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LIPIDOMIC ANALYSIS, CAROTENOIDS CONTENT, AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF DIFFERENT PARTS OF *GANODERMA LUCIDUM* AND *GANODERMA ATRUM*

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ABSTRACT

This study determined fatty acid compositions of two cultivated *Ganoderma*. The canopy and stalk lipids of the *Ganoderma* were analyzed for fatty acid composition, β-carotene content and antioxidant activities. The results showed that *Ganoderma* samples contained 51 fatty acids. Their lipid extraction yields ranged between 0.45% and 1.09%. The β-carotene content of the red *Ganoderma* canopy extract was higher than its stalk extract. The canopy extract also had the highest DPPH hydroxyl radical scavenging activity. The data of the overall heat map showed that over 30 fatty acids were positively and highly correlated to the lipid in the black *Ganoderma* stalk. The main types of fatty acids in these *Ganoderma* samples were palmitic, oleic, and linoleic acids. The concentration of linoleic acid in the red *Ganoderma* canopy was as high as 164.02 mg/g lipid. These results suggested that these *Ganoderma* lipids are lipid-based antioxidants and potential sources of dietary supplements.

1. Introduction

Ganoderma (Ga) mushroom is a medicinal fungus that promotes good health. It has been used as traditional medicine to promote health in China for over 2000 years (Li *et al.*, 2019). The fungus is used as a dietary supplement in the United States. It is listed in the U.S. Pharmacopoeia of Dietary Supplements and Herbal Medicines, and its subsidiary entities are commonly used as primary medicinal parts (Xu and Yu, 2021). Some bioactive ingredients in Ga mushrooms that affect human physiology have been studied and developed as active ingredients in cosmetics and nutraceuticals (El Sheikha, 2022). The mushroom is a source of micronutrients and bioactive substances such as terpenoids and polysaccharides. Unsaturated fatty acids (UFAs) are also the natural bioactive components of Ga mushrooms, but the studies on FAs and bioactivities of this mushroom are still lacking.

Ga lipid contains a variety of FAs, including linoleic and α-linolenic acids. These FAs cannot be synthesized in the human body and must be obtained from foods. The FAs in foods are mainly in the form of phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. They constitute major lipid components and cell membranes. Saturated fatty acids (SFAs) possess adverse health effects, whereas unsaturated FAs are thought to have protective effects. Trans FAs have been shown to possess detrimental effects on human health, but

conjugated FAs may be beneficial (Tvrzicka *et al.*, 2011).

Monounsaturated fatty acids (MUFAs) predominate in the Ga sample (Stojkovic *et al.*, 2014). Researchers have found large amounts of oleic and palmitic acids in the lipid extracts of *G. lucidum* (Salvatore *et al.*, 2020). Oleic acid is thought to have various physiological functions; a previous study has confirmed its cancer-preventive effect (Carrillo *et al.*, 2012). On the other hand, unsaturated FAs have the function of regulating immune response, which can reduce wound inflammation and enhance repair response (Cardoso *et al.*, 2011). Studies have also shown that the Ga spore oil has excellent antioxidant capacity, which can effectively scavenge free radicals and extend the life span of fruit flies (Zhang *et al.*, 2021). The unsaturated FAs in the fruiting bodies of Ga are effective antioxidants, but the study on the antioxidant activities of Ga lipids is limited.

In this study, we evaluated the fat extraction rates and β-carotene content in fat from different parts of four Ga species. We also determined the DPPH free radical scavenging ability and ferric-reducing power of the lipid extracts, quantitatively analyzed 51 kinds of FAs in the extract, and discussed the differences of different varieties and parts of Ga regarding the extraction rate, *in vitro* antioxidant activity and FA content. Reviewing the results of previous studies, we have not seen similar reports. The results obtained have the potential to use Ga lipid as a functional food for the prevention of age-related diseases and provide guidance to industrialists and manufacturers who are looking for the best excipients for their products.

2. Materials and methods

2.1. Materials

Cultivated red (*G. lucidum*) and black (*G. atrum*) Ga mushroom samples were obtained from the Guilin Chinese medicinal wholesales market in Guangxi. The red Ga was of Guilin-origin, whereas the black Ga was cultivated in the Yunnan Province of China. The fruiting bodies of all Ga samples were separated into canopy and stem parts, and their base-part was discarded. The canopy part of the cultivated red Ga was named RCC, and its stem part was RCS. The canopy and stem of the black Ga were BCC and BCS.

The chemicals used in experiments are analytical grade unless otherwise specified. The n-hexane, ethanol, chloroform and sulfuric acid was purchased from Xilong Science Co., Ltd. (Sichuan, China); 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), β-carotene, potassium ferricyanide, trichloroacetic acid, ferric chloride and methanol from Yien Chemical Technology Co., Ltd. (Shanghai, China); ascorbic acid from Jingchun Biochemical Technology Co., Ltd. (Shanghai, China). The methyl salicylate was obtained from Sigma-Aldrich Shanghai Trading Co, Ltd. (Shanghai, China) and anhydrous sodium sulfate from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). Also, the 0.1 M phosphate buffer saline (PBS) of pH 7.4 were from Solarbio Biotechnology Co., Ltd. (Shanghai, China).

