EXTRACTION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM GINGER *(ZINGIBER OFFICINALE)*

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The paper presents the extraction of the bioactive compounds of ginger by ultrasounds. Extraction tests were performed by variation of extraction time and also the load of ginger powder used, in order to determine the duration of the operation and the necessary quantity of ginger to obtain the highest amount of active ingredients in the extract. Thin layer chromatography was used, in order to separate components of the extract. Characterization of their structural properties was made by UV-Vis and FTIR spectroscopic techniques, confirming the isolation of the bioactive compounds in the extract. Antioxidant activity of the extracts was determined using DPPH method.

Keywords: ginger extract, bioactive compounds, ultrasonic extraction

1. Introduction

Plant extracts are multicomponent mixtures of natural dyes and biologically active compounds. Plants may produce aromatic compounds in practically infinite quantities, the majority of which are phenols or their oxygen-substituted derivatives [1]. The existing literature data show that until now, a significant number of plants have been used for the extraction of active compounds, which are subsequently used for their biological activity, antibacterial properties and impregnation of textiles [2]. Some of these include: salvia *(Salvia sclarea)*

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[3], St. John's wort *(Hypericum perforatum)* [4], chamomile *(Matricaria recutita L)* [5] and marigolds *(Calendula officinalis)* [6].

Ginger *(Zingiber officinale)* is an herbaceous perennial plant with a meter- high annual pseudostem bearing narrow leaf blades and a rhizome that is extensively used as a spice [7]. Ginger contains phenolic compounds, terpenes, polysaccharides, lipids, organic acids, and crude fiber, among other chemical elements. The phenolic chemicals in ginger, primarily gingerol and shogaol [8] (Fig.1), are responsible for the multiple bioactivities of ginger, including antioxidant [9], anti-inflammatory [10], antibacterial [11], and adjuvant in cancer treatment [12]. Furthermore, research has indicated that ginger has the ability to prevent and control a variety of disorders, including neurodegenerative diseases [13], cardiovascular disease [14], obesity [15], and diabetes [16].

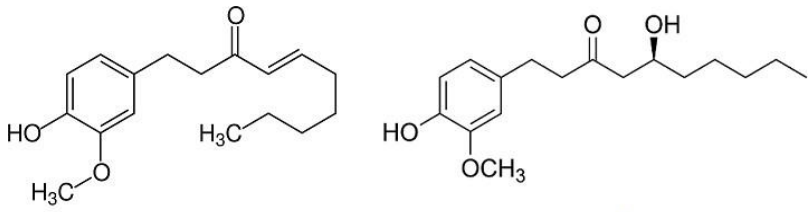

Fig.1. Structural formula of (a) 6-Shogaol and (b) 6-Gingerol

Due to these special medical effects, ginger has great potential for use in the medical and pharmaceutical industry. Ginger can be extracted using various methods, including usual processes such as maceration and Soxhlet extraction [17]. Modern extraction methods include ultrasonic assisted extraction [18] and microwave extraction [19]. Separation and identification of the active compounds from the extract (gingerol and shogaol) is done by Flash Chromatography [20].

This study is intended to provide data on the extraction and separation of bioactive ginger compounds, using a modern extraction method, ultrasonic assisted extraction, as well as the characterization of their structural properties and the antioxidant activity of the extracts.

2. Experimental

a. Materials

The organic ginger powder was purchased from Organic India (India). Isopropyl alcohol and n-hexane were obtained from Chimreactiv S.R.L. (Romania). All chemicals used were of laboratory reagent grade and they are used without further purification.

b. Equipment

The extraction took place in a jacketed beaker cooling system. The organic ginger powder and solvent were stirred using an ultrasonic probe processor Sonics 750W. Thin layer chromatography was performed using as a support Silicagel F_{254} deposited on plastic and a mixture of solvents as mobile phase. Absorbance and reflectance spectra were recorded by UV–Vis spectrophotometry. Total color differences in CIELAB system, using a 10-degree standard observer and illuminant D65 and diffuse reflectance spectra of powders were measured with a Jasco V570 UV–Vis– NIR spectrophotometer equipped with a Jasco ILN-472 (150 mm) integrating sphere, using Spectralon as reference. IR spectra of all samples analyzed were recorded on a Jasco FTIR 6300 spectrometer equipped with a Specac Golden Gate ATR (KRS5 lens), in the 400-4000 cm^{-1} range (32 accumulations at a resolution of 4 cm^{-1}). Antioxidant activity was determined using DPPH method.

