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Research Article

Chemical Composition, Antibacterial Test, and Antioxidant Activity of Essential Oils from Fresh and Dried *Stropharia rugosoannulata*

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The essential oils, respectively, from fresh and dried *Stropharia rugosoannulata* fruiting bodies, an important edible mushroom, have been studied for their chemical composition, antibacterial capacity, and antioxidant activity. The essential oils were obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS) combined with Kovats retention index. The oils' antibacterial test was evaluated by the microdilution method against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and antioxidant activity was determined through DPPH radical scavenging activity and ferric reducing power. Twenty-nine components were identified from the fresh mushroom, and the compositions were mainly dominated by hydrocarbons (54.72%), acids (32.99%), esters (5.07%), and terpenic compounds (0.96%). Thirty-five components were identified from the dried sample, and acids (31.22%), terpenic compounds (28.7%), alcohols (12.7%), and ketones (10.48%) were the major compounds. Strong antibacterial capacity and obvious antioxidant activity were observed for both essential oils from the fresh and dried mushrooms.

1. Introduction

Mushrooms have a long and rich history that prehistoric humans likely used mushrooms collected from the wild as foods or medicines to fight illnesses [1, 2]. Now, the cultivation of edible mushrooms worldwide has become a respectable development of economic importance. *Stropharia rugosoannulata* (Strophariaceae), better known as "Chisongrong" in China, is a type of precious edible mushroom recommended by the United Nations Food and Agriculture Organization (FAO) to be cultivated in developing countries [3]. It has been reported that *S. rugosoannulata* is a good source of protein, fibre, amino acids, mineral elements, polysaccharides, phenols, and various biologically active substances [4] and shows important pharmacological activities such as antitumor, antihyperglycemic, antibacterial, antioxidant, and coronary heart disease preventative effects [5, 6]. Therefore, *S. rugosoannulata* has become one of the top ten mushrooms in the international mushroom market.

In China, *S. rugosoannulata* is cultivated in many places, including Yunnan, Shandong, Fujian, and Henan provinces [7]. It is becoming more and more popular with a pleasant and refreshing fragrance when fresh, which has a closely connection with its volatile compounds. Even so, considerable fresh *S. rugosoannulata* is dried and circulated in the market. On the one hand, the dried mushroom can be preserved for long in case of going rotten due to its high moisture content and strong postharvest respiration [8]. On the other hand, it can be used as an important ingredient in the broad range of food formulations such as stuffing, instant soup premix, snack seasoning, pizza, pasta, salad, and rice dishes [9]. In fact, the dried *S. rugosoannulata* also has a special smell that some people like and the other people dislike. Nevertheless, to the best of our knowledge, there are

no reports about the investigation on the volatile chemical composition of fresh or dried *S. rugosoannulata*.

Multiresistant bacterial infection is one of the significant public health problems. Multidrug resistance of bacteria causes infections that cannot be treated with most conventional antibiotics [10]. Among those bacteria, *Escherichia coli* have resistance to several drugs such as carbapenemases and cephalosporins [11]. *Staphylococcus aureus* may cause infections ranging from simple skin diseases to severe conditions such as bacteremia and endocarditis [12, 13]. *Pseudomonas aeruginosa* with high virulence acts as opportunistic microorganisms that can cause severe infections and even sepsis [14]. Their multidrug resistance has driven the development of research aimed at identifying new antimicrobial agents through chemical synthesis or isolation from natural products. We have reported that the aqueous and ethanolic extracts of *S. rugosoannulata* both showed strong antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* with MIC from 0.0625 to 1 mg/mL [4]. However, the antibacterial activity of *S. rugosoannulata* essential oil (SEO) has not been studied up to now.

The research of antioxidants has become a hotspot in the pharmaceutical and food industries [15–17]. It has been reported that some types of essential oils from natural biomaterials have strong antioxidant activities compared to the classical standard antioxidants, butylated hydroxytoluene, butylated hydroxyanisole, and ascorbic acid [18, 19], and the essential oil from mushrooms including *Ganoderma pfeifferi* Bres [20] and *Tremella fuciformis* [21] showed good antioxidant activity. However, little is known about the antioxidant activity of SEO.

For the reasons mentioned above, this study aimed to investigate the chemical composition of essential oils, respectively, from fresh and dried *S. rugosoannulata* fruiting bodies through hydrodistillation coupled with gas chromatography-mass spectrometry (GC-MS) and Kovats retention index. In addition, their antibacterial and antioxidant activity was tested, which could provide a reference for the research of natural bactericides and antioxidants.

