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Chemical composition and bioactivities of *Phellinus pini* extracts, and quality evaluation of healthy drinks prepared from the mushroom

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Abstract: Phellinus pini, a mushroom species found in East Asian countries, is commonly consumed as a medicinal beverage known for its stomach-warming effects and purported ability to alleviate pain and tumors. In this study, *P. pini* was extracted using different methods (hot water, sonication, microwave, and soaking). The extracts were analyzed for phenolic and polysaccharide contents. Additionally, the extracts were evaluated for their antioxidant potential and ability to inhibit albumin denaturation. The results demonstrated that the extract obtained with hot water extraction contained the greatest amount of phenolics (105.98 ± 0.53 mg GAE/mL). The hot water and microwave extraction methods showed more effective in extracting polysaccharide from the mushroom. Moreover, the extract from the ultrasound extraction method presented the strongest antioxidant activity by scavenging DPPH and ABTS radicals by 41.26 and 97.84 %, respectively while the hot water extract exhibited the most potent ability to inhibit albumin denaturation by 96.40 %. Among the four healthy drinks formulated, the formulation with the greatest proportion of *P. pini* extract contained the highest total phenolic content, antioxidant activity, and the most favorable sensory overall liking. The findings deepen our understanding of the chemical composition and potential health-promoting properties of *P. pini*, as well as revealing new potential applications for the mushroom in the food and nutraceutical industries.

Keywords: mushroom; phenolics; antioxidant activity; healthy drinks; *Phellinus pini*.

INTRODUCTION

Phellinus, a genus of mushrooms from the family Hymenochaetaceae, is primarily found in tropical regions of the America, Africa, and Asia, especially in Uzbekistan, China, Vietnam, Japan, and South Korea.[1,2] In Vietnam, this genus is

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composed of 26 species that can be found in humid mountainous forests from the north to the south.[3] *Phellinus* mushrooms are highly valued for their medicinal properties and are widely used in traditional medicine. They are known to promote and improve health, as well as to prevent or treat various conditions, such as oral ulcers, certain gastrointestinal disorders, and blood-related diseases, as reported in studies.[4,5] Previous studies have revealed that *Phellinus* mushrooms contain phytochemical constituents such as polyphenols, terpenoids, and polysaccharides, all of which exhibit antioxidant, anticancer, antiviral, immunomodulatory properties, and other biological effects.[6]

Phellinus pini is one of the commonly found species in Vietnam, particularly in the Central and Central Highlands regions. It is also prevalent in various regions worldwide, such as the Americas, Africa, and East Asia, with significant prevalence and extensive use in countries like China, South Korea, and Japan.[6] People in Asian countries commonly use *P. pini* as a beverage with medicinal effects, including warming the stomach and treating pain and tumors. Similar to other species in the genus *Phellinus*, *P. pini* contains numerous biologically active compounds, including lignan, (+)-pinoresinol, sterol, stirypirol, ergosterol peroxide, ceramide, and polysaccharides.[7] Among these compounds, polysaccharides extracted from this mushroom possess multiple health-promoting activities that have been demonstrated through numerous studies, such as antioxidant, antiviral, anticancer, and immune-enhancing properties.[8,9] Moreover, extracts from *P. pini* have shown inhibitory effects on enzymes like α-glucosidase and α-amylase, suggesting potential benefits in managing blood sugar levels.[10] Several solvents were employed for crude extraction of bioactive compounds from *Phellinus* species. Various extraction techniques, such as decoction, soaking, ultrasound, and microwave-assisted methods, are commonly utilized for isolating natural products from *Phellinus* species. Zhang *et al.* demonstrated that ultrasonic treatment enhanced the antioxidant activities of polysaccharides extracted from *Phellinus linteus* mycelia.[11] Recently, decoction technique was used to extract bioactive components from dried powder of fruiting bodies of various *Ganoderma* species to prepare tea.[12] Despite numerous studies investigating the bioactivities of *Phellinus* extracts from different species, limited data are available regarding the applications of *P. pini* extracts in developing healthy beverage formulations. Therefore, this study aimed to determine the chemical composition, bioactivities, and sensory acceptability of *P. pini* extracts incorporated into beverage formulations. The objectives of this study were:

1) Compare phenolic and polysaccharide contents, antioxidant activity, and albumin denaturation inhibitory effect of *P. pini* extracts obtained through various extraction methods;

2) Assess the quality of healthy beverages formulated from the selected *P. pini* extract.

