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Effect of microwave irradiation on polyphenolic compounds from *Satureja hortensis* L.

Research Article

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Abstract: Summer savory (*Satureja hortensis* L.) is most often used as a culinary herb, but it also has medicinal benefits. The extracts from control and irradiated savory were obtained by ultrasound extraction for 30 minutes in an ethanol – water (80:20, v/v) mixture. Polyphenolic compounds from savory were identified and characterized by high-performance liquid chromatography coupled with a photodiode array detector and mass spectrometer. The separation was performed using an Altima C18 column (100×3 mm, 3 μm) and as mobile phase two solvent mixture: A – acetonitrile and B – water-formic acid (99.9:0.1, v/v). Peaks were identified with authentic standards in accordance to retention time, UV spectra and molecular mass. It was identified as caffeic acid, rosmarinic acid, luteolin, naringenin and apigenin. A quantitative determination of polyphenolic compounds was performed applying the external standard method. Our study showed large quantitative differences between the control plant and the irradiated plant.

Keywords: *Satureja hortensis L. • Polyphenolic compounds • HPLC • Microwave • Irradiation effects on plant growth*

1. Introduction

The progress in molecular biology and genetic manipulation offers a promising perspective to improving the biosynthesis of secondary compounds that act on regulatory genes. Recent studies indicate that the presence of microwave irradiation has an effect on living systems, even at power levels below the norms [1-4].

Marc Tafforeau and his collaborators showed an increase in meristerms in flax seedlings that were exposed to microwave radiation for a single exposure of 2 hours at a radiation emitted by a GSM. Also, they investigated flax seedlings subjected to radiation at 105 GHz from a Gunn oscillator. The result was the same for the seedling irradiation with a GSM [5].

Davies [6] reported an increase in weight and size for radishes exposed to a weak electromagnetic field of 60 Hz and 40 μT. There was a decrease in cell division in three types of algae [7] subject to an electromagnetic field of 7.8 Hz, amplitude varying between 50 and 200 μT.

Cotton seeds were exposed to microwaves at 45 kW and 2450 MHz for four minutes at a temperature of 94°C. At the end of the experiment, the seed temperature was 76°C; the, microwave treatment caused a decrease in humidity by 20%. Immediately after the microwave experiment, there was no difference in the total soluble protein content, quality or color of oil from the seeds treated with microwave compared with those untreated [8]. The effect of different radiation types (gamma, UV, X-ray, electron beam) on phytochemicals from plant was studied [9,10].

The consumption of products/dietary supplements phytochemicals, particularly polyphenolic compounds is correlated with a number of beneficial effects in the body, such as reducing the risk of diabetes, obesity, heart disease, colon cancer and gastrointestinal disorders [11]. Because polyphenolic compounds are

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found in plants under various forms, their extraction raises special problems [12,13]. The existing analytical protocols currently use classic techniques of solid - liquid extraction maceration, refluxing and Soxhlet extraction. Due to their disadvantages, these techniques tend to be replaced with others that occur with the consumption of reactive energy and less time, but with similar, if not higher efficiency. And so, techniques such as ultrasound-assisted extraction (UAE) [14-16], microwave-assisted extraction (MAE) [17], accelerated solvent extraction / pressurized liquid extraction dynamic or static mode (AES / PLE) [18] were implemented. The extracts obtained can be analyzed by spectrophotometry and high-performance liquid chromatography. For identification and the quantification of polyphenolic compounds, the most used are high-performance liquid chromatography with a photodiode array detector (HPLC-PAD) coupled with a mass spectrometer (HPLC-MS).

This study followed the influence of a microwave field on polyphenolic compounds from *Satureja hortensis* L. Summer savory (*Satureja hortensis* L.) is the better known as a savory species. It is an annual plant, most often used as a culinary herb, but also has marked medicinal benefits, especially upon the whole digestive system. The whole herb, and especially the flowering shoots, is antiseptic, aromatic, carminative, digestive, expectorant and stomachic. Taken internally, it is said to be a sovereign remedy for colic and a cure for flatulence, while it is also used to treat nausea, diarrhea, bronchial congestion, sore throat and menstrual disorders [19,20].

The important polyphenolic compounds that have been identified in *Satureja hortensis* L. in the literature are: rosmarinic acid, chlorogenic acid, caffeic acid, apigenin, luteolin and their glycosides [21,22].

This study determines the effect of irradiation in a microwave field on polyphenolic compounds from *Satureja hortensis* L. The results obtained from the irradiated plant were compared to the results obtained from the control plant (non-irradiated plants).