2. Materials and Methods

2.1. Materials. Both fresh and dried *S. rugosoannulata* fruiting bodies were purchased from Xiangzhu Planting Professional Cooperative in Bo'ai County of Henan Province in China, and the fresh sample was thoroughly washed with water and kept on filter papers to soak up excess water; the dried sample had been dehydrated through the air drying method by the professional cooperative. Three kinds of bacteria, including *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), and *Pseudomonas aeruginosa* (ATCC 27853) were purchased from the Nanjing Bianzhen Biological Technology Co., Ltd. C_7-C_{40} saturated alkane standard was purchased from Shanghai Yuanye Biological Technology Co., Ltd.

2.2. Extraction of Essential Oil. The fresh *S. rugosoannulata* (total 10 kg) was cut into about $1 \times 1 \times 1\ cm^3$ blocks and the dried one (1 kg) was crushed into about $2 \times 2\ cm^2$ irregular pieces. The essential oils were isolated by hydrodistillation using a big Clevenger-type apparatus for 6 h in distilled water and then extracted with n-hexane for 2 h. After liquid-liquid extraction, sodium sulfate was added into the n-hexane phase, and the solvent was evaporated in vacuo. The yields of the oils from fresh and dried samples were 0.0109% (W/W) and 0.0841% (W/W), respectively.

2.3. Chemical Composition Analysis. Chemical analysis of essential oils, respectively, from fresh and dried *S. rugosoannulata* was carried out on a Shimadzu (Kyoto, Japan) GC-MS QP2010 ultra system equipped with a Shimadzu AOC-20i autoinjector and a Rtx-5ms fused silica capillary column ($30\ m \times 0.25\ mm$ inner diameter $\times$ film thickness $0.25\ \mu m$, 5% phenyl/95% dimethyl polysiloxane, Restek, USA). Helium was employed as the carrier gas at a flow rate of 3.0 mL/min and the injector port temperature was set at 280°C in a split mode with a ratio of 1 : 5. The GC oven temperature was initially held at 35°C for 5 min, increased to 190°C at the rate of 7°C/min for 5 min, then increased to 230°C at the rate of 5°C/min for 10 min, then increased to 240°C at the rate of 5°C/min for 5 min, then increased to 260°C at the rate of 5°C/min for 5 min, then increased to 280°C at the rate of 5°C/min for 8 min, finally increased to 310°C at the rate of 4°C/min for 15 min. The mass spectrometer was operated in the electron impact (EI) ionization mode with an ion source temperature of 230°C. The GC-MS interface temperature was kept at 220°C and the energy of electrons was kept at 70 eV. The NIST14 was used in the GC-MS databank. The relative contents of the constituents in the essential oils were assumed to be proportional to the areas under the corresponding chromatogram peaks. The Kovats retention index (KI) was calculated by using the retention times of C_7-C_{40} n-alkanes that were injected under the same chromatographic conditions. The KI calculation formula was as follows:

$$\mathrm{KI}_{(x)} = 100n + \frac{tR(x) - tR(n)}{tR(n+1) - tR(n)} \times 100, \quad (1)$$

where n and $n+1$ are the number of normal alkane carbon atoms before and after the outflow; $t_{R(n)}$ and $t_{R(n+1)}$ are the retention time of the corresponding normal alkane, respectively; and $t_{R(x)}$ is the retention time of the unknown substance in the gas chromatography and $t_{R(n)} < t_{R(x)} < t_{R(n+1)}$.

2.4. Antibacterial Activity Test. The broth microdilution method to determine the minimum inhibitory concentration (MIC) values was employed in reference to the Clinical and Laboratory Standards Institute (CLSI) [22]. Briefly, the essential oil was dissolved in ethyl alcohol using the double dilution method. The concentrations included 24, 12, 6, 3, 1.5, 0.75, 0.375, 0.1875, 0.09375, and 0.046875 mg/mL. Cephalothin was used as a positive control. The three bacteria aforementioned were regulated to an $OD_{600} = 0.5$ in the beef-protein broth. 100 μL SEO solution (or cephalothin solution) and 100 μL bacterial broth were added to each well in 96-microwell plates. These plates were covered with lids

and incubated at 37°C for 24 h. The presence of turbidity and a pellet on the bottom suggest microbial growth. The MIC value is the lowest concentration of tested sample with clarified solution. The lowest concentration at which 99.9% of the bacteria have been killed is considered to be the minimal bactericidal concentration (MBC).

