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Choline chloride: lactic acid as a green solvent for the extraction of eugenol, eugenol acetate, and β-caryophyllene of clove buds

Petra Strižincová [1], Igor Šurina [1,*] Michal Jablonský [1], Veronika Majová [1], Aleš Ház [1] and Katarína Hroboňová [2]

[1] Institute of Natural and Synthetic Polymers, Department of Wood, Pulp and Paper, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK-812 37, Bratislava, Slovakia; petra.strizincova@stuba.sk; igor.surina@stuba.sk; michal.jablonsky@stuba.sk; veronika.majova@stuba.sk; ales.haz@stuba.sk;

[2] Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK-812 37, Bratislava, Slovakia; katarina.hrobonova@stuba.sk

* Correspondence: igor.surina@stuba.sk;

Abstract: In this study, the effect of extraction conditions on the total phenolic content of clove buds was investigated. Clove buds were extracted using a deep eutectic solvents like mixtures choline chloride: lactic acid in the 1:2 molar ratio. Additionally, the HPLC-DAD method was used to determine the main components of the clove extracts, including eugenol, eugenol acetate, and β-caryophyllene. The conditions used in the extraction with choline chloride: lactic acid (molar ratio 1:2) included extraction temperature (t = 40 – 80°C), water addition (5.6 – 40%), and extraction time (30 – 90 min). Determination of the phenolic compounds was done by the Folin-Ciocalteu method. The optimum operating conditions for the total phenolic content were identified at 77°C, 30 min, and a water addition of 40%. Based on the results, the highest amount of eugenol (307.26 ± 8,44 mg/g dry raw material) was determined in clove extracts which were extracted under condition of 60°C, 22.8%, and 30 min.

Keywords: clove buds; choline chloride: lactic acid; extraction; design of experiment; eugenol

1. Introduction

Syzygium aromaticum commonly known as *Eugenia caryophyllata L.* or clove represents a valuable medicinal plant currently being investigated for its antiviral and antioxidant activity. It is a significant source of a mixture of aliphatic and cyclic volatile terpenes and phenylpropanoids [1]. Clove has been used since ancient times in traditional medicine as an herbal remedy for respiratory ailments, as a warming and stimulating agent, helping to control digestive problems and to improve circulation and metabolism [2–6].

Clove buds contain 15 to 20% of essential oil by weight. The main component of essential oil is eugenol (70 - 95%), with others being eugenol acetate (up to 20%) and β-caryophyllene (12 - 17%) at lower amount [7]. According to numerous scientific research, clove and its essential oil have exceptional biological qualities that are beneficial to human health [4–6,8–13]. The authors [13–18] described the potential role of clove extractives for the prevention and treatment of the SARS-CoV-2 associated disease, focusing on the antiviral, antioxidant, antimicrobial, antifungal, antinociceptive, cytotoxicity, anti-inflammatory, and antithrombotic activity [19–25]. From a molecular perspective, some computational studies [15,16] suggested phytocompounds extracted from cloves as effective anti-COVID-19 medications, and one of them, kaempferol, was shown in silico to bind the substrate binding pocket of the main protease of SARS-CoV-2 with high affinity and interact with the active site residues like Cys145 [14]. In the work [26] have evaluated clove phytochemicals (eugenol, eugenin, syzyginineB, and causaricin) as potential candidates for antiviral drugs targeting the major protease of SARS-CoV-2 using computational

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techniques. In general, a wide range of extractive chemicals found in plants may be able to combat viruses. Plant extracts have the ability to inhibit $3CL^{pro}$, the primary protease of the SARS-CoV-2 virus (6WQF) [27,28].

The extraction of bioactive compounds from natural sources is an important technological process that produces materials capable of replacing petrochemical-based feedstocks. These products are used for the preparation of food additives, pharmaceutical products, and nutraceuticals. The conventional extraction methods have several drawbacks, including using toxic solvents, possible thermal degradation, and hydrolysis of some of the constituents of interest. Therefore new, modern, environmentally friendly techniques extraction of natural chemicals offer new opportunities for process development to improve extraction efficiency [29–31]. A key technological step in obtaining extractable compounds with the desired product yield is to find the appropriate method and conditions for their isolation since the conditions of isolation affect the quantitative and qualitative representation of the individual components. One of the subjects of interest in the selection of extraction conditions is the replacement of organic solvents with environmentally friendly alternatives, green solvents [32–34].

Many authors have investigated the chemical composition and biological activity of cloves using green extraction techniques with non-toxic solvents. Several studies [35–42]have evaluated extraction with supercritical CO_2 as a green extraction technique of clove buds, in which high yields (13 – 23.9 wt%) and eugenol content (56.97 – 87.41%) were obtained. Using conventional extraction technology, the eugenol yields were as follows 11.5 - 21.2 wt% and eugenol concentrations were 50.3 - 87.26%. Clove essential oil obtained by the conventional extraction technique of hydrodistillation was examined by Gonzales-Rivera et al. [43]. From the results, they found the concentration of eugenol (66.9%), β-caryophyllene (24.8%), alpha-humulene (3.1%), and eugenol acetate (2.7%). Oliviera et al. [44] have performed extraction with supercritical CO_2 in the experimental conditions of 40°C and 50°C, with extraction pressure of 10, 20, and 30 MPa. The results showed quantitative chemical composition feature mainly eugenol (62.88%), E-caryophyllene (17.13%), eugenol acetate (21.20%), and alfa-humulene (2.62%), with eugenol being obtained at the highest concentrations in all essential oil fractions analyzed. It is further evident from the results that the major constituent eugenol plays an active role as a phytotoxic activity-promoting substance.

