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Optimization of extraction methods for total polyphenolic compounds obtained from rhizomes of *Zingiber officinale*

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ABSTRACT

In an alternative medicine, aqueous extracts and decoctions of the ginger root (Ginger-*Zingiber officinale* Roscoe L., Ginger family *Zingiberaceae*) are used as antibacterial, diaphoretic, analgesic, anti-emetic and anti-inflammatory drugs. The aim of this study was to determine the optimal method and conditions of total polyphenolic compounds (TPC) extraction from ginger roots using ethanol as the solvent. The ethanol extracts were obtained by several extraction methods involving maceration, Soxhlet apparatus and ultrasound. TPC and total flavonoids (TF) were determined spectrophotometrically using the Folin-Ciocalteu method. It was found that the highest content of TPC and TF is obtained after sonication followed by subsequent maceration for a period of ten for TPC and seven days for TF, with a yield of 2.48 ± 0.029% and 1.3 ± 0.08% (per 1 g of dry substance), respectively. Optimal extraction conditions were found with ethanol (70%) and a ratio raw material/solvent of 1:1.

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1. Introduction

The use of medicinal plants has increased immeasurably in recent years. This is due to many reasons, including the fact that excessive increase of medicines of synthetic origin has led to the emergence of a new nosological form, called a drug disease (Lubberts and Berg, 2003; Kim et al., 2008; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017; Nunes and Miguel, 2017). Currently, according to the data by World Health Organization (WHO), around 2.5-5% of the hospitalized are the patients with medical complications.

This predetermined the necessity of a more detailed study of the medicinal plants, namely their chemical composition and process conditions of extraction optimization of Biologically Active Substances (BAS) from the herbal drugs for further rational use in medical purposes.

One of the least studied species of plants is medicinal ginger-*Zingiber officinale*. Ginger (*Zingiber officinale* Roscoe L.), Ginger family (*Zingiberaceae*) is cultivated in India, China, Africa, from where it flows to the world market. In a non-traditional medicine, aqueous extracts and decoctions of the ginger root are used as antibacterial, diaphoretic, analgesic, anti-emetic and anti-inflammatory drugs. A brief literature survey shows that different essential oils and extracts from *Z. officinale* Roscoe L. have promising antibacterial (Snuossi et al., 2016), anti-inflammatory (Funk et al., 2016), antimicrobial (Santo Grace et al., 2017; Sharma et al., 2016), antioxidant (Noori et al., 2018; Tung et al., 2017; Snuossi et al., 2016), neuroprotective (Sutalangka and Wattanathorn, 2017) and antifungal (Nerilo et al., 2016) properties and activities.

Among the variety of methods that can be used for dissolution of joints salts deposits, the preparations based on ginger are often used and have the most pronounced effect (Ravidran and Nirmal Babu, 2005; Sasidharan and Menon, 2010; Padalia et al., 2011; Pragasam et al., 2011; Mishra and Kumar, 2012; Hasan et al., 2012; Rahmani et al., 2014). Ginger rhizome is

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composed of a variety of nutrients including essential oils, to which it owes its spicy and tart flavor (about 3%), monocyclic sesquiterpenes and zingiberenes (70%), as well as bisabolene, borneol and farnesene (Zhou et al., 1998). The pungent taste is due to resinous substances-gingerols (5-8%). There are also vitamins (nicotinic acid, vitamin A), amino acids, lipids (6-8%) and starch (50%) (Ghasemzadeh et al., 2010; Hasan et al., 2012; Kumar et al., 2013).

The diversity of species composition of biologically active substances (BAS) in ginger rhizome is described by many authors (Ghasemzadeh et al., 2010; Kumar et al., 2013; Zahraa et al., 2013), however, some information about these bioactives still remains unexamined. In addition, there is a relevant question about optimization of the target extraction conditions adapted to a specific group of BAS. For example, some authors (Wattanathorn et al., 2001) have described the quantitative content of flavonoids and other polyphenolic compounds. However, sample preparation and extraction methods have not been indicated. Therefore, their results are not comparable. This suggests that the quantitative extraction of biologically active substances depends on many factors like climatic growing conditions and completeness of the extraction from the raw materials (Masullo et al., 2015).

This work has been focused on polyphenolic compounds (PC) as a very large group of biologically active substances with a wide range of pharmacological activity. From this point of view, PC are promising object of research.

Due to the fact that various factors influence the yield and quality of the target product from the raw materials, the optimization of the extraction process and the yield of the target product are provided by selection of optimal conditions of the extraction process.