2.5. Antioxidant Activity Assay

2.5.1. DPPH Radical Scavenging Activity. The two kinds of essential oils were dissolved in ethyl alcohol at concentrations of 1, 0.5, 0.25, 0.125, and 0.0625 mg/mL, respectively. The reaction mixtures included 50 μL of sample solutions and 150 μL of 1,1-diphenyl-2-picrylhydrazyl (DPPH, 0.05 mM in ethyl alcohol) in a 96-microwell plate. The reaction plates were incubated in a 25°C water bath for 30 min in the dark, and then the absorbance was measured at 517 nm according to the report [23] with slight modifications. A reference commercial synthetic antioxidant (ascorbic acid) was tested with the same method. The results were expressed as the percentage reduction in absorbance shown by the sample with respect to DPPH solution. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity}\,(\%) = \left[\frac{1-(A_s - A_e)}{A_c}\right] \times 100, \quad (2)$$

where A_s was the absorbance value of sample, A_e was the absorbance value of ethyl alcohol instead of sample, and A_c was the absorbance value of sample only (ethyl alcohol instead of DPPH).

2.5.2. Ferric Reducing Power (FRP). The FRP was modified slightly according to the method previously reported [24]. The fresh FRP reagent was prepared as described previously. 20 μL of the diluted sample and 180 μL of the FRP solution were fully mixed and reacted. The absorbance was measured at 593 nm after a reaction at room temperature in the dark for 40 min. The reducing power of sample was expressed using sample absorbance against ultrapure water (a blank), and ascorbic acid was used as the positive control at the same concentration (0.25–4 mg/mL).

2.6. Statistical Analysis. All trials were carried out in triplicate. The results were expressed as mean value ± standard error (SE). Data differences were carried out by analyses of variance (ANOVA) using the *t*-test by the SPSS software. *P* values below 0.05 were considered to be statistically significant.

3. Results and Discussion

3.1. Fresh SEO Analysis. The various chemical constituents identified in the essential oil from fresh *S. rugosoannulata* fruiting bodies are shown in Table 1, in order of their elution from an Rxi-5mS column. The corresponding chromatogram is shown in Figure 1. Twenty-nine components were identified, comprised 93.74% of the total essential oil, which indicated that hydrocarbons were a major class (54.72%), followed by acids (32.99%), ester compounds (5.07%) and terpenic compounds (0.96%). Diisobutyl phthalate (R_T = 29.949 minutes) was seen as plasticizers. Three predominant components identified were n-hexadecanoic acid (R_T = 32.598 minutes; percentage total = 17.68%), pentacosane (R_T = 45.603 minutes; percentage total = 14.26%), and linoleic acid (R_T = 37.016 minutes; percentage total- = 14.23%). Among them, n-hexadecanoic acid, a saturated fatty acid, has various functions such as cancer prevention, antioxidant, hypocholesterolemic, nematicide, pesticide, insecticide, lubricant, antiandrogenic, hemolytic, and 5-alpha reductase inhibitory properties [25]. Oils rich in n-hexadecanoic acid have been used for the treatment of rheumatic symptoms [26], which has been attributed to its ability to inhibit phospholipase A_2. The enzyme kinetics proved that n-hexadecanoic acid inhibits phospholipase A_2 in a competitive manner and this may help in the design of specific inhibitors of phospholipase A_2 as anti-inflammatory agents [27, 28]. A high content of n-hexadecanoic acid was also found in the constituent oil of the edible mushroom *Ganoderma lucidum* (24.275%) [29]. Pentacosane exists widely in the essential oils of plants like *Calotropis procera* [30] and *Rosa damascena* Mill. [31], but rarely in mushrooms including *Lentinus boryanus*, *Lentinus edodes* [32], *Boletopsis leucomelas* [33], *Ganoderma lucidum* [29], *Tremella fuciformis* [21], *Trametes suaveolens* [34], *Agaricus bisporus*, *Boletus edulis*, *Cantharellus cibarius*, or *Hericium erinaceus* [35]. Therefore, this is the first report that high content of pentacosane (14.26%) exists and can be detected in the essential oil of an edible mushroom. Linoleic acid is a functional polyunsaturated fatty acid, which is reported to play important role in reducing serum cholesterol level, inhibiting arterial thrombosis, preventing cancer, participating in the control of human cardiovascular diseases, immune regulation, and cell growth and apoptosis. In addition, it can be used to mitigate joint inflammation and alter cartilage turnover following an inflammatory insult [36], and also induce new vessel formation and improve skin wound healing [37]. Therefore, the essential oil of fresh *S. rugosoannulata* may have a good medicinal value and broad prospects of development and application.

