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Green Extraction of Antioxidants from *Lentinula edodes* Using 2-Hydroxyethylammonium Acetate Ionic Liquid assisted by an ultrasonic bath

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ABSTRACT

Shiitake mushrooms (*Lentinula edodes*) are valued for their nutritional and therapeutic properties and are an important source of bioactive antioxidant compounds. However, the application of ionic liquids (ILs) for extracting these compounds from fungal matrices remains underexplored. This study evaluated 2-hydroxyethylammonium acetate as a green solvent for extracting antioxidant and phenolic compounds from shiitake mushrooms. Extraction was conducted by solvent–biomass contact followed by vortex agitation and ultrasonic treatment. A 2³ factorial design was employed to investigate the effects of ionic liquid concentration (0.4–2.0 mol L⁻¹), temperature (25–60 °C), and ultrasonic extraction time (30–60 min). Total phenolic content was quantified using the Folin–Ciocalteu method, and antioxidant capacity was assessed via DPPH and ABTS radical scavenging assays. Spectrophotometric results indicated total phenolic contents ranging from 18.43 to 32.71 mg 100 g⁻¹. Antioxidant activity varied between 0.09 and 0.73 mM TE g⁻¹ for DPPH and from 1.48 to 2.20 mg 100 g⁻¹ for ABTS. Statistical analysis demonstrated that ionic liquid concentration and temperature significantly affected ($p < 0.05$) total phenolic content and DPPH activity, whereas no significant effects were observed for

ABTS under the studied conditions. The findings confirm that 2-hydroxyethylammonium acetate is an effective and environmentally favorable solvent for recovering antioxidant compounds from shiitake mushrooms. This approach contributes to sustainable biomass valorization and supports the development of greener extraction strategies with potential applications in food, nutraceutical, and pharmaceutical sectors

Keywords: shiitake; extraction; ionic liquid, antioxidant activity.

41 INTRODUCTION

42 The growing awareness of the importance of health and well-being has driven
43 the search for natural sources of bioactive compounds, especially those with
44 antioxidant properties[1, 2]. Among these sources, edible and medicinal mushrooms,
45 such as shiitake (*Lentinula edodes*), have gained prominence for their nutritional and
46 therapeutic properties. Native to East Asia, Shiitake mushrooms are cultivated globally
47 and have been valued for centuries in both Asian cuisine and traditional medicinal
48 practices, where they are prized for their properties that are believed to promote health,
49 longevity, and blood circulation[3, 4]

50 *Lentinula edodes* is rich in macronutrients, consisting of approximately 90%
51 water when fresh, and on a dry basis, mostly carbohydrates (67.5 to 78%, of which
52 44% is dietary fiber), as well as proteins (13.4 to 17.5%)[5]. In terms of micronutrients,
53 shiitake mushrooms are an excellent source of minerals such as phosphorus,
54 magnesium, potassium, zinc, iron, and copper, and vitamins such as C, B1, B2, B3,
55 B9, B12, and D[6, 7]. In addition to its nutritional value, shiitake contains bioactive
56 compounds such as polysaccharides, fiber, and ergosterol, which confer antimicrobial,
57 antitumor, antiviral, anti-inflammatory, and antioxidant properties[8].

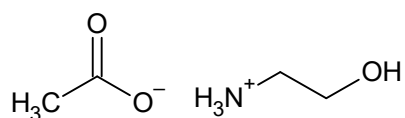
58 The antioxidant properties of shiitake mushrooms are primarily attributed to the
59 presence of various compounds, including polyphenols, flavonoids, beta-glucans, and
60 ergosterol[2]. It was identified the presence of ergothioneine, gallic acid (a type of
61 phenolic acid), carvacrol, protocathechuic acid, catechin, and tocopherols (vitamin E) in
62 shiitake extracts[8]. Antioxidants are molecules that preferentially oxidize other
63 molecules, interact with free radicals, and inhibit chain reactions that could lead to
64 cellular damage and oxidative stress, such as lipid peroxidation[9]. These antioxidants
65 can be categorized as either enzymatic or non-enzymatic (including minerals, vitamins,
66 carotenoids, and polyphenols), with many natural antioxidants possessing phenolic
67 structures akin to those synthetic antioxidants[10].

68 Traditionally, the extraction of these bioactive compounds from natural matrices
69 involves the use of organic solvents[11], which can be inefficient, time-consuming, and
70 harmful to the environment. In this scenario, the search for alternative solvents that are
71 more efficient and environmentally friendly has intensified recently[12]. Ionic liquids
72 (ILs) have emerged as a promising solution to these challenges[13].

Ionic liquids are organic salts that are liquid at temperatures below 100 °C. They are composed of ions and possess unique and desirable properties for extraction processes, high thermal stability, ability to act as good solvents for a wide range of compounds, high electrical conductivity, and very low vapor pressure. These characteristics qualify them as “green solvents,” minimizing the environmental impact of their production and use[14, 15]. Consequently, ionic liquids are gaining recognition as promising solvents for extracting bioactive compounds[16].