The aim of this work was screening and determination of optimal conditions for the extraction of BAS (polyphenolic compounds) from ginger roots in order to increase their usage by using a different extraction methods and conditions.

2. Experimental

2.1. Chemicals and reagents

All chemicals used in this study were of analytical reagent grade or higher purity. Gallic acid was obtained from Sigma Chemical Co., St Louis, MO, USA. The Folin-Ciocalteu phenol reagent was purchased from Fluke Chemie AG, Buchs, Switzerland. All other solvents and chemicals were of analytical grade.

2.2. Apparatus

The quantitative content of the total polyphenolic compounds (TPC) and total flavonoids (TF) were determined using the following extraction methods: i) maceration method (separately for fresh and dried samples), ii) extraction under reflux, extraction method in Soxhlet apparatus, as well as iii) a complex extraction method that included a 15 minutes processing of vegetable raw materials with an ultrasonic disintegrator, followed by maceration (Bartley and Jacobs, 2000; Balanchandarn et al., 2006; Yang et al., 2009; Supardan et al., 2011; Offei-Oknye et al., 2015). Studying of release kinetics for polyphenolic compounds and flavonoids from the raw material in the solvent system was measured after 2 h, 3 days, 7 days, 10 days, 14 days and 1 month for all extraction methods.

2.2.1. Maceration method

Before the maceration, ginger roots were ground to fine pieces of 2 mm size and than extracted with 70% ethanol in the raw material/solvent ratio of 1:1 (Mandana et al., 2011; Mesomo et al., 2012; Kumar et al., 2013).

2.2.2. The ultrasound extraction

The ultrasound extraction was carried out using ultrasonic disintegrator type UD-11 (US-generator power in the range 0.1-2.0 W/cm^2). The extraction process was performed by adding 100 g of ground ginger (2 mm size pieces) to 100 mL solvent (70% ethyl alcohol) in a glass tube. Then, the resulting solution was continuously sonicated with ultrasound (frequency 25 kHz) for 15 min at 20 °C (Supardan et al., 2011; Kumar et al., 2013; Zahraa et al., 2013).

2.2.3. Soxhlet apparatus

For extraction of TPC in Soxhlet apparatus, the raw material was chopped to 2 mm size pieces and placed into the extractor. Then, 100 mL of solvent (70% ethanol as extragent) was poured into a round bottom flask and the extraction was conducted at the boiling point of the solvent. The duration of the extraction was 5 h in the circulation of the solvent at least 5 times (Singleton et al., 1999; Balanchandarn et al., 2006; Chen et al., 2011).

2.2.4. Extraction with reflux condenser

When extracted under reflux, the weighed plant material (100 g) was put into a flask and 100 mL of ethanol (70%) was added (the ratio of the raw material:solvent was 1:1) and boiled under reflux for 30 minutes (Wattanathorn et al., 2001).

2.2.5. Photocolorimetric determination

Quantitative determination of TPC content and the TF content was carried out photocolorimetrically. Quantitative content was determined at 765 nm for TPC and at 415 nm for TF on CPC-3 Photocolorimeter,

Table 1

Optimal conditions for the extraction of total polyphenolic compounds (TPC) and flavonoids (TF) from the rhizomes of *Z. officinale* Roscoe L.

Compounds	Plant / solvent ratio (g/g)	Grinding of plant (mm)			
		2	4	8	10
TPC content, %	1:1	**1.80±0.02**	1.72±0.03	1.64±0.04	1.58±0.05
	1:3	1.78±0.03	1.73±0.03	1.61±0.05	1.54±0.03
	1:5	1.75±0,11	1.76±0.07	1.54±0.12	1.48±0.06
	1:10	1.67±0.05	1.60±0.04	1.42±0.09	1.36±0.07
TF content, %	1:1	**1.14±0.03**	1.11±0.08	0.97±0.05	0.84±0.07
	1:3	0.95±0.06	0.88±0.04	0.76±0.06	0.67±0.04
	1:5	0.86±0.07	0.80±0.07	0.71±0.08	0.63±0.05
	1:10	0.72±0.05	0.64±0.03	0.52±0.05	0.46±0.02

Data expressed as mean ± SEM; n = 5 for all groups.

Ukraine.

2.3. Plant and extraction

Ginger (*Z. officinale* Roscoe L.) was procured from the local market of Odessa city, Ukraine for experimental work.