Deep eutectic solvents (DES) like mixtures have become increasingly popular as promising green solvents [45]. DES-like mixtures prepared of natural components (sugars, organic acids, amino acids) contain a quaternary ammonium salt as a hydrogen bond acceptor and a hydrogen bond donor, which bond to each other by hydrogen bonding. DES like mixtures has unique physicochemical properties which are closely related to ecological requirements and that make it an attractive green solvent – biodegradability, non-toxicity, easy to prepare, renewable, sustainable, tailorable properties, miscibility with water, low flammability, biocompatibility, stability, and low-cost. The other important step in extraction is the determination of extraction conditions. The extraction efficiency is dependent on many factors such as the type and volume of extracting solvents, temperature, ionic strength, extraction time, analyte properties, agitation, etc. These conditions and the interactions between them have a significant effect on the quantity and quality of the yield of individual compounds [46–49].

The objective of the present study was to evaluate extraction using deep eutectic solvents like mixtures of choline chloride: lactic acid (molar ratio 1:2), describe the effect of varying the working parameters of extraction (temperature, addition of water, extraction time) on the total phenolic content and determine the main constituent of clove buds extracts namely eugenol, eugenol acetate, and β-caryophyllene by HPLC-DAD method.

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2. Materials and Methods

2.1. Chemicals

All the solvents, chemicals, and reagents used in this study were analytical grade. Choline chloride (ChCl) (≥ 98.0%), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl radical, gallic acid, anhydrous sodium carbonate (Na_2CO_3), analytical standard eugenol (≥98.0%), eugenol acetate (≥98.0%), and β-caryophyllene (≥80.0%), were purchased from Sigma-Aldrich. Lactic acid (LacA) (90.0%) solution was obtained from VWR International (Bratislava, Slovakia). Choline chloride was dried under a vacuum.

2.2. Plant materials

Clove buds were purchased from the store Svet plodu (Czech Republic) [50]**.** Organic cloves were grown and dried in Molucca (Indonesia). Before each experimental assay, the raw material was ground in a knife mill and separated sieves into 0.02-0.04 mesh. The moisture content of the material was determined by drying approximately 1g of clove buds at 105°C for 6 hours until complete moisture removal according to ISO 3130:1975.

2.3. Preparation of deep eutectic solvents like mixtures

The DES like mixtures were prepared by mixing and stirring choline chloride and lactic acid (molar ratio 1:2) in a water bath (60°C; 30 min) to form a homogeneous liquid. Upon mixing, the hydrogen-bond donor and hydrogen-bond acceptor components interact with each other through hydrogen bonding.

2.4. Design of experiment

The method of the planned experiment was used for a comprehensive description of the influence of the studied physical parameters of extraction on the total phenolic content. A full 2^3 factorial design of experiments was performed for a comprehensive description of selected physical parameters (temperature, water addition, extraction time) affecting the extraction process. The complete experiment design consisted of twenty combinations including five replicates of the center point for reported standard deviation from the overall mean. The design of the experiment (DOE) method was used to describe mathematical-statistical data of complex formation. Analysis of variance (ANOVA) was employed to assess the statistically significant factors.

2.5. Extraction using deep eutectic solvents like mixtures

The extraction was carried out under constant stirring (speed of the blender 12; Tube Revolver Rotator Thermo Fisher Scientific) in a closed flask under the conditions of the design of the experiment. DOE was applied for the study the effect of the extraction temperature (t = 40 - 80°C), water addition (the added water was above 5.6 up to 40%), and extraction time (30 – 90 min) on total phenolic content. The value of the lowest water content (5.6%) represents the amount of water that is bound in the choline chloride: lactic acid (1:2). The ratio of clove buds to extraction solvent 1:10 (w/v) was kept constant during the experimental procedures.

2.6. Determination of Total Phenolic Content

The total phenolics content (TPC) of the clove extracts was determined by the Folin-Ciocalteu assay, based on the redox reactions of phenolic compounds [51]. A volume of 0.5 mL of Folin-Ciocalteu reagent and 0.5 mL of the extract or extraction mixture (as a blank sample) were pipetted into a test tube. After 3 min, 1.5 mL of 20% sodium carbonate solution and distilled water were added into the test tube. After stirring, the mixture was incubated in a closed dark-colored flask at room temperature for 120 min, and then the absorbance of the solution was recorded at 765 nm. The TPC in the extracts was determined using the calibration curve based on the absorbance at 765 nm and expressed as gallic acid equivalent (GAE) in mg per 1g of dry raw material.

