UPLC-MS Metabolite Profiling and Antioxidant Activity of *Sanghuangporous sanghuang* Extract

Weike Wang[1] , Na Lu[1] , Chen Jiang[2] , Guanping Chen[2]

[1] Hangzhou Academy of Agricultural Sciences, Hangzhou, China
[2] Cancer Institute of Integrated Traditional Chinese and Western Medicine, Zhejiang Academy of Traditional Chinese Medicine, Tongde Hospital of Zhejiang Province, Hangzhou, China

Corresponding Author:
Guanping Chen
No. 234 Gucui Road, Hangzhou, Zhejiang, 310012, China
Email address: beyond_cgp@163.com

Abstract

Background. The present study aims to explore the total phenolic and flavonoid present in ethanol extract of *Sanghuangporus sanghuang* (Sheng H. Wu, T. Hatt. & Y.C. Dai) Sheng H. Wu, L.W. Zhou & Y.C. Dai ~~*Sanghuangporous sanghuang*~~ and to assess its phytochemical properties, antioxidant activity, and its DNA damage protection activities. This pharmaceutical/food resource mushroom may serve as a new substitute functional food for health-conscious purchasers owing to its promising source of phenolics and flavonoids.

Methods. *S. sanghuang* powder was prepared into ethanol extract (SEE), SEE was evaluated for its total phenolic and flavonoid contents. Additionally, UPLC-MS analysis of SEE, component identification, and function prediction were performed. SEE was assessed for antioxidant and anti-DNA damage activities.

Results and Conclusion. Total phenolic content (TPC) in SEE amounting to 385.38 ± 1.36 mg GA/g Extract. And total flavonoid content (TFC) in SEE amounting to 298.22±2.38 mg QE/g Extract. The extracts exhibited high antioxidant and free radical scavenging activities with relatively stronger free radical scavenging activity. A total of 491 metabolites were investigated by Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Most of the top 20 compounds were predicted to have various functions like antioxidants, anticancer and anti-inflammatory. This study highlighted *S. sanghuang* ~~*S. Sanghuang*~~ was a beneficial source of phenolics and flavonoids. It contains potential natural antioxidants that could be used as a lead contender in the development of antioxidant medicines for the treatment of a wide range of oxidative stress-related illnesses.

Açıklamalı [SK1]: Please use correct name

Sanghuangporus sanghuang

Açıklamalı [SK2]: This article can be publish with minor revision

Please see comments

Introduction

Sanghuangporus sanghuang~~*Sanghuangporous sanghuang (S. sanghuang)*~~ is a useful edible fungus, which belongs to Basidiomycota, Hymenomycetes, Aphyllophorales, Hymenochaetaceae, and Sanghuangporus (Li et al., 2020). It has been used as a Traditional Chinese Medicine for at least 2000 years and recent researches showed that *S. sanghuang* have various bioactivities, such as anti-oxidative, anti-inflammatory, anti-carcinogenesis, anti-fungal and immunomodulatory activities, as well as anti-diabetic, hepatoprotective and neuroprotective effects (Lu et al., 2024; Lin et al., 2017).

Previous phytochemical studies have shown that *S. sanghuang* mainly contains polysaccharides, flavonoids, triterpenoids, polyphenols and alkaloids (Yuan et al., 2023; Zuo et al., 2021). Besides to these well known compounds, there are also numerous unknown metabolites to be studied. Given the complications and advance effects of natural products, there is a serious consideration of using chinese herbal medicines. In fact, the use of herbal medicines has significantly increased in recent years (Hao et al., 2022; Sevindik et al., 2024).

The medicinal active ingredient site of *S. sanghuang* is mainly its fruiting bodies, the extraction of bioactive compounds from fruiting bodies using various solvents is a key focus of research (Li et al., 2020). This step is crucial in the production of products that are rich in metabolites. Utilizing this low-cost technology to extract molecules from fruiting bodies, is a suitable strategy for producing food additives and nutraceutical products (Zuo et al., 2021; Gu et al., 2022).