The findings of this study will contribute to our understanding of the chemical composition and bioactivities of *P. pini*, while also unveiling new potential applications of the mushroom within the food and nutraceutical industries.

EXPERIMENTAL

Sample collection

Phellinus pini was collected at the Pu Mat National Park in Nghe An province, Vietnam. The sample was thoroughly cleaned with water, air dried, sliced, and ground to 1 mm size. The powder was carefully stored in a polyethylene bag at a refrigerator (4 °C) for further analysis.

Chemicals

Phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol and water (HPLC grade) were obtained from Fisher Scientific (Pittsburg, Pennsylvania, USA). ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) and were purchased from Sigma-Aldrich and Sisco Research Laboratories (Maharashtra, India), respectively. Food ingredients, including honey, dried jujube, and licorice, were purchased at a local grocery store.

Extraction

Briefly, 5 g of the sample were mixed with 500 mL of deionized water, and the extraction was carried out using different extraction methods, including hot water (closed lid), sonication (closed lid, Sonica 3200 EP S3, 360 W, 39 kHz), microwave (closed lid, Sanyo EM-S2052W, 700 W, 2.45 MHz), and soaking. The parameters of each extraction method were shown in Table 1. After the extraction step, the mixture was then filtered by a Whatman filter paper (110 mm in diameter, GE Healthcare, Illinois, USA). The filtrate obtained were analyzed for phenolic contents, total polysaccharide content, and bioactivities.

TABLE I. Parameters of the various extraction methods of *Phellinus pini*

Extraction methods	Hot water	Sonication	Microwave	Soaking
Time, min	5	15	2	60
Temperature, °C	95 – 100	50	30	30

Determination of phenolics

Total phenolic content (TPC) of extracts was determined by the Folin-Ciocalteu approach.[13] A volume of an extract (0.5 mL) was pipetted into a test-tube containing 2.5 mL of 10 % Folin-Ciocalteu solution, followed by shaking and 5 min incubation in darkness. 2 mL of 7.5 % Na_2CO_3 were added, and the mixture was then incubated in darkness for 60 min. The absorbance was measured by a UV–VIS spectrophotometer (Thermo GENESYS 20 UV-VIS) at 765 nm. Gallic acid was used as a reference standard.

To identify and quantify individual phenolic acids and flavonoids, the extracts were injected into a high performance liquid chromatography system equipped with a UV detector (Prominent, Shimazu, Japan), following the method of Nguyen *et al.* (2023).[14] The separation was performed using a VertiSep GES C18 reverse-phase column (250 mm × 4.6 mm × 5 µm). The mobile phases were methanol (A) and 2 % formic acid (B) with the elution gradient as follows: from 0 to 3 min, 25 % A; 3 to 10 min 25-40 % A; 10 to 20 min 40-60 % A; 20 to 30 min 60-80 % A; 30 to 40 min 80 % A; from 40 to 48 min 80-25 %. The flow rate was set at 1.0 mL/min. Detection wavelengths were 295 and 340 nm. The column was operated at room

temperature (30^0C). Sample injection by system was automatically set with a sample volume of 10 µL. Quantification of phenolics was based on calibration curves of the external standards at the same condition.

Determination of total polysaccharide content (TPSC)

Total polysaccharide content of each extract was determined by Nielsen's method with minor modifications.[15] Five grams of the ground mushroom were combined with water at a ratio of 1:10 (g/mL). The extraction methods were the same as those described in the ealier section. The extracts were collected after removing the sample solids. 80 % ethanol in water was added in the extracts at a ratio of 1:4 (v/v), and the mixture was kept under low temperature (4 °C) for 12 h, followed by centrifugation (5000 rpm, 10 min). The resulting residue (i.e., polysaccharide) was dried and dissolved in 5 mL of sodium hydroxide. Water was added to the solution to make a volume of 10 mL. 1 mL of this mixture was combined with 1 mL of 5 % phenol and 5 mL of concentrated sulfuric acid, followed by storage at room temperature for 10 min. Absorbance was spectrophotometrically measured at 490 nm. Glucose served as a reference standard.