2.2. Lipid extraction

All Ga parts were washed, oven-dried, and pulverized to 50-mesh particles. The lipid in two different parts of the Ga fruiting body was extracted using hexane (Salvatore *et al.*, 2020). In brief, 2.0 g Ga powder was added into 50 mL of n-hexane and subjected to ultrasonic assisted treatment for 30 min. After putting to stand in a water bath at 60 ℃ for 6 h, the hexane was removed using a rotary evaporator. The Ga lipid was collected and stored at -20 ℃ before FA analysis.

2.3. FA analysis

Trace 1300 gas chromatography equipped with an ISQ LT mass selective detector (Thermo Fisher Scientific, USA) was used to analyze the FAs in Ga lipids. The column used was the Thermo TG-FAME capillary column (50 m × 0.25 mm, 0.20 μm). The injection volume was 1 μL, and the shunt ratio was 8:1. The lipid sample (1 mg/mL) was weighed and added with 1 mL of chloroform-methanol (2:1, v/v) solution. The lipid solution was homogenized at 60 Hz for 1 min and then ultrasonicated for 30 min at a room temperature (RT) of 25 ℃. After centrifugation

at 10,000 rpm for 5 min at 4 ℃, the supernatant was collected. The supernatant was added with 2 mL of 1% methanol sulfate solution, mixed thoroughly, and esterified in an 80 ℃ water bath for 30 min. After cooled to RT, the esterified FAs were extracted with n-hexane for 5 min and then added to the chilled distilled water (4 ℃) for washing and centrifuged for 10 min before adding 500 ppm methyl salicylate as the internal standard (Hoving *et al.*, 2018). The esterified FAs were analyzed using the GC-MS. Fifty-one FA methyl ester mixtures (4000 μg/mL) were diluted with n-hexane at concentrations ranging between 1 and 2000 μg/mL for plotting standard calibration curves. The GC-MS data was analyzed using multivariate statistics, and the receiver operating characteristic (ROC) analysis was also performed.

2.4. Extraction and analysis of β-carotene

The β-carotene in the Ga lipid samples was extracted using dimethyl sulfoxide (DMSO) (Kozłowska *et al.*, 2016). In brief, a 0.1-g lipid sample was weighed and added with 2.5 mL of n-hexane and 2.5 mL of DMSO. The solution was homogenized and ultrasonicated for 20 min at RT and centrifuged at 4000 rpm for 5 min. The DMSO layer containing β-carotene was collected. The β-carotene content of the Ga lipid samples was determined based on a spectrophotometric method (Khoo *et al.*, 2009).

2.5. Determination of *in vitro* antioxidant activities

DPPH radical scavenging activities of the carotenoid extracts of Ga lipids were determined according to a previously reported method with slight modification (Farooq *et al.*, 2023). The DPPH reagent (0.02 mg/mL) was prepared by dissolving it in an anhydrous ethanolic solution. The DPPH reagent (2 mL) was mixed with 2.0 mL of the extract at concentrations of 0.25, 0.5, 1.0, 2.0, and 4 mg/mL. The mixture was stirred and left to stand at RT for 30 min in the dark, and the absorbance was measured at 517 nm. The scavenging activities of the extracts were then calculated, and the results were expressed at EC_{50} values. Ascorbic acid (Vc) was used for comparison.

The reducing power of the carotenoid extracts was evaluated according to a previous method (Mota *et al.*, 2022), also known as ferric-reducing antioxidant capacity (FRAP). The 0.5-mL extracts (0.25, 0.5, 1.0, 2.0, and 4 mg/mL) were added with 0.5 mL of 1% potassium ferricyanide solution and 0.5 mL phosphate buffer (0.2 M, pH 6.7) in test tubes. The test tubes containing the reagent mixture were incubated in a 50 ℃ water bath for 20 min. After cooling to RT, 0.5 mL of 10% trichloroacetic acid solution (TCA) was added. The supernatant was collected after centrifugation, mixed with 0.5 mL of 0.1% ferric chloride solution and 2.0 mL distilled water, and left to stand at RT for 10 min. The absorbance was measured at 700 nm. The standard curve was plotted by applying a ferrous sulfate solution of five different concentrations (0 to 6.58 μM). FRAP value was expressed as μM Fe^{2+}/g extract. Vc was used for comparison.

2.6. Statistical analysis

All data were expressed as mean±standard error (SE). The results were statistically analyzed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance coupled with the Tukey range test was used to compare the mean differences between different groups, and $p<0.05$ was considered statistically significant. The FAs in the lipid samples were also analyzed based on the principal component and ROC analysis.