The use of hydroxylamine-based ionic liquids for extracting bioactive compounds has surged in recent years because of their affordability and low toxicity and its reuse compared to imidazolium-based ILs[17]. Oliveira et al.[18] reported that 2-hydroxyethylammonium acetate (2HEAA) exhibited the lowest toxicity among protic solvents when tested against various microorganisms. Phycobiliproteins were extracted from *Spirulina (Arthrospira platensis)* using IL microwave-assisted[19]. The authors discovered that the combination of 2HEAA and 2-hydroxyethylammonium formate (2HEAF) was both efficient and rapid, with minimal energy consumption. Additionally, 2-hydroxyethylammonium has been employed to precipitate lignin from eucalyptus wood Villarino et al.[20, 21] and in the pretreatment of sugarcane bagasse to decompose cellulose into smaller sugars[20, 21]. The chemical structure of the ionic liquid 2-hydroxyethylammonium acetate is illustrated in Figure 1, where functional groups are identified; for the cation, the numbering denotes the ethyl chain bridging the hydroxyl and ammonium groups.

Figure 1 - 2-hydroxyethylammonium acetate



This study aimed to assess the ability of the ionic liquid 2-hydroxyethylammonium acetate to solubilize bioactive compounds from shiitake mushrooms (*Lentinula edodes*) and to compare its efficiency with that of conventional solvents. This study contributes to advancing more sustainable extraction processes and to the valorization of a natural resource with significant medicinal and nutritional potential.

2. Materials and methods

2.1. Materials

All chemical reagents used in this study were purchased from Sigma-Aldrich and used as received unless otherwise noted. Dehydrated shiitake mushroom samples were purchased online from São Paulo, Brazil. The samples were ground in a domestic blender until they became flour and then passed through a 30-mesh sieve. After processing, the samples were packaged and stored in sterilized glass containers.

2.2. Synthesis of 2-hydroxyethylammonium acetate ionic liquid

The ionic liquid (IL), 2-hydroxyethylammonium acetate, was synthesized by reacting ethanolamine with glacial acetic acid, following the methodology of Álvarez et al. [22] with a minor modification. Ethanolamine was added to a 100 mL round-bottom flask in an ice bath, which was then placed on a magnetic stirrer. Next, the acetic acid solution slowly dripped into the flask containing ethanolamine while stirring magnetically for 2 h. At the end of the synthesis, IL was rotary evaporated (Fisatom, 801, Brazil) at 60 °C, resulting in a viscous, amber-colored liquid.

2.3. Experimental Factorial Design

To evaluate the influence of ionic liquid concentration, extraction time, and temperature on the extraction of total antioxidant and phenolic compounds, a $2^{(3-0)}$ factorial experimental design was applied. The independent variables and their respective levels were IL concentration (0.4, 1.2, and 2.0 Mol L⁻¹), called ionic liquid solution, extraction time (30, 45, and 60 min), and temperature (25, 45, and 65 °C). A total of 11 trials were conducted, including central points, to verify repeatability.

2.4. Antioxidant Compounds Extraction.

Extraction was performed using the procedure described by Qin et al.[23] with a slight modification. Initially, 0.3 g of shiitake flour was weighed and added to Falcon tubes. Then, 5 mL of ionic liquid solution was added according to the factorial design. The samples were stirred in a vortex mixer and subjected to an ultrasonic bath (SolidSteel, Brazil), under the time and temperature conditions for each experimental

point. The entire procedure was performed in triplicates. After extraction, the samples were ultracentrifuged (Hermle, Germany), at 8.000 rpm for 10 min to obtain the supernatant phase (liquid extract). The liquid extracts were stored in a freezer until analysis.

2.5. Total Phenolic Compounds Quantification

Quantification was performed using the Folin-Ciocalteu method, as described by Singleton et al.[24]. A standard gallic acid curve was generated. A 10% 2M Folin-Ciocalteu solution and 4% sodium carbonate solution were added. The blank was distilled water, and the tests were performed in the dark. The absorbance was measured at 740 nm using a spectrophotometer (Femto, 800XI, Brazil), and the results were expressed in milligrams of gallic acid per 100 grams (mg GAE 100 g⁻¹). The reducing capacity of the antioxidant was measured by electron transfer, with the color of the solution ranging from light blue (lower concentration) to dark blue (higher concentration).

2.6. DPPH - Method for Determining Antioxidant Activity

Antioxidant activity was determined using the DPPH method according to Brand-William et al.[25], with adaptations. Solutions of 0.5 mM DPPH (in P.A. ethanol), 2000 µM Trolox, and 1000 µM Trolox (both in P.A. ethanol) were prepared. A standard curve was prepared using 1000 and 2000 µM Trolox. The absorbance of the samples was measured at 517 nm using a spectrophotometer (Femto, 800XI, Brazil), with ethanol as the blank. The results were expressed in mM TEAC g⁻¹ of extract (TEAC = Trolox equivalent antioxidant activity). The DPPH solution is purple and, when reduced, turns yellow, indicating the consumption of the reagent by the decrease in absorbance.