Antioxidant activity

Antioxidant activity evaluated by DPPH and ABTS methods was described by Tuan *et al.* (2023).[16] One mL of an extract obtained in Section 2.2 was pipetted into a tube containing 5 mL of 0.1 mM DPPH solution, the mixture was thoroughly shaken and kept in darkness for 30 min, and absorbance was determined at 517 nm. Regarding ABTS assay, 38.4 mg of ABTS were mixed in methanol, 6.6 mg potassium persulfate were added, followed by vigorous shaking and storage in darkness for 16 h. The ABTS solution was adjusted in methanol to absorbance at 0.7 ± 0.02 at the wavelength of 734 nm. 100 µL of an extract combined with 3 mL of the ABTS solution were shaken and kept in darkness for 6 min. Afterwards, absorbance of the mixture was measured using a UV-VIS spectrophotometer.

Inhibition of albumin denaturation

The inhibitory activity of the extracts was evaluated and compared with that of diclofenac, a nonsteroidal anti-inflammatory drug.[17] Each *P. pini* extract obtained from the various extraction methods or diclofenac sodium solution (100 µL) was combined with 100 µL of bovine serum albumin solution (0.16 %) and 200 µL of sodium acetate buffer (25 mM, pH 5.5). The mixture was subjected to incubation at 37 °C for 45 min, followed by a heating step to 67 °C for 3 min. After cooling down to room temperature, absorbance of the mixture was measured at 660 nm.

Healthy drink formulation and quality evaluation

The *P. pini* extract with the highest TPC was selected to be applied to drink formulation. First, the extract was diluted with deionized water at the extract/water ratios of 1:3, 1:2, 1:1, and 2:1 (v/v), resulting in four drink formulae (S13, S12, S11, and S21), respectively. Afterwards, each of the mixtures (1,000 mL) was combined with honey, jujube juice, and licorice juice as shown in the diagram. The resulting mixtures were evaluated for TPC, antioxidant activity, soluble solids, pH, color, and sensory overall linking.

Licorice and jujube juices were prepared by mixing 5 g of dried licorice and 12 g of dried jujube in 200 and 600 mL of water, respectively. The mixtures were then boiled for 5 min, followed by cooling for 30 min before use.

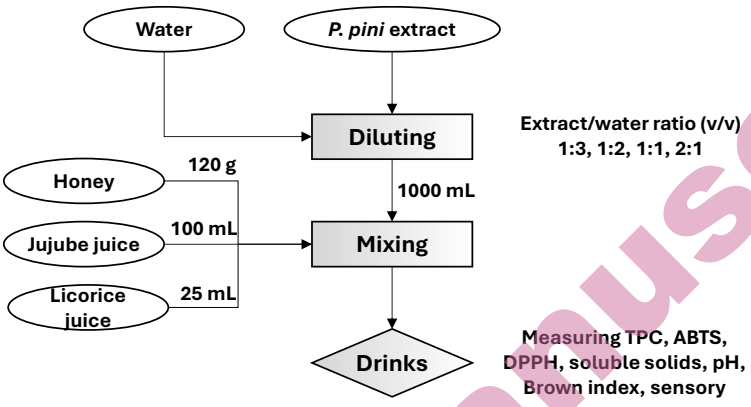

Fig. 1. The diagram of healthy drink formulation.

The color (CIE L*, a*, b*) of the drink was measured using a colorimeter (Konica Minolta, CR-400 Ramsey). Brown index was calculated according to previous studies (Pathak *et al.*, 2016). The pH measurement was conducted using a benchtop pH meter (Hanna Instruments, German), and Brix value was recorded by a portable refractometer (Cole-Palmer). Overall sensory acceptance was run on the four drink formulations (S13, S12, S11, S21) prepared as described in Fig. 1. The drinks were served at room temperature and in red light. Overall liking of each sample was recorded from 50 panelists, using a nine-point hedonic scale (1-Extremely Dislike, 5- Neither Like nor Dislike, 9-Extremely Like). All samples were coded with 3-digit numbers and served in a random order.

