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Scientific Research

Optimization of the effect of pulsed electric field on antioxidant and phenolic properties of extract extracted from fragrant geranium leaves

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ABSTRACT

In this study, the optimization of pulsed electric field and its effect on antioxidant and phenolic properties of the extract extracted from Geranium aromaticum leaves was investigated. This process was investigated by applying pulsed electric field pretreatment at 3 electric field intensities (1000, 2000 and 3000) and 3 pulse levels (50, 85 and 120). Then, the phenolic compounds, fatty acid composition of the extract and its antioxidant properties were investigated. Also, gas chromatography/spectrometry was used to identify chemical compounds and the results showed that the total phenol content and antioxidant properties of the extract increased with increasing the number of pulses and increasing the intensity of the pulsed electric field applied to the sample. Also, according to the GC results, the most abundant compounds in the extract of Geranium aromaticum leaves were Glycidyl oleate and Oleic Acid, respectively. According to the process optimization results, it can be stated that the electric field intensity of 2000 kV/cm and the application of a pulse level of 85 increased the antioxidant properties and phenolic compounds in the extract extracted from the leaves of Geranium aromaticum.

1.Introduction

Geranium is a slow-growing, perennial plant that has flowers composed of five sepals, five petals, and ten stamens connected together. The round leaves with wavy margins contain active ingredients in its essential oil, which has made it one of the plants of interest in the production of medicine. This plant is also rich in natural antioxidants and can neutralize the activities of free radicals. Therefore, it is recommended to use this plant and plants with these characteristics as an effective combination in the production of knowledge-based and functional food products in the prevention of various diseases and help in the treatment of some diseases (Upadhyay et al., 2016). The majority of the essential oil of the aromatic geranium plant is geraniol, which is associated with beta-citronellol, which is the main smell of the essential oil. In the essential oil of geranium, the amount of linalool is high, as well as the amount of citronyl formate and geraniol formate, and it is possible to identify these compounds by gas chromatography and spectrophotometry (UV) methods (Bijalwan et al., 2014).

Plant extracts constitute a source of useful chemical compounds that have potential applications in medicine and other fields. Extraction is the first important step in the preparation of plant extracts. New and modern extraction methods are effective in the development of these plant extracts. The use of modern preparation methods with significant advantages over conventional extraction methods plays an important role in the production of plant extracts and ensuring the availability of high-quality products to consumers worldwide. In addition to the conventional and conventional methods, numerous new methods have been developed, but so far no single method has been considered as a standard for the extraction of plant extracts. The efficiency of conventional and modern extraction methods mainly depends on important input parameters, understanding of the nature of the plant matrix, the chemistry of the bioactive compounds and scientific expertise. Each of these methods has its own advantages, considering efficiency, ease, time used, costs, energy and environmental impacts. (Negi., 2012). Solvent extraction of plant compounds is known as one of the oldest separation methods, so that the extraction of bioactive compounds from plants has mainly been carried out by steam distillation and extraction with organic solvents using percolation, maceration, and Soxhlet methods. (Olszewska et al., 2020). The use of methods to extract valuable compounds that can obtain the most functional material with the least impurities and minimal destruction is of great importance. (Armendáriz-Barragán et al., 2016). Long extraction time, low yield, toxicity of the extracted phase with solvent, and harsh operating conditions have made the use of such methods challenging. There are also other methods to extract or assist in the extraction of bioactive compounds, such as microwave, supercritical fluid extraction, or ultrasound, which result in faster and more effective extraction and, as a result, a higher amount of output sample. Due to the lower consumption of organic solvents, these methods are considered environmentally friendly (Yu et al., 2015).

In recent years, numerous studies have been conducted on the ability of treatment with strong pulsed fields, and the effects of this method on the safety and shelf life of treated products have been investigated. In recent decades, the advancement of pulsed electric field technology with its application in non-thermal food processing has been considered as an important research area and alternative study of energy-efficient applications. (Arshad et al., 2021). Electric fields cause damage to plant tissue, which accelerates the outflow of liquid from the inner parts of the cell and increases the efficiency of juice extraction. Although some researchers have found that

the plant cell wall remains undamaged, others have obtained evidence of cell wall damage due to increased permeability. Researchers have found that PEF causes non-thermal disruption of the cell membrane, whereby the extensibility of the cell membrane is reduced or destroyed, especially when PEF is applied after the initial pressing process. The combination of PEF and heat also reduces cell recovery. The application of PEF to mild heat treatment increases tissue damage in carrots, potatoes and apples and increases the extraction efficiency of the extract by pressing devices. PEF has also been useful in coconut processing, causing a 20% increase in extraction of coconut milk, a 50% increase in protein content and a 58% increase in fat content (after 20 pulses at 2.5KV/CM). The yield of sugar beet extract using the PEF process was significantly increased to 88%. Pretreatment of sugar beet using PEF has increased the sugar content by about 25% compared to conventional methods of extraction with hot water. (Raso et al., 2022).
Given that no research has been conducted so far on extracting from the leaves of the Geranium aromaticum plant, the present study attempted to investigate the effect of these pretreatments on the antioxidant and phenolic properties of the leaf extract of the Geranium aromaticum plant.