2. Experimental procedures

2.1. Plant materials

The plants were grown in a laboratory from ARO seeds (Romania). Three weeks after seeding the vessels with plants, they were placed in two identical anechoic chambers. Both chambers were placed under the same temperature and humidity conditions and fully closed. The chambers presented a 60 dB radio frequency isolation between the interior and exterior. The control plants were placed in one chamber, and the plants for microwave irradiation were placed in the second chamber. Microwave radiation was used to stimulate and was modulated by a specific WLAN communications protocol, in the 2.4...2.49 GHz frequency band, at a power density in the plants of 70 mW m^{-2}. Irradiation was conducted over a two-week period. The plants were then removed from the chambers and analyzed.

Fresh leaves were manually excised from the plants and stored in the dark at room temperature (~25°C) until completely dry. Dry leaves were powdered in a handle mixer.

2.2. Reagents and standards

Ethanol used for polyphenolic compounds extraction was purchased from Chimopar, Romania. Caffeic acid, rosmarinic acid, rutoside, luteolin, apigenin, naringenin standards were employed from Sigma-Aldrich, Germany. Acetonitrile and methanol of HPLC grade were obtained from Merck, Germany.

All applied reagents and chemicals were of analytical grade.

2.3. Extraction procedure

The powdered material (500 mg) obtained from the irridiated *Satureja hortensis* L. and the control plants was extracted in a 40 mL ethanol-water mixture (80:20 v/v) for 30 minutes using an ultrasound device (Elmasonic S 15H, 37 kHz). Before ultrasound extraction, the powdered material was macerated for 30 minutes. The extracts were evaporated till dry, and the residues were re-dissolved in a 10 mL extraction solvent. Each extraction was performed in five parallel samples. In all cases, samples were filtered by nylon syringe filters (0.45 µm) before use.

2.4. HPLC-DAD-MS analysis

HPLC separation was performed with a LC2010 Shimadzu system (Shimadzu, Kyoto, Japan) consisting of a LC-20AD pump, DGU-20A5 degasser, SIL-20A auto sampler and CTO-20AC column oven. The instrument was equipped with a SPD-M20A photodiode array detector and LCMS-2010EV mass spectrometer.

The separation was performed on an Altima C18 column (100 × 3 mm, 3 µm), maintained at 30°C. A gradient of acetonitrile (eluent A) and formic acid in water (1%, eluent B) was applied at a flow rate of 0.43 mL min^{-1} flow rate. The elution gradient consisted in: 0 min: 5% eluent A, 5 min: 42% eluent A, followed by a linear gradient elution with eluent A to 35% in 25 minutes. UV-spectra were recorded between 220 and 480 nm and chromatograms were acquired at 330 nm. Injection volume was 10 µL.

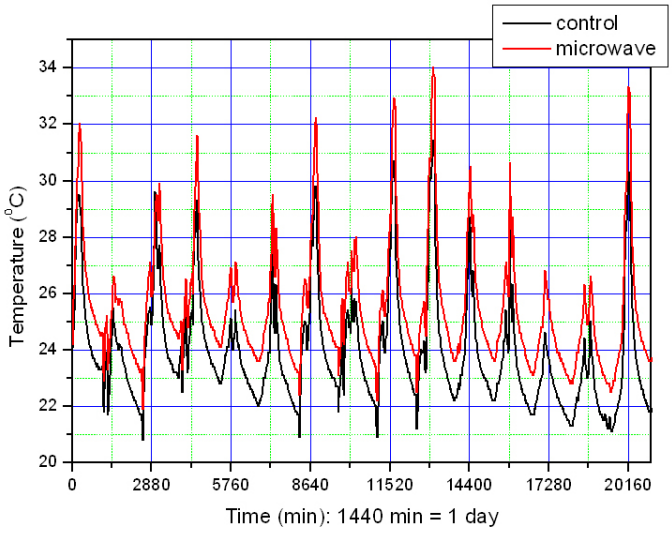

Figure 1. Temperature variation depending on the time in both chambers.