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2.7. HPLC determination of eugenol, eugenol acetate, β-caryophyllene

Eugenol, eugenol acetate and β-caryophyllene were determined by HPLC-DAD method [46–49]. The analysis was performed using Agilent Technologies HPLC system (series 1100) consisting of degasser, binary solvent delivery pump, an autosampler, a column thermostat and a diode array detector (DAD). Separation of analytes was carried out in reverse phase mode using a Nucleodur 100-5 C18ec (250 x 4.6 mm i.d., 5 μm particles) column (Macherey-Nagel, Germany). The mobile phase consisted of acetonitrile (A) and water (B). The gradient program was 0 – 10 min linear gradient for (A) component from 40 to 80% then to 100% of (A) over 0.5 min and held at 100% of (A) for 10 min. This was followed by a reverse gradient over 0.5 min and held at 40/60 (A)/(B) for 4 min. The flow rate was 1.0 mL/min, injection volume was 20 μL, and column temperature was maintained at 25°C. DAD was operated in the wavelength range of 190 – 400 nm, and detection wavelengths was set at 210 nm for β-caryophyllene. Eugenol and eugenol acetate were detected at 280 nm. The cloves extracts were diluted 1:10 (v/v) with water before injecting in to HPLC.

Quantification of target analytes was performed by the calibration curve method. Calibration curve was constructed as dependence of average peak areas of analyte versus concentration of analyte in standard solution. Mixed analytes calibration solutions at six concentration levels ranged from 100 to 1000 μg/mL for eugenol and eugenol acetate, or from 15.5 to 100 μg/mL for β-caryophyllene were analysed in triplicate for construction of calibration curve. The values of the retention time, resolution, peak symmetry factor, high equivalent of theoretical plate, linearity, limit of detection (LOD), and limit of quantification (LOQ) are summarized in Table 1.

Table 1. System suitability parameters and validation parameters of HPLC-DAD method for determination of eugenol, eugenol acetate and β-caryophyllene.

	eugenol	**eugenol acetate**	**β-caryophyllene**
HPLC system suitability parameters [a]			
Retention time (t_R, min)	7.36	9.09	17.54
Repeatability RSD (%) t_R	0.21	0.25	0.22
Repeatability RSD (%) *A*	1.65	1.05	1.59
Peak symmetry	1.16	1.14	1.15
HEPT (μm)	17.27	9.33	2.26
R_S	7.53	39.12	
Validation parameters			
Calibration curve	A = 145.32 + 18809.18 × c	A = 101.13 + 10523.43 × c	A = 23.55 + 46563.62 × c
R^2	0.9998	0.9989	0.9990
Linear range (μg/mL) [b]	100 - 1000	100 - 1000	15.5 - 100
LOD (μg/mL) [c]	7.7	16.6	5.1
LOQ (μg/mL) [c]	23.2	50.3	15.5

[a] the concentrations of eugenol, eugenol acetate, and beta-caryophyllene in standard solution were 0.1 mg/mL, [b] expressed as the concentration of the analyte in the reference solution, [c] the values of LOD and LOQ were calculated according to equations 1 and 2, t_R – retention time, *A* – peak area, HEPT - height equivalent of a theoretical plate, c – concentration of analyte (in mg/mL), RSD – relative standard deviation, (n=6)

Equations (1), (2) for calculation LOD and LOQ:

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$$\mathbf{LOD} = \frac{\mathbf{3.3\ x\ Standard\ error\ of\ the\ calibration\ curve}}{\mathbf{Slope\ of\ the\ calibraton\ curve}} \quad (1)$$

$$\mathbf{LOQ} = \frac{\mathbf{10\ x\ Standard\ error\ of\ the\ calibration\ curve}}{\mathbf{Slope\ of\ the\ calibration\ curve}} \quad (2)$$

3. Results and discussion

There are several factors that could have independent effects or combined effects on the extraction efficiency. Modification of extraction factors affects the transport and solubilization of biological macromolecules, kinetics of extraction, and also physicochemical properties of deep eutectic solvents like mixtures [52,53]. In this experiment, extraction temperature, water addition, and extraction time were investigated on the effect of the total amount of phenolic compounds obtained by extraction using choline chloride and lactic acid (molar ratio 1:2). Modification of water addition was carried out to improve the diffusion of polyphenols. The ranges of the factors were selected based on previous scientific knowledge from a literature search. Experimental design conditions are listed in Table 2. To comprehensively describe the selected physical parameters affecting the extraction process, a full 2^3 factorial design of experiments were carried out and twenty experiments were conducted. The results of the measured values of the total amount of phenolic compounds are listed in

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Table *3*.

Table 2. Conditions of Experimental Design

Factor	-1.682	-1	0	1	1.682
x_1	40	48.11	60	71.89	80
x_2	5.63	12.6	22.8	33.0	40
x_3	30	42	60	77	90

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Table 3. Measured parameter TPC; (mg GAE/g dry raw material) and extraction factors - temperature (factor x_1; °C), water addition (factor x_2; %), time of extraction (factor x_3; min.)

Trial no.	Sample no.	Factor x_1	Factor x_2	Factor x_3	TPC (mg GAE/g dry raw material)
1	Clove_1	48.11	12.6	42	48.8
2	Clove_2	71.89	12.6	42	33.4
3	Clove_3	48.11	33.0	42	89.1
4	Clove_4	71.89	33.0	42	80.5
5	Clove_5	48.11	12.6	78	40.7
6	Clove_6	71.89	12.6	78	34.6
7	Clove_7	48.11	33.0	78	70.3
8	Clove_8	71.89	33.0	78	75.9
9	Clove_9	40.00	22.8	60	43.4
10	Clove_10	80.00	22.8	60	31.6
11	Clove_11	60.00	5.63	60	39.5
12	Clove_12	60.00	40.0	60	111.9
13	Clove_13	60.00	22.8	30	55.8
14	Clove_14	60.00	22.8	90	56.8
15	Clove_15	60.00	22.8	60	59.8
16	Clove_16	60.00	22.8	60	55.9
17	Clove_17	60.00	22.8	60	59.4
18	Clove_18	60.00	22.8	60	74.3
19	Clove_19	60.00	22.8	60	67.4
20	Clove_20	60.00	22.8	60	76.2