3. Results and discussions

3.1. Ga lipid yields

The lipids in different Ga species and parts of their fruiting bodies were extracted with hexane. As shown in Figure 1, the extraction yield of RCC was the highest (1.09%). The extraction yields of RCS, BCC, and BCS were 0.96%, 0.92%, and 0.84%, respectively. There was no significant difference in the extraction yields among RCS, BCC, and BCS ($p>0.05$). The total lipid content of the Ga samples was estimated based on their extraction yields. The results showed that the Ga canopies had total lipid content higher than that of their stalks.

The difference in Ga lipid content could be related to their growing environment. The high-temperature treatment during extraction might also cause a loss of volatile components. These volatile substances were trans anisinol, R-(−)-linalsol, S-(+)-carvone, and sesquiterpenol (Ziegenbein *et al.*, 2006). The lipid content of Ga could be varied among different species (Chen *et al.*, 2023).

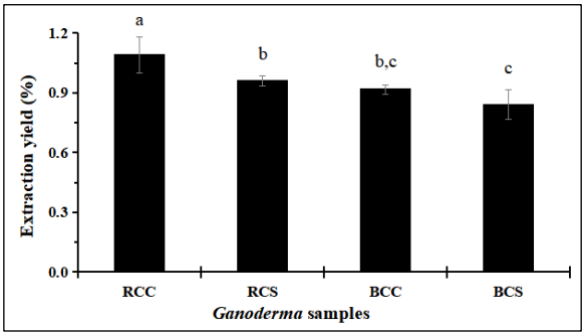

Figure 1. Lipid extraction yields of the Ga samples. *RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga.

3.2. FA composition of Ga lipids

The FA composition of Ga lipids was determined using a gas chromatographic system coupled with a mass spectrometer (GC-MS). The chromatographic parameters are shown in Table S1. The TICs of the samples and fatty acid (FA) standards are depicted in Figure S1. As shown in the overall heat map (Figure 2), the types and levels of FAs in different Ga samples are varied. These FAs were divided into four categories. The results showed that RCC, RCS, BCC, and BCS had 0.28%, 0.1%, 0.24%, and 0.1% total FAs in the Ga lipids (Table 1). The black Ga samples had higher total SFA levels than the red Ga sample, whereas the total unsaturated FA levels were higher in the red Ga samples. Among the Ga samples, BCC had the highest total MUFA level, followed by RBC, BCS, and RCS; RCC had the highest total polyunsaturated FA (PUFA) level, whereas RCS had the lowest. The SFAs were highly distributed in the canopy of red Ga but not for the black Ga canopy. Moreover, the trans FA levels in the black Ga samples were higher than in the red Ga samples.

The result showed that 47 FAs were detected in the Ga samples (Table 1). They are 16 SFAs, 10 MUFAs, 11 PUFAs, and 12 trans FAs. Among the trans FAs, 7-trans nonadecenoate and 10-trans-nonadecenoate were not detected in all these Ga samples. The major FA was palmitic acid (C16:0), oleic acid (C18:1n9c), and linoleic acid (C18:2n6). They were the main SFA, MUFA, and PUFA, respectively, detected in the Ga lipids.

Table 1. Quantitative analysis of fatty acids

No.	Compound	RCC	RCS	BCC	BCS
Saturated fatty acid (μg/g)					
1	**C6:0**	68.60±4.93	148.17±5.04	59.36±1.65	1141.57±16.76
2	**C8:0**	12.51±0.35	32.67±0.97	68.94±2.38	275.95±7.61
3	C10:0	16.58±0.84	21.14±0.70	10.79±0.86	62.71±2.87
4	**C11:0**	7.22±0.32	11.64±0.24	2.83±0.32	11.72±1.17
5	**C12:0**	104.77±5.31	77.81±0.91	52.41±1.96	82.92±1.46
6	**C13:0**	26.64±0.63	22.24±0.41	18.01±0.42	32.56±0.81
7	**C14:0**	647.73±11.74	328.45±4.92	504.13±6.19	668.91±1.43
8	**C15:0**	5500.96±90.09	2206.63±12.99	3437.25±27.20	3328.16±87.70
9	C16:0	38414.06±286.31	12198.40±32.83	22754.51±310.53	27286.88±393.37
10	**C17:0**	1933.18±14.86	431.97±3.38	1000.47±28.29	2060.77±25.10
11	**C18:0**	4057.22±51.87	1125.55±12.60	1714.70±17.03	3313.34±21.39
12	**C20:0**	499.06±4.02	118.30±7.24	153.17±3.16	208.77±9.60
13	**C21:0**	177.94±3.91	172.42±3.46	189.95±7.04	159.22±10.42
14	**C22:0**	1005.60±15.88	405.136±9.92	776.26±26.11	685.34±26.58
15	**C23:0**	660.66±56.06	634.16±5.75	1805.91±32.68	1207.65±44.63