Statistical analysis

The measurements were performed three times, and the findings were presented as mean value ± standard deviation. Data were analyzed using one-way ANOVA alongside Tukey's HSD test at a significance level of 0.05. Statistical analysis was carried out using XLSTAT version 2016 (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Total phenolic content

Phenolic compounds are prevalent in mushrooms and play a crucial role in their antioxidant properties.[18-20] The hydroxyl groups of phenolics, confirmed in extracts from various mushroom species such as *Ganoderma* spp. and *Hydnum repandum*, enhance the efficiency of scavenging free radicals and reducing ferrous ions. The results presented under Table 2 indicated that the examined *P. pini* extracts are abundant in phenolic compounds (25.00 to 105.98 mg GAE/mL). The highest TPC was detected in the hot water extract (105.98 ± 0.53 mg GAE/mL) while the ultrasonic extract has the lowest TPC (25.00 ± 0.10 mg GAE/mL). Hot water extraction was shown to be among the most effective methods in recovering phenolics. For example, one study on shiitake mushroom showed that extracts obtained with hot water had significantly higher TPC than with conventional organic solvents, and equivalent to that of microwave extraction.[21] In another

study, hot water extraction produced the lemon-byproduct extracts richest in phenolics compared to ultrasound and organic solvent extractions.[22] The higher TPC in hot water extracts could be due to the combined effects of breaking bonds, increasing solubility, disrupting cell walls, enhancing diffusion, and hydrolyzing complexes, all of which help release and dissolve more phenolic compounds from the mushroom material. In comparison with some medicinal mushrooms, such as turkey tail (*Trametes versicolor*) and other *Phellinus* species,[23, 24] total phenolic content in the *P. pini* extracts were higher.

TABLE II. The total phenolic and total polysaccharide content of *P. pini* extracts

	Hot water extract	Microwave extract	Ultrasonic extract	Soaking extract
TPC (mg GAE/mL)				$25.00^{b} \pm 0.10$
TPSC (mg GE/g)	$5.60^{a} \pm 0.03$	$5.67^{a} \pm 0.03$	$1.69^{c} \pm 0.02$	$1.93^{b} \pm 0.02$

GAE: gallic acid equivalents; GE: glucose equivalents. Different letters (a, b, c) indicate significant differences in concentrations of phenolics among the extracts.

Phenolic compound contents

TABLE III. Phenolic compound contents (μg/mL) of *P. pini* extracts obtained with different extraction methods

Compounds	Soaking extract	Microwave extract	Ultrasonic extract	Hot water extract
Gallic acid	$19.89^{b} \pm 0.00$	$20.33^{b} \pm 0.58$	$19.38^{b} \pm 0.23$	$40.55^{a} \pm 0.40$
Chlorogenic acid	$0.71^{b} \pm 0.08$	$0.76^{b} \pm 0.22$	$0.48^{c} \pm 0.08$	$7.39^{a} \pm 1.00$
Caffeic acid	$0.44^{c} \pm 0.18$	$0.43^{c} \pm 0.03$	$0.85^{b} \pm 0.04$	$2.09^{a} \pm 0.92$
p-Coumaric acid	n.d.	$2.56^{a} \pm 0.31$	n.d.	$0.85^{b} \pm 0.21$
Ferulic acid	$0.44^{c} \pm 0.28$	$1.80^{a} \pm 1.65$	$1.25^{b} \pm 0.13$	$1.52^{a} \pm 0.15$
Salicylic acid	n.d.	n.d.	n.d.	n.d.
Cinnamic acid	n.d.	n.d.	n.d.	n.d.
HBA*	n.d.	$1.76^{b} \pm 0.32$	n.d.	$15.18^{a} \pm 2.97$
Rutin	n.d.	n.d.	n.d.	3.11 ± 0.50
Quercetin	n.d.	n.d.	n.d.	n.d.