2- Materials and Methods

2-1- Materials and Equipment

Geranium aromaticum leaves were obtained from local centers in Shiraz. The chemicals used were obtained from Merck (Germany).

2-2- Methods

The method of strong pulsed electric fields is a process that is performed by applying high voltage pulses between two electrodes (20-80 kV/cm) to food materials. The use of PEF as a membrane permeability technique has received considerable attention over the past two decades due to its potential to increase extraction or create alternatives to conventional methods in food extraction and processing. (Aghai Shalmani and Salehi, 2014). In order to optimize the extraction method, extraction was performed by the electric pulse method using RSM software by changing the two factors of pulse intensity and time, in 21 rounds.

3-2- Relevant experiments

1-3-2- Investigation of phenolic compounds of the extract

A mass chromatography (GC-mass) device was used to determine the percentage of phenolic compounds of the extract. The preparation of the compounds was carried out according to the method of Mason et al. (1996). First, 50 µl of the sample was poured into a capped test tube and 1 ml of hexane was added to it. After the oil was completely dissolved, 100 µl of methanolic sodium methoxide was added to it and shaken for 15 minutes at room temperature. After the required time, the hexane layer was transferred to another test tube containing amounts of anhydrous sodium sulfate (this compound removes excess water from the sample).

2-3-2- Investigation of antioxidant properties using the DPPH method

The amount of free radicals was evaluated using the Son and Lewis method with some modifications. For this purpose, 4 ml of ethanolic solution, 1 mM DPPH was added to the treatments. Then the volume of the final solutions was brought to 10 ml with ethanol and stirred vigorously. After 30 minutes of darkness, the absorption of the samples was measured by spectrophotometer at 515 nm. (Serlak et al., 2018).

3-3-2- Examination of phenolic compounds using the Folin-Ciocaltio method

The basis of this method is the reduction of the Folin reagent by phenolic compounds in an alkaline medium and the formation of a blue complex that will show maximum absorption at 760 nm. In this method, 20 microliters of the sample solution were mixed in a test tube with 1.160 ml of distilled water and 100 ml of Folin-Ciocaltio reagent. After 8 minutes, 300 microliters of sodium carbonate solution (20% w/v) was added to the contents of the tube and after shaking it was placed in a 40°C water bath for 30 minutes and then the absorbance was read by a spectrophotometer at 760 nm. (Serlak et al., 2018).

4-3-2- Statistical analysis

In this study, the response surface methodology with a central composite design was used to optimize the process and evaluate the parameters under study. Statistical analysis of the data was performed at a probability level of 95% using Design-Expert-10 software, where the laboratory parameters and their ranges used, and the designed experiment for solution R1 and solution R2 are given.

Table 1. Laboratory parameters and their ranges of use.

		Factor 1	Factor 2	Response 1	Response 2
Std	Run	Pals number	Voltage	R1	R2
7	1	85	1000	3210.67	70.0602
6	2	120	2000	3481.34	48.9875
8	3	85	3000	23788.1	71.3002
4	4	120	3000	13770.1	33.76
2	5	120	1000	3400.99	50.65
1	6	50	1000	11518.7	88.7156
5	7	50	2000	21432.8	89.9156
9	8	85	2000	13371.9	70.2156
3	9	50	3000	31346.8	91.1156

3- Results and Discussion

1-3- Results of HPLC analysis of extract compounds

Geranium aromaticum leaf extract extracted with electric pulse pretreatment was evaluated by gas chromatography-mass spectrometry. As shown in Table 2, the most abundant compounds in the geranium aromaticum leaf extract were Glycidyl Oleate and Oleic Acid, respectively.

Table 2. Results of the study of the fatty acid composition of Geranium aromaticum leaf extract using GC-mass spectrometry.

