Bioprocesses and Sustainability

Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

Microwave hydrodiffusion and gravity and pressurized-liquid extraction for obtaining bioactive compounds from *Solanum viarum*

Hidrodifusão por micro-ondas e extração por gravidade e líquido pressurizado para obtenção de compostos bioativos de *Solanum viarum*

Tássia Carla Confortin[1], *Izelmar Todero*[2], *Luciana Luft*[2], *Silvana Schmaltz*[2], *Daniele de Freitas Ferreira*[2], *Juliano Smanioto Barin*[2], *Maicon Sérgio Nascimento dos Santos*[1], *Marcio Antonio Mazutti*[2], *Giovani Leone Zabot*[1], *Marcus Vinícius Tres*[2]

ABSTRACT

Brazilian biodiversity is considered a source of bioactive substances, and one of the species found is *Solanum viarum* Dunal, which is mainly composed of pyrrolizidine alkaloids. The purpose of this study was to evaluate two non-conventional extraction techniques — microwave hydrodiffusion and gravity (MHG) and pressurized-liquid extraction (PLE) — in obtaining bioactive compounds from *S. viarum*. Different parameters were assessed that directly influenced the yield and chemical composition of extracts. For PLE, the percentage of ethanol and temperature were evaluated on yield and composition. For MHG, temperature and pressure were evaluated on the same responses. PLE presented the highest extract yield (26.11 wt.%) and bioactive compounds concentration, while the highest extract yield of MHG was 1.68 wt.%. Both techniques indicated efficiency in extracting integerrimine, senecionine, and quinic acid. Knowing the compounds present in plants, using different extractive methods, enables the development of research that addresses their possible potential in the future.

Keywords: active substances; pyrrolizidine alkaloids; plant secondary metabolites; vegetable extracts.

RESUMO

A biodiversidade brasileira é considerada fonte de substâncias bioativas, e uma das espécies encontradas é a *Solanum viarum* Dunal, que é composta, principalmente, por alcaloides pirrolizidínicos. O propósito deste estudo foi avaliar duas técnicas de extração não convencionais — micro-ondas de hidrodifusão e gravidade (MHG) e extração em líquido pressurizado (ELP) — na obtenção de compostos bioativos de *S. viarum*. Foram examinados diferentes parâmetros que influenciaram diretamente o rendimento e a composição química dos extratos. Para ELP, foram avaliadas a porcentagem de etanol e a temperatura sobre os rendimentos e composição. Para MHG, temperatura e pressão foram avaliadas nas mesmas respostas. O ELP apresentou o maior rendimento de extrato (26,11% em peso) e concentração de compostos bioativos, enquanto o maior rendimento de extrato de MHG foi de 1,68% em peso. Ambas as técnicas indicaram eficiência na extração de integerrimina, senecionina e ácido quínico. Conhecer os compostos presentes nas plantas utilizando diferentes métodos extrativos possibilita o desenvolvimento de pesquisas que abordem seu possível potencial no futuro.

Palavras-chave: substâncias ativas; alcaloides pirrolizidínicos; metabólitos secundários vegetais; extratos vegetais.

[1]Federal University of Santa Maria – Cachoeira do Sul (RS), Brazil.

[2]Federal University of Santa Maria – Santa Maria (RS), Brazil.

Correspondence author: Marcus Vinícius Tres – Federal University of Santa Maria (UFSM) – Rodovia Taufik Germano, 3013 – Universitário II – CEP: 96503-205 – Cachoeira do Sul (RS), Brazil. E-mail: marcus.tres@ufsm.br

Conflicts of interest: the authors declare no conflicts of interest.

Funding: This work was supported by the National Council for Scientific and Technological Development (CNPq) under grant number 302593/2023-3, 428180/2018-3; Coordination for the Improvement of Higher Education Personnel (CAPES) under grant number 001; and Research Support Foundation of the State of Rio Grande do Sul (FAPERGS) under grant number 21/2551-0002253-1.

Received on: 03/27/2024. Accepted on: 05/04/2024.

https://doi.org/10.5327/Z2176-94782070

Introduction

Bioactive substances are functional ingredients or molecules with potential applicability, which can be naturally found in plants (Santos et al., 2022). In addition to important primary metabolites such as lipids, carbohydrates, and amino acids, plants also synthesize a significant diversity of secondary metabolites (Qaderi et al., 2023). The plant secondary metabolism comprises a variety of metabolites that have evolved to promote plant survival by providing protection against general stresses such as environmental factors, insects, herbivores, predators, pathogens, and ultraviolet radiation (Jan et al., 2021). Some secondary metabolites can interfere with the growth and development of biological systems, considered allelopathic compounds. These compounds (allelochemicals) can be used directly for the formulation of agricultural pesticides or even altered in order to improve their biological action (Gajger and Dar, 2021).

Some aspects must be considered when working with bioactive compounds from plants, including the environmental condition of the habitat where the species grow and the extraction technique applied (Krakowska-Sieprawska et al., 2022). Appropriately, various techniques have been explored for extracting bioactive compounds and these are structured into two categories: conventional or traditional and non-conventional. For a long time, extractions were performed by traditional methods such as Soxhlet and maceration, but due to certain disadvantages, the development and use of new methods have arisen (Ilyas et al., 2021). Non-conventional technologies such as microwave hydrodiffusion and gravity (MHG) and pressurized-liquid extraction (PLE) showed advantages such as low toxicity, high efficiency, and reduced extraction time when compared to techniques such as Soxhlet and maceration (Ali et al., 2021).