Therefore, the present study aims to explore the total phenolic and flavonoid present in ethanol extract of *S. sanghuang* and to assess its phytochemical properties, antioxidant activity, and its DNA damage protection activities. GC-MS study was also performed to find out functional groups and active compounds in the ethanol extract of *S. sanghuang*.

Materials & Methods

Preparation of the S. sanghuang ethanol extract (SEE)

S. sanghuang was authenticated by the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. The fruiting body were dried and ground, then the powder was extracted three times with 80% ethanol (1:10 w/v) at room temperature, each time for 2 h. Three extractions were combined and was evaporated at 40℃, then freeze-dried for 72 h.

Determination of total phenolic content (TPC)

1 mg of SEE was dissolved in 1 mL of methanol. Then, 0.5 mL of SEE solution was combined with 1 mL of Folin-Ciocalteu reagent. After 5 minutes, Na_2CO_3 (7% w/v) was added to the mixture, and the volume was increased to 10 mL with water before shaking vigorously. A calibration curve was created for gallic acid at concentrations of 20, 40, 60, 80, and 100 µg/mL. Following 1 h of incubation at room temperature, absorbance was measured at 760 nm with a UV-VIS spectrophotometer. Total phenolic content was quantified in gallic acid equivalents (mg GA/g of dw).

Determination of total flavonoid content (TFC)

Açıklamalı [SK3]: Please add reference

Sevindik, M., Gürgen, A., Khassanov, V. T., & Bal, C. (2024). Biological Activities of Ethanol Extracts of Hericium erinaceus Obtained as a Result of Optimization Analysis. *Foods*, *13*(10), 1560.

SEE was diluted with methanol to 1 mg/mL, and quercetin (20, 40, 60, 80, 100 μg/mL) was used as the standard curve. 2 mL of diluted SEE or quercetin was combined with 0.1 mL (10% w/v) $AlCl_3$ and 0.1 mL (0.1 mmol/L) CH_3COOK. Following a 30 min incubation at 25°C, absorbance was measured at 415 nm. The total flavonoid concentration of the extracts was expressed as mg quercetin equivalent per gram dry weight of extracts (mg Q/g of dw).

Ferric Reducing Antioxidant Power (FRAP) Assay

Total antioxidant activity (TAA) of SEE was taked using the classic Fe^{3+} reducing power test. SEE and standard (1 mL) were combined with 5 mL of PBS (0.2 mol/L, pH 6.6) and 1% $K_3[Fe(CN)_6]$ (5 mL). The mixture was incubated at 50°C for half an hour. Then, 5 mL of TCA was joined and centrifuged for 10 min. Lastly, 5 mL of supernatant was collected and combined with 5 mL of ddH_2O and 1 mL of $FeCl_3$ (0.1%). The absorbance was measured at lmax=700 nm by a UV-spectrometer.

~~Antioxidant Capacity by~~ DPPH Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) test was performed to assess antioxidant activity of SEE. 0.1 mL of DPPH in methanol (0.1 mmol/L) was combined with 0.1 mL of SEE solutions (10, 20, 30, 40, 50 μg/mL each). After 1 h of incubation at room temperature in the dark, the absorbance at 517 nm was measured with a UV-spectrometer.

ABTS Scavenging Assay

The 2,2'-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid (ABTS) scavenging assay uses the radical cation decolorization technique. $K_2S_2O_8$ (2.45 mmol/L) and ABTS (7 mmol/L), were produced in ddH_2O and combined in a ratio of 1:1 before being incubated in the dark for 24-48 h. After diluting the ABTS at a ratio of 1:25, 300 μL of SEE was introduced to 3.0 mL of the $ABTS^+$ solution. Subsequently, the absorbance of this uniform mixture was assessed at a wavelength of 745 nm.

DNA Damage Protection Activity

A final volume of 50 μL reactant solution, consisting of 10 μL vector DNA (0.1 μg/μL), 25 μL Fenton's testing agent (0.2 mmol/L $FeCl_3$, 0.1 mmol/L Vitamin C and 0.03 mol/L H_2O_2), and 15 μL SEE (1 mg/mL), was prepared. The formula mix was then incubated at a temperature of 37°C for a duration of 45 min. 6 μL loading buffer was then added into the mixture. DNA cleavage and protective efficacy were assessed by 1% agarose gel electrophoresis.