16	**C24:0**	1426.71±54.43	488.03±12.88	2908.04±67.16	2814.08±11.92
Trans unsaturated fatty acids (μg/g)					
1	C14:1t	25.06±1.08	19.67±1.76	21.91±1.62	97.97±7.19
2	**C15:1t**	45.87±3.67	43.95±4.40	34.33±2.46	77.11±8.25
3	**C16:1t**	70.31±4.86	53.69±4.70	55.88±4.44	133.84±11.39
4	**C17:1t**	235.28±6.42	186.36±17.00	150.60±4.07	291.42±8.51
5	C18:1n12t	342.51±12.72	421.86±8.67	254.38±1.85	948.89±28.63
6	C18:1n9t	185.05±2.57	165.56±10.74	234.26±3.90	368.11±14.37
7	C18:1n7t	136.60±4.23	148.00±8.48	89.95±1.49	276.78±7.74
8	C18:2n6t	117.95±8.72	101.63±2.58	81.78±4.01	186.16±5.45
9	**C20:1t**	133.34±3.31	86.54±3.91	82.41±3.27	179.59±3.16
10	C22:1n9t	128.46±12.08	138.42±7.42	117.42±2.14	219.39±3.52
11	C19:1n12t	ND	ND	ND	ND
12	C19:1n9t	ND	ND	ND	ND
Monounsaturated fatty acids (μg/g)					
1	C14:1	14.48±1.03	19.47±2.39	13.23±1.06	26.66±1.19
2	**C15:1**	55.43±7.58	49.79±4.18	40.78±2.30	74.82±4.14
3	**C16:1**	792.54±16.37	797.00±2.60	1649.53±24.95	703.50±33.20
4	**C17:1**	604.57±24.01	394.38±8.33	628.36±72.81	440.23±16.29
5	C18:1n12	5518.17±283.62	2187.24±111.10	6159.64±890.72	3971.72±351.34
6	C18:1nN9c	41705.70±201.66	13746.63±278.06	106148.21±973.26	18594.50±177.39
7	C18:1n7	5174.58±41.92	4596.26±51.44	3337.64±43.98	2713.32±32.66
8	C20:1	239.65±10.12	138.73±7.65	345.06±5.23	155.31±2.67
9	C22:1n9	327.93±7.66	287.10±13.12	247.85±8.43	324.52±16.38
10	C24:1	345.11±34.02	361.93±35.99	492.03±10.44	506.22±18.39
Polyunsaturated fatty acids (μg/g)					
1	C18:2n6	164024.74±2096.30	56918.17±1694.32	83024.11±1209.12	27072.35±362.11
2	**C18:3n6**	95.40±1.18	80.26±8.41	65.56±1.40	96.29±2.31
3	**C18:3n3**	307.71±11.12	205.75±7.08	243.78±16.76	152.16±8.34
4	**C20:3n6**	95.93±2.23	76.25±2.63	77.52±3.75	99.23±4.30
5	**C20:3n3**	66.11±1.71	58.34±7.77	52.53±3.07	85.05±0.81
6	**C20:4n6**	66.46±2.59	58.77±0.93	56.25±3.64	84.68±3.90
7	**C20:5n3**	78.80±6.58	57.59±6.25	48.38±3.60	95.10±6.63
8	**C22:4**	16.68±1.38	42.09±0.71	34.62±3.78	55.12±8.04
9	**C22:5n6**	15.89±0.42	60.95±3.92	52.52±6.97	143.87±19.19
10	**C22:5n3**	266.60±7.99	303.02±25.04	170.03±7.78	651.70±33.77
11	**C22:6n3**	131.70±3.44	109.11±8.81	125.22±9.78	204.01±9.49

* All values are expressed as mean±standard deviation of three replicates. The fatty acids content is presented as mg/g lipid. ND indicates that not detected.

These FAs were the highest in RCC. The other main SFAs were pentadecanoic (C15:0), heptadecanoic (C17:0), stearic (C18:0), and lignoceric acids (C24:0). Petroselaidic acid (C18:1n12t) was the highest trans FA in the Ga samples among all trans FAs. The total trans FAs content of these Ga samples ranged between 1 and 3 mg/g. Moreover, long-chain and very long-chain FAs were the main SFAs in the Ga samples. The very long-chain FAs content of the black Ga samples were higher than the red Ga samples, especially the canopy part of Ga.

The variations in Ga FA compositions could be due to the influence of the growing environment, especially environmental stress

and soil composition (Upchurch, 2008). The literature supports our findings that the red-purplish varieties of Ga contained palmitic, oleic, and linoleic acids as the main FAs (Lv et al., 2012). Palmitic acid and oleic acid are important FAs; they are involved in cell growth and apoptosis. Oleic acid can convert palmitic acid into inert triglycerides for storage.