Contextually, the MHG procedure is a new technology with huge potential for a variety of applications (Farias et al., 2022). The use of microwaves influences the textural properties of the plant material and increases the diffusion of secondary metabolites, improving tissue softness and cell permeability. It emerges as an energy-saving technology since microwaves can also improve cell rupture due to their high penetration power, resulting in increased mass transfer inside and outside the plant tissues (Chouhan et al., 2019). On the other hand, PLE is based on the use of solvents at elevated pressures and temperatures, but not above the critical point. The main purpose of this technique is to promote the extraction of compounds from solid or semi-solid matrices in a short time and using a small amount of solvent (Wianowska and Gil, 2019). A main advantage of ELP compared to conventional extraction methods is that the solvents pressurized remain in liquid state when brought to temperatures above their boiling points. These conditions improve the solvation power of liquids and the kinetics of solid matrix desorption. Additionally, it features higher automation, extraction yields at higher levels, shorter extraction time, and lower toxicity of solvents than other conventional methods (Kang et al., 2016).

Many plants of the Solanaceae family, including *S. viarum*, present in their chemical constitution a large number of alkaloids and steroids such as solasodine, which has several medicinal properties, specifically for cardiotonic, antifungal, antispermatogenic, antiandrogenic, immunomodulatory, anticancer, anti-inflammatory, contraceptive, antimicrobial, and antipyretic applications (Pandey et al., 2018). Kausar and Singh (2018) reported other compounds present in the leaves of *S. viarum* as caffeoylquinic acid derivatives, 5-caffeoyl acid, 3-malonyl-5-caffeoyl-[4-(1-beta-[6-(5-caffeoyl) quinate] glucopyranosyl)], and quinic acid. Only one study with *S. viarum* fruits was observed in the literature so far, where high percentages of polyphenols and tannins were indicated.

Based on the aforementioned aspects and the importance of such bioactive compounds, and considering upcoming agricultural applicability of the obtained extracts, the interest in the extraction research of compounds from *S. viarum* with two different non-conventional techniques arose. The yield and chemical composition of the extracts were evaluated using PLE and MHG. There are few studies about the extraction yield and chemical composition involving the fruits and roots of *S. viarum*, and so far, no references have been noticed using PLE and MHG as extraction techniques.

Material and Methods

Samples preparation

The fruits and roots of *S. viarum* were collected in southern Brazil (27°55'39.43S, 52°7'37.14W). The samples were dried (40°C) until constant mass and refrigerated (-4°C) until extraction procedures. The samples were dried at 40°C until reaching a moisture content of approximately 10%. Then, they were ground (Marconi, SP, Brazil) and particles were classified by the Sauter Mean Diameter by the Tyler series. Sizes varied from 8 to 48 mesh (0.3–2 mm) and were used for subsequent steps. The samples were maintained at -4°C until extraction (Confortin et al., 2019). Moreover, the assays were performed in triplicate. For a better comprehension of the steps performed in this study, a structural flowchart is indicated in Figure 1.

Extraction techniques

The PLE and MHG extraction were performed according to the procedures described by Confortin et al. (2021). PLE is one of the main modern extraction techniques and its operation involves the use of high pressure and temperature to improve the extraction process. In this study, the solid sample was ground and dried to increase the surface area and facilitate extraction. Subsequently, the sample was placed in a sealed extraction cell, and the solvent was pumped from the reservoir to the extraction cell by an HPLC pump. Afterward, the extraction cell was heated and the extraction process was established, increasing the solubility of the target compounds in the solvent. Thus, the solvent extracted the bioactive compounds from the sample matrix.

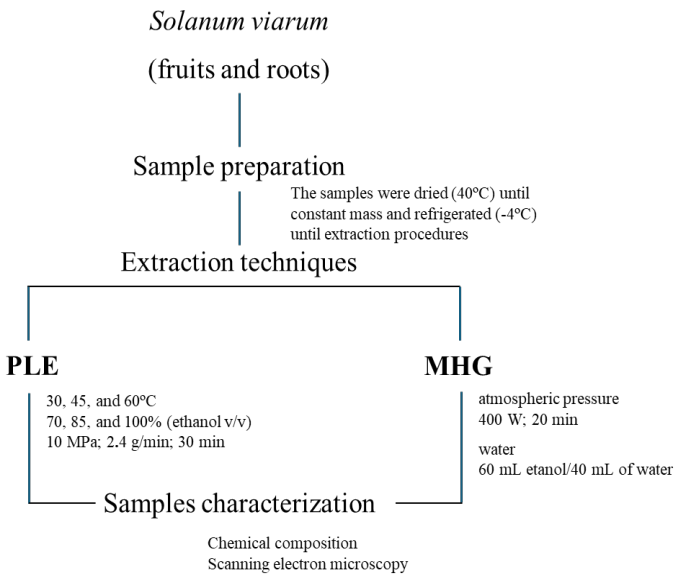

Figure 1 – Comprehensive structural flowchart of the steps conducted in this study.

The main reason for choosing the method was its highly efficient extraction strategy, which allows rapid extraction of target compounds. Furthermore, PLE generally results in higher extraction yields than traditional methods, due to the compounds' greater solubility at high pressure and temperature. In summary, PLE is an advanced method that uses high pressure and temperature to increase the efficiency, selectivity, and yield of extracting bioactive compounds from solid samples, offering several benefits over traditional extraction techniques. On the other side, MHG extraction was performed in a 2.45 GHz microwave equipment (Milestone multimode NEOS-GR, Bergamo, Italy). The operational process was established by Confortin et al. (2021), under atmospheric pressure (0.1 MPa) and 400 W of power for 20min. Approximately 100 g for each matrix plant were humidified before extraction, by immersing the plant in the solvent for 1h. Extractions were evaluated by soaking the matrices only in water or hydroalcoholic solution (60 mL of ethanol and 40 mL of water). After extraction, the extract was collected and the solvent was evaporated. For the extraction procedures, approximately 10 g of the sample were used. For PLE, the temperatures used in the tests were 30, 45, and 60°C, and the ethanol percentages were 70, 85, and 100% (v/v). Thepressure was held constant at 10 MPa. The solvent flow rates were 3.0, 2.8, and 2.7 mL/min for ethanol at 100, 85, and 70%, respectively, in order to maintain the mass flow rate of the solvent constant (2.4 g/min), and the extraction time was 30min. Finally, extractions with MHG were kept at atmospheric pressure under 400 W power for 20 min. They were then evaluated by soaking the matrix only in water and in a mixture of hydroalcoholic solution (60 mL ethanol and 40 mL of water).