2.4. Total phenolic compounds content determination

Quantitative determination of TPC content was carried out photocolorimetrically by the Folin-Ciocalteu method (Magalhães et al., 2010). Accordingly, a volume of 0.1 mL of each sample solution was mixed with 0.4 mL of ethyl alcohol (70%). Then, 2.5 mL of Folin-Ciocalteu reagent was added to each sample (diluted 10 times with water) and well stirred. After 3 minutes, 2 mL of sodium carbonate (7.5% Na_2CO_3) was added and the resulting solution was stirred well. After incubation for 2 h at 20 °C, the absorbance was determined at 765 nm on CPC-3photocolorimeter. Gallic acid was used as a standard for the calibration curve. Accordingly, a volume of calibration solutions of gallic acid (50, 100, 150, 200 and 250 mg.L^{-1} were mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) and well stirred. After 3 minutes, 2 mL of sodium carbonate (7.5% Na_2CO_3) was added to the obtained mixture and well stirred. After incubation for 2 h the absorbance was determined at 765 nm.

TPC was expressed in mg of gallic acid equivalent per gram of dry substance. All measurements were performed in triplicate. Data were expressed as mean ± standard derivation (±SD).

2.5. Total flavonoids content determination

The TF content was determined using the differential spectrophotometry (Antolovich et al., 2000; Chen et al., 2002; Feng et al., 2002; Aizam and Ibrahim, 2006; Ding et al., 2012). In this regard, 4.0 mL of 5% $AlCl_3$ in methanol was mixed with 15 mL of the extract solution and 6 mL ethyl alcohol 70%. Absorbance records at 415 nm using CPC-3 photocolorimeter were made after 30 min against a control solution consisting of extract solution (15 mL) with 10.0 mL of ethanol 70% without $AlCl_3$. The TF content was determined using a rutin calibration curve. The results were expressed in mg of rutin per gram of ginger rhizome dry weight both of fresh and dry samples.

2.6. Statistical analysis

All definitions were determined in three parallels with subsequent statistical data processing to determine the reproducibility and accuracy of the results.

The results of statistical analysis were expressed as mean ± standard deviation of the mean. The differences between the means were determined by student t-criteria. In all cases, differences were considered significant if $p \leq 0.05$.

3. Results and Discussion

3.1. Optimization of the extraction conditions

To increase the yield of the TPC and TF from the ginger rhizomes, we studied different extraction conditions: depending on the degree of grinding of plant raw materials, concentration of solvent, ratio of raw material and solvent, as well as the extraction time and different methods of extraction. At each stage of the study (in certain intervals), we determined the amount of TPC and the amount of TF as the main criteria to select the optimal extraction conditions.

The most effective solvent for the extraction of the TPC, according to the literature, was 70% ethyl alcohol (Hu et al., 2011; Kumar et al., 2013). Dry residue content for *Z. officinale* Roscoe L. root was 7.76%.

The maximum content of the extracted compounds was achieved using a fraction of the raw material with a 2 mm particle size by maceration (Table 1).

In the experiment, we have investigated the optimal conditions and methods of extraction of TPC and TF from the rhizomes of *Z. officinale* Roscoe L. The most effective extraction method was chosen based on the quantitative yield of polyphenolic compounds as well as flavonoids and extraction time. The results are presented in Table 1. The most effective extraction for the separation of polyphenols and flavonoids was at a ratio of raw materials and solvent 1:1. When determining

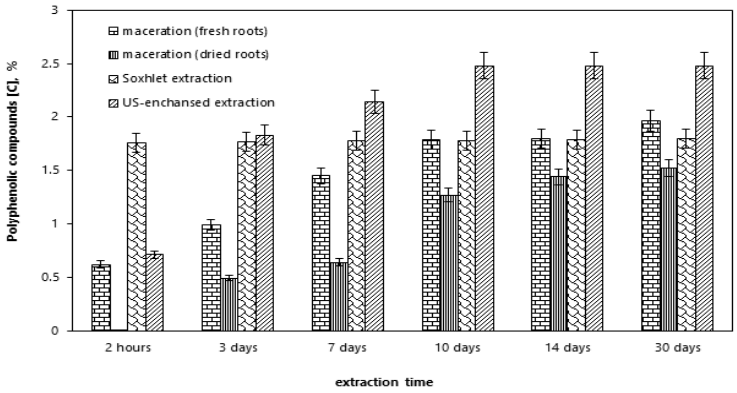

Fig. 1. Total amount of polyphenolic compounds from the ginger rhizome under different methods of extraction (%).