UPLC-MS Analyses of SEE

Chromatography was performed using an ACQUITY UPLC I-Class HF system (Waters, USA). ACQUITY UPLC HSS T3 (100 mm ×2.1 mm, 1.8 um; Waters, USA) was used as a separation column. The elution solution consisted of deionized water contains 0.1% formic acid (A) and acetonitrile (B). The rate was set at 0.35 mL/min, and the injection volume was 5 μL. Mass spectrometric experiments were performed using Obritrap-QE (Thermo, USA) in negative mode.

Statistical Analysis

All analyses were conducted using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). Data were presented as mean±standard deviation (SD). The statistical significance of the

difference between the SEE and Control was assessed by the Student's t-test. Differences with p values<0.05 were considered statistically significant.

Results

Total Phenolic and Flavonoid Contents of SEE

SEE was evaluated to determine the content of phenolics and flavonoids, as presented in Table 1. Total phenolic content (TPC) in SEE amounting to 385.38 ± 1.36 mg GA/g Extract. Total flavonoid content (TFC) in SEE amounting to 298.22±2.38 mg QE/g Extract. A larger concentration of these phytochemicals in SEE increases its radical scavenging action.

Ferric-Reducing/Antioxidant Power (FRAP) Assay

The antioxidant function of flavonoids and phenolic acids were assessed by measuring their capability to reduce iron from Fe^{3+} to the Fe^{2+} state, absorbance is directly proportional to reducing capability of the extract. In this study, SEE exhibited strong antioxidant capacity of 380.46±2.12 µmol/g Extract (Table 2), which suggested that SEE possesses the ability to interact with free radicals, transforming them into a stable and non-reactive state. We deduce that flavonoids and phenolic acids in SEE act as free radical scavengers by donating electrons or hydrogen, thereby endowing SEE with reducing power function.

DPPH Radical Scavenging Activity

DPPH assay reveals the existence of phenolic and flavonoid components in phytoextracts, serving as a prevalent method to examine the antioxidant characteristics of phytoextracts. Our findings demonstrated notable antioxidant activities in SEE. DPPH activity values for SEE as presented in Table 2. The SEE was able to reduce DPPH radicals with a percentage of 72.09±1.27% at the concentration of 1 mg/mL.

ABTS Radical Scavenging Activity

SEE showed an inhibition of 57.25±1.03% at the concentration of 10 µg/mL (Table 2). The significant impact suggests that SEE is an effective suppressor of free radicals. The elevated scavenging ability of SEE is associated with increased levels of flavonoids, polyphenols, and proanthocyanins. This further substantiated that SEE has the potential to eliminate free radicals. SEE might serve as a preferable alternative for inhibiting the sequential reaction in lipid peroxidation and could also be considered as health supplements.

Inhibition of Fenton's Reagent Induced Strand Breaks in Plasmid DNA

In this study, SEE was examined for their protective ability against oxidative damage to plasmid DNA (pGEM-T) caused by Fenton's reagent (Fig. 1). Result showed that SEE have significant antioxidant activity. The inclusion of SEE in conjunction with Fenton's reagent demonstrated the existence of super coiled plasmid DNA, akin to the native pGEM-T plasmid, indicating a diminished impact of DNA damage induced by Fenton's reagent.

Identify metabolites of SEE

A total of 491 metabolites were identified based on their MS/MS spectra (Table S1). Mass spectra of all identified compounds were matched with LuMet-TCM and Herb databases. The top 20 compounds were shown in Table 3. Most of the top 20 compounds like Phytosphingosine,

Hispidin, Sorbitol, Withaferin A, Salvianolic acid A identified have been reported to have antioxidants, anticancer and anti-inflammatory properties. In addition, 2 compounds that are not reported in plant extracts yet have been identified (siraitic acid D and Withametelin C). Extraordinary, the structures of top 20 compounds were determined by comparing the retention time, MS, and MS data with the references (Fig. 2).