Samples characterization

The chemical composition and scanning electron microscopy (SEM) analysis were determined according to the procedures described by Confortin et al. (2021). The injector temperature was maintained at 320°C. A volume of 1 µL of each sample was injected at a split ratio of 1:40. The oven temperature program used was 5°C/min from 80 to 300°C (waiting 15 min). The interface temperature was maintained at 320°C and the ion source temperature at 260°C. Mass spectra were recorded over 35-500 amu at 3.33 scan/s with an ionization energy of 70 eV. The SEM analysis was employed since it plays a crucial role in identifying the main compounds of raw material due to its ability to provide detailed insights into their microstructure, elemental composition, and surface characteristics.

Statistical evaluation

A factorial experimental design was utilized to investigate the effect of different independent variables on one or more dependent variables. This made it possible to verify the isolated effect of each variable and the interactions between them. For this study, the specified variables were A (plant extract from *S. viarum* fruits and roots), B (extraction methods PLE and MHG), and C (PLE temperatures of 30, 45, and 60°C, and the ethanol percentages of 70, 85, and 100% v/v). Finally, data analysis was performed using a mean comparison test using the Tukey method ($p<0.050$) and the software Statistica 8.0 (Statsoft Inc., USA). The Tukey test was applied to determine significant differences between yields at the 5% uncertainty level. This test enabled the identification of which groups were significantly different from each other after performing the analysis of variance (ANOVA) test.

Results and Discussion

Extraction yields

PLE is widely used to extract plant compounds as it is a faster, more efficient, and economical methodology (Dobroslavić et al., 2022). This technique uses solvents always below their critical points, maintaining their liquid phase throughout the extraction process and at high temperatures, as well as indicating higher yields than MHG (Lama-Muñoz et al., 2019).

The yields for the studied matrices are presented in Table 1. For both matrices of *S. viarum*, the behavior was similar. When a hydroalcoholic mixture and higher temperatures were used, the yields increased considerably. The highest yields were obtained in the condition at 60°C and 70% ethanol (assay 3), while the lowest yields were attained in the condition with the lowest temperature (30°C) and highest ethanol percentage (100%). Notably, the yields using pressurized liquids were higher than the yields using the ultrasound strategy.

Regarding the fruit extract yield, the most promising results were indicated for samples 3 (temperature 60°C and ethanol 70%; 26.11 wt.%), 1 (temperature 30°C and ethanol 70%; 20.29 wt.%), and 5 (temperature 45°C and ethanol 85%; 14.74 wt.%). These results differed statistically from each other and from all other samples. The least promising results were observed for samples 2 (temperature 30°C and ethanol 100%; 1.26 wt.%), 4 (temperature 60°C and ethanol 100%; 4.79 wt.%), and 7 (temperature 45°C and ethanol 85%; 13.74 wt.%). Additionally, root extract yields provided the most promising results for samples 3 (temperature 60°C and ethanol 70%; 11.22 wt.%), 1 (temperature 30°C and ethanol 70%; 7.92 wt.%) and 7 (temperature 45°C and ethanol 85%; 5.36 wt.%). These results differed statistically from each other and from all other samples. The least promising results were observed for samples 2 (temperature 30°C and ethanol 100%; 1.66 wt.%), 4 (temperature 60°C and ethanol 100%; 2.36 wt.%), and 7 (temperature 45°C and ethanol 85%; 5.02 wt.%).

The linear, quadratic, and interaction terms of the studied variables over the responses were calculated using data from Table 1, and the effects were expressed as a Pareto chart (Figure 2). The linear effects of ethanol percentage and temperature were statistically significant ($p<0.050$) for the two matrices of *S. viarum*. The ethanol percentage presented a negative effect. This means that the higher the percentage of ethanol in the solvent, the lower the yield. The temperature presented a positive effect, the increase of which can raise yields. Comparing tests 1 and 3 for the two matrices, the highest yields were obtained at the highest temperature, while the percentage of ethanol was kept constant. For all the ethanol percentages investigated, the increase in temperature increases yields.

The increase in temperature and binary mixture of solvents satisfactorily influences the extraction yield when it comes to pressurized liquids (Getachew et al., 2022). The highest global yields are reached when the mass transfer rate increases (Dias et al., 2021). The surface tension and viscosity of the solvents decrease and the diffusivity increases as the temperature increases, augmenting the capacity of the solvent to penetrate the matrix and accelerating the dissolution of analytes in the extract (Chaves et al., 2020; Dias et al., 2021). Extraction yields are increased with the use of solvent mixtures, improving the solubility and enhancing the interaction between the extraction solvent and the target components (Nawaz et al., 2020). Moreover, the presence of water in the extractions causes swelling of plant cells, improving their permeability and facilitating cell wall rupture, consequently increasing yields (Lasta et al., 2019). Water is also essential for breaking down the matrix and solute-matrix (hydrogen) bonds (Hammami and Issaoui, 2022).

In the recovery of phenolic compounds from passion fruit peel initially using PLE, the results affirmed the influence of temperature on global yield (Pereira et al., 2021). Also, some studies indicated increased yield when using higher temperatures and a solvent mixture (80°C/ethanol-water) (Santos et al., 2021). Furthermore, reports showed the condition of ethanol+water as solvent at 100°C as the best condition for PLE, which provided higher yields of blackberries (Machado et al., 2015).

The MHG extraction boosted microwave-assisted extraction even further towards an innovative, fast, and eco-friendly process (Fernandes et al., 2021). This technique, which combines microwave heating and terrestrial gravity at atmospheric pressure, was originally designed for essential oils, but later extended to the extraction of other compounds present in plant matrices (Ferreira et al., 2020).