NO.	Name	Retention Time (min)	Area Sum%
1	2,6-Nonadienal, 3,7-dimethyl	8.564	1.44
2	Cyclohexanone, 2-methyl	9.689	3.29
3	4-Cyclopropylcarbonyloxytridecane	15.121	0.57
4	7,10-Epoxytricyclo[4.2.1.1(2,5)]decane, 1-trimethylsilyl	16.188	0.93
5	Dodecanoic acid, 3-hydroxy	18.846	0.4
6	1,7-Octanediol, 3,7-dimethyl	19.516	1.34
7	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	20.233	0.25
8	2-Nitrohept-2-en-1-ol	20.542	1.44
9	Octanal, 7-hydroxy-3,7-dimethyl	23.707	13.76
10	1-Heptatriacotanol	29.582	1.15
11	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	30.771	0.41
12	trans-13-Octadecenoic acid	32.77	0.5
13	cis-13-Octadecenoic acid	32.986	0.4
14	Oleic Acid	33.604	18.7
15	Banisterin	34.851	7.82
16	Harmine, ethenyl(ester)	34.869	6.56
17	Hexadecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aα,2α,5β,5aβ,6β,8aα,9α,10aα)]-	35.481	2.27
18	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	36.023	1.34
19	Ethyl iso-allocholate	36.373	1.49
20	Glycidyl oleate	36.419	22.26
21	Erucylamide	38.786	4.49
22	15,17,19,21-Hexatriacontatetrayne	39.747	1.92
23	3,5,9-Trioxa-5-phosphaheptacos-18-en-1-aminium, 4-hydroxy-N,N,N-trimethyl-10-oxo-7-[(1-oxo-9-octadecenyl)oxy]-, hydroxide, inner salt, 4-oxide, (R)	40.103	1.42
24	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5β)	41.863	2.22
25	7,8-Epoxylanostan-11-ol, 3-acetoxy	44.393	3.63

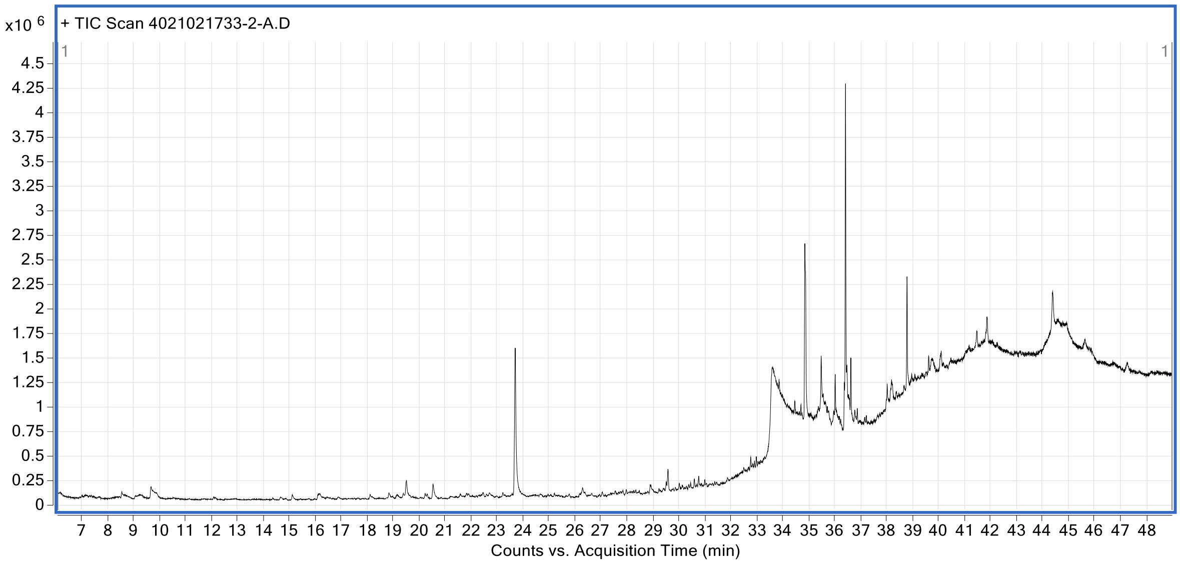

Figure 1. GC spectrum of Geranium aromaticum leaf extract.