Classification of SEE

UPLC-MS analysis of SEE, component identification, and function prediction were performed. In terms of the number of components, the compounds with the highest numbers were Terpenoids (19.75%), Phenylpropanoid (17.20%), Flavonoids (15.29%), Steroidal (7.64%), and Phenols (6.37%) (Fig. 2A); In terms of compositional content, the most abundant compounds were Sugars and glycosides (44.48%), Alkaloid (11.31%), Sphingolipid (10.73%), Amino acids and peptides (7.85%), Flavonoids (3.51%), and Phenylpropanoids (3.08%) (Fig. 2B).

Discussion

Natural products and derivatives account for more than half of all clinically used medications worldwide (Bhattacharya, 2017; Krupodorova et al., 2024). It is also true that 25% of all pharmaceutical prescriptions are formulations based on chemicals derived from plants or plant-derived synthetic analogs (Dewanjee et al., 2023). These plants' medicinal value stems from their bioactive phytochemical components, such as phenolics, flavonoids, terpenes, tocopherols and carotenoids, which are together known as antioxidants (Mehta et al., 2018; Sevindik et al., 2023). Oxidative stress, which is frequently generated by an excess of free radicals, has been linked to a variety of degenerative illnesses, including cancer, ischemic heart disease, atherosclerosis, diabetes, and neurological disorders (Wang et al., 2020; Uysal et al., 2023). The biological role that antioxidants play in medicinal plants is very important. Numerous research have shown that medicinal plants possess a wide range of pharmacological properties, such as antibacterial, anti-inflammatory, anticancer, and anthelmintic properties (Marín et al., 2023; El-Chaghaby et al., 2024; Mohammed et al., 2024). Thus, natural compounds generated from medicinal plants, including extracts, provide several prospects for novel medication development.

Phenolic and flavonoid compounds are the main active ingredient in the alcoholic extract of *S. sanghuang* (Seephonkai et al., 2024). They are known for their radical scavenging activity, that helps to reduce oxidative stress which has been implicated as a major cause of several diseases (Dong et al., 2023). For example, Saffron extract is helpful in the treatment of cardiovascular disease and shows protective effects on the myocardium (Su et al., 2021). Supplementing with green tea extract can reduce oxidative stress, body mass (BM), body mass index (BMI), and body fat percentage (BFP), all of which are deemed to be detrimental to human health (Asbaghi et al., 2024). Thus, incorporating *S. ~~Sanghuang~~ sanghuang* composition (food and supplements) into our food can act as protective medicament and provide innumerable health advantage.

Açıklamalı [SK4]: ??

Açıklamalı [SK5]: Please add reference

Açıklamalı [SK6]: Please add reference
Sevindik, M., Mohammed, F. S., & Uysal, I. (2023). Autism: plants with neuro-psychopharmacotherapeutic potential. *Prospects in Pharmaceutical Sciences*, *21*(3), 38-48.

Açıklamalı [SK7]: Please add reference

Uysal, I., Koçer, O., Mohammed, F. S., Lekesiz, Ö., Doğan, M., Şabik, A. E., ... & Sevindik, M. (2023). Pharmacological and nutritional properties: Genus Salvia. *Advances in Pharmacology and Pharmacy*, *11*(2), 140-155.

Açıklamalı [SK8]: Please add references

El-Chaghaby, G. A., Mohammed, F. S., Rashad, S., Uysal, I., Koçer, O., Lekesiz, Ö., ... & Sevindik, M. (2024). Genus Hypericum: General Properties, Chemical Contents and Biological Activities. Egyptian Journal of Botany, 64(1), 1-26.

Mohammed, F. S., Sevindik, M., Uysal, İ., Çesko, C., & Koraqi, H. (2024). Chemical Composition, Biological Activities, Uses, Nutritional and Mineral Contents of Cumin (Cuminum cyminum). *Measurement: Food*, 100157.