2.3. The procedure for the extraction of active compounds

Extraction tests were performed by variation of extraction time and also the amount of ginger powder used, in order to determine the duration of the operation and the necessary quantity of ginger to obtain the highest amount of active ingredients in the extract. Extraction time was varied from 15 min-1h, using the same amount of ginger powder. Also, the quantity of raw material was also varied (1-18g) according to a fixed extraction time, 30 min. The quantity of solvent used remained the same throughout al experiments (80 ml). Extracts of different concentrations were obtained according to the proportion of raw material used. The product obtained from the extractions is filtered. The residue obtained from the extractions was dried in air, at room temperature, for 24 h, followed by drying at 105°C for 1h.

2.4. General method for the separation of the main compounds in the extract by thin layer chromatography

A series of tests were performed with solvents which have different polarity, mixed in different proportions. A polar solvent (isopropyl alcohol) and a non-polar solvent (n-hexane) were mainly used in a ratio of (1.5:8). The proportion in which each solvent was used was varied in order to determine the best system for separating the active compounds. Three spots were separated. The retention factor (Rf) was calculated for each spot obtained. The Rf values obtained were similar to those presented in the literature for 6-gingerol [21] 6-shogaol [22] and 6-paradol [23]. They were associated with the compounds of interest in the extract as follows: 0.45 (gingerol), 0.56 (shogaol) and 0.71 (paradol), a secondary compound.

2.5. The method for determining the antioxidant activity

The antioxidant activity of the extracts was determined as a measurement of radical scavenging using the DPPH (1,1-diphenil-2-picrylhydrazyl) radical. Because the radical molecule is stable and does not need to be manufactured, DPPH is regarded as an accurate, simple, and cost-effective approach for evaluating antioxidant radical scavenging activity. When DPPH absorbs an electron from an antioxidant chemical, it decolorizes, which may be quantified using changes in absorbance [24]. Briefly, an alcoholic dilution of the extracts was mixed with a DPPH solution. The mixture was incubated for 30 min in dark at room temperature.The absorbance was determined by UV-Vis spectrometry at 517 nm. Ascorbic acidwas used as reference antioxidant compound.

3. Results and discussions

The concentration of the extracts (Fig.2) is determined by the difference between the amount of dry raw material and the amount of dry residue obtained after each extraction. (Table 1). From the results obtained it is concluded that the concentration of extracts increases with increasing amount of raw material used in the same volume of solvent.

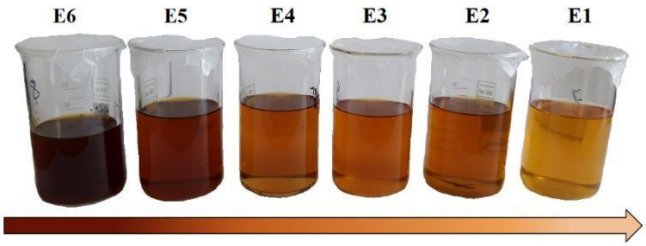

Fig.2. Ginger extracts

Table 1

Concentration of ginger extracts

Ginger extract	Extraction time	Quantity of raw material (g)	Residue quantity (g)	Extract volume (mL)	Concentration (mg/mL)
1	30 min	2	1.58	70	6
2	15 min	4	3.30	68	10.3
3	30 min	4	3.27	68	10.7
4	1 h	4	3.27	68	10.7
5	30 min	8	6.48	66	23
6	30 min	16	13.07	50	58.6

The extracts were analyzed by spectrophotometry to determine the UV–Vis absorbance. Dilutions of each extract were prepared. Samples of different concentrations were obtained by increasing the amount of raw material in solution. The samples obtained by varying the extraction time (15 min-1 h) were also subjected to analysis. In the latter case, no significant change was observed, the extraction time having no direct influence on the absorbance. (Fig.3) The peaks of the polyphenols fall within the UV portion of the spectrum, more precisely at 278 nm. In order to respect the Lambert-Beer law, halving the concentration of the samples, the absorbance of each extract must be half of the previous one. This rule is not followed at these concentrations in the UV domain (absorbance becomes closer in the UV zone as the concentration of extracts decreases) but is observed in the VIS range of the spectrum, related to the content of dyes (Fig.4).