* dihydroxybenzoic acid. Different letters (a, b, c) indicate significant differences in concentrations of phenolics among the extracts. n.d.: not detectable

Ten phenolic compounds, including gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, DHBA (dihydroxybenzoic acid), salicylic acid, cinnamic acid, rutin and quercetin in the four *P. pini* extracts were analyzed by HPLC-DAD, and the results were presented in Table III. In total, seven phenolics were identified and quantified in the extracts. Among these, four compounds, namely gallic acid, chlorogenic acid, caffeic acid, and ferulic acid, were present in all the extracts. Quercetin, salicylic acid, and cinnamic acid were not detected in any of the extracts. The hot water extract with the highest TPC was shown to

contain all the seven phenolic compounds. The microwave extract consisted of six compounds while the soaking and ultrasound extracts were found with four compounds. Gallic acid was the most dominant compound in all the extracts, with the concentrations ranging from 19.38 to 40.55 μg/mL. The hot water extract contained twice as much of this compound as the others. Gallic acid was previously reported to be much more abundant in an aqueous extract of *Ganoderma lucidum* compared to extracts prepared from organic solvents.[25] The hot water extract also contained the highest amount of chlorogenic acid (7.39 ± 1.00 μg/mL), followed by the microwave extract (0.76 ± 0.22 μg/mL), and the soaking extract (0.71 ± 0.08 μg/mL). The ultrasonic extract had the lowest chlorogenic acid content (0.48 ± 0.08 μg/mL). The other phenolic acids that had the greatest levels in the hot water extract than in the others were caffeic acid and HBA. Previously, hydroxybenzoic acids were reported to be abundant in extracts of *Flammulina velutipes*, *Trametes versicolor*, and *Ganodema* spp.[18, 26] Rutin was the only flavonoid that was found to be present in the extract, which is the hot water extract. Unlike the aforementioned compounds, ferulic acid and *p*-coumaric acid were detected at the highest levels in the microwave extract (1.80 ± 1.65 and 2.56 ± 0.31, respectively).

The results demonstrated the superiority of hot water and microwave extractions over soaking and ultrasonic methods for extracting phenolics from *P. pini*. The effectiveness of hot water and microwave extractions can be attributed to several factors, including thermal effects, increased solubility, inactivation of enzymes, and microwave effects. The high temperature involved in the methods can break down the plant cell walls and disrupt the hydrogen bonds and hydrophobic interactions that bind phenolic molecules to plant matrices,[1] facilitating their extraction. The high temperature also increases the solubility and diffusion rates of phenolics in the extraction solvent, leading to more efficient extraction.[27] Inactivation of enzymes like polyphenol oxidases can occur under high temperature, preventing degradation of phenolics during extraction. Microwave irradiation can cause molecular motion and dipole rotation, leading to the disruption of hydrogen bonds and increased mass transfer of phenolic compounds from the sample to the solvent.[28]

Total polysaccharide content

Total polysaccharide contents of the four different extracts from *P. pini* were examined and shown in Table II. The TPSC ranged between 1.69 and 5.67 mg GE/g. Due to the high temperature, the microwave extract (5.67 mg GE/g) and hot water extract (5.60 mg GE/g) samples had the highest TPSC. The two samples with the lowest concentrations were soaking and ultrasonic extracts (1.93 and 1.69 mg GE/g, respectively). Mushroom polysaccharides primarily belong to the category of β-glucans, similar to the digestive enzymes secreted by the pancreas that aid in the digestion process.[29] In addition to their significance as prebiotics, mushroom polysaccharides have been shown to possess a wide range of other

bioactivities, including antitumor, antimicrobial, antioxidant, antiviral, and immunomodulatory activities.[30, 31]

Antioxidant activity

TABLE IV. Antioxidant activity of the *Phellinus pini* extracts

	Hot water extract	Microwave extract	Soaking extract	Ultrasonic extract
DPPH, %	$26.21^{b} \pm 2.37$	$36.21^{a} \pm 1.26$	$37.01^{a} \pm 1.61$	$41.26^{a} \pm 2.07$
ABTS, %	$96.09^{d} \pm 0.07$	$97.57^{b} \pm 0.07$	$97.19^{c} \pm 0.07$	$97.84^{a} \pm 0.00$

Different letters (a, b, c) indicate significant differences in concentrations of phenolics among the extracts.