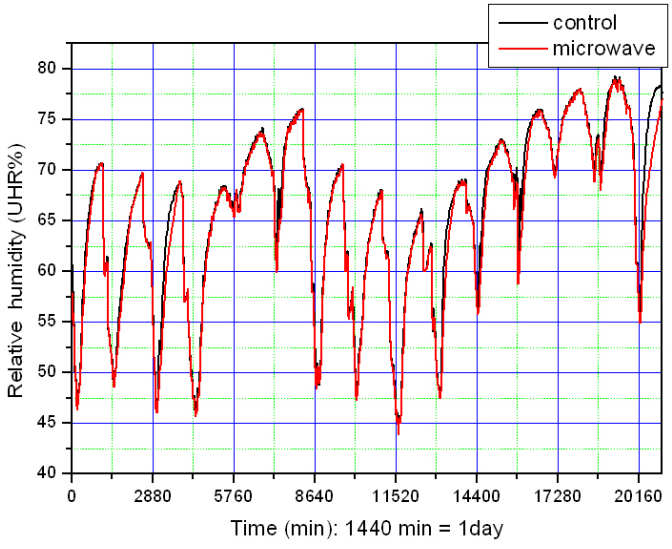

Figure 2. Humidity variation depending on the time in both chambers.

For high-performance liquid chromatography – mass spectrometry used a single quadrupole MS instrument (Shimadzu, Kyoto, Japan). The temperature of CDL was 250°C and the heat block was 200°C, the nebulizing gas flow being 1.5 L min^{-1}. Full scan spectra were recorded in negative ion mode over an m/z range of 50-700.

Triple analyses were performed for each sample. The identification of each compound was established by comparing the retention time, UV spectra and molecular mass of the peaks from plant extracts with those of the reference standards.

Standard solutions containing caffeic acid, rosmarinic acid, luteolin, naringenin and apigenin with different concentration were prepared in methanol and 10 µL of each standard solution were injected in triplicate. Calibration curves for caffeic acid, rosmarinic acid, luteolin, naringenin and apigenin were plotted using flavonoid peak area versus concentration.

3. Results and discussion

The purpose of our work was to determine the influence of the microwave field on polyphenolic compounds from irradiated plants.

The temperature and humidity in both chambers were permanently registered and showed a good correlation. The temperature increase caused a decrease in humidity with the same percentage in both chambers. The growing conditions were almost identical in both chambers (Figs. 1 and 2).

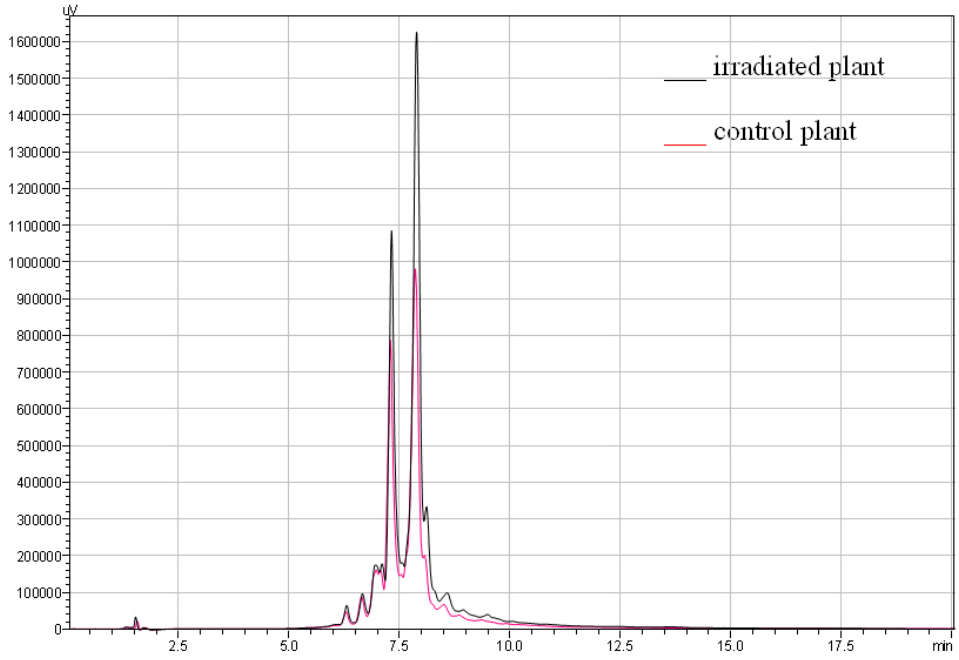

Figure 3. HPLC chromatogram of polyphenolic compounds from *Satureja hortensis* L.