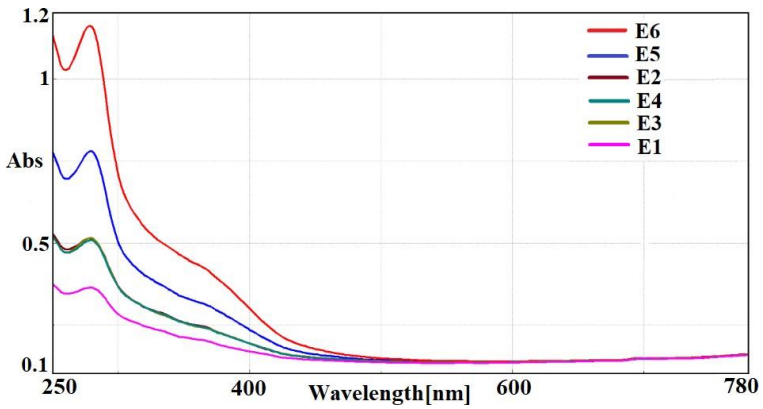

Fig.3. Absorbance spectra of ginger extracts (E1-E6)

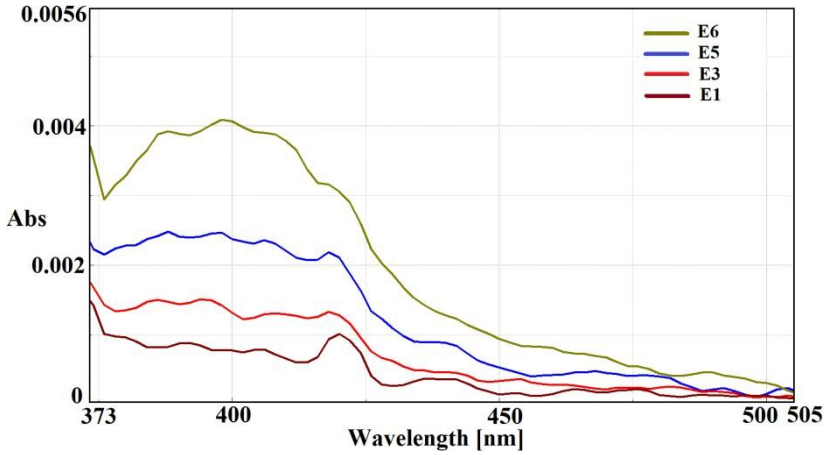

Fig.4. Absorbance spectra in the VIS region in relation to the Vis range

According to the parameters varied, the efficiency of the extraction process, was followed by recording the reflectance spectra of the remaining plant residues (R1-R6) and color measurements (Fig.5). The raw material (ginger powder) has a lower reflectance on the entire visible range and therefore a more intense color, compared to the residues obtained from extractions. In the case of residues, the increase in reflectance confirms that a considerable amount of colored compounds has been extracted from the raw material. It should be noted that the reflectance of the residue from extraction 5 (E5), shows a slight increase compared to the rest of the samples. According to the spectra, the highest reflectance is obtained in this case, so the residue is the lightest in color. Therefore, it can be concluded that this extraction was the most efficient: the largest amount of ginger-colored compounds is extracted, in relation to the quantity of raw material and solvent used.

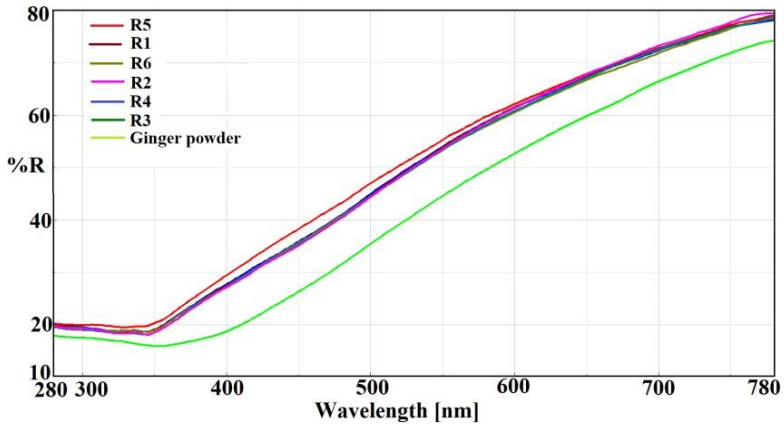

Fig.5. Reflection spectra of ginger powder and residues form extractions (R1-R6)