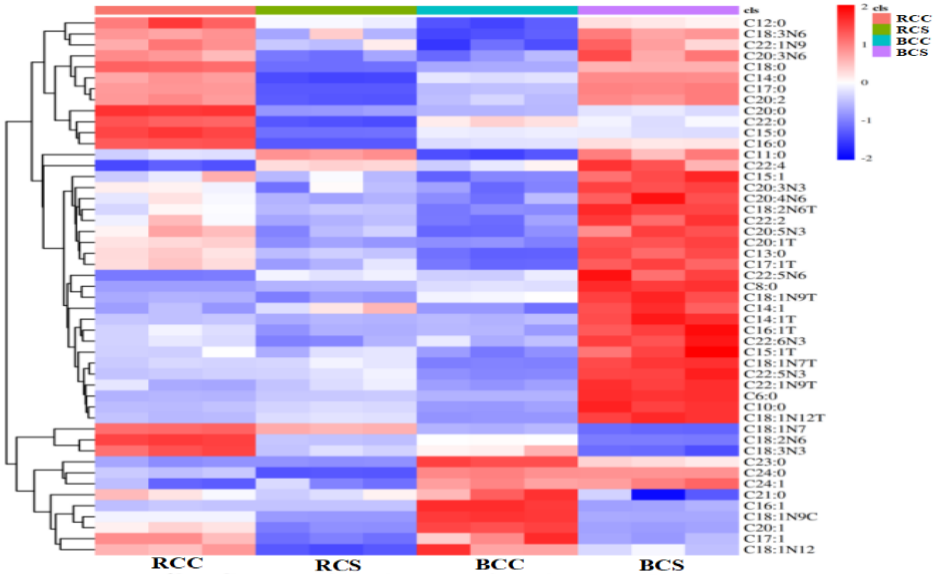

Figure 2. Overall heat map of fatty acids in the Ga samples.
*RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga

On the other hand, the lower amount of trans FAs and higher unsaturated FAs in the red Ga showed that it is a potent source of nutraceuticals for disease prevention. In this study, the types and parts of Ga are not only important for quality evaluation but also help to clarify their pharmacological activities.

3.3. Multivariate analysis of FA composition

The FA composition of Ga samples (RCC, RCS, BCC, and BCS) was analyzed based on the principal component analysis (PCA). It has been a popular multivariate statistical technique used to compare the FAs in the Ga samples. The PCA represented the similarity pattern of observations and variables by displaying them as points on a map (Abdi and Williams, 2010). As shown in Figure 3A, the PCA score plot reflects the distribution of the Ga samples. The distribution points of each sample are close to each other. It indicates that the FA composition and concentration in these samples are similar. The differences between the first and second principal components were 56.6% and 23.7%, respectively. The result also showed that 80.3% of the variation can be explained by these two components. Moreover, less than 1/5 of the variation remained in the other components (Bro and Smilde, 2014). In this study, the Ga samples had R^2X (cum) of 0.803. The R^2X and PCA score plots were the main reference parameters for the model cross-validation, and R^2X was also the interpretability of the model. The R^2X (cum) of higher than 0.5 indicated that the cross-validation and interpretability of the test samples were good.

The model's independent variable explainability (R^2X), model dependent variable explainability (R^2Y), model predictability (Q^2), and PLS-DA score plot were used as parameters for the cross-validation of partial least squares discriminant analysis (PLS-DA) models. The results showed that the Ga samples had R^2X (cum), R^2Y (cum) and Q^2 (cum) of 0.972, 0.998, and 0.994, respectively. The differences between R^2 (cum), and Q^2 (cum) were between 0.004 and 0.022. The R^2 (cum) close to 1 showed that the experimental repeatability was high. It showed that the background noise was either absent or very small. There were also a

few abnormal samples in the model. The orthogonal partial least squares discriminant analysis (OPLS-DA) can evaluate the classification performance of the model by using the independent variable explainability (R^2X), model dependent variable explainability (R^2Y), model predictability (Q^2), and OPLS-DA score plot (Yao *et al.*, 2019). The OPLS-DA score plot is shown in Figure 3C. The R^2X (cum), R^2Y (cum), and Q^2 (cum) of the Ga samples was 0.771, 0.99, and 0.982. We observe that the differences between R^2 (cum) and Q^2 (cum) ranging from 0.008 to 0.0.211. The R^2 (cum) of OPLS-DA was significantly lower than that of the PLS-DA, which indicated that the its experimental repeatability was worse than the PLS-DA. However, its R^2 (cum) was higher than 0.5, and the Q^2 (cum) was 0.982. This results indicated that the experimental set was repeatable, and there were only a few abnormal samples in the model.