3.2. Dried SEO Analysis. The constituents of essential oil from dried *S. rugosoannulata* fruiting bodies were different from those of fresh ones. Exclusion of the silicone and plasticizer constituents, thirty-five components were identified and comprised 89.66% of the total essential oil. The largest content substance was acids (31.22%), followed by terpenic compounds (28.7%), alcohols (12.7%), ketones (10.48%), esters (5.14%), nitrides (0.84%), and aromatic compounds (0.58%), as shown in Table 2 and Figure 2. Four predominant components identified were linoleic acid (R_T = 37.024 minutes; percentage total = 12.18%), n-hexadecanoic acid (R_T = 32.557 minutes; percentage total- = 9.36%), valeranone (R_T = 26.603 minutes; percentage total = 8.03%), and caryophyllene oxide (R_T = 25.11 minutes;

Table 1: Compound detected in the GC-MS analysis of the essential oil from fresh *S. rugosoannulata* fruiting bodies.

S/N	Compound	RT	RI	RC	MF
1	Undecane	15.356	1100	0.37	$C_{11}H_{24}$
2	Tetradecane	21.602	1400	0.13	$C_{14}H_{30}$
3	Nonanoic acid, 9-oxo-, 1-methylethyl ester	23.536	1506	0.18	$C_{12}H_{22}O_3$
4	E-nerolidol	24.59	1568	0.96	$C_{15}H_{26}O$
5	Hexadecane	25.131	1599	0.13	$C_{16}H_{34}$
6	Heptadecane	26.756	1699	0.18	$C_{17}H_{36}$
7	Tetradecanoic acid	27.76	1770	0.44	$C_{14}H_{28}O_2$
8	Octadecane	28.433	1810	0.16	$C_{18}H_{38}$
9	Pentadecanoic acid	29.704	1859	0.37	$C_{15}H_{30}O_2$
10	Diisobutyl phthalate	29.949	1870	0.12	$C_{16}H_{22}O_4$
11	Nonadecane	30.607	1910	3.72	$C_{19}H_{40}$
12	n-hexadecanoic acid	32.598	1971	17.68	$C_{16}H_{32}O_2$
13	Hexadecanoic acid, ethyl ester	33.264	1995	1.68	$C_{18}H_{36}O_2$
14	Eicosane	33.4	1999	0.38	$C_{20}H_{42}$
15	Heneicosane	36.015	2100	4.01	$C_{21}H_{44}$
16	Linoleic acid	37.016	2143	14.23	$C_{18}H_{32}O_2$
17	Octadecanoic acid	37.521	2165	0.27	$C_{18}H_{36}O_2$
18	Docosane	38.308	2199	1.04	$C_{22}H_{46}$
19	Tricosane	40.431	2301	13.29	$C_{23}H_{48}$
20	Tetracosane	42.664	2399	2.11	$C_{24}H_{50}$
21	Pentacosane	45.603	2501	14.26	$C_{25}H_{52}$
22	Hexacosane	49.202	2599	1.27	$C_{26}H_{54}$
23	Heptacosane	53.193	2700	7.24	$C_{27}H_{56}$
24	2-methylhexacosane	56.199	2771	3.79	$C_{27}H_{56}$
25	Octacosane	57.401	2798	0.39	$C_{28}H_{58}$
26	Nonacosane	61.048	2899	1.41	$C_{29}H_{60}$
27	Triacontane	64.268	2999	0.22	$C_{30}H_{62}$
28	Hentriacontane	67.909	3099	0.62	$C_{31}H_{64}$
29	Benzenepropanoic acid, 3,5-bis(1,1- dimethylethyl)-4-hydroxy-, octadecyl ester	83.521	3605	3.09	$C_{35}H_{62}O_3$

Notes. RT: retention time (min); RI: retention index; RC: relative content (%); MF: molecular formula; -: not recorded in database.