The influence of the extraction time on the color of the residues was studied. The ginger powder, the residue obtained after extraction 4 at 1h (R4-1), and the residue (R4-2) obtained after a second extraction (in the same conditions) on R4-1, were analyzed. (Fig.6) In the case of the first extraction, an increase in reflectance can be observed, associated with a decrease in the amount of dyes in R4-1. After the second extraction, the reflectance decreases slightly, which may be the result of the beginning of the phenols oxidation process which determined the formation of colored quinones. The residue contains, among others, antioxidant compounds that could not be extracted and are oxidized at air, over time. Therefore, the reflectance analysis highlights this aspect. Also, another important information that emerges from the UV-Vis reflectance spectra analysis are the trichromatic coordinates of the ginger residues (Table 2). Using these coordinates, the chromaticity diagram was figured and the position in the CIELab color space can be observed. This information explains the process of loss

of color intensity and theprocess of oxidation of certain compounds in the residue of the second extraction.

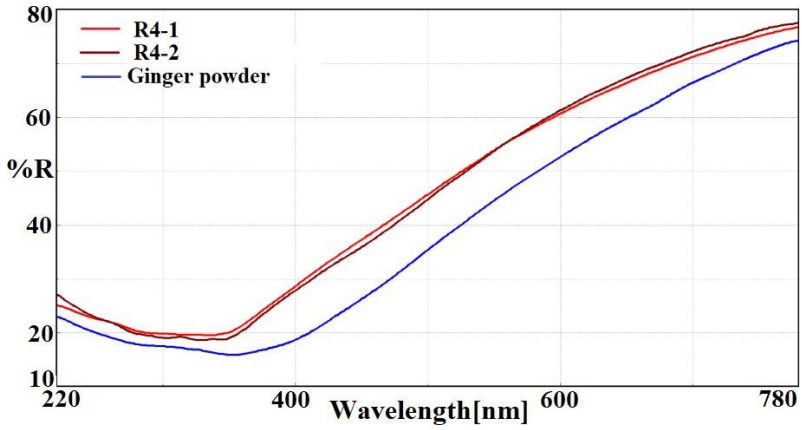

Fig.6. Reflectance spectra of ginger powder and residues after two consecutive extractions: R4-1 (residue after extraction 4 at 1h) R4-2 (residue after extraction 4 at 2h)

Table 2

Chromaticity coordinates of the residues obtained after extraction

Analyzedmaterial	**Trichromatic coordinates**			**CIEL*a*b***		
				*	*	**b***
Raw material	3.78	4.30	8.70	2.43	29	23.61
R4-1	2.07	3.47	0.38	8.15	.64	17.94
R4-2	2.16	3.34	9.06	8.08	.18	19.41

Color measurements are used to determine the efficiency of the extractions.The residues obtained from the extractions were analyzed compared to the raw material. The raw material has a darker color while the residues have a lighter colorwhich results in the fact that they lost the colored substances, which passed into theextract. The data show that the brightness (L*) of the residues has an increasing tendency towards the raw material. The higher the brightness, the lighter the color showing loss of colored compounds. Consequently, as the extraction time increases,the raw material loses its intensity. From the correlation of the coordinates with theCIEL*a*b* diagram, it appears that the position of the colors related to the ginger residues after extraction is around the yellow-brown colors, located in a plane of the diagram, situated at L*=78 and at different distances from the origin (Fig.7). Hence, the premise that the compounds present in the R4-1 powder are oxidized toquinones was confirmed by the color change

(Δa*, Δb*>0), from yellow (R4-1) tobrown (R4-2).