Antioxidant-rich plant extracts, characterized by their DPPH free radical scavenging ability, can donate hydrogen to lipid peroxyl or hydroperoxyl radicals, which are pivotal in the propagation of lipid autoxidation. It also produces non-radicals that disrupt lipid peroxidation chain reaction (Sowndhararajan et al., 2013; Korkmaz et al., 2021). Free radicals have the potential to facilitate DNA damage, thereby significantly decreasing the carcinogenic, mutagenic, and cytotoxic effects of reactive oxygen species (ROS) (Fischer et al., 2018). The findings from our research compellingly demonstrated that SEE mitigated oxidative stress and protected the DNA from the harmful effects of hydroxyl radicals generated by Fenton's reagent. The shielding effect of SEE against DNA damage induced by hydroxyl radicals might be ascribed to the presence of various flavonoids and phenolic compounds. Similar protective effect on DNA were observed in various plant extracts (Salar et al., 2017). For example, Yen et al. (2000) reported that water extracts of Hsian-tsao could lessen DNA damage brought on by UV-C, and it was more effective against UV-C than H2O2-induced DNA damage. Hsieh et al. (2018) demonstrated that *Lycium barbarum* extract exhibit a higher level of preventive action against UVB-induced growth arrest in ARPE-19 cells, protect cells from UVB-induced apoptosis. Furthermore, it demonstrated a dose-responsive reduction in the activation of γH2AX, a sensor of DNA damage in ARPE-19 cells. Consequently, there is a great deal of medical potential for the study of active chemicals in pricey medicinal supplies.

Therefore, the findings provide the reader with a more appropriate way for assessing active components in valuable medical goods. It offers a potential path for future research and development regarding the examination and quality assurance of *S.~~Sanghuang~~ sanghuang* and the identification of bioactive substances.

Conclusions

In conclusion, the findings of this study gain an insight into ethanol extract of *S. sanghuang*. This study confirms that *S. ~~Sanghuang~~ sanghuang* was a beneficial source of phenolics, flavonoids and terpenoids. This is reflected in its high antioxidant ability. According to the findings, this pharmaceutical/food resource mushroom contains potential natural antioxidants that could be used as a lead contender in the development of antioxidant medicines for the treatment of a wide range of oxidative stress-related illnesses.

Acknowledgements

The authors gratefully acknowledge the supports of the Westlake Univergity, Weiyan Jin.

References

Asbaghi O, Rezaei Kelishadi M, Larky DA, Bagheri R, Amirani N, Goudarzi K, Kargar F, Ghanavati M, Zamani M. 2024. The effects of green tea extract supplementation on body composition, obesity-related hormones and oxidative stress markers: a grade-assessed systematic review and dose-response meta-analysis of randomised controlled trials. *The British journal of nutrition* **131**(7):1125-1157

Açıklamalı [SK9]: Please add reference

Korkmaz, N., Dayangaç, A., & Sevindik, M. (2021). Antioxidant, antimicrobial and antiproliferative activities of Galium aparine. *Journal of Faculty of Pharmacy of Ankara University*, *45*(3), 554-564.

Biçimlendirdi: Yazı tipi: İtalik

Bhattacharya S. 2017. Medicinal plants and natural products in amelioration of arsenic toxicity: a short review. *Pharmaceutical biology* **55**(1):349-354

Dewanjee S, Sohel M, Hossain MS, Ansari F, Islam MT, Sultana F, Al Mamun A, Islam MM, Amin MN. 2023. A comprehensive review on clinically proven natural products in the management of nerve pain, with mechanistic insights. *Heliyon* **9**(5):e15346

Dong Z, Wang Y, Hao C, Cheng Y, Guo X, He Y, Shi Y, Wang S, Li Y, Shi W. 2023. *Sanghuangporus sanghuang* extract extended the lifespan and healthspan of *Caenorhabditis elegans via* DAF-16/SIR-2.1. *Frontiers in pharmacology* **14**:1136897

Fischer N, Seo EJ, Efferth T. 2018. Prevention from radiation damage by natural products. *Phytomedicine* **47**:192-200

Gu X, Hao D, Xiao P. 2022. Research progress of Chinese herbal medicine compounds and their bioactivities: Fruitful 2020. *Chinese herbal medicines* **14**(2):171-186