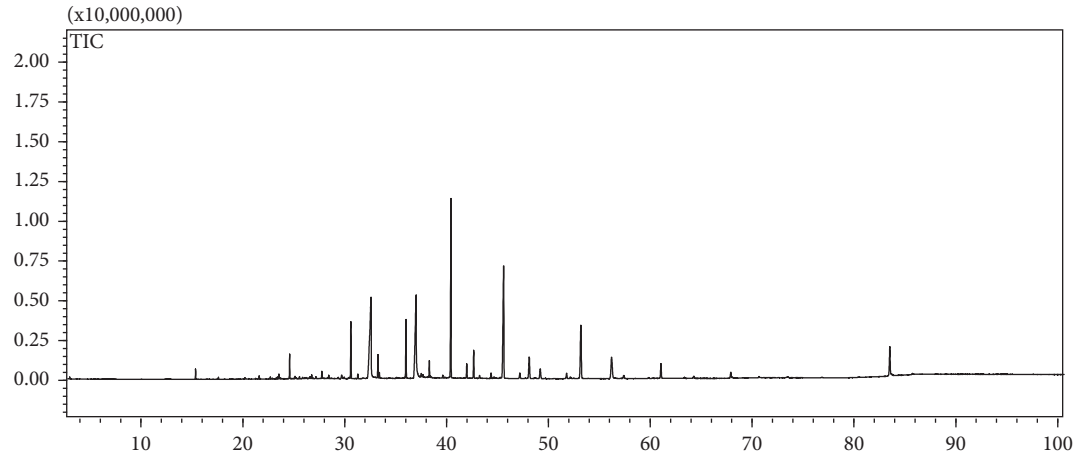

Figure 1: GC-MS of the essential oil from fresh *S. rugosoannulata* fruiting bodies.

percentage total = 6.67%). Linoleic acid and n-hexadecanoic acid have multiple functions as described above. Valeranone was the main compound in the essential oils of some plants such as *Cistanche tubulosa* [38] and *Nardostachys jatamansi* [39], but not found in edible mushrooms. Caryophyllene oxide presents some pharmacological activities, including cytotoxic [40], analgesic, anti-inflammatory [41], and gastroprotective [42] activities, as well as a synergistic effect of terpenoids against the epimastigote forms of *Trypanosoma cruzi* [43]. Furthermore, caryophyllene oxide enhances the cytotoxic and proapoptotic effects of the chemotherapeutics paclitaxel and doxorubicin in human multiple myeloma and human prostate cancer cells [44]. So the dried *S. rugosoannulata* fruiting bodies or its essential oil may be considered as a good source of health-promoting food or an ingredient in the preparation of food formulation.

Table 2: Compound detected in the GC-MS analysis of the essential oil from dried *S. rugosoannulata* fruiting bodies.