As presented in Table IV, the ultrasonic extract may show the strongest DPPH and ABTS scavenging activities (41.26 and 97.84 %, respectively). In contrast, the hot water extract could have the weakest ability to remove the radicals. The microwave and soaking extraction methods resulted in the extracts with comparable scavenging activities. The findings are in agreement with previous studies reporting that ultrasound-assisted extraction can enhance antioxidant potential and other bioactivities of *Phellinus* medicinal mushroom extracts, such as *Phellinus igniarius* and *P. linteus*.[11, 32] Ultrasound-assisted extraction can break down the tough and woody structure of *Phellinus* mushrooms into smaller particles, increasing the surface area available for solvent penetration. This finer particle size exposes more fungal cells to the solvent, promoting the release of antioxidants and other bioactive compounds.

Albumin denaturation inhibitory activity

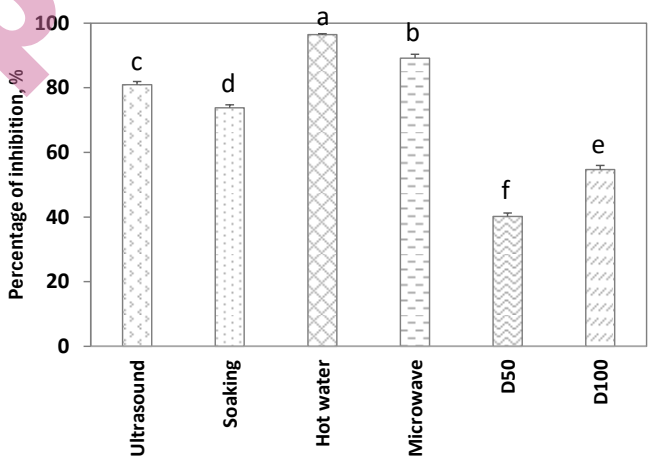

Fig. 2. Inhibitory effect (%) of the extracts and diclofenac solutions on albumin denaturation. D50 and D100 denote the solution of diclofenac at 50 and 100 μg/mL, respectively. Different letters (a, b, c, d, e, f) indicate significant differences in concentrations of phenolics among the extracts.

Fig. 2 shows that the extract obtained with hot water may possess the most potent inhibitory activity against albumin denaturation, followed by the extract from the microwave extraction method, with the percentages of inhibition as high as 96.40 and 89.10 %, respectively. The extracts from the ultrasound and soaking methods showed lower effects to inhibit albumin denaturation. The results also demonstrated that all the extracts could have higher capacities to protect albumin from denaturation than the 50 and 100 μg/mL diclofenac solutions. In the present study, the ability of *P. pini* to inhibit albumin denaturation was used to understand potential anti-inflammatory properties of the mushroom species. Research has shown that *Phellinus* species may possess strong anti-inflammatory activity.[33] This could be due to the presence of chemical constituents in their composition. For example, polysaccharides of *P. linteus* reportedly reduce the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-2, IL-6 and IL-12) in lipopolysaccharide-induced inflammatory cell model.[34] Another study revealed that inotilone isolated from *P. linteus* inhibited LPS-induced MMP-9 expression by inactivation of NF-κB via ERK, p38, and JNK signaling pathways in RAW 264.7 cells.[35]

Healthy drink formulation

TABLE V. Total phenolic content, ABTS and DPPH radical scavenging activities, physicochemical properties and sensory acceptance of the four healthy drink formulae from the *P. pini* extract.