2.7. ABTS - Method for Determining Antioxidant Activity

The ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) method was applied to the extracts following the procedures described by Re et al.[26]. A 2000 µM

Trolox solution was used to generate the standard curve, and ethanol was used as the blank. The absorbance was measured at 734 nm, using a spectrophotometer (Femto, 800XI, Brazil). and the results were expressed in mM TEAC g⁻¹ of extract.

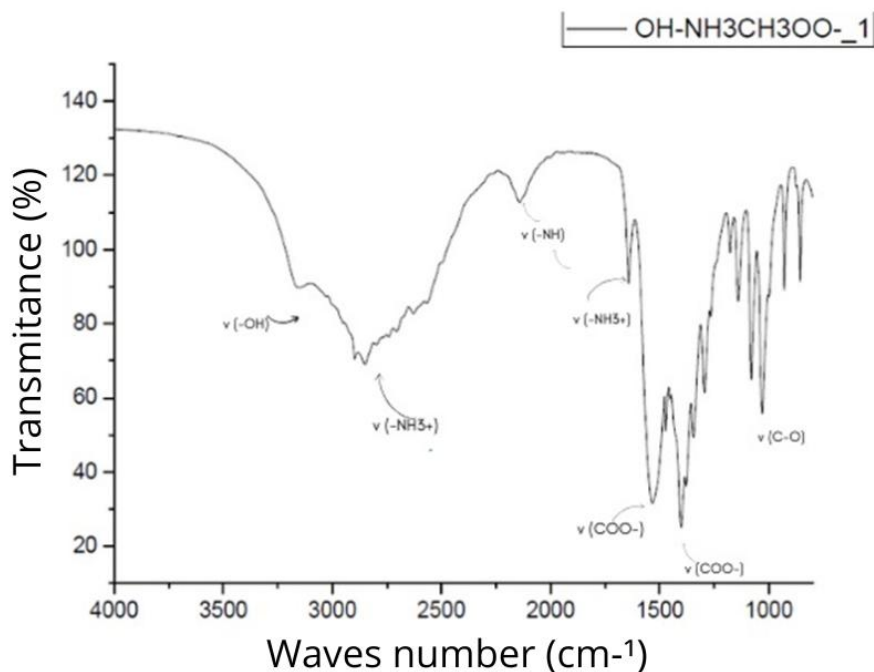
3. RESULT AND DISCUSSION

This section presents and discusses the results obtained from the extraction antioxidant compounds from Shiitake mushrooms using 2-hydroxyethylammonium acetate ionic liquid. The discussion will focus on the influence of independent variables (IL concentration, time, and temperature) on the extraction of total phenolic compounds and antioxidant activity evaluated by the DPPH and ABTS methods.

3.1. Characterization of 2-Hydroxyethylammonium Acetate Ionic Liquid

The synthesis of the ionic liquid 2-hydroxyethylammonium acetate was confirmed using infrared spectroscopy (IR) techniques, which allowed the identification of the main functional group's characteristic of the IL structure, as shown in Figure 2.

Figure 2 - InfraRed spectra of 2-hydroxyethylammonium acetate



The following bands were identified: -OH group stretching around 3,500 and 3,400 cm^{-1} ; -NH₃⁺ group stretching around 3,100 cm^{-1} ; -N-H group stretching around 2,100 cm^{-1} ; -NH₃, asymmetric stretching around 1,550 cm^{-1} ; -(C=O)₂ group, asymmetric stretching around 1500 cm^{-1} , -(C=O)₂ group, symmetric stretching around 1450 cm^{-1} ; -(C-O) group, stretching at 1000 cm^{-1} [27].

3.2. Total phenolic compounds

The quantification of Total Phenolic Compounds in Shiitake extracts provided crucial information on the effectiveness of different extraction conditions. The results (Table 1) revealed significant variation, with levels ranging from 18.43 to 32.71 mg GAE 100 g⁻¹.

Table 1 - Total phenolic compounds of Shiitake extracts based on integral factorial design 2⁽³⁻⁰⁾

Trial	IL concentration (L mol ⁻¹) X1	Time (min) X2	Temperature (°C) X3	Total Phenolic Compounds in Shiitake extracts (mg GAE/100 g)*
1	0.4	30	25	28.12 ± 0.09
2	2.0	30	25	21.98 ± 0.62
3	0.4	60	25	32.71 ± 0.05
4	2.0	60	25	24.19 ± 0.46
5	0.4	30	65	27.58 ± 0.09
6	2.0	30	65	21.35 ± 1.04
7	0.4	60	65	24.39 ± 0.34
8	2.0	60	65	18.43 ± 0.18
9	1.2	45	45	23.28 ± 0.39
10	1.2	45	45	23.35 ± 0.11
11	1.2	45	45	23.25 ± 0.18