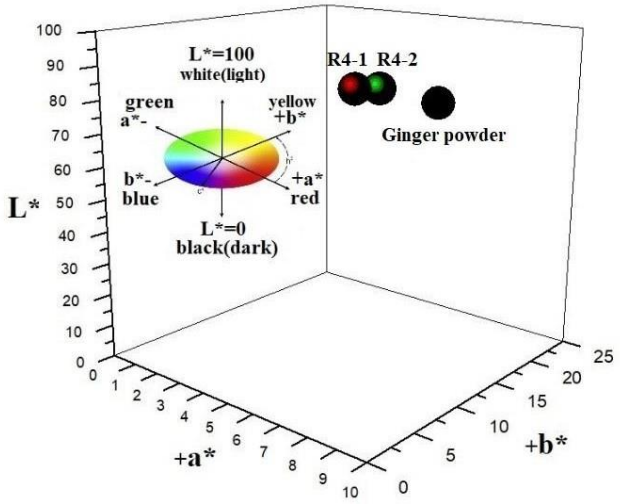

Fig.7. Position of ginger powders (raw material, R4-1 and R4-2) in the CIEL*a*b* diagram

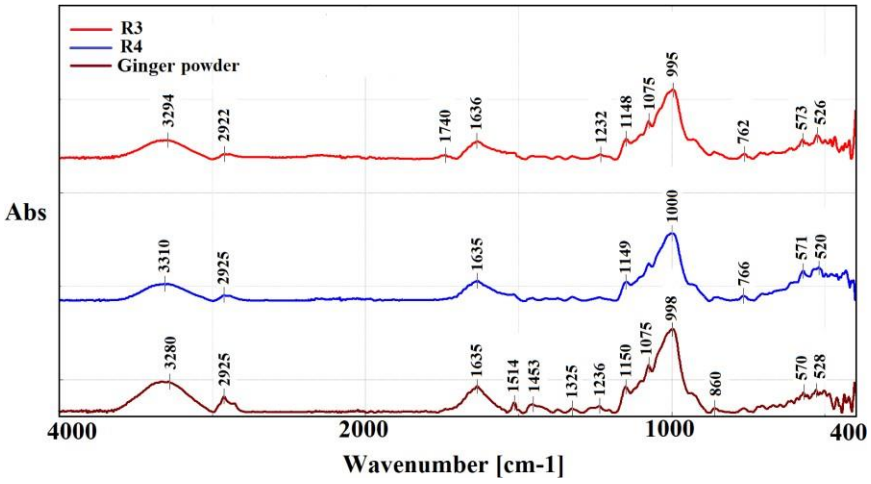

Fig.8. Infrared spectra of ginger powder and residues: R3 (residue after extraction 3 at 30 min), R4 (residue after extraction 4 at 1h)

The ginger raw material and the residues obtained after extraction performed during 30 min (R3) were compared to the residue obtained at 1h (R4) using the same amount of raw material (Fig.8). In the spectrum related to the raw material, stretching vibrations of the groups (–OH) associated within the O–H–O bonds can be observed around 3280 cm^{-1}, vibrations that are also present in the residue spectra but being significantly reduced in intensity. Also, there are bands characteristic of asymmetric (2925 cm^{-1}) and symmetric (2854 cm^{-1}) stretching vibrations, related to the presence of C–H bonds in the composition of organic

substances, which are very weak in the residue spectra. Hence, the amount of organic compounds has been reduced. Asymmetrical and wide bands characteristic of the rocking vibration of water molecules are located around 1634–1635 cm^{-1}. Bands between 1514–1326 cm^{-1} are stretching bands associated with C–H bonds and the aromatic nucleus of phenols. In the case of residues, the intensity of these bands decreases. At 1236 cm^{-1}, there are bands of stretching vibrations of the groups (–OH) in the phenol structure, which diminished in intensity as the extraction time increased. In the range 1150-998 cm^{-1} there are bands of tensile vibrations associated with C–O bonds, which lose their intensity in the case of residues.