Table 1 – Extract yields of fruit and root from Solanum viarum obtained by pressurized-liquid extraction*.

Assay	Temperature (°C)	Ethanol (%)	Yield (wt.%)	
			Fruit	Root
1	(-1) 30	(-1) 70	20.29^{b}	7.92^{b}
2	(-1) 30	(+1) 100	1.26^{f}	1.66^{e}
3	(+1) 60	(-1) 70	26.11^{a}	11.22^{a}
4	(+1) 60	(+1) 100	4.79^{e}	2.36^{d}
5	(0) 45	(0) 85	14.74^{c}	5.33^{c}
6	(0) 45	(0) 85	14.52^{c}	5.02^{c}
7	(0) 45	(0) 85	13.74^{d}	5.36^{c}
Average			13.66	5.50
CV (%)			0.86	2.58

CV: coefficient of variation.
*Groups sharing identical letters do not exhibit statistically significant differences ($p>0.05$ based on ANOVA with Tukey's test).

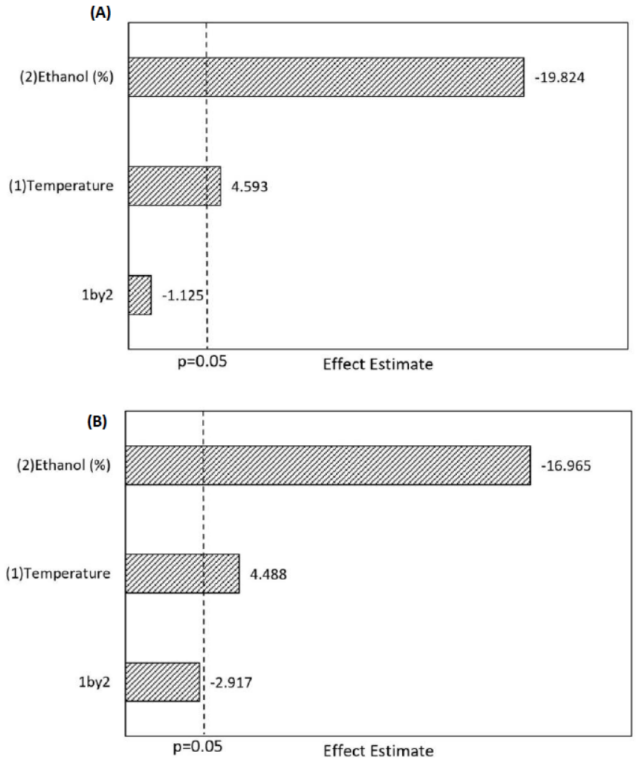

Figure 2 – Pareto's charts of process variables effects on the extraction yield of Solanum viarum using PLE for (A) fruit and (B) root.

Only different solvents for plant humidification were evaluated for MHG extraction. The power output was 450 W and the time was 15 min, which was sufficient for complete extraction. The yields obtained from plant humidification with the hydroalcoholic solution and water were very similar. Nevertheless, the hydroalcoholic solution presented a slight increase in results. The matrix that resulted in the highest yields was the fruit with 1.68% (wt.%), while the root resulted in the lowest yields with 0.11% (wt.%) (Table 2). Considering root and fruit extracts, treatments differed statistically for both samples.

Comparing the results of this technique with PLE, lower yields for both matrices were obtained. There are no reports in the literature using this technique for this plant, but when compared to the extraction using supercritical carbon dioxide ($CO_{2)}$, the yields for fruits were higher and for roots were similar, but with reduced extraction time (Confortin et al., 2019). When correlating with the extraction of *Rosmarinus officinalis* leaves, where the yield was 0.33% under conditions of 1000 W and 15 min (Bousbia et al., 2009), the results found for the fruit matrix were superior. Additionally, a study involving the extraction of *Rosmarinus officinalis* essential oil in the same conditions used in this scientific work (power of 400 W and 20 min), resulted in a yield of 2.32%, reporting that MHG extraction recovered significant volatile and non-volatile fractions in low reaction time and can be explored as an eco-friendly strategy for extraction of antioxidants without the application of conventional solvents (Ferreira et al., 2020).

The low yields can be explained by the excessive use of microwave irradiation power. A shorter time was used and these conditions were not sufficient to recover completely the *S. viarum* extract. Due to the high irradiation power, the pyrolysis of some volatile components may occur, causing a decrease in yield (Gogoi et al., 2023). In order to improve the extraction yield, the best extraction conditions should be investigated in future continued studies. Considering the importance of the technique applied, it is suggested to consider MHG in combination with sequential extractive technologies to improve yields because the extracts obtained may be suitable for applications in a wide range of fields, with the main idea of obtaining new products using green processes.

Chemical composition by gas chromatography-mass spectrometry

The compounds identified in each extraction technique are represented in Tables 3 and 4. Both were efficient for the extraction of bioactive compounds reported in the literature. The PLE was demonstrated to be more efficient since it extracted more compounds (29), while the MHG extracted a smaller number (7). The root matrix allowed recovering 12 compounds with PLE and five with MHG, while the fruit matrix allowed recovering 11 with PLE and seven with MHG. The solvent used for compound extractions determines which compound will be extracted. In the case of the extractions used in this study, ethanol can dissolve moderately polar compounds, whereas water can dissolve highly polar compounds. When a binary mixture is prepared, the behavior is positive based on the amount of extracted compounds (Barrales et al., 2018). The composition of the extracts obtained also changes with temperature because it causes an increase in solubility when it is high (Onyebuchi and Kavaz, 2020). This scenario breaks the analyte-matrix bonds, stimulating the diffusion of the analyte on the matrix surface (Chaves et al., 2020). These statements are in agreement with the findings of this study, since in the extractions with PLE, the condition of 60°C and 70% ethanol for both matrices extracted a higher number of compounds, and for MHG when using the hydroalcoholic solution, the results were more promising.