Formulae	S13	S12	S11	S21
TPC (mg GAE/mL)	$112.50^{c} \pm 0.00$	$118.4^{b} \pm 0.26$	$86.49^{d} \pm 0.06$	$126.53^{a} \pm 0.59$
ABTS, %	$93.76^{d} \pm 0.06$	$99.11^{c} \pm 0.06$	$99.61^{a} \pm 0.06$	$99.54^{a} \pm 0.06$
DPPH, %	$65.64^{c} \pm 0.62$	$73.01^{b} \pm 0.39$	$66.14^{c} \pm 0.16$	$82.52^{a} \pm 0.31$
Soluble Solids (°Brix)	7.60 ± 0.14	8.00 ± 0.57	7.70 ± 0.00	7.80 ± 0.28
Brown index	$82.47^{c} \pm 0.03$	$84.36^{b} \pm 0.30$	$86.73^{e} \pm 0.38$	$88.02^{a} \pm 0.31$
pH	6.11 ± 0.01	6.21 ± 0.16	6.13 ± 0.03	6.16 ± 0.06
Overall liking	$5.62^{a} \pm 1.62$	$5.40^{a} \pm 1.77$	$5.52^{a} \pm 1.85$	$5.62^{a} \pm 1.83$

Total phenolic content and antioxidant activity

In the present study, the cook extract with the highest TPC was used for healthy drink formulation. In traditional folk medicine, mushroom decoctions frequently entail immersing crushed or small pieces of the fruiting body in hot water to extract soluble components. The resulting decoction is then ingested. Additionally, mushrooms are typically not consumed raw but undergo various food processing methods to enhance digestion and assimilation. Consequently, preparing hot water extracts replicates cooking conditions, imitating the typical culinary practice for consuming edible mushrooms. Table V shows total phenolic content and the antioxidant activity measured by the ABTS and DPPH radical scavenging assays for four healthy drink formulae (S13, S12, S11, S21) containing *P. pini* extract. Among these, S21 was the phenolic-richest drink (126.53 ± 0.59

mg GAE/mL), followed by S13 (112. 5 ng GAE/mL) and S12 (118.4 ± 0.26 mg GAE/mL). An increase in TPC indicated that as the level of the extract proportion increased, the antioxidant activity (through ABTS and DPPH values) increased in most cases. Notably, S11, formulated with half the proportion of the *P. pini* extract, demonstrated the lowest phenolic content (86.49 ± 0.06 mg/mL GAE) and thus possessed the lowest DPPH value (66.14 %). As described earlier, honey, jujube juice, and licorice were used in the formulation, it is important to note that the various ingredients might interact with each other and other components in the extract, resulting in changes in TPC of the formulated drinks. Previously, research showed that both honey and fruit juice rich in phenolics and vitamin C, when used as ingredients, can impact the final phenolic content of beverages.[36]

The results also revealed that all the formulated drinks had higher ABTS percentage of inhibition, falling within 93.76 – 99.61 %. S11 and S21 exhibited the strongest activity while S13 showed the lowest activity to remove ABTS radicals. S21 also had the most potent capacity to inhibit the formation of DPPH radicals (82.52 ± 0.31 %). Similarly, S13 exerted the weakest DPPH scavenging activity (65.64 ± 0.62 %). Studies have consistently demonstrated a positive correlation between TPC and antioxidant potential in various foods, beverages, and plant extracts.[17, 37] Phenolic compounds, including flavonoids, phenolic acids, and other polyphenols, are well-known antioxidants that can scavenge free radicals and inhibit oxidative stress. These can partly explain the high TPC of S21 accompanying its free radical scavenging activities. In comparison with the mushroom extract obtained with the hot water extraction as described earlier, the formulated drinks all had a higher potential to scavenge DPPH radicals. This means that adding honey, jujube juice, and licorice led to an increase in TPC of the final drinks. The mechanism underlying this phenomenon has been poorly elucidated, primarily due to the intricate composition of the mixtures, especially plant extracts. Possible explanations could be formation of stable dimers, oligomers, adducts, and/or even new phenolics with elevated antioxidant activity compared to the parent components during the formulation of the drinks.

Soluble solids

The observed soluble solids content ranging from 7.60 to 8.00 °Brix can be attributed to the combined contributions of the sugars present in the extract, honey, jujube juice, and licorice juice used in the formulations. The slight variations could be due to differences in the proportions of the extract across the formulations.