Hao D, Liu C. 2022. Chinese herbal medicines will illuminate the post-epidemic era. *Chinese herbal medicines* **14**(2):169-170

Hsieh FC, Hung CT, Cheng KC, Wu CY, Chen YC, Wu YJ, Liu W, Chiu CC. 2018. Protective Effects of Lycium barbarum Extracts on UVB-Induced Damage in Human Retinal Pigment Epithelial Cells Accompanied by Attenuating ROS and DNA Damage. *Oxidative Medicine and Cellular Longevity* **2018**:4814928

Li IC, Chen CC, Sheu SJ, Huang IH, Chen CC. 2020. Optimized production and safety evaluation of hispidin-enriched *Sanghuangporus sanghuang* mycelia. *Food science & nutrition* **8**(4):1864-1873

Li T, Chen L, Wu D, Dong G, Chen W, Zhang H, Yang Y, Wu W. 2020. The Structural Characteristics and Biological Activities of Intracellular Polysaccharide Derived from Mutagenic *Sanghuangporous sanghuang* Strain. *Molecules* **25**(16):3693

Lin WC, Deng JS, Huang SS, Wu SH, Chen CC, Lin WR, Lin HY, Huang GJ. 2017. Anti-Inflammatory Activity of *Sanghuangporus sanghuang* Mycelium. *International Journal of Molecular Sciences* **18**(2):347

Lu J, Su M, Zhou X, Li D, Niu X, Wang Y. 2024. Research Progress of Bioactive Components in Sanghuangporus spp. *Molecules* **29**(6):1195

Marín R, Abad C, Rojas D, Chiarello DI, Alejandro TG. 2023. Biomarkers of oxidative stress and reproductive complications. *Advances in Clinical Chemistry* **113**:157-233

Mehta J, Rayalam S, Wang X. 2018. Cytoprotective Effects of Natural Compounds against Oxidative Stress. *Antioxidants (Basel)* **7**(10):147

Salar RK, Purewal SS, Sandhu KS. 2017. Relationships between DNA damage protection activity, total phenolic content, condensed tannin content and antioxidant potential among Indian barley cultivars. *Biocatalysis and agricultural biotechnology* **11**:201-206

Seephonkai P, Theerapong T, Jaikhan S, Klinhom U, Kaewtong C. 2024. Characterisation of indole alkaloids and phenolic acids from wild mushroom *Tropicoporus linteus* and its chemical profiles compared with other Sanghuang mushrooms. *Natural product research* **38**(2):198-205

Sowndhararajan K, Kang SC. 2013. Evaluation of in vitro free radical scavenging potential of Streptomyces sp. AM-S1 culture filtrate. *Saudi Journal of Biological Sciences* **20**(3):227-233

Su X, Yuan C, Wang L, Chen R, Li X, Zhang Y, Liu C, Liu X, Liang W, Xing Y. 2021. The Beneficial Effects of Saffron Extract on Potential Oxidative Stress in Cardiovascular Diseases. *Oxidative medicine and cellular longevity* **2021**:6699821

Wang W, Kang PM. 2020. Oxidative Stress and Antioxidant Treatments in Cardiovascular Diseases. *Antioxidants (Basel)* **9**(12):1292

Yen GC, Hung YL, Hsieh CL. 2000. Protective effect of extracts of Mesona procumbens Hemsl. on DNA damage in human lymphocytes exposed to hydrogen peroxide and UV irradiation. *Food and chemical toxicology* **38**(9):747-754

Yuan W, Yuan W, Zhou R, Lv G, Sun M, Zhao Y, Zheng W. 2023. Production of hispidin polyphenols from medicinal mushroom *Sanghuangporus vaninii* in submerged cultures. *Chinese herbal medicines* **15**(4):594-602

Zuo K, Tang K, Liang Y, Xu Y, Sheng K, Kong X, Wang J, Zhu F, Zha X, Wang Y. 2021. Purification and antioxidant and anti-Inflammatory activity of extracellular polysaccharopeptide from sanghuang mushroom, Sanghuangporus lonicericola. *Journal of the science of food and agriculture* **101**(3):1009-1020