The measured values for each parameter were processed according to design of experiment theory using the computer program STATIS, written by Pavel Alexy et al.[54] in MS Excel Visual Basic. Output from the program statis was in the form of full regression and statistical analysis, where the ANOVA was used. All statistical tests were carried out at a probability level of 95%. For each parameter the following type of regression equation (3) was obtained:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}\,x_3^2 \quad (3)$$

where y is an output parameter, x_1, x_2, and x_3 are levels of factors in coded coordinates, and b_0 - b_{33} are regression coefficients, b_0 is a constant, b_1, b_2 and b_3 are the linear coefficients, b_{12}, b_{13} and b_{23} are the interaction or crossed coefficients, and b_{11}, b_{22} and b_{33} are the quadratic coefficient.

Based on the results obtained, the effect of the water addition choline chloride: lactic acid (molar ratio 1:2) greatly influences the total amount of phenolic compounds extracted from clove buds as well as according to the value of regression coefficient b_2 (Table 4). The solubility of compounds is related to the polarity of the deep eutectic solvents like mixtures and can be further increased by adding water. According to Figure 1, TPC varies in dependency on the water addition from low values 36 mg GAE/g dry raw material up to about 112 mg GAE/g dry raw material. Many reviews [48,49,55–58] have demonstrated the effect of water addition on the physicochemical properties of solvents, and

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consequently on their behavior in biological structures. The presence of a high polar solvent such as water can probably affect their extraction capability such as viscosity. High viscosity is one of the main obstacles in the analytical use of deep eutectic solvents like mixtures because it limits the mass transfer between the sample and the extraction phase. The water addition leads to a decrease in the viscosity of the reaction medium, thereby increasing mass transfer and improving extraction efficiency and facilities polyphenol diffusion [49,59].

Table 4. Results of ANOVA

Coeficient	TPC
*b_0	65.164
b_1	0.869
*b_2	24.646
*b_3	-6.214
*b_{11}	-7.745
*b_{12}	9.337
b_{13}	-4.086
*b_{22}	5.780
*b_{23}	-9.064
b_{33}	-1.080
*statistically significant coefficients	

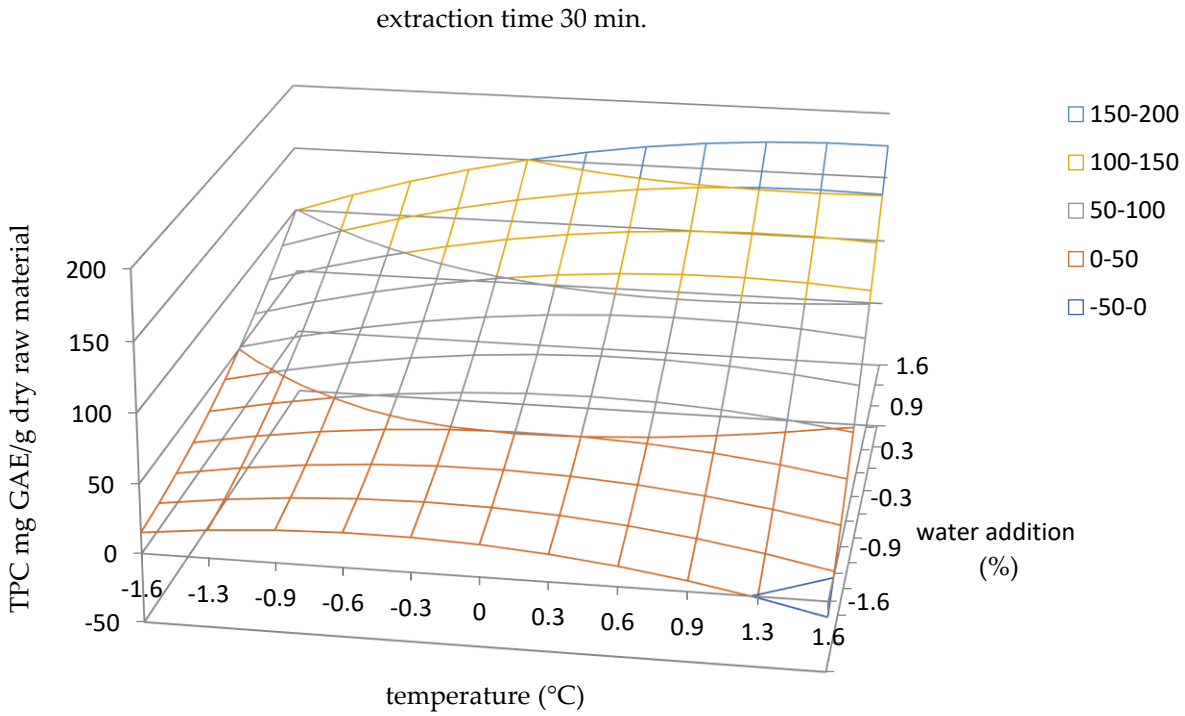

Figure 1. Response surface of total phenolic content in dependency on factor x_1 (temperature, °C) and factor x_2 (water addition %) in coded coordinates at constant factor x_3, (extraction time 30 min.)