The use of new technologies such as: electric pulse waves, microwave waves, etc. causes rapid breakdown of the walls of the secretory glands, including the extract, and as a result, increases the yield in a short time. This high yield of essential oil is due to the penetration of pulse waves into the essential oil cells and intensifies the process of extracting essential oil from them. This method also reduces water, time, and energy consumption, and as a result, reduces costs and increases extraction efficiency. The low yield of essential oil at low powers can be related to the loss of a large amount of volatile compounds over long periods of time. (Bozinou et al., 2019). Jafar Azad et al. (2019) studied the effect of extraction method on the quantity, quality, chemical composition and antioxidant nature of the essential oil of the medicinal plant Ferulago angulata (Schlechat) Boiss. The results of their research also showed that using microwave and increasing its power leads to a reduction in the extraction start time, a reduction in the time required to complete the extraction, and also the time required to stabilize the extraction curve, from four hours to one hour. Also, ultrasonic pretreatment at different temperatures and the use of different extraction methods, by Clevenger, and extraction with the help of microwave at different powers, as well as changing the plant to water ratio, did not have much effect on the refractive index of the essential oil, antioxidant activity, and specific gravity of the chive plant. (Jafar Azad et al., 2019).

2-3- Investigating antioxidant properties using the DPPH method

The DPPH test is widely used as a method to determine antioxidant activity in a relatively short period compared to other methods. (Delfanian et al., 2015). According to the results (Figure 2), there is a nonlinear relationship between DPPH and the independent variables studied; in such a way that with increasing the number of pulses (85), the amount of this compound increased. With increasing the electric field intensity to 85 kV/cm and voltage of 2000, the ability to inhibit DPPH free radicals increased linearly. The reason for this increase is that the ability to inhibit DPPH free radicals can be attributed to the greater release of antioxidant compounds such as tocopherols and flavonoids, and its decrease can be attributed to the destructive effect of increasing the pulse.

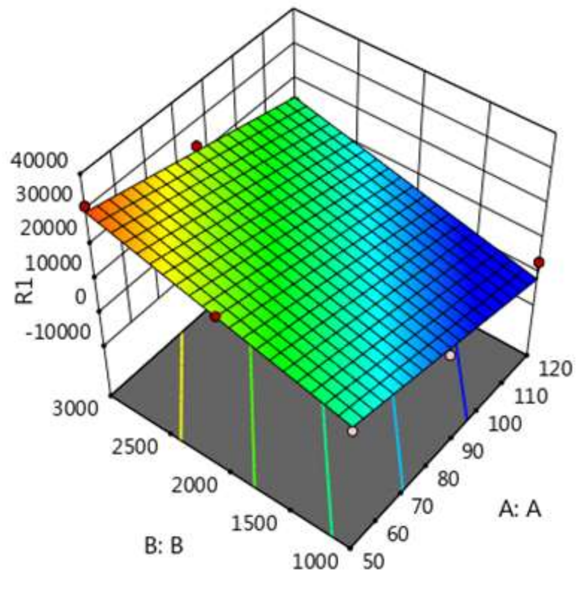

Figure 2. Three-dimensional diagram of the effect of electric field intensity and pulse number on DPPH.

The results of this section were consistent with the results of Guderjan et al. (2007). Arab-Shirazi et al. (1400), by investigating the antioxidant properties of aqueous and alcoholic extracts extracted from two plants, Noroozak and Cotton under the influence of pulsed electric field, showed that by increasing the intensity of pretreatment and choosing an alcoholic solvent, the antioxidant activity of the extracts increased significantly at the 95% confidence level. Also, Zhang et al. (2015), by investigating the effect of pulsed electric field on vitamin C and antioxidant properties, showed that pulsed electric waves change the molecular weight, spatial structure and polarity of molecules, and this causes a change in the inhibition of DPPH free radicals. Also, the pulsed electric field increased the amount of antioxidants, which is due to the increase in the maintenance of the electrochemical reaction and the formation of metal-anthocyanin complexes that have a higher antioxidant capacity;

3-3- Results of the study of phenolic compounds using the Folin-Ciocaltio method

Polyphenolic compounds in plants are widely available and reports show that there is a positive relationship between total phenolic content and antioxidant activity in many plant species. Phenolic compounds in plants are known as potent endogenous antioxidants, due to their ability to donate hydrogen or electrons and convert into a stable radical form, intermediates. Phenols are important groups of secondary metabolites of plant compounds, because they have the capacity to trap free radicals due to the presence of hydroxyl groups. Therefore, the phenolic content of plants can directly contribute to its antioxidant activity. (Sánchez-Moreno et al., 2005). According to the results (Figure 3). It was observed that the total phenolic content of the extract increased with increasing the number of pulses and increasing the intensity of the pulsed electric field applied to the sample. Also in Figure 3, it was observed that the total phenolic content of the extract decreased with increasing number of pulses and decreasing the intensity of the pulsed electric field applied to the sample. Due to the complexity of the chemical reactions present in natural systems, increasing the separation of the total phenolic content is difficult. Total phenolics are formed in plant products through the activity of phenylalanine ammonia lyase (PAL) of phenylpropanoid metabolism. (Sánchez-Moreno Sánchez-Moreno et al., 2005). The decrease of these compounds is probably due to the destructive effect of the electric field intensity at high pulses on these compounds.