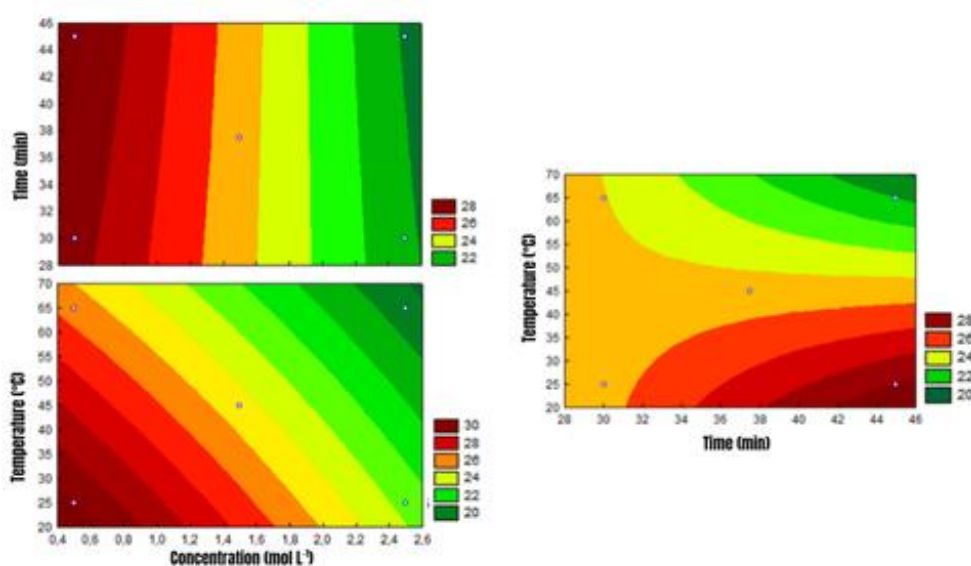
GAE: gallic acid

* Average values followed by standard deviation.

To gain a more detailed understanding of the influence of the variables, response surface graphs were generated (Figure 3) with the aid of the Statistica 8.0 computer program. These graphs indicated that the most favorable conditions for the extraction of total phenolic compounds, resulting in values ≥ 28 mg GAE 100 g⁻¹, were obtained when the concentration of the ionic liquid (IL) and the extraction temperature were at their minimum values (0.4 Mol L⁻¹ and 25 °C, respectively), while the extraction

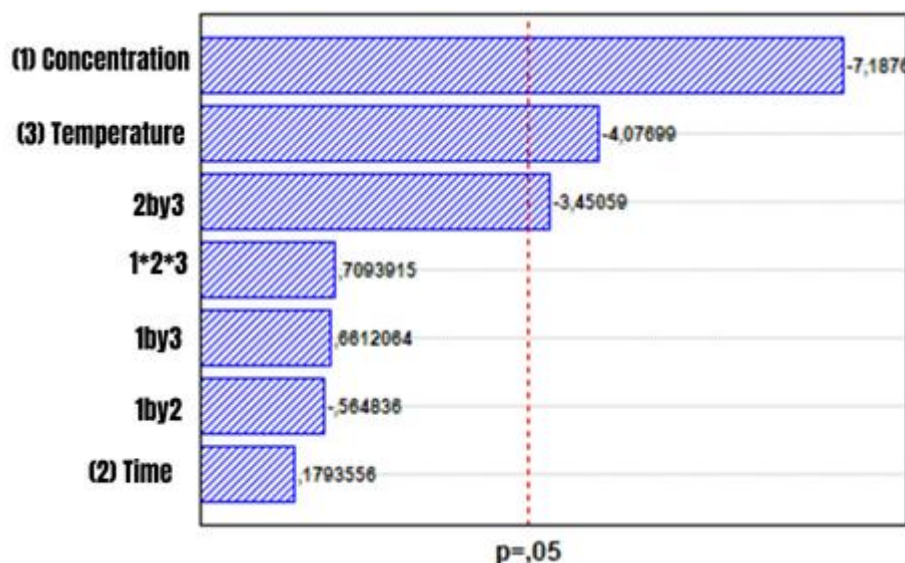
time approached its maximum value (60 minutes). In contrast, the least favorable conditions, which led to the lowest levels of total phenolic compounds (≤ 22 mg GAE 100 g^{-1}), occurred when the three variables (IL, temperature and time) were close to their maximum values. Intermediate values (24 to 26 mg GAE 100 g^{-1}) were observed in conditions close to the central points of the experimental plan.

Figure 3 - Response surface graphs of the effects of time, temperature, and IL concentration variables on total phenolic compounds in Shiitake extracts



Analysis of variance (ANOVA) by the Statistica 8.0 computer program was performed to identify statistically significant effects. The Pareto chart (Figure 4) clearly showed that temperature and IL concentration were the most influential and statistically significant independent variables ($p < 0.05$) in determining total phenolic compound content. The interaction between temperature and time also showed a significant effect. The R^2 value of 0.964 for the ANOVA model indicates an excellent fit of the experimental data to the proposed model, explaining approximately 96.4% of the variability in the total phenolic compound results.

