, 536,686.62 ± 3,721 µg of K, and 928,876.31 ± 5,862 µg of Ca.

Conclusion

The comprehensive analytical investigation conducted in this study demonstrates that the walnut (Juglans regia L.) fruit septum is an exceptional reservoir of essential mineral constituents and trace elements. Through the application of high-precision Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), several critical scientific conclusions have been established:

– **Elemental Diversity and Profiling**: Out of the 68 chemical elements screened during the analysis, 35 elements were identified at concentrations significantly exceeding the Limit of Detection (<LOD). This diverse elemental profile underscores the biological complexity of the walnut septum and its potential as a multi-mineral source.

– **Macro-element Hierarchical Distribution**: The study identified a dominant cluster of five macro-elements that constitute the bulk of the mineral mass. Their concentrations follow a distinct ascending hierarchy: Na < P < Mg < K < Ca. The prevalence of Calcium (Ca) and Potassium (K) as the most abundant elements highlights the potential of this material in supporting bone density and cardiovascular homeostasis.

– **Pharmaceutical and Nutraceutical Potential**: Given the rich presence of 35 distinct macro- and micro-elements, the walnut fruit septum represents a highly viable and cost-effective raw material for the pharmaceutical industry. It offers

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a natural substrate for the formulation of advanced dietary supplements, mineral-fortified functional foods, and therapeutic additives aimed at addressing micronutrient deficiencies.

– **Industrial and Ecological Implications**: The rational utilisation of the walnut septum—often treated as a secondary byproduct of walnut processing—presents an opportunity for "zero-waste" agricultural technology. Developing standardisation protocols for this raw material could significantly enhance the production of indigenous, bio-available mineral complexes.

In summary, the walnut fruit septum is not merely a botanical byproduct but a sophisticated mineral matrix. Its high concentration of essential elements, combined with its natural origin, positions it as a promising candidate for further pharmacological standardisation and large-scale application in the health and wellness sectors.

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