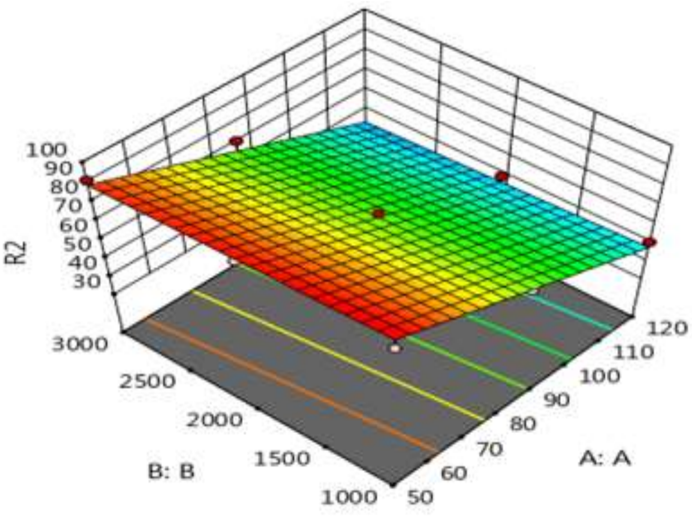

Figure 3. Three-dimensional diagram of the effect of electric field intensity and pulse number on phenolic compounds.

Bakhshabadi et al. (2017) examined the changes in total phenolic compounds of black seed oil and showed that the intensity of the electric field had the greatest effect on the amount of total phenolic compounds. This means that with increasing the intensity of the electric field and increasing the number of pulses, a similar effect is also created on the total phenolic compounds. In such a way that with increasing the intensity of the electric field and the number of pulses, the amount of total phenolic compounds increases due to the release of these compounds and the greater intensity of these two variables, due to the destructive effect of high electric field intensities on the amount of these compounds. (Bozinou et al., 2017). Also, with increasing total phenolic compounds, the antioxidant property increases. Phenolic compounds with high molecular weight have a high ability to scavenge free radicals. This ability depends more on the number of aromatic rings and the nature of the hydroxyl groups that can be displaced (Sarkis et al., 2015).

4- Conclusion

Pulsed electric field is a non-thermal process in which food is exposed to a pulsed field with variable voltage. This method, while having little effect on the sensory characteristics and nutritional value of the products, as a pre-process increases the diffusion of intracellular substances and increases the drying rate. The results obtained by the software were compared and evaluated and showed that the use of pulsed electric field, under optimal conditions, increased the antioxidant properties and phenolic compounds in the extract, which shows the effectiveness of this method in the processing of food products. On the other hand, it was found that the use of pulsed electric field increases the extraction of more compounds in the pre-treated sample, which shows the effectiveness of this method in the processing of food products.

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چکیده

در این تحقیق بهینه‌سازی میدان الکتریکی پالسی و تاثیر آن بر خصوصیات آنتی‌اکسیدانی و فنولی عصاره استخراج شده از برگ شمعدانی عطری بررسی شد. این فرآیند با استفاده از اعمال پیش‌تیمار میدان الکتریکی پالسی، در ۳ شدت میدان الکتریکی (۱۰۰۰، ۲۰۰۰ و ۳۰۰۰) و ۳ سطح پالس (۵۰، ۸۵ و ۱۲۰) مورد بررسی قرار گرفت. سپس ترکیبات فنولی، ترکیب اسیدهای چرب عصاره و خواص آنتی اکسیدانی آن بررسی شد. همچنین برای شناسایی ترکیبات شیمیایی از کروماتوگرافی گازی/طیف‌سنجی استفاده و نتایج نشان داد که محتوای فنل کل و خواص آنتی اکسیدانی عصاره با افزایش تعداد پالس و افزایش شدت میدان الکتریکی پالسی اعمال شده به نمونه، افزایش یافته است. همچنین طبق نتایج GC بیشترین ترکیب موجود در عصاره برگ شمعدانی عطری، به ترتیب Glycidyl oleate و Oleic Acid بود. با توجه به نتایج بهینه‌سازی فرایند می‌توان بیان نمود که شدت میدان الکتریکی ۲۰۰۰ کیلوولت بر سانتی‌متر و اعمال سطح پالس ۸۵، موجب افزایش خواص آنتی‌اکسیدانی و ترکیبات فنولی در عصاره استخراج شده از برگ شمعدانی عطری شد.