Figure 4 - Pareto chart of the effects at $p \leq 0.05$ of the total phenolic compounds in Shiitake extracts



An increase in IL concentration (variable X1), while the variables time (X2) and temperature (X3) were kept constant, led to a reduction in total phenolic compound results, with an average decrease of approximately 1.30 times. This phenomenon can be attributed to the increase in the viscosity of the IL solution with the decrease in the proportion of water, which theoretically would hinder mass transfer. Temperature (variable X3) was also statistically significant, with a slight reduction in results at higher temperatures. This may be due to the thermal degradation of some heat-sensitive antioxidant compounds or changes in the properties of IL that decrease its affinity with phenolics at higher temperatures. Interestingly, extraction time was not statistically significant in this analysis, which can be explained by the relatively narrow range of variation between the minimum and maximum times used in the factorial design, suggesting that at times above 30 minutes, extraction saturation may already have been reached.

Analyzing the individual results in Table 1, test 3, which used 0.4 Mol L^{-1} of IL, 60 minutes of time, and 25°C temperature, presented the best extraction result ($32.71 \text{ mg GAE } 100 \text{ g}^{-1}$). On the other hand, test 8 (2.0 Mol L^{-1} of IL, 60 minutes, and 65°C) resulted in the lowest total phenolic compound content ($18.43 \text{ mg GAE } 100 \text{ g}^{-1}$). It is important to note that samples 1, 3, and 5, all using the lowest concentration of IL (0.4 Mol L^{-1}), had total phenolic compounds values higher than or similar to those found by

Baptista et al.[28], who reported results between 6.391 and 25.092 mg GAE 100 g⁻¹ in their Shiitake substrate extracts. This finding suggests that lower concentrations of ionic liquid, combined with milder temperatures, may be more efficient for the extraction of phenolics in Shiitake with IL.

Other studies also indicate this diversity Garcia et al.[29] reported 4.9 mg 100 g⁻¹ for aqueous extracts of Shiitake Koshin and 2.12 mg 100 g⁻¹ for Donko, after extractions with distilled water or 80% methanol. Nam et al.[30] found significantly higher levels in methanolic extracts (506 mg 100 g⁻¹ and 486 mg 100 g⁻¹), while Sharpe et al.[31] reported 90.16 mg 100 g⁻¹ for hydroalcoholic extract and 243.27 mg 100 g⁻¹ for aqueous extract. This disparity in results highlights the importance of extraction conditions in the recovery of phenolic compounds.

3.3. Antioxidant activity – DPPH method

The evaluation of the antioxidant activity of Shiitake extracts using the DPPH radical scavenging method provided insight into the ability of these extracts to neutralize free radicals. The results in Table 2 for antioxidant activity via DPPH ranged from 0.09 mM TE g⁻¹ to 0.73 mM TE g⁻¹.

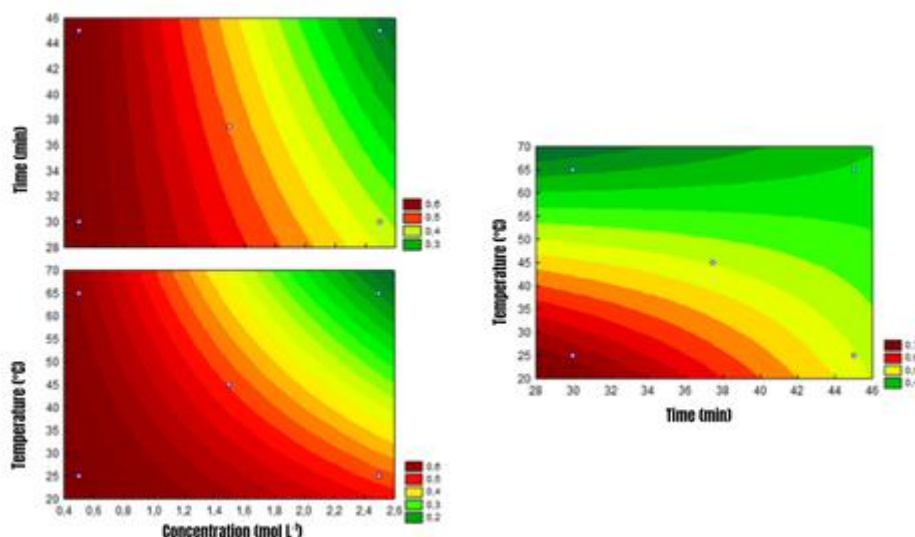
Table 2 - Antioxidant activity of shiitake extracts using the DPPH method based on integral factorial design 2⁽³⁻⁰⁾.

Trials	IL concentration	Time	Temperature	<i>Antioxidant activity (DPPH)</i>
	(L mol ⁻¹) X1	(min) X2	(°C) X3	(mM TE g ⁻¹)*
1	0.4	30	25	0.73 ± 0.02
2	2.0	30	25	0.51 ± 0.02
3	0.4	60	25	0.54 ± 0.04
4	2.0	60	25	0.37 ± 0.02
5	0.4	30	65	0.47 ± 0.11
6	2.0	30	65	0.21 ± 0.04
7	0.4	60	65	0.67 ± 0.02
8	2.0	60	65	0.09 ± 0.04
9	1.2	45	45	0.51 ± 0.14
10	1.2	45	45	0.53 ± 0.12
11	1.2	45	45	0.55 ± 0.03

* Average values followed by standard deviation.