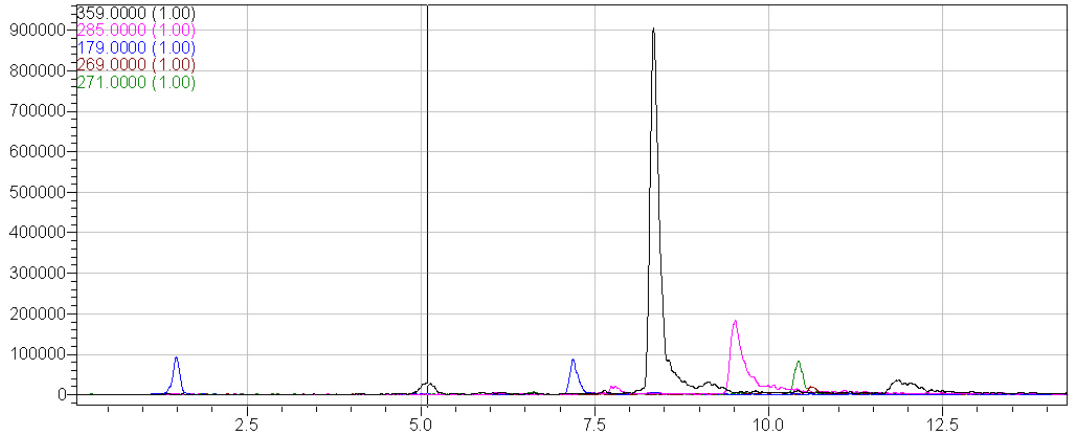

Figure 4. LC-MS (scan) chromatogram of the analyzed extract from the irradiated plant.

The HPLC-DAD method was used to identify and quantify some polyphenolic compounds from the irradiated and control *Satureja hortensis* L. The detection wavelength at 330 nm gave the best abundance for the identified compounds. Figs. 3 and 4 report the cromatogram obtained for extracts of the *Satureja hortensis* L. irradiated and control plants and the LC-MS (scan) chromatogram of the analyzed extract from the irradiated plant.

Caffeic acid, rosmarinic acid, luteolin, naringenin and apigenin were identified in extracts of the irradiated and control plants after comparing the retention time, UV spectra and molecular mass of standards and compounds from extracts. The irradiated and control plant extracts showed no change in quality.

The HPLC conditions used to separate the polyphenolic compounds ensured reproducible retention time and peak areas for investigated compounds (Table 1). Table 1 presents maximum wavelength and m/z specific for the compounds identified in extracts. The identified compounds were quantified at maximum wavelength (Fig. 5).

The polyphenolic compounds were quantified by an external standard method at the maximum wavelength corresponding to each standard. Linear calibration curves with good correlation coefficients were obtained for the identified compounds (Table 2). The limit of detection was between 0.02-1.16 μg mL^{-1}, while the limit of quantification was between 0.30-1.57 μg mL^{-1} for all polyphenolic compounds identified.

Table 1. Polyphenolic compounds identified in *Satureja hortensis* L. extracts.

Compound	t_R (min)	Maximum wavelength (nm)	RSD (%)	M - H$^+$
Caffeic acid	7.16	324	0.18	179
Rosmarinic acid	8.33	330	0.50	359
Luteolin	9.59	348	0.63	285
Naringenin	10.37	289	0.24	271
Apigenin	10.78	336	0.37	269

Table 2. Parameters of linear regression for polyphenolic compounds identified in *Satureja hortensis* L.

Compound	Range (μg mL^{-1})	Linear regression equation[a] y = ax + b	Correlation coefficient	LOQ (μg mL^{-1})	LOD (μg mL^{-1})
Caffeic acid	0.05-10	y = 110181x + 12398	0.9987	0.35	0.02
Rosmarinic acid	0.1-10	y = 50202x - 2219	0.9995	0.30	0.17
Luteolin	1-20	y = 11260x – 8429.8	0.9988	1.57	1.16
Naringenin	0.05-10	y = 273363x + 42167	0.9989	0.31	0.16
Apigenin	1-20	y = 10274x – 4331.5	0.9989	1.23	0.82