The metabolome data is multidimensional, and some variables are highly correlated. The traditional univariate analysis cannot rapidly and accurately mine the potential information in the dataset. Therefore, it is necessary to apply the principle of chemometrics and multivariate statistical methods in the metabolomic analysis for reducing and classifying the multidimensional data. As a results, the most applicable information can be mined and extracted.

3.4. ROC analysis

The ROC results showed that the area under curve (AUC) values of 10-trans pentadecenoic acid (C15:1t) and tricosanoic acid (C23:0) between RCC and RCS were ≤ 0.5. Their differential content was 44.96 and 637.10 μg/g, respectively. The finding suggests that the method used to identify these two FAs is ineffective to predict the occurrence of the events and has no predictive value. The AUC value of palmitoleic acid (C16:1) was 0.556, and its differential content was 797.49 μg/g, which indicates that the differential prediction accuracy of this FA was low. The AUC value of 10-pentadecenoic acid (C15:1), nervonoic acid (C24:1), and brassidic acid (C22:1n9t) ranged from 0.7 to 0.9.

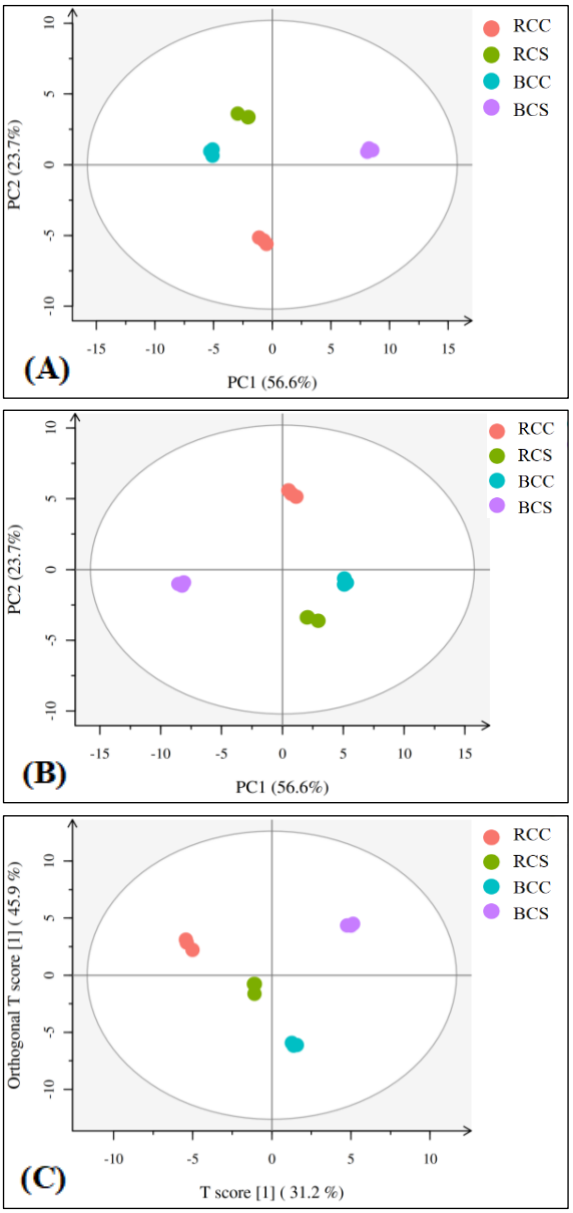

Figure 3. Multivariate statistical analysis diagrams of FAs in the Ga samples. (A) PCA, (B) PLS-DA, and (C) OPLS-DA score plots.

Their differential content was 48.42, 331.81, and 126.49 μg/g, respectively. The high AUC values indicated that the method used to identify these three biomarkers had some predictive accuracy. The AUC values of all remaining FAs were 1. It showed that the identification of these FAs was highly accurate.

The AUC values of pentadecanoic acid (C15:0) and nervonoic acid (C24:1) between

BCC and BCS were 0.889 and 0.778, respectively, and their differential content was 3436.73 and 505.25 μg/g, respectively. This result indicates that the method used has some predictive accuracy. The AUC values of the remaining FAs were 1, which shows that this method has high predictive accuracy.

The comparison of the AUC values of 10-heptadecenoic acid (C17:1) and docosahexaenoic acid (C22:6n3) between RCC and BCC was also performed. Their AUC values were 0.667, and their differential content was 619.46 and 125.84 μg/g, respectively. It showed that the predictive accuracy of identifying these two biomarkers was low. The AUC values of myristoleic acid (C14:1) and petroselinic acid (C18:1n12) were 0.778, and their differential content was 13.96 and 5531.62 μg/g, respectively. The AUC values of the remaining FAs were 1, which indicates that the identification of these biomarkers had high predictive accuracy. By comparing RCS and BCS, their AUC value of unndecanoic acid (C11:0) was 0.667, and its differential content was 12.10 μg/g, indicating that the identification of this biomarker had low predictive accuracy. The AUC value of heneicosanoic acid (C21:0) was 0.889, and its identification content was 170.86 μg/g. It indicates that the identification of this FA was predictively accurate.