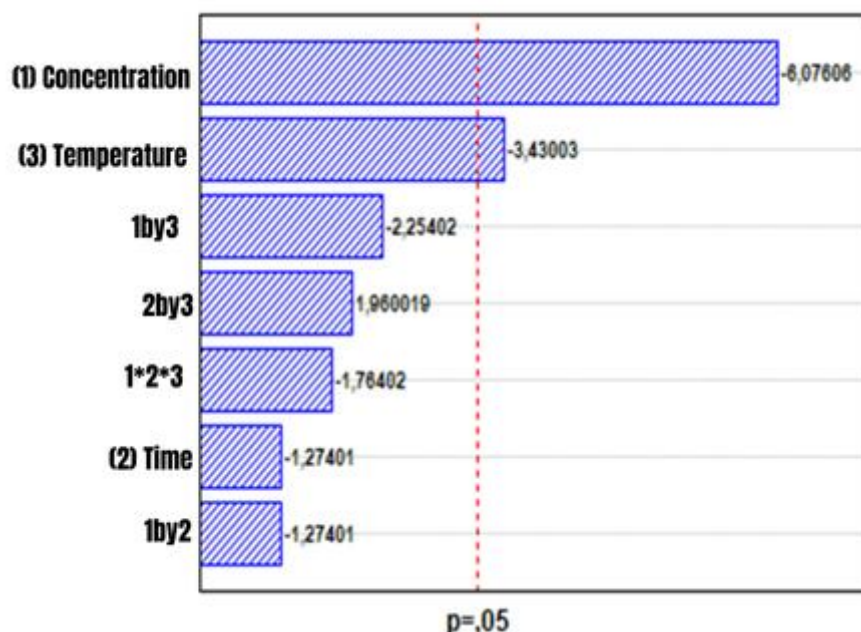
When analyzing the response surface graphs for DPPH (Figure 5), it was observed that the most favorable conditions for obtaining greater antioxidant activity were those in which the IL concentration and temperature were at their minimum values (0.4 Mol L⁻¹ and 25 °C, respectively), while the extraction time was at its maximum value (60 minutes). This trend is remarkably similar to that identified for the extraction of Total Phenolic Compounds, reinforcing the correlation between the presence of phenolic compounds and antioxidant capacity.

Figure 5 - Response surface graphs of the effects of variables on the antioxidant activity of Shiitake extracts using the DPPH method



The Pareto chart for the DPPH method (Figure 6) confirmed that IL concentration and temperature were the statistically significant independent variables ($p < 0.05$) that most influenced the antioxidant activity of the extracts. This suggests that, to maximize DPPH radical scavenging capacity, it is preferable to use lower concentrations of ionic liquid and milder temperatures during the extraction process. The interaction between temperature and IL concentration was also found to be relevant, indicating that the combination of these factors is crucial for antioxidant performance. The coefficient of determination (R^2) of the DPPH model was 0.948, indicating that the model explains approximately 94.8% of the variability in the results, which demonstrates a good fit and predictive ability of the model.

Figure 6 - Pareto chart of the effects at $p \leq 0.05$ on the antioxidant activity of Shiitake extracts using the DPPH method



Lower concentrations of the 2-hydroxyethylammonium acetate ionic liquid proved to be more efficient. This superiority, especially in IL/water mixtures, is explained by the reduction in IL viscosity and the increase in the solubility of hydrophilic compounds, as observed in research by Wu et al.[32, 33] and Yu et al.[32, 33] on hybrid ionic liquids. The formation of micelles by ammonium-based ionic liquids in aqueous media is a relevant factor, as it creates environments favorable to the solvation of hydrophobic compounds. This characteristic directly benefits the extraction of antioxidants, such as tocopherols and carotenoids, found in *Lentinula edodes* by Lim et al.[16].

Studies in the literature demonstrate variations in the antioxidant activity of *Lentinula edodes* extracts depending on the extraction method. Baptista et al.[28] observed DPPH values between 0.240 and 1.197 mM TE g⁻¹ in the Shiitake substrate, with extraction optimized by 50% ethanol (v/v) at 50 °C. Comparatively, Bach et al.[34] obtained a lower result of 0.01148 mM TE g⁻¹ using 25% ethanol (V/V). Other studies also provide points of comparison. Jimenez et al.[35] reported antioxidant activity of 0.321 to 0.331 mM TE g⁻¹ for methanolic extracts of Shiitake (best result with methanol/water 90:10), these values being close to those of sample 4 in the present

work. In turn, Ayar-Sümer et al.[36], when investigating free and bound antioxidant compounds in fermented Shiitake (extracted with 80% (V/V) methanol), presented significantly lower values, ranging from 0.00883 to 0.02613 mM TE g⁻¹. The comparative analysis suggests that the ionic liquid extraction method used in this study demonstrates comparable or superior efficacy in the recovery of antioxidant compounds from Shiitake.