The results (Table 4) show that temperature did not significantly affect the yield of phenolics in the given range of values. An increase in the temperature of extraction as well as water addition caused an increase in TPC according to the response surface (**Figure *1***).

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According to the x-axis (extraction temperature) total phenolic content shows a maximum, which can be observed on the response surface as well as according to the negative value of coefficient b_{11} (Table 4). The mutual interaction is confirmed in this case between water addition and temperature by a significant interaction coefficient b_{12} (Table 4). According to theory, when temperatures are high, plant tissues become softer and the weak contacts have an impact on the cell membranes. Thus, it is simple to extract phenolic chemicals into the solvent. [60] The mutual interaction is confirmed also between water addition and extraction time by interaction coefficient b_{23} (Table 4). In chemistry and technology DES, water is the most influential chemical compound [61]. Water reduces the viscosity of DES due its viscosity is lower than that of DES. The effect of both temperature and water content on viscosity is shown in **Figure 2** [62]. Francisco et al. [63] also investigated the effect of water on density and found that the density of the studied DES decreases with water content, but this effect is less pronounced.

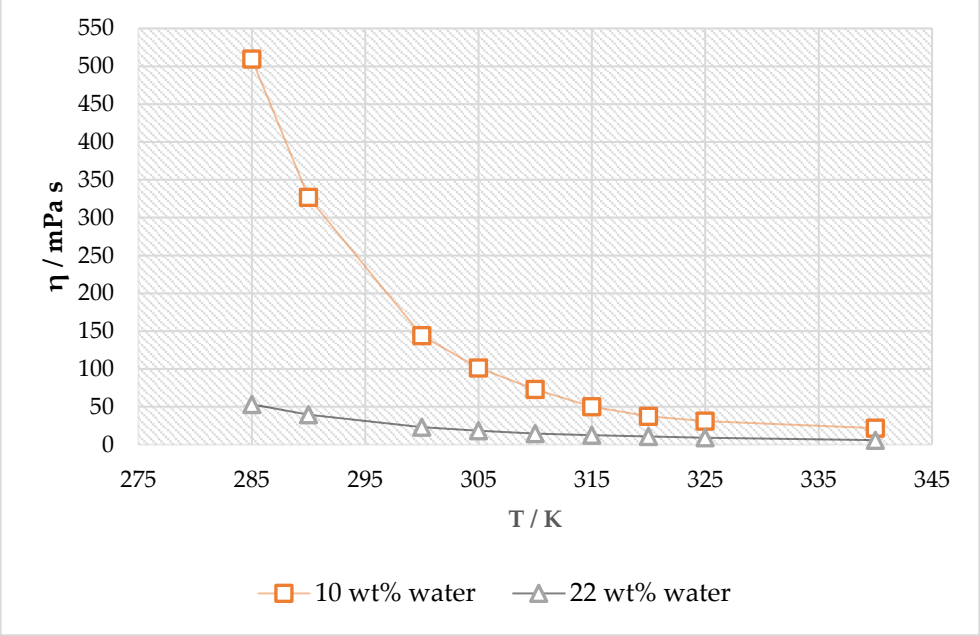

Figure 2. Dependence of viscosity on temperature and water content in DES composed of lactic acid and choline chloride in 2:1 molar ratio.

Water's effect on DES polarity was examined by Craveiro et al. [52]. They noticed that the solvent's polarity increased with water quantity. Given that water has one of the highest polarities of all solvents, this fact is expected.

As shown in Figure 3 extraction time was interpreted in the ranges of 30 – 90 min. The results summarized in Figure 3 show the influence of extraction time on the response surface of phenols, where it was observed that response surface reach the maximum value already at 30 min. This behaviour proves the significant role of the extraction time, also confirmed by our regression analysis results (Table 4). The amount of phenolics obtained was decreasing when the extraction time was increased from 30 to 90 min. Longer extraction times increase the chance of oxidation of phenolics unless reducing agents are added to the solvent system.

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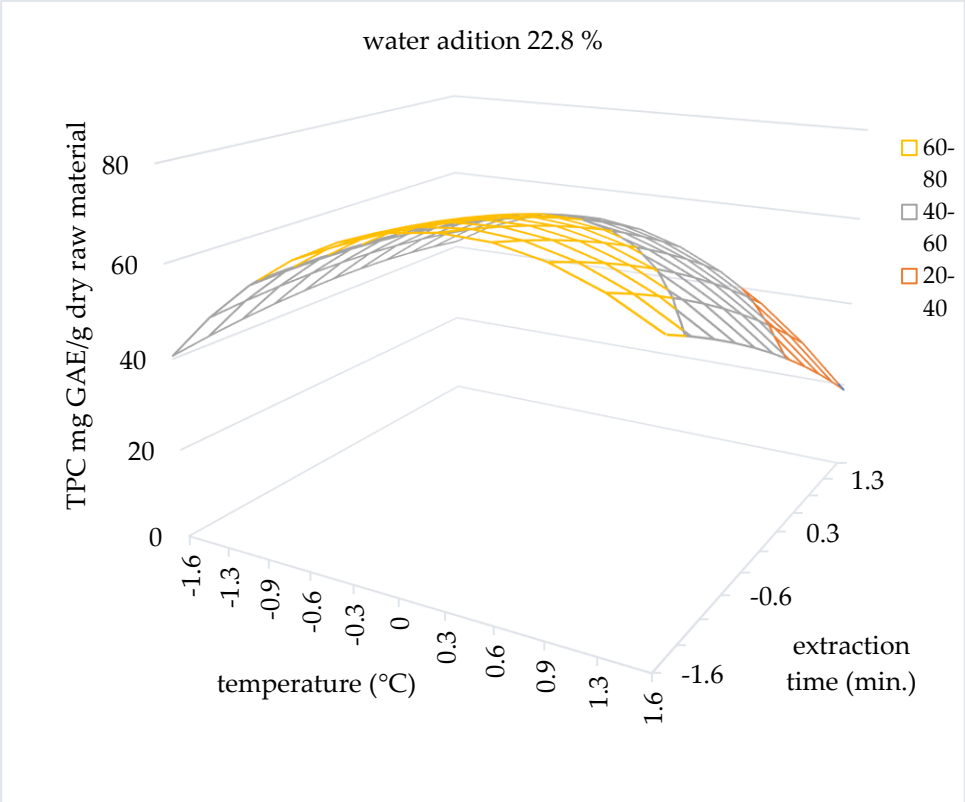