The AUC is used to assess the sensitivity and specificity of the biomarker for predicting the occurrence of the event. The sensitivity and specificity of each metabolite are determined by the optimal threshold of the ROC curve (Carter *et al.*, 2016). If the AUC value is between 0.5 and 1.0, the AUC value closer to 1 denotes a higher prediction accuracy. The prediction accuracy is low when the AUC value ranges between 0.5 and 0.7; the prediction accuracy is moderate to high when the AUC value is between 0.7 and 0.9; the prediction accuracy is higher when the AUC value is above 0.9. The biomarker does not effect the occurrence of events and has no predictive value if the AUC value is 0.5. In this study, the GC-MS analysis and quantification of most FAs in the Ga samples was highly accurate, especially their palmitic, oleic, and linoleic acids. It is because the AUC values of these FAs were 1. These analytical data were precise and highly reliable, especially determining the principal FAs in the Ga samples.

3.5. β-carotene content

As shown in Figure 4, the RCC extract had the significantly highest β-carotene content ($p<0.05$), followed by BCS, BCC, and RCS extracts. There was no significant difference in β-carotene content among these Ga extracts, except for RCC extract ($p>0.05$). The results showed that the β-carotene in the extracts of black Ga stalk extract was somehow higher than that of the red Ga stalk extract.

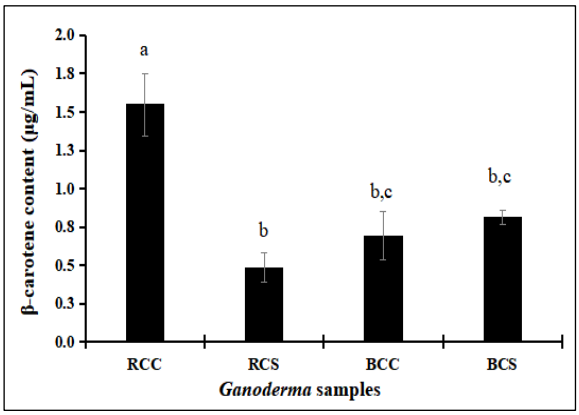

Figure 4. β-carotene content of the Ga lipids. *RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga.

β-carotene content in the RCS extract was lower than in the RCC extract, probably due to its larger canopy. The literature showed that the carotenoid-rich extracts, even stored in the dark at an ambient atmosphere of -20 ℃, had a carotenoid decomposition rate of about 5% per day in the presence of antioxidants (Rodriguez-Amaya, 2010). As plant-based lipid is rich in carotenoids, it is a potent free radical scavenger (May, 1994). Carotenoids have been reported to be beneficial to human health. These colored compounds play a role in disease prevention besides their antioxidative effect (Khoo *et al.*, 2011). Therefore, the in vitro antioxidant activity of the Ga extracts could be attributed to the β-carotene content.

3.6. *In vitro* antioxidant activity

The antioxidant activities of the Ga extracts are depicted in Figure 5. Both DPPH radical scavenging activity and FRAP values were according to the electron transfer reaction pathway. The activities of these antioxidant assays were dose-dependent, with linear R^2 values greater than 0.9. The results showed that the EC_{50} values of the red Ga extracts were significantly lower than the black Ga extracts ($p<0.05$). It shows that the red Ga extract, especially RCC, had the highest antioxidant activity, followed by RCS, BCS, and BCC extracts. The low DPPH radical scavenging activity of BCC could be due to its small black canopy part, where it might contain a low amount of water-soluble carotenoids. Only BCC had an EC_{50} value greater than 5 mg/mL. The EC_{50} value of Vc was 5.63±0.09 μg/mL. The results also showed that the DPPH radical scavenging activity of Vc was about 1000 times better than the BCC extract.

The Ga extracts had the significantly highest FRAP values, except for the RCC extract ($p>0.05$). The FRAP value of the RCC extract was about one time lower than the FRAP values of the other Ga extracts. Vc had an FRAP value of 19.92±1.75 μM Fe^{2+}/g. The value was 50 times higher than those reported for the Ga extracts. The low FRAP value of the Ga extract is attributed to the antioxidants with a low reducing ability. On the other hand, the RCC extract had the highest DPPH radical scavenging activity with a low FRAP value. It shows that the DPPH radical scavenging activities of the Ga extracts are not dependent on their FRAP values. The contradicting results between these two antioxidant assays could be due to the antioxidant components in the extracts. Although RCC extract had a high β-carotene content with a high DPPH radical scavenging activity, its reducing activity was low. Therefore, β-carotene is a weak reducing agent.