3.4. Antioxidant activity – ABTS method

The antioxidant activity of Shiitake extracts was also evaluated by the ABTS cation radical scavenging method, complementing the DPPH results. The values obtained in Table 3 for antioxidant activity via ABTS ranged from 1.48 mM TE g⁻¹ to 2.20 mM TE g⁻¹.

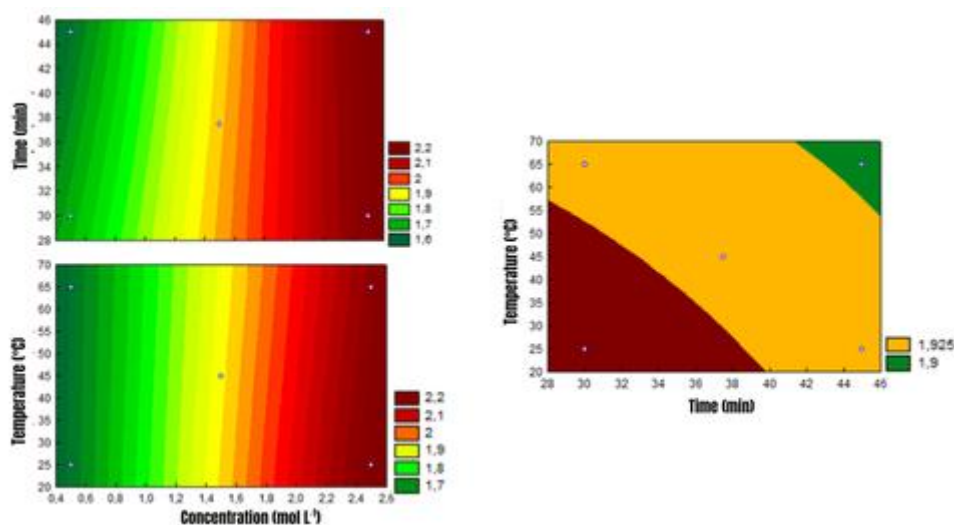
Table 3 - Antioxidant activity of Shiitake extracts using the ABTS method based on integral factorial design 2⁽³⁻⁰⁾

Trials	IL concentration	Time	Temperature	<i>Antioxidante activity (ABTS)</i> (mM TE/g)
	(Mol L ⁻¹)	(min)	(°C)	
	X1	X2	X3	
1	0.4	30	25	1.65 ± 0.05
2	2.0	30	25	2.05 ± 0.05
3	0.4	60	25	1.48 ± 0.11
4	2.0	60	25	2.16 ± 0.07
5	0.4	30	65	1.54 ± 0.03
6	2.0	30	65	2.11 ± 0.02
7	0.4	60	65	1.58 ± 0.03
8	2.0	60	65	2.03 ± 0.12
9	1.2	45	45	2.13 ± 0.04
10	1.2	45	45	2.20 ± 0.01
11	1.2	45	45	2.16 ± 0.01

* Average values followed by standard deviation.

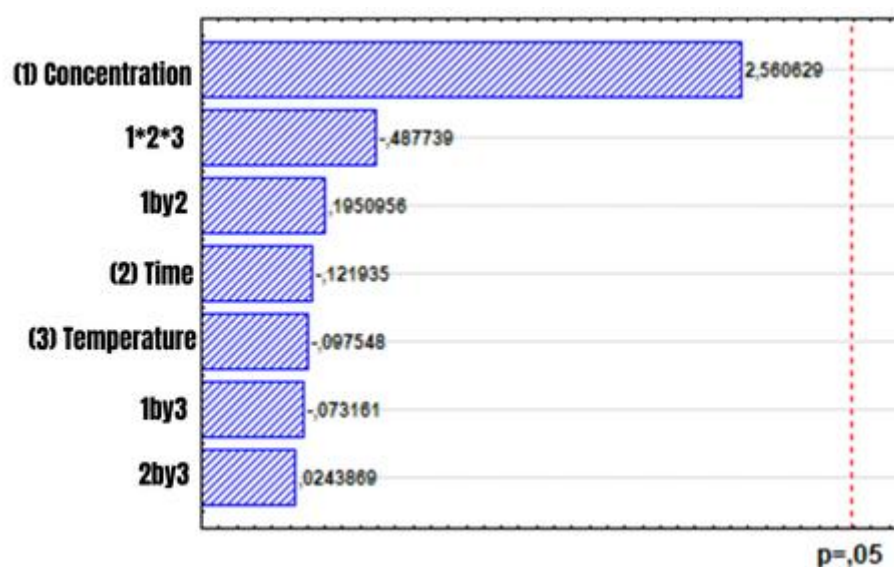
The response surface graphs (Figure 7) for antioxidant activity by ABTS showed that the conditions that tended to generate higher antioxidant activity values were when time and temperature were at their minimum values, while the IL concentration approached its maximum value.

Figure 7 - Response surface graphs of the effects of variables on the antioxidant activity of Shiitake extracts using the ABTS method



However, detailed statistical analysis using the Pareto chart (Figure 8) revealed a distinct result compared to Total Phenolic Compounds and the DPPH method. For the ABTS analysis, none of the independent variables studied (IL concentration, extraction time, or temperature) were considered statistically significant in the extraction process ($p \geq 0.05$).