Color measurement

The brown index, a measure of browning or color intensity, varied significantly among the formulations. S21 had the highest brown index (88.02 ± 0.31), indicating a darker color, followed by S11 (86.73 ± 0.38), S12 (84.36 ± 0.30), and S13 (82.47 ± 0.03). The significant differences in the brown index

values can be explained by the varying proportions of the *P. pini* extract in the drink. Although jujube juice and licorice juice used in the formulations are known to contain natural pigments and can contribute to the brown coloration of the drinks, their ratios are constant across the formulations. Therefore, their contribution to brown color intensity should be the same.

pH

The pH values of the formulations ranged from 6.11 (S13) to 6.21 (S31), indicating slightly acidic conditions. The pH values were relatively similar across the formulations, suggesting comparable acidity levels.

Sensory evaluation

The sensory overall liking revealed insignificant differences among the formulations. These results highlighted that the contribution of different proportions to the overall liking of the sample were not significant. Generally, data have shown a moderate sensory acceptability in overall liking. The data suggest that up to two thirds of the extract may be substituted by water in drink formulation with acceptable sensory properties. A study by Thumrongchote found that adding honey and lemon juice to a drink extract of *Schizophyllum commune* Fr., an edible macro-fungus, helped increasing aroma, taste, and overall acceptability.[38] Similarly, the ratio of apple juice and honey significantly influenced color, pH, and sensory attributes of apple-honey beverages.[39] A study by Azami *et al.* (2018) also reported that the addition of licorice extract in beverages affected their overall acceptability.[40]

CONCLUSION

This study represents the first investigation into the comparative effects of various extraction techniques on the phenolic and polysaccharide content, as well as the bioactivities of *Phellinus pini*. Additionally, it assesses the quality of healthy beverages formulated from the mushroom extract. The results indicated that the hot water extract exhibited the highest levels of phenolics, polysaccharides, and albumin denaturation inhibitory activity. Conversely, the ultrasound extract displayed the most potent antioxidant activity, as determined by DPPH and ABTS assays. Notably, the beverage formulated with the greatest proportion of *P. pini* extract showcased the highest total phenolic content, antioxidant activity, and garnered moderate overall liking. Future investigations should focus on formulating beverages with Phellinus mushroom extract combined with other nutritious ingredients to enhance their health-promoting properties. Studies should also aim to optimize the taste, texture, and shelf-life of the formulations to ensure consumer acceptance and marketability.

И З В О Д

ХЕМИЈСКИ САСТАВ И БИОАКТИВНОСТ ЕКСТРАКТА *PHELLINUS PINI* И ЕВАЛУАЦИЈА КВАЛИТЕТА ЗДРАВИХ НАПИТАКА ПРИПРЕМЉЕНИХ ОД ПЕЧУРАКА

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Phellinus pini, врста печурака која се налази у земљама источне Азије, обично се конзумира као медицински напитак познат по својим ефектима загревања стомака и наводној способности да ублажи бол и туморе. У овој студији, *P. pini* је екстрахован различитим методама (топла вода, сонификација, микроталасна дигестија и квашење). У екстрактима је одређиван садржај фенола и полисахарида. Додатно, екстракти су испитивани због њиховог антиоксидативног потенцијала и способности да инхибирају денатурацију албумина. Резултати су показали да екстракт добијен екстракцијом топлом водом садржи највећу количину фенола (105,98 ± 0.53 mg GAE/mL). Екстракција топлом водом и микроталасна дигестија су се показале ефикаснијим поступцима у екстраховању полисахарида из печурака. Штавише, екстракт добијен ултразвучном екстракцијом је показао највишу антиоксидативну активност уклањањем ДППХ и АБТС радикала за 41,26 и 97,84 %, респективно, док је екстракт добијен екстракцијом топлом водом показао најснажнију способност да инхибира денатурацију албумина 96,40 %. Међу четири формулисана здрава напитка, формулација са највећим уделом екстракта P. pini садржи највећи укупни садржај фенола, највећу антиоксидативну активност и најповољнију сензорну укупну допадљивост. Добијени резултати продубљују наше разумевање хемијског састава и потенцијалних здравствених својстава *P. pini*, као и откривање нових потенцијалних примена гљиве у прехрамбеној индустрији.

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