As reported in the literature, the reducing ability of lycopene is higher than β-carotene (Edge *et al.*, 1998). The study reveals that lycopene is easily oxidized to protect the eye macular, but not for β-carotene. This result confirms that β-carotene was the main carotenoids in the Ga extracts. The high amount of β-carotene could be found in the spongy canopy of the Ga, and RCC had the most spongy proportion. The high reducing ability of the other Ga extracts could also be due to these lipid extracts having high amounts of lipid-soluble terpenoids. The high free radical scavenging activity of the Ga extracts makes them potent nutraceuticals for preventing diseases. The related pharmacological effects are immune regulation, anti-tumor, hypotensive, hypoglycemia, and anti-aging (Lin and Deng, 2019).

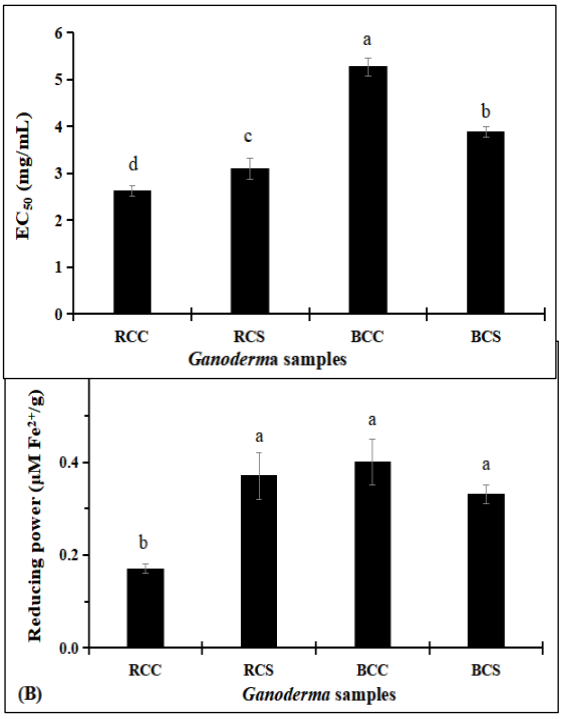

Figure 5. In vitro antioxidant activities of the Ga lipid extracts. (A) DPPH radical scavenging assay (EC50 value); (B) FRAP assay (reducing power). *RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga.

The free radical scavenging activity of the Ga extract could be involved the electorn transfer from an oxygen molecule of the hydroxyl group to the carotenoid structure with unpaired electrons or hydrogen during oxidative stress (Khoo *et al.*, 2017). The carotenoids in the Ga extracts could scavenge free radicals through hydrogen atom transfer reaction besides the electron transfer pathway (Gharib *et al.*, 2022).

The carotenoids also have antidiabetic effects besides their antioxidative ability (Sayahi and Shirali, 2017). Determining antioxidant activity in the carotenoid-rich extracts of Ga samples indicates that these extracts are potential edible fats with pharmacological effects.

4. Conclusions

The lipid content of the Ga canopies was higher than that of the stalk part. GC-MS analysis found 49 FAs in the Ga lipids, and their major FAs were palmitic acid, oleic acid, and linoleic acid. Multivariate analysis showed that the FA measurements were precise. The Ga lipids were composed of β-carotene as one of the main carotenoids. These carotenoid-rich extracts possessed antioxidant activities, especially the RCC extract, possessing the highest DPPH radical scavenging activity with a low FRAP value. The results also showed that the lipid extracts of black Ga samples had lower DPPH radical scavenging activity with high reducing ability, but these results were not comparable to Vc. Based on these findings, the red and black Ga are sources of functional lipids with β -carotene as their main bioactive substances. These lipids can also be used as functional excipients in dietary supplements and cosmetic products in the future.

5. References

Bro, R., Smilde, A. K. (2014). Principal component analysis. *Analytical Methods*, 6(9), 2812-2831.

Cardoso, C. R., Favoreto Jr, S., Oliveira, L. L., Vancim, J. O., Barban, G. B., Ferraz, D. B., Silva, J. S. (2011). Oleic acid modulation of the immune response in wound healing: A new approach for skin repair. *Immunobiology*, 216(3), 409-415.

Carrillo, C., Cavia M. D. M., Alonso-Torre, S. R. (2012). Antitumor effect of oleic acid; mechanisms of action. A review. *Nutrición Hospitalaria*, 27(5), 1860-1865.

Carter, J. V., Pan, J., Rai, S. N., Galandiuk, S. (2016). ROC-ing along: Evaluation and interpretation of receiver operating characteristic curves. *Surgery*, 159(6), 1638-1645.

Chen, B. J., Liu, Y., Yang, K., Li, X., Dong, X., Guan, Y., Ismail A., Khoo, H. E. (2023). Amylase-assisted extraction alters nutritional and physicochemical properties of polysaccharides and saponins isolated from *Ganoderma* spp. *Food Chemistry: X*, 20, 100913.

Edge, R., Land, E. J