[a]*y = area, x = concentration (μg mL^{-1})*

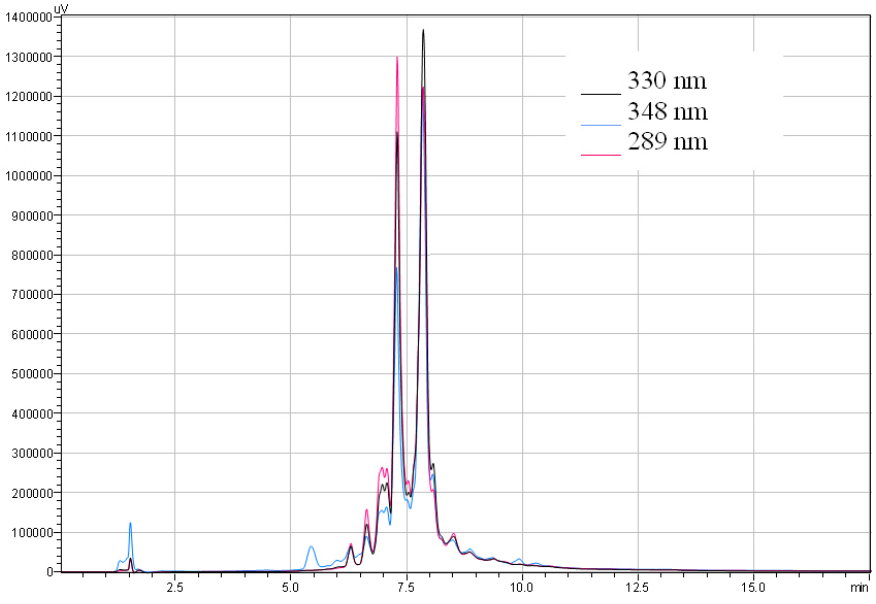

Figure 5. Chromatograms at different wavelengths of extract from the irradiated plant.

The repeatability was determined on five analysis of control plant extract of *Satureja hortensis* L. The first sample solution was measured five times repeatedly, to determine the content of caffeic acid, rosmarinic acid, luteolin, naringenin and apigenin (Table 3). Good results were obtained, the relative standard deviation (RSD) being 0.46-1.28%.

The apigenin was under quantification limit. Quantitative analysis of polyphenolic compounds from irradiated and control plants were compared.

Among of the polyphenolic compounds identified in *Satureja hortensis* L. rosmarinic acid is found in highest amount, followed by luteolin, caffeic acid, apigenin, and naringenin (Fig. 6).

It has been found that in the irradiated plant, the content of polyphenolic compounds was higher than in the control plant.

It can be observed that in the irradiated plant, the increase varied in the quantity of polyphenolic compounds depending on the compound type.

Table 3. Repeatability of determination of polyphenolic compounds in control plant extract of *Satureja hortensis* L.

No. of measurement	Content ($\mu g\ mL^{-1}$)			
	Caffeic acid	Rosmarinic acid	Luteolin	Naringenin
1	1.11	5.50	2.81	0.32
2	1.02	5.51	2.99	0.33
3	1.10	5.45	2.95	0.31
4	1.03	5.36	2.90	0.33
5	1.01	5.40	3.00	0.31
Mean	1.05	5.44	2.93	0.32
RSD (%)	1.79	0.46	1.07	1.28

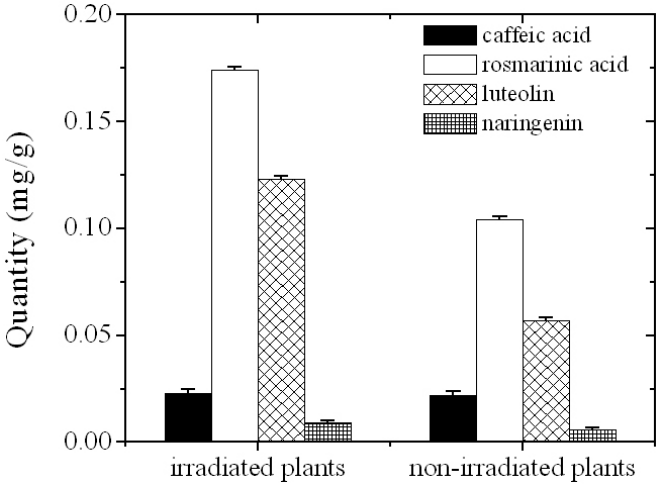

Figure 6. The comparative diagram of the polyphenolic compounds quantity from *Satureja hortensis* L.

4. Conclusions

The present work reports the influence of irradiation with low power microwave, derived from a wireless router (WLAN) type, on the polyphenolic compounds from Satureja hortensis L. The polyphenolic compounds from *Satureja hortensis* L. were identified and quantified by high-performance liquid chromatography with gradient method, using photodiode array and mass spectrometer detectors. Important differences were identified in the caffeic acid, rosmarinic acid, luteolin, naringenin and apigenin amount in the irradiated plant compared with the control plant. Microwave irradiation had a significant influence on luteolin which amounts increased by 116.5% in the irradiated plant and on rosmarinic acid and naringenin, which amounts increased by 67.34%, 34.37% .

Acknowledgments

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