Figure 3. Response surface of total phenolic content in dependency on factor x_1 (temperature, °C) and factor x_3 (extraction time, min.) in coded coordinates at constant factor x_2 (water addition 22.8%).

The subprogram SOLVER from Microsoft Excel was used for optimization computation. The main purpose of this optimization was to obtain a composition with a phenolic content as high as possible. Based on the results obtained, it can be assumed that the total phenolic content was the most obtained for an extraction temperature of 77°C, an extraction time of 30 min, and a water addition of 40%.

The work further determined the representation of phytogenic bioactive components. Clove extract is a mixture of various compounds, with three main compounds namely eugenol, eugenol acetate and β-caryophyllene. Eugenol and eugenol acetate has shown excellent antimicrobial activity in studies, being active against fungi and a wide range of gram-negative and gram-positive bacteria [64,65]. Eugenol has been endorsed as a substance that encompasses a number of beneficial aspects against a vast array of life-threatening diseases including inflammation, oxidative stress, hyperglycemia, nerve disorders, elevated, cholesterol, and cancer [66–68]. Antiviral activity of eugenol against the influenza A virus was showed in research Dai et al. [69] Eugenol has the potential to limit viral infection and replication categorically against herpes simplex viruses, i.e., HSV-1 and HSV-2, exhibiting IC50 values between 16.2 mg/mL and 25.6 mg/mL, as examined by the plaque reduction assay (PRA). Eugenol has been validated as suitable against clinical isolates of herpesimplex virus-1 (HSV-1) [70,71]. Musthafa et al. [72] evaluated eugenol acetate for its antiviral potential against both gram-negative and gram-positive pathogens. The results of the work reveal the potential of eugenol acetate as an alternative candidate to control the pathogenicity of both gram-negative and gram-positive organisms. Eugenyl acetate at a concentration of 150 µg/ml significantly inhibited the production of virulence factors, such as pyocyanin and pyoverdin, by Pseudomonas aeruginosa. In recent years, the pharmacological effects of β-caryophyllene have been demonstrated in many organs such as the liver [73], kidney [74] and brain. β-caryophyllene has been reported to have therapeutic effects as an antioxidant, anti-inflammatory [75], and anticancer [76,77].

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HPLC-DAD method was applied to quantitatively determine eugenol, eugenol acetate, and β-caryophyllene in 20 samples obtained by extraction using deep eutectic solvents like mixtures. The results of the determination of target analytes by HPLC-DAD method are summarized in

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Table *5*. Representative chromatograms of a standard solution of eugenol, eugenol acetate, and β-caryophyllene are documented in **Figure *4***. Chromatograms of sample extracts are shown in **Figure *5***.

Eugenol was present in the extracts in the highest concentration among the target analytes, according to the HPLC results obtained. The major component of clove extract, eugenol, increased from 168.61 mg/g dry raw material to 307.26 mg/g dry raw material, which is several times lower compared to that of eugenol acetate (11.63 – 32.70 mg/g dry raw material) and β-caryophyllene (0.57-0.87 mg/g dry raw material). Eugenol was more abundant in the extract obtained at higher temperatures (70°C) and lower water addition (15%).

A comparison of the published results shows large variability in the composition of the chemical of clove essential oils. Alma et al. [78] tested the chemical composition and content of essential oil from the clove bud by steam distillation. They found in total 18 identified components in clove bud essential, with eugenol 87% being the main constituent, followed by eugenol acetate 8.01% and β-caryophyllene 3.56%. Razafimamonjison et al. [79]obtained essential oil by steam distillation method for 12 hours. They detected the essential oil composition of bud clove from Indonesia, Madagascar, and Zanzibar, the major constituent eugenol (77.50 – 79.87%) and β-caryophyllene (4.06 – 6.91%). In another publication, Fichi et al. found a eugenol content of 59.3%, with a concentration of β-caryophyllene and eugenyl acetate was 24.9% and 4.2%, respectively. Chatterjee et al. [80] isolated eugenol from dried clove buds using supercritical carbon dioxide extraction. The optimized conditions that provided the optimum yield (129.86 mg/g dry clove buds) of eugenol were at 60°C, 25 MPa, 90 min with flow rate 2 L/min of CO_2. Yazdani et al. [38] provided the optimum yield (86.7%) at 80°C, 19 MPa. Also, a comparison of eugenol yields in extracts obtained by hydrodistillation, microwave-assisted extraction, and supercritical extraction is reported in the publication. The percentage of eugenol obtained by supercritical extraction (86.70%) was higher than that in extracts obtained by steam distillation (81.47%) and microwave-assisted extraction (79.08%). Differences in components and composition depend on variety, agroecological conditions, pretreatments, processing, and extraction methods, and working parameters of extraction.