S/N	Compound	RT	RI	RC	MF
1	Dihydro-5-pentyl-2(3H)-furanone	20.964	1367	2.21	$C_{10}H_{18}O_2$
2	2,4-di-tert-butylphenol	23.726	1518	0.58	$C_{14}H_{22}O$
3	Cyclohexyl ketone	23.863	1526	0.62	$C_{13}H_{22}O$
4	Dodecanoic acid	24.541	1565	0.75	$C_{12}H_{24}O_2$
5	E-nerolidol	24.591	1568	0.81	$C_{15}H_{26}O$
6	Spathulenol	24.995	1592	2.17	$C_{15}H_{24}O$
7	Caryophyllene oxide	25.11	1598	6.67	$C_{15}H_{24}O$
8	Globulol	25.255	1607	2.08	$C_{15}H_{26}O$
9	Ledol	25.447	1619	0.65	$C_{15}H_{26}O$
10	Humulene epoxide ii	25.544	1625	1.51	$C_{15}H_{24}O$
11	Junenol	25.714	1635	2.22	$C_{15}H_{26}O$
12	11,11-dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	25.981	1652	1.06	$C_{15}H_{24}O$
13	.tau-muurolol	26.034	1655	2.69	$C_{15}H_{26}O$
14	.alpha-acorenol	26.096	1659	1.47	$C_{15}H_{26}O$
15	.alpha-cadinol	26.254	1669	5.73	$C_{15}H_{26}O$
16	Neointermedeol	26.288	1671	5.22	$C_{15}H_{26}O$
17	5.beta.,7.beta.H,10.alpha.-Eudesm-11-en-1.alpha.-ol	26.365	1675	0.34	$C_{15}H_{26}O$
18	9-methoxycalamenene	26.46	1681	0.23	$C_{16}H_{24}O$
19	Valeranone	26.603	1690	8.03	$C_{16}H_{24}O$
20	2-methylenecyclododecanone	26.732	1698	1.85	$C_{13}H_{22}O$
21	Isolongifolol	26.78	1701	0.52	$C_{15}H_{26}O$
22	Acetic acid,1-[2-(2,2,6-trimethyl- bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester	26.98	1713	0.31	$C_{16}H_{26}O_2$
23	(S,E)-6-hydroxy-6-methyl-2-((2S,5R)-5-methyl-5-vinyltetrahydrofuran-2-yl) hept-4-en-3-one	27.159	1724	3	$C_{15}H_{24}O_3$
24	1-(7-hydroxy-1,6,6-trimethyl-10-oxatricyclo[5.2.1.0(2,4)]dec-9-yl)ethanone	27.42	1740	1.99	$C_{14}H_{22}O_3$
25	4-(2-hydroxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one	27.649	1753	0.79	$C_{13}H_{22}O$
26	Tetradecanoic acid	27.817	1763	4.81	$C_{14}H_{28}O_2$
27	1,8-dimethyl-8,9-epoxy-4-isopropyl-spiro[4.5]decan-7-one	28.357	1795	1.86	$C_{15}H_{24}O_2$
28	(3R,3aR,5R,6R,7aR)-3,6-dimethyl-5-(prop-1-en-2-yl)-6-vinylhexahydrobenzofuran-2(3H)-one	29.607	1858	0.37	$C_{15}H_{22}O_2$
29	Pentadecanoic acid	29.758	1865	3.54	$C_{15}H_{30}O_2$
30	n-hexadecanoic acid	32.557	1970	9.36	$C_{16}H_{32}O_2$
31	Linoleic acid	37.024	2136	12.18	$C_{18}H_{32}O_2$
32	(Z,Z,Z)-9,12,15-octadecatrienoic acid methyl ester	37.095	2147	2.1	$C_{19}H_{32}O_2$
33	Octadecanoic acid	37.529	2166	0.58	$C_{18}H_{36}O_2$
34	(Z)-9-octadecenamide	41.735	2359	0.84	$C_{18}H_{35}NO$
35	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	83.501	3604	0.52	$C_{35}H_{62}O_3$

Notes. RT: retention time (min); RI: retention index; RC: relative content (%); MF: molecular formula; -: not recorded in database.

GC-MS analysis of fresh and dried SEO indicated that E-nerolidol (R_T = 24.59 minutes and 24.591 minutes, respectively), tetradecanoic acid (R_T = 27.76 minutes and 27.817 minutes, respectively), pentadecanoic acid (R_T = 29.704 minutes and 29.758 minutes, respectively), n-hexadecanoic acid (R_T = 32.598 minutes and 32.557 minutes, respectively), linoleic acid (R_T = 37.016 minutes and 37.024 minutes, respectively), octadecanoic acid (R_T = 37.521 minutes and 37.529 minutes, respectively), and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester (R_T = 83.521 minutes and 83.501 minutes, respectively) were detected in both of them. Compared with the fresh mushroom, ingredients identified in the dried one were more abundant, which could be attributed to the drying process. In reality, the chemical compounds and biological activities of dried and fresh mushrooms or plants always have a significant difference. An et al. reported that there were considerable differences in flavor compounds detected by GC-MS between the fresh and dried *Lentinus edodes* and there were ten more compounds in the dried sample than in the fresh one [45]. Antioxidant metabolites were detected and included ascorbic acid at 2.395 and 0.6204 g/100 g DW in fresh and dried *Pleurotus ostreatus*, respectively, free phenols were 23.99 and 163.515, and bound phenols were 2.85 and 1.96 μg/100 g in fresh and dried samples, respectively [46]. Similarly, essential oils extracted from the aerial parts of fresh *Pallenis spinosa* contained 36 different compounds, while dried one contained 53 molecules. Besides, the dried sample showed a 2-fold greater antioxidant activity than the fresh one and 2-3-fold stronger cytotoxicity against several tested tumor cells [47].