Results of the extraction of bioactive compounds from passion fruit peel reported by Viganó et al. (2016) corroborate this study. The authors obtained phenolic content (3.186±0.025 mg GAE/g dry mass) and higher antioxidant capacity under the conditions of 60°C and 70% ethanol. Also, the extraction kinetic analysis indicated that the target compounds can be recovered in a short time under PLE, evidencing the economic viability and reaction efficiency of PLE extraction. Furthermore, a study exploring the recovery of phenolic compounds in a mixture of solvents (50% ethanol-water), with higher temperatures (100°C) in grape pomace indicated PLE with the conditions ethanol-water pH 2.0 (50% p/p) with an excellent technique for recovering monomeric anthocyanins (up to 10.21 mg of malvidin-3-O-glycoside/g of dried grape pomace/dr), as well as the condition ethanol-water (50% p/p) as a solvent at 100°C showed a total phenolic content of up to 65.68 mg GAE/gdr and antioxidant capacity of up to 772.11 µmolTE/gdr (Pereira et al., 2019).

Table 2 – Extract yields of fruit and root from Solanum viarum obtained by microwave hydrodiffusion and gravity extraction*.

Assay	Microwave Power (W)	Solvent	Yield (wt.%)	
			Root	Fruit
1	450	Water	0.11[b]	1.37[b]
2	450	Hydroalcoholic solution	0.15[a]	1.68[a]
Average CV (%)			0.14 8.35	1.53 1.36

CV: coefficient of variation.
*Groups sharing identical letters do not exhibit statistically significant differences (p>0.05 based on ANOVA with Tukey's test).

Table 3 – Chemical compounds obtained by pressurized-liquid extraction (PLE) from the fruit and root of Solanum viarum.

Assay	Relative area (%)									
	Fruit					Root				
	PLE					PLE				
	1	2	3	4	5/6/7	1	2	3	4	5/6/7
Compounds										
2,3-butanediol	2.36	-	9.65	24.23	23.63					
Dl-glyceraldehyde	9.45	8.16	2.78	4.55	24.79					
5H-1-Pyrindine		9.62	5.36	18.23	20.13					
9-octadecenamide	1.05						4.09			
2-Propanone, 1,3-dihydroxy										
Pentadecane								3.06		
Neophytadiene		8.21						3.75		3.97
1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester		16.10	6.23	19.36			4.16	3.52		1.48
Benzoic acid, 2-hydroxy, phenylmethyl ester		9.22	5.69				6.94			
Heptadecanoic acid, ethyl ester		8,17				13.09	-	0.64	6.0	
Ethyl linoleate		9.93								
Bicyclo[10.1.0]tridec-1-ene										
Hexadecadienoic acid, methyl ester		18.13	10.36	9.45				8.25		
2-Butanone, 3-hydroxy-										
9,12-Octadecadienoic acid (Z,Z)-, methyl ester					21.94					
Methane, sulfinylbis							2.89			
N-Methyl-L-prolinol							9.51	6.42		
Quinic acid	38.33	7.84	10.56	8.26	6.25	23.03	16.52	5.05	23.91	
Ergost-5-en-3-ol, (3 beta,24R)	2.15		14.02			12.05	13.72	7.42	7.34	3.84
Spirosol-5-en-3-ol (Solasodine)	33.36	4.62	16.35	8.56	3.26	14.40	13.12	13.58	19.51	19.53
Cytidine	13.30		13.36	7.36		30.84	21.39	42.78	27.49	31.87
5H-1-pyrindine										7.14
Neophytadiene										
Integerrimine			5.64				7.66		6.59	33.64
Methyl commate B										
Ethyl linoleate						6.59			7.07	
9,12,15-Octadecatrien-1-ol (CAS)									2.10	
9,12-Octadecadien-1-ol (CAS)								2.39		
Cholest-5-ene, 3-bromo-, (3 beta)-								2.92		

Finally, a study explored the performance of different alternative extractions, including PLE, on rosemary (*Rosmarinus officinalis*) leaves, and it was reported that PLE using ethanol at 200°C was highly efficient in the synthesis of extracts with significant antioxidant activity, and yields were up to 40% extraction of high potential antioxidants, such as carnosic and rosmarinic acids (Herrero et al., 2010).

Both techniques presented promising results regarding the extracted compounds, having as main compounds quinic acid, cytidine, and solasodine. These compounds were also found in the matrices of *S. viarum* by Confortin et al. (2019), using as an extraction technique ultrasound and supercritical CO_2. These compounds have important activities reported in the literature.

Table 4 – Chemical compounds obtained by microwave hydrodiffusion and gravity from the fruit and root of Solanum viarum.

Assay	Relative area (%)			
	Fruit		Root	
	Water	Water/Ethanol	Water	Water/Ethanol
Compounds				
2,3-Butanediol	10.03	1.33		
1,3-Butanediol	21.40	1.08	9.95	
5H-1-Pyrindine	7.57	9.58		10.01
Spirosol-5-en-3-ol (Solasodine)	12.43	13.43	5.36	17.54
Cytidine	20.35	40.41	14.38	51.07
Quinic acid	18.40	22.11	8.26	11.15
Integerrimine	9.52	12.06	62.05	10.23

Quinic acid has been reported for its anti-inflammatory and antioxidant activities (Valanciene and Malys, 2022). Martín et al. (2017) attributed antiviral action to cytidine, which is a compound with a pyrimidine nucleus that plays a vital role in biological activities such as antifungal. Cytidine is also described as a compound responsible for antifungal action against *Aspergillus niger*, *Fusarium culmorum*, *Penicillium expansum*, and *Penicillium roqueforti* (Pawlowska et al., 2012). Some other compounds, such as esters, can probably be considered contaminants of anthropogenic origin. The investigation of the true origin of some compounds from natural products is very important (Thiemann, 2021). According to Venditti (2020), the use of methanol (ethanol and butanol) as an extractive or eluting solvent in the analytical procedure can lead to the isolation of a methyl (ethyl or butyl) ester, which can produce other compounds.