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Table 5. HPLC determination of eugenol, eugenol acetate, β-caryophyllene

Number of experiment	Eugenol	Eugenol acetate	β-caryophyllene
	(mg/g) *		
1	168.61	11.63	0.87
2	200.70	18.93	0.89
3	216.14	15.23	0.96
4	218.51	13.96	0.46
5	196.01	19.64	1.05
6	205.67	18.08	0.64
7	222.11	14.22	0.76
8	217.64	13.08	0.22
9	222.99	20.45	0.46
10	211.89	18.51	0.37
11	188.14	19.63	1.09
12	218.41	32.70	0.57
13	307.26	28.64	0.56
14	221.18	18.53	0.27
15	211.18	17.87	0.51
16	215.14	18.23	0.37
17	210.66	17.60	0.45
18	218.22	19.29	0.31
19	209.85	17.55	0.22
20	208.67	17.37	0.39
*expressed for dried sample			

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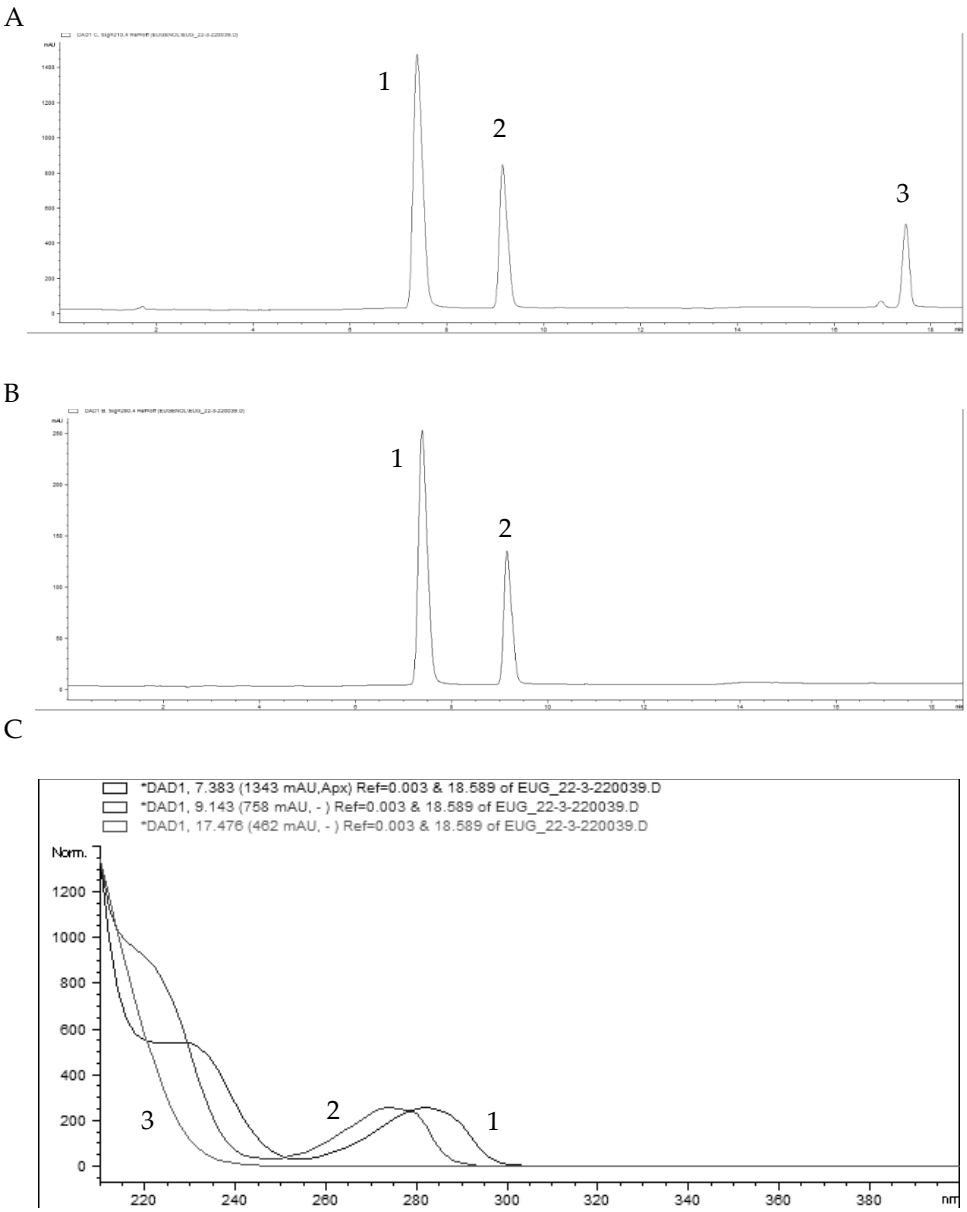