3.3. MIC and MBC of the Essential Oils. We used the microdilution method to determine the MIC of fresh and dried SEOs. As shown in Table 3, the MIC range of the positive control was 8–32 μg/mL. The results also revealed that fresh and dried SEO both had varying degrees of antibacterial activity against strains of *E. coli*, *S. aureus*, and

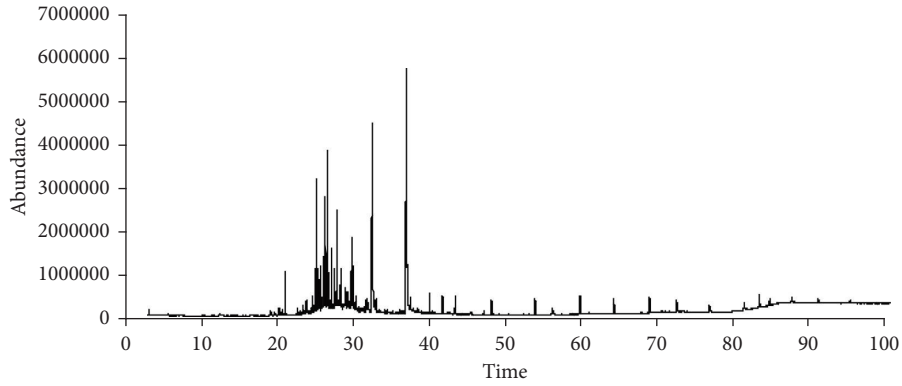

Figure 2: GC-MS of the essential oil from dried *S. rugosoannulata* fruiting bodies.

Table 3: MIC and MBC of essential oils from fresh and dried *S. rugosoannulata*.

Strains	Cephalothin		FSEO		DSEO	
	MIC[a]	MBC[a]	MIC[b]	MBC[b]	MIC[b]	MBC[b]
E. coli	16	32	1.5	3	3	3
S. aureus	8	16	3	6	1.5	3
P. aeruginosa	32	32	0.375	1.5	0.1875	0.375

Notes. *E. coli*: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; FSEO: essential oil from fresh *S. rugosoannulata*; DSEO: essential oil from dried *S. rugosoannulata*; MIC: minimum inhibitory concentration; MBC: minimal bactericidal concentration; a: μg/mL; b: mg/mL.

P. aeruginosa. The MIC and MBC values for the tested bacterial strains were in the range of 0.1875–3 mg/mL and 0.375–6 mg/mL, respectively. In antibacterial activity test, MIC of rude solvent extracts less than 8 mg/mL or isolated phytochemicals less than 1 mg/mL can be considered possessing potential therapeutical applications [48, 49]. In this study, the MIC values ranging from 0.1875 to 3 mg/mL were less than 8 mg/mL, which confirmed the existence of significant activity against the three bacteria strains tested.

Only a few kinds of essential oils of edible mushrooms were evaluated for antibacterial activity. The essential oil of the common edible mushroom-*Lentinus edodes* had a wide antibacterial spectrum, and the MICs against *E. coli* and *S. aureus* were both 5 mg/mL according to Zhang et al. [50]. Yang et al. reported that the MICs of *Pleurotus abalonus* essential oil against *E. coli* and *S. aureus* were both 10 mg/mL [51]. Compared with these two kinds of mushrooms, SEO had a stronger antibacterial effect on *E. coli* and *S. aureus*, which demonstrated a promising prospect for exploitation.

Among these bacteria, the essential oil from dried *S. rugosoannulata* performed at a minimum MIC of 0.1875 mg/mL and a minimum MBC of 0.375 mg/mL against *P. aeruginosa*, which indicated that it was the most effective bacterial inhibitor and bactericide against *P. aeruginosa*. *P. aeruginosa* is an important cause of infection in patients with compromised host defense mechanisms, and this infection is complicated and can be life-threatening [52]. However, this strain is resistant to many antibiotics and disinfectants, which makes it difficult to treat. Our study showed that the essential oil of dried *S. rugosoannulata* could be used to develop new antibacterial drugs against *P. aeruginosa*.

3.4. Antioxidant Activity of the Essential Oils. Two types of tests were performed to evaluate the antioxidant activities of fresh and dried SEOs, including scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers) and ferric reducing power (measuring the conversion of a Fe^{3+}/ferricyanide complex to the ferrous form). The results were presented in Figure 3.

Free radicals are harmful by-products generated during normal cellular metabolism, which could initiate oxidative damage to the body [53], and the DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants [54]. As shown in Figure 3(a), FSEO and DSEO both scavenged the DPPH radicals in a dose-dependent manner with the test concentration range (0.0625–1 mg/mL), which was similar to the scavenging effects on DPPH radicals of *Tremella fuciformis* oil [21]. When the concentration was 1 mg/mL, the scavenging rates reached to 80.5 ± 2.9 and $82.2 \pm 2.4\%$, respectively. The IC_{50} values of FSEO and DSEO were 0.424 mg/mL and 0.372 mg/mL, respectively, which were a little higher than *Tremella fuciformis* oil (0.176 mg/mL). Even though, they both showed strong antioxidant activity in the DPPH assay. Furthermore, DSEO possessed higher DPPH radical scavenging activity compared with FSEO, which was in accordance with the previous report [47].