Finally, solasodine is a compound found in the Solanaceae family and is described as highly toxic in many scientific studies. It is also described as the main compound in the root extract of *S. viarum* (Confortin et al., 2019). Nonetheless, it can be extracted from different vegetative parts of *S. viarum* plants such as the leaf and stalk (Patel et al., 2021). This solasodine is reported to be an excellent insecticide and anthelmintic agent (Khaserao and Somani, 2017). Previously, the importance of solasodine on the death of the fifth instar larvae of *Tribolium confusum* was emphasized (Lingampally et al., 2014). Lastly, it has been widely employed for a number of medically based treatments, such as anti-oxidant, neuroprotective, anti-cancer, and anti-tumor anticonvulsant activity. This panorama indicates the importance of compounds originating from *S. viarum* and a strong spectrum of potential applications for a series of fields of study that involve the use of bioactive compounds from plants.

Furthermore, *S. viarum* extracts contain a wide range of phytochemicals, including alkaloids, flavonoids, phenolic compounds, and steroids, which contribute to its antioxidant potential. Nevertheless, scientific studies focus on the evaluation of *S. viarum* leaves. They indicated the elimination of DPPH radicals (2,2-diphenyl-1-picrylhydrazyl), ABTS radicals (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric ion reducing antioxidant power) to evaluate the antioxidant capacity of *S. viarum* extracts, and demonstrate the antioxidant activity of extracts (Silva et al., 2023).

Conclusions

Considering this study as a pioneer in the use of MHG extraction and PLE for extracting compounds from *S. viarum*, the results obtained were extremely satisfactory, indicating that the plant has important compounds in its composition with possible applicability. The PLE technique demonstrated to be more efficient in obtaining higher yields (up to 26.11% for fruit and up to 11.22% for root) and concentration of chemical compounds. Nonetheless, the MHG technique was found to be effective in concentrating quinic acid, cytidine, and solasodine in the extracts. Finally, it can be concluded that innovative extraction technologies based on green approaches can be an excellent alternative to conventional extraction methods. The *S. viarum* extracts indicated promising results for extraction yields and its components can be applied in a diversity of applications. This scenario can positively impact the encouragement of future research that proposes to study in detail the potential of compounds obtained from *S. viarum*, mainly optimization and performance of toxicity tests, potential for industrial and agricultural use, optimization of microbial tests by testing different application dosages of extracts, etc. Furthermore, understanding the compounds extracted from plants using different methods opens up an avenue for further research exploring their potential applications in the future. This work highlights the importance of innovative extraction techniques in unlocking the bioactive potential of Brazilian biodiversity, paving the way for the development of novel bioactive compounds with diverse applications in pharmaceutical, agricultural, and other industries.

Authors' Contributions

CONFORTIN, T.C.: formal analysis, investigation, methodology, software, validation, visualization, writing – original draft. TODERO, I.: formal analysis, investigation, methodology. LUFT, L.: formal analysis, investigation, methodology, validation. SCHMALTZ, S.: formal analysis, investigation, methodology. FERREIRA, D.F.: formal analysis, methodology. BARIN, J.S.: formal analysis, methodology. NASCIMENTO DOS SANTOS, M.S.: methodology, validation, visualization, writing – original draft. MAZUTTI, M.A.: supervision, validation, writing – review & editing. ZABOT, G.L.: supervision, validation, writing – review & editing. TRES, M.V.: project administration, funding, resources, supervision, validation, writing – review & editing.

References

Ali, A.; Wei, S.; Liu, Z.; Fan, X.; Sun, Q.; Xia, Q.; Liu, S.; Hao, J.; Deng, C., 2021. Non-thermal processing technologies for the recovery of bioactive compounds from marine by-products. Lebensmittel-Wissenschaft & Technologie, v. 147, 111549. https://doi.org/10.1016/j.lwt.2021.111549

Barrales, F.M.; Silveira, P.; Barbosa, P.P.M.; Ruviaro, A.R.; Paulino, B.N.; Pastore, G.M.; Macedo, G.A.; Martinez, J., 2018. Recovery of phenolic compounds from citrus by-products using pressurized liquids — an application to orange peel. Food and Bioproducts Processing, v. 112, 9-21. https://doi.org/10.1016/j.fbp.2018.08.006

Bousbia, N.; Abert, M.; Ferhat, M.A.; Petitcolas, E.; Meklati, B.Y.; Chemat, F., 2009. Comparison of two isolation methods for essential oil from rosemary leaves: hydrodistillation and microwave hydrodiffusion and gravity. Food Chemistry, v. 114, (1), 355-362. https://doi.org/10.1016/j.foodchem.2008.09.106

Chaves, J.O.; de Souza, M.C.; da Silva, L.C.; Lachos-Perez, D.; Torres-Mayanga, P.C.; Machado, A.P.F.; Forster-Carneiro, T.; Vázquez-Espinosa, M.; González-de-Peredo, A V.; Barbero, G.F.; Rostagno, M.A., 2020. Extraction of flavonoids from natural sources using modern techniques. Frontiers in Chemistry, v. 8, 507887. https://doi.org/10.3389/fchem.2020.507887

Chouhan, K.B.S.; Tandey, R.; Sem, K.K.; Mehta, R.; Mandal, V., 2019. Critical analysis of microwave hydrodiffusion and gravity as a green tool for extraction of essential oils: Time to replace traditional distillation. Trends in Food Science & Technology, v. 92, 12-21. https://doi.org/10.1016/j.tifs.2019.08.006

Confortin, T.C.; Todero, I.; Luft, L.; Teixeira, A.L.; Mazutti, M.A.; Zabot, G.L.; Tres, M.V., 2019. Valorization of *Solanum viarum* dunal by extracting bioactive compounds from roots and fruits using ultrasound and supercritical CO_2. Brazilian Journal of Chemical Engineering, v. 36, (4), 1689-1702. https://doi.org/10.1590/0104-6632.20190364s20190267