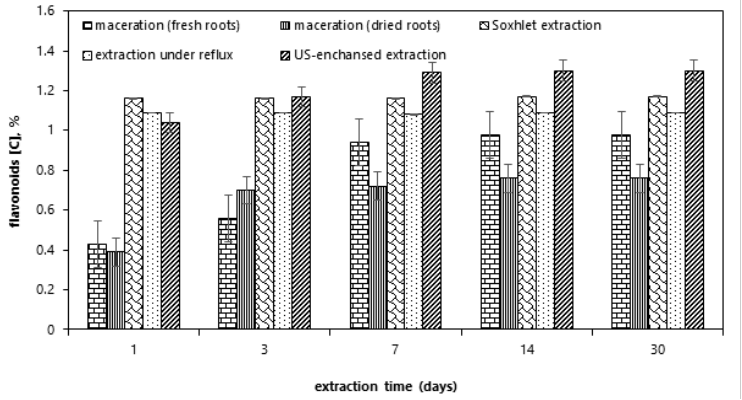

Fig. 2. Total flavonoids content from the rhizome of ginger under different methods of extraction (%).

the optimum degree of grinding of raw materials, we used fractions passing through a sieve with a whole diameter of 2; 4; 8 and 10 mm.

The simplest and the most inexpensive in performance is a technique of maceration of a dry and fresh ginger root. In the results of our study, the yield of biologically active substances from raw materials into the solvent system has a linear dependence in time. According to the results obtained by maceration method of the rhizome of ginger within one month, the quantitative content of TPC and TF in extracts of fresh roots was higher by 22% than in the extracts from the dry roots (Fig. 1 and Fig. 2). Due to the literature data, it can be explained by the fact that the cell wall of the roots of many plants are impregnated with hydrophobic substances, which when drying lead to block the microspores and the narrow capillaries in the cell walls. As a result, diffusion is hindered (or it is absent) between the solvent and the plant material that deteriorates the process and the duration of the extraction of the dried material (Zahraa et al., 2013).

Due to the fact that the dry roots maceration method is a static method of extraction and does not always provide the maximum yield of extractive substances, at the next stage of the study we have performed the extraction of the fresh roots of ginger in Soxhlet apparatus with further maceration during 1 month. As a result, the TPC and the quantitative content of TF after 5 h extraction in Soxhlet apparatus was equal to the results after maceration of the fresh roots for 1 month. The advantage of this extraction method is to reduce the duration of extraction and to achieve high release of active substances at a short period of time (Fig. 1 and Fig. 2).

3.2. Selection of the best extracting method

According to the obtained results, the sonication method followed by maceration at room temperature (20 ºC) is the best method of active substances extraction from ginger rhizomes.

It should be noted that in the initial period of time (by the 3rd day of maceration), we observed acceleration of the extraction process due to the enlarging of the surface phases boundaries and particles disparaging of plant material, which resulted in TPC accumulation quantitatively equal to the extraction in Soxhlet apparatus (Fig. 1 and Fig. 2).

After sonication of raw material, it was additionally extracted using the maceration technique for 10 days. By the tenth day of maceration, the maximum yield of the studied biologically active substances (TPC: -2.48 ± 0.14%, and TF: -1.3 ± 0.08%) was observed, which did not change for the next two weeks of the extraction process. According to the literature data, the sonication of the plant material provides an effective

extraction of substances due to the ultrasound waves. The result is the acceleration of material impregnation and dissolution of the cell contents, increasing the speed of raw material particles flow. Molecular diffusion inside the plant material and in the diffusion layer is almost turned into the convection that produces the intensification of mass transfer processes (Bager and Ovesen, 2012; Pavlyuk et al., 2015).

4. Concluding remarks

This study gives some new information about the optimization of extraction conditions of bioactives (TPC and TF) from rhizome of *Z. officinale* Roscoe L. Results show that use of extraction conditions with ethanol solvent 70% and a ratio raw material/solvent of 1:1 give a maximum content of TPC and TF from ginger rhizome. Also, this study prove that the highest yield of BAS from ginger rhizome is obtained after sonication followed by subsequent maceration for a period of ten for (TPC) and seven for TF, with a yield of 2.48 ± 0.029% and 1.3 ± 0.08 %, respectively.

Conflict of interest

The authors declare that there is no conflict of interest.

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