The reducing power assay is often used to evaluate the ability of a natural antioxidant to donate electrons [55]. According to Figure 3(b), the reducing power of FSEO and DSEO was moderate and increased with the concentrations from 0.25 to 4 mg/mL, which also showed a dose-dependent manner. Meanwhile, DSEO also showed stronger reducing

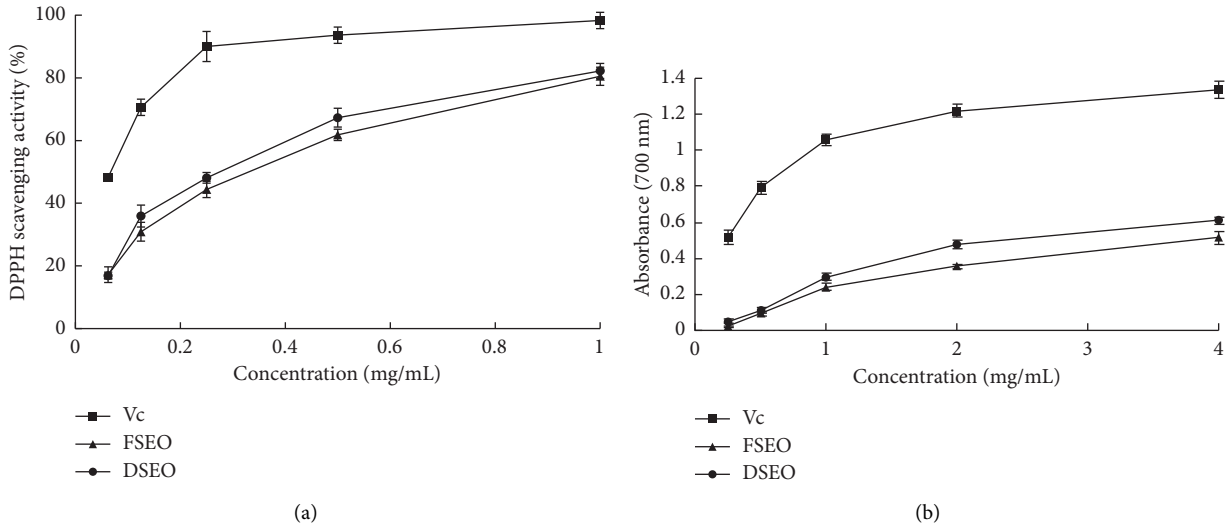

Figure 3: Antioxidant activities of fresh and dried SEOs. *Notes*. (a) DPPH radical scavenging activity of FSEO and DSEO; (b) ferric reducing power of FSEO and DSEO. FSEO: essential oil from fresh *S. rugosoannulata*; DSEO: essential oil from dried *S. rugosoannulata*; Vc: ascorbic acid.

power than FSEO, which was in agreement with the DPPH result. This could be explained by difference in the chemical composition [24].

4. Conclusion

In this study, we investigated the chemical composition of essential oils from fresh and dried *S. rugosoannulata* fruiting bodies, respectively. It revealed that hydrocarbons and acids such as n-hexadecanoic acid, pentacosane, linoleic acid, and tricosane were the major components in the fresh mushroom. While in dried one, acids, terpenic compounds, and alcohols were predominant with linoleic acid, n-hexadecanoic acid, valeranone, and caryophyllene oxide as main components. Such a difference in chemical composition was probably due to the drying process. It should also be mentioned that only seven compounds were detected in both fresh and dried samples. Meanwhile, the antibacterial and antioxidant activity assay indicated that the essential oils from fresh and dried mushrooms both exhibited potent growth inhibitory activity against the three multiresistant bacteria, including *E. coli*, *S. aureus*, and *P. aeruginosa*, strong DPPH radical scavenging activity and moderate ferric reducing power.

In conclusion, this is the first study on the essential oils from fresh and dried *S. rugosoannulata*, an important edible mushroom. The results of this research can provide some references and a basis for a better understanding and utilization of this mushroom.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

Authors' Contributions

All authors have read and approved the final manuscript.

Acknowledgments

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