Confortin, T.C.; Todero, I.; Luft, L.; Schmaltz, S.; Ferreira, D.F.; Barin, J.S.; Mazutti, M.A.; Zabot, G.L.; Tres, M.V., 2021. Extraction of bioactive compounds from *Senecio brasiliensis* using emergent technologies. 3 Biotech, v. 11, 284. https://doi.org/10.1007/s13205-021-02845-1

Dias, A.L.B.; de Aguiar, A.C.; Rostagno, M.A., 2021. Extraction of natural products using supercritical fluids and pressurized liquids assisted by ultrasound: current status and trends. Ultrasonics Sonochemistry, v. 74, 105584. https://doi.org/10.1016/j.ultsonch.2021.105584

Dobroslavić, E.; Elez Garofulić, I.; Šeparović, J.; Zorić, Z.; Pedisić, S.; Dragović-Uzelac, V., 2022. Pressurized liquid extraction as a novel technique for the isolation of *Laurus nobilis* L. leaf polyphenols. Molecules, v. 27, (16), 5099. https://doi.org/10.3390/molecules27165099

Farias, C.A.A.A.; Moraes, D.P.; Neuenfeldt, N.H.; Zabot, G.L; Emanuelli, T.; Barin, J.S.; Ballus, C.A.; Barcia, M.T., 2022. Microwave hydrodiffusion and gravity model with a unique hydration strategy for exhaustive extraction of anthocyanins from strawberries and raspberries. Food Chemistry, v. 383, 132446. https://doi.org/10.1016/j.foodchem.2022.132446

Fernandes, P.A.R.; Bastos, R.; Calvão, J.; Neto, F.; Coelho, E.; Wessel, D.F.; Cardoso, S.M.; Coimbra, M.A.; Passos, C.P., 2021. Microwave hydrodiffusion and gravity as a sustainable alternative approach for an efficient apple pomace drying. Bioresource Technology, v. 333, 125207. https://doi.org/10.1016/j.biortech.2021.125207

Ferreira, D.F.; Lucas, B.N.; Voss, M.; Santos, D.; Mello, P.A.; Wagner, R.; Cravotto, G.; Barin, J.S., 2020. Solvent-free simultaneous extraction of volatile and non-volatile antioxidants from rosemary (*Rosmarinus officinalis* L.) by microwave hydrodiffusion and gravity. Industrial Crops and Products, v. 145, 112094. https://doi.org/10.1016/j.indcrop.2020.112094

Gajger, I.T.; Dar, S.A., 2021. Plant allelochemicals as sources of insecticides. Insects, v. 12, (3), 189. https://doi.org/10.3390/insects12030189

Getachew, A.T.; Holdt, S.L.; Meyer, A.S.; Jacobsen, C., 2022. Effect of extraction temperature on pressurized liquid extraction of bioactive compounds from *Fucus vesiculosus*. Marine Drugs, v. 20, (4), 1-16. https://doi.org/10.3390/md20040263

Gogoi, D.; Kumar, M.; Gruha, Y., 2023. A comprehensive review on "pyrolysis" for energy recovery. BioEnergy Research, v. 16, 1417-1437. https://doi.org/10.1007/s12155-023-10568-9

Hammami, F.; Issaoui, N., 2022. A DFT study of the hydrogen bonded structures of pyruvic acid–water complexes. Frontiers in Physics, v. 10, 1-9. https://doi.org/10.3389/fphy.2022.901736

Herrero, M.; Plaza, M.; Cifuentes, A.; Ibáñez, E., 2010. Green processes for the extraction of bioactives from rosemary: chemical and functional characterization via ultra-performance liquid chromatography-tandem mass spectrometry and in-vitro assays. Journal of Chromatography A, v. 1217, (16), 2512-2520. https://doi.org/10.1016/j.chroma.2009.11.032

Ilyas, T.; Chowdhary, P.; Chaurasia, D.; Gnansounou, E.; Pandey, A.; Chaturvedi, P., 2021. Sustainable green processing of grape pomace for the production of value-added products: an overview. Environmental Technology & Innovation, v. 23, 101592. https://doi.org/10.1016/j.eti.2021.101592

Jan, R.; Asaf, S.; Numan, M.; Lubna; Kim, K.-M., 2021. Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. Agronomy, v. 11, (5), 968. https://doi.org/10.3390/agronomy11050968

Kausar, M.; Singh, B.K., 2018. Pharmacological evaluation of *Solanum viarum* Dunal leaves extract for analgesic and antipyretic activities. Journal of Drug Delivery and Therapeutics, v. 8, (4), 356-361. https://doi.org/10.22270/jddt.v8i4.1812

Kang, J. H.; Kim, S.; Moon, B., 2016. Optimization by response surface methodology of lutein recovery from paprika leaves using accelerated solvent extraction. Food Chemistry, v. 205, 140-145. https://doi.org/10.1016/j.foodchem.2016.03.013

Khaserao, S.; Somani, R., 2017. Evaluation of anti-obesity activity of solasodine in high fat diet-induced obesity in rat. International Journal of

Pharmaceutical Sciences and Research, v. 9, (3), 23. https://doi.org/10.22159/ijpps.2017v9i3.16025

Krakowska-Sieprawska, A.; Kiełbasa, A.; Rafińska, K.; Ligor, M.; Buszewski, B., 2022. Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. Molecules, v. 27, (3), 730. https://doi.org/10.3390/molecules27030730

Lama-Muñoz, A.; Del Mar Contreras, M.; Espínola, F.; Moya, M.; de Torres, A.; Romero, I.; Castro, E., 2019. Extraction of oleuropein and luteolin-7-O-glucoside from olive leaves: optimization of technique and operating conditions. Food Chemistry, v. 293, 161-168. https://doi.org/10.1016/j.foodchem.2019.04.075

Lasta, H.F.B.; Lentz, L.; Mezzomo, N.; Ferreira, S.R.S., 2019. Supercritical CO_2 to recover extracts enriched in antioxidant compounds from beetroot aerial parts. Biocatalysis and Agricultural Biotechnology, v. 19, 101169. https://doi.org/10.1016/j.bcab.2019.101169

Lingampally, V.; Solanki, V R.; Anuradha, D.L.; Raja, S.S., 2014. Effect of solasodine against last instar larvae of *Tribolium confusum*. Journal of Entomology and Zoology Studies, v. 2, (2), 118-120.