Figure 4. HPLC-DAD chromatograms of standard mixture of eugenol, eugenol acetate, and β-caryophyllene (concentration of each standard: 0.1 mg/mL) detected at two wavelengths, 210 nm (A), 280 nm (B), and UV spectra of eugenol, eugenol acetate, and β-caryophyllene (C). Legend: 1- eugenol, 2- eugenol acetate, 3- β-caryophyllene

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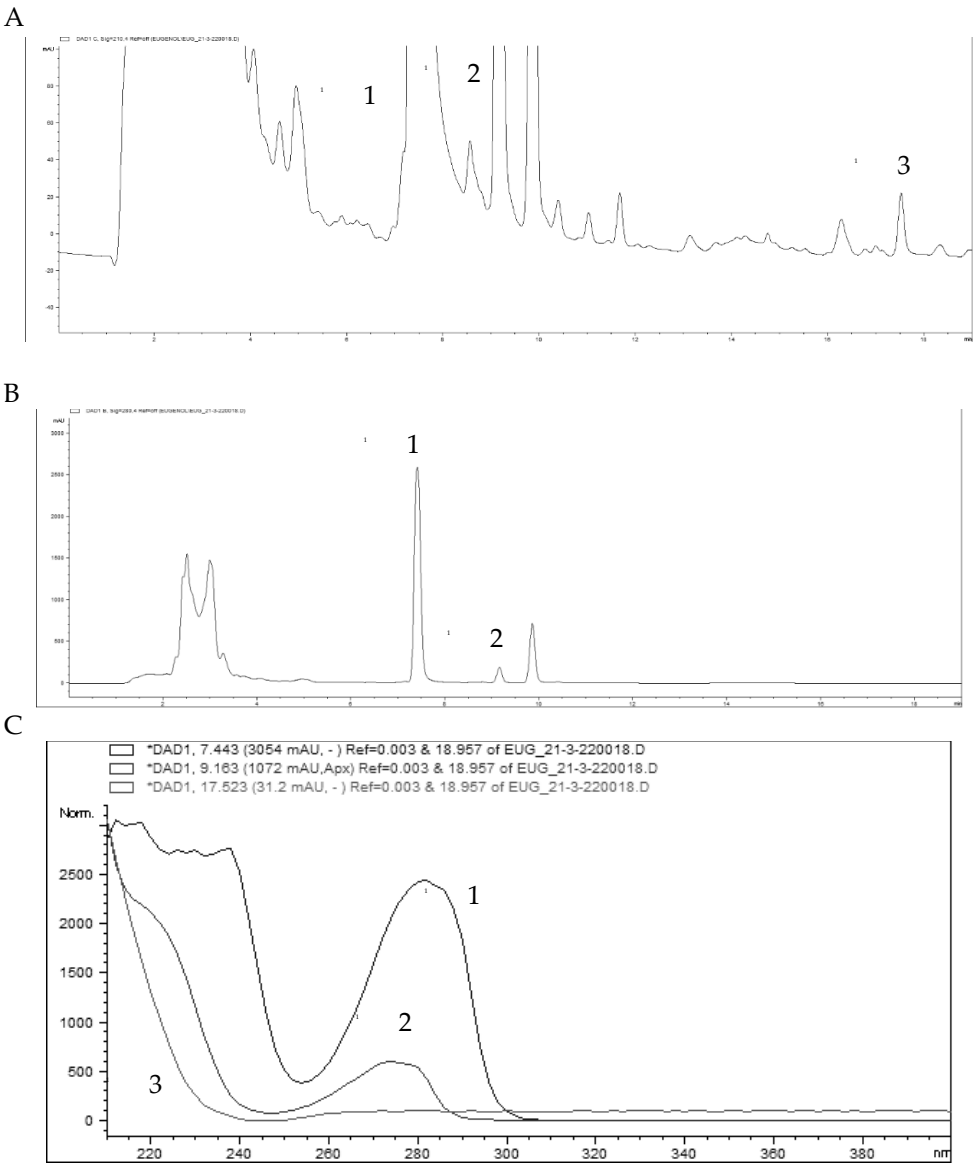

Figure 5. HPLC-DAD chromatograms of sample extract (sample 5, extraction temperature 48°C, water addtition 12,6%, extraction time 78 min.) detected at two wavelengths, 210nm (A), 280 nm (B), and UV spectra of eugenol, eugenol acetate, and β-caryophyllene (C). Legend: 1- eugenol, 2- eugenol acetate, 3- β-caryophyllene

5. Conclusions

Based on the analysis of the experiment, our findings lead to the conclusion that the extraction conditions have a significant impact on the total phenolic content, especially the water addition and extraction time. The water addition had the most significant effect on the total phenolic content, followed by the extraction time. The significance of extraction temperature as a factor on TPC was confirmed in combination with the addition of water. Based on the results obtained, it can be assumed that the total phenolic content was the most obtained for an extraction temperature of 77°C, an extraction time of 30 min, and a water addition of 40%. The high proportion of eugenol was confirmed by HPLC analysis. Eugenol increased from 168.61 mg/g dry raw material to 307.26 mg/g dry raw material, which is several times lower compared to that of eugenol acetate (11.63 – 32.70 mg/g dry raw material) and β-caryophyllene (0.57-0.87 mg/g dry raw material). Based on the results,

choline chloride: lactic acid (molar ratio 1:2) was shown to be an efficient solvent for the extraction of essential oil from clove buds.

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