Figure 8 - Pareto chart of the effects at $p \leq 0.05$ on antioxidant activity using the ABTS method



This lack of statistical significance for the ABTS method may indicate that, within the range of variation of the variables tested, the ability of the extracts to sequester the ABTS⁺ radical is not as sensitive to changes in these conditions as the extraction of total phenolics or DPPH activity. This may be attributed to differences in the reaction mechanisms of DPPH and ABTS radicals, or to the nature of specific antioxidant compounds that react preferentially with each radical. In addition, it may suggest that the extraction of the compounds responsible for ABTS activity is less dependent on IL conditions, time, and temperature within the evaluated ranges, or that other factors not considered in this factorial design exert a greater influence on this response. The R^2 of the model for ABTS was 0.696, a value lower than those observed for total phenolic compounds and DPPH, corroborating the lesser influence of the variables tested.

The analysis of the antioxidant activity of Shiitake extracts using the ABTS method revealed a variation between 1.65 mM TE g⁻¹ (test 1, least favorable) and 2.20 mM TE g⁻¹ (test 10, most favorable). Although the independent variables of the factorial design (IL concentration, time, and temperature) were not statistically significant for this response, a minimal variation in the results was observed, and the intermediate tests (9, 10, and 11) tended to perform better.

Although not statistically significant, the analysis of variable X1 (IL concentration) indicated an increase in results from minimum to maximum concentration, with optimized performance at the average concentration (1.2 Mol L⁻¹). Similarly, for temperature and time, the results decreased from minimum to maximum conditions but were higher at intermediate points. This characteristic, where the best results are concentrated at intermediate points and the absence of significance, suggests that, for ABTS activity, the variables may operate at a plateau or that the complexity of the interactions has not been fully captured. Compared to literature, the results of the present study are promising. Baptista et al. [28] reported ABTS values between 0.391 and 0.906 mM TE g⁻¹ for extracts from the Shiitake substrate, with the best performance obtained by extraction with 50% ethanol. Garcia et al. [29] presented results ranging from 0.77 to 1.53 mM TE g⁻¹ for aqueous and methanolic extracts. The study by Bach et al.[34] showed a value of 0.02885 mM TE g⁻¹, notably lower than those obtained in this work.

In addition, Ayar-Sümer et al. [36] investigated methanolic extracts (80% v/v) of Shiitake, reporting ABTS results of 0.09605 mM TE g⁻¹ for bound antioxidant compounds and 0.02513 mM TE g⁻¹ for free ones. The ABTS antioxidant activity values achieved in this study, especially in assay 10, are competitive with or superior to many of those reported in the literature, indicating that 2-hydroxyethylammonium acetate ionic liquid is an effective solvent for the extraction of these compounds from Shiitake.

4. CONCLUSION

This study demonstrated the feasibility of extracting antioxidant compounds from Shiitake mushrooms using 2-hydroxyethylammonium acetate ionic liquid. The IL synthesis was successful, and factorial planning allowed the identification of ideal conditions for the extraction of different classes of bioactive compounds.

For the quantification of total phenolic compounds and antioxidant activity assessed by the DPPH method, the ideal conditions were characterized by low IL concentration (0.4 Mol L⁻¹) and temperature (25 °C), combined with a prolonged extraction time (60 minutes). IL concentration and temperature were identified as the most significant variables, directly influencing extraction efficiency and antioxidant capacity. However, for antioxidant activity measured by ABTS, none of the variables studied showed statistical significance within the parameters investigated, indicating a lower sensitivity or dependence of these variables on the extraction of the compounds responsible for this activity.

The results obtained for total phenolic compounds exceeded or were comparable to previous studies using traditional solvents, reinforcing the potential of ionic liquids as “green” and efficient solvents in bioactive compound extraction processes. The use of protic ionic liquids, such as 2-hydroxyethylammonium acetate, offers additional advantages in terms of cost, sustainability, and ease of synthesis compared to other types of ILs.

This work contributes significantly to the field of natural product extraction, providing valuable data on the application of ionic liquids for the valorization of fungal

resources such as shiitake. The findings pave the way for future research into the development of more sustainable and efficient extraction processes, as well as for the application of these antioxidant-rich extracts in various industries, such as pharmaceuticals, food, and cosmetics, with a view to developing new products with health benefits.

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Declaration of Interests Statement

The authors declare that they have no known competing financial interests or personal relationships.

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Author's contribution.

Rafael Marques -Methodology; Investigation; Writing – Original Draft; Irede Dalmolin Formal Analysis; Data Curation; Claudia Castro - Supervision, Conceptualization; Validation; Ellen.Porto.-Supervision, Methodology; Validation; Marcelo Bortoli- formal analysis, Visualization; Tânia Cassol.-Supervision; Conceptualization, Writing – Review & Editing; Project Administration. All authors reviewed and approved the final manuscript .

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

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