Machado, A.P.D.F.; Pasquel-Reátegui, J.L.; Barbero, G.F.; Martínez, J., 2015. Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. Food Research International, v. 77, 675-683. https://doi.org/10.1016/j.foodres.2014.12.042

Martín, S.; Cuevas, J.M.; Elena, S.F.; Grande-Pérez, A., 2017. A putative antiviral role of plant cytidine deaminases. F1000Research, v. 6, 622. https://doi.org/10.12688/f1000research.11111.1

Nawaz, H.; Shad, M. A.; Rehman, N.; Andaleeb, H.; Ullah, N., 2020. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. Brazilian Journal of Pharmaceutical Sciences, v. 56. https://doi.org/10.1590/s2175-97902019000417129

Onyebuchi, C.; Kavaz, D., 2020. Effect of extraction temperature and solvent type on the bioactive potential of *Ocimum gratissimum* L. extracts. Scientific Reports, v. 10, 1-11. https://doi.org/10.1038/s41598-020-78847-5

Pandey, S.; Shukla, P.; Misra, P., 2018. Physical state of the culture medium triggers shift in morphogenetic pattern from shoot bud formation to somatic embryo in *Solanum khasianum*. Physiology and Molecular Biology of Plants, v. 24, 1295-1305. https://doi.org/10.1007/s12298-018-0582-8

Patel, P.; Prasad, A.; Gupta, S.C.; Niranjan, A.; Lehri, A.; Singh, S.S.; Misra, P.; Chakrabarty, D., 2021. Growth, phytochemical and gene expression changes related to the secondary metabolite synthesis of *Solanum viarum* Dunal. Industrial Crops and Products, v. 166, 113464. https://doi.org/10.1016/j.indcrop.2021.113464

Pawlowska, A.M.; Zannini, E.; Coffey, A.; Arendt, E.K., 2012. "Green preservatives": combating fungi in the food and feed industry by applying antifungal lactic acid bacteria. Advances in Food and Nutrition Research, v. 66, 217-238. https://doi.org/10.1016/B978-0-12-394597-6.00005-7

Pereira, D.T.V.; Tarone, A.G.; Cazarin, C.B.B.; Barbero, G.F.; Martínez, J., 2019. Pressurized liquid extraction of bioactive compounds from grape marc. Journal of Food Engineering, v. 240, 105-113. https://doi.org/10.1016/j.jfoodeng.2018.07.019

Pereira, D.T.V.; Zabot, G.L.; Reyes, F.G.R.; Iglesias, A.H.; Martínez, J., 2021. Integration of pressurized liquids and ultrasound in the extraction of bioactive compounds from passion fruit rinds: impact on phenolic yield, extraction kinetics and technical-economic evaluation. Innovative Food Science & Emerging Technologies, v. 67, 102549. https://doi.org/10.1016/j.ifset.2020.102549

Qaderi, M.M.; Martel, A.B.; Strugnell, C.A., 2023. Environmental factors regulate plant secondary metabolites. Plants, v. 12, (3), 447. https://doi.org/10.3390/plants12030447

Santos, M.S.N.; Wancura, J.H.C.; Oro, C.E.D.; Dallago, R.M.; Tres, M.V., 2022. Opportunities and challenges of plant bioactive compounds for food and agricultural-related areas. Phyton-International Journal of Experimental Botany, v. 91, (16), 1105-1127. https://doi.org/10.32604/phyton.2022.020913

Santos, P.H.; Kammers, J.C.; Silva, A.P.; Oliveira, J.V.; Hense, H., 2021. Antioxidant and antibacterial compounds from feijoa leaf extracts obtained by pressurized liquid extraction and supercritical fluid extraction. Food Chemistry, v. 344, 128620. https://doi.org/10.1016/j.foodchem.2020.128620

Silva, A.F.G.; Gomes, P.F.; Damião, P.D.; Oliveira, M.F., 2023. Toxicity and antioxidant activity of extracts from the leaves, bark and green fruits of *Solanum viarum* Dunal (Solanaceae). Cuadernos de Educación y Desarrollo, v. 15, (6). https://doi.org/10.55905/cuadv15n6-017

Thiemann, T., 2021. Isolation of phthalates and terephthalates from plant material–natural products or contaminants? The Open Chemistry Journal, v. 8, 1874-8422. https://doi.org/10.2174/1874842202108010001

Valanciene, E.; Malys, N., 2022. Advances in production of hydroxycinnamoyl-quinic acids: from natural sources to biotechnology. Antioxidants, v. 11, (12), 2427. https://doi.org/10.3390/antiox11122427

Venditti, A., 2020. What is and what should never be: artifacts, improbable phytochemicals, contaminants and natural products. Natural Product Research, v. 347, 1014-1031. https://doi.org/10.1080/14786419.2018.1543674

Viganó, J.; Brumer, I.Z.; Braga, P.A.C.; da Silva, J.K.; Maróstica Júnior, M.R.; Reyes Reyes, F.G.; Martínez, J., 2016. Pressurized liquids extraction as an alternative process to readily obtain bioactive compounds from passion fruit rinds. Food and Bioproducts Processing, v. 100, (Part A), 382-390. https://doi.org/10.1016/j.fbp.2016.08.011

Wianowska, D.; Gil, M., 2019. Critical approach to PLE technique application in the analysis of secondary metabolites in plants. TrAC Trends in Analytical Chemistry, v. 114, 314-325. https://doi.org/10.1016/j.trac.2019.03.018