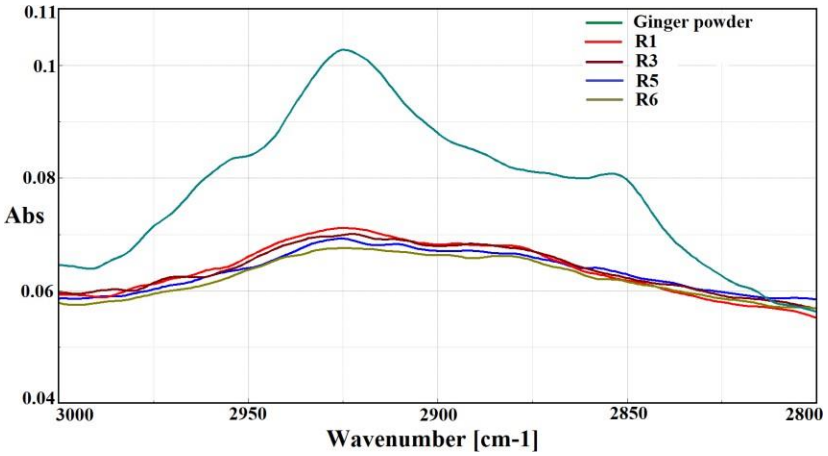

Fig.9. FTIR spectra related to the specific range for alkyl residues

Thus, from the comparison of the FT-IR spectra of the raw material and the residues it can be concluded that the extractions were effective, and extracts incorporated a significant amount of organic substances from the plant, as evidenced by the FTIR spectra in the range related to the presence of organic substances (Fig.9).

The radical scavenging activity (%) of ginger extracts is determined in relation to that of ascorbic acid (Fig.10). The extracts decolorize the DPPH solution depending on the amount of antioxidants present in each dilution. The results of the DPPH test compared to those of ascorbic acid demonstrate that ginger extracts have a significant antioxidant activity reaching a free radical inhibition rate of about 97% in the most concentrated sample. The ability to scavenge DPPH radical was calculated using formula (1).

$$Radical\ scavenging\ activity(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \cdot 100 \quad (1)$$

$Abs_{control}$ = absorbance of the DPPH solutionAbs_{sample}= absorbance of the sample

The radical scavenging activity of the extracts increases with the increasing of their concentration (Table 3).

Table 3

Antioxidant activity of the ginger extracts

Extract	Concentration(mg/mL)	Radical scavengingactivity (%)
E1	6	73.49
E3	10.7	84.09
E5	23	93.81
E6	58.6	97.35

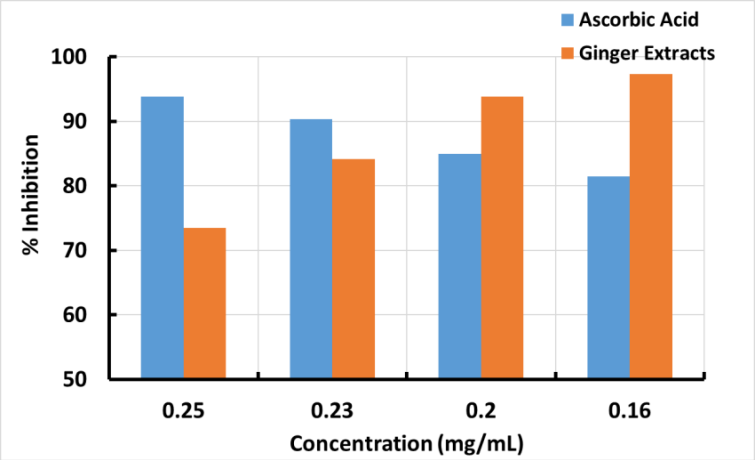

Fig.10. Radical scavenging activity of ginger extracts in relation to Ascorbic Acid

6. Conclusions

Extraction of bioactive colored compounds from ginger was performed by a modern extraction method, ultrasonic assisted extraction. Evaluation of the extraction parameters was performed, and results obtained for ultrasonic assisted extraction proved to be more efficient than the classical methods due to the shorter extraction time and the use of a smaller amount of solvent. Thin layer chromatography was used in order to separate components of the extract, establishing optimal conditions for an efficient separation of the main-colored compounds. Characterization of their structural properties confirm the isolation of the bioactive compounds in the extract. The results of the DPPH assay prove that ginger extracts have a significant antioxidant activity reaching a free radical inhibition rate of about 97% in the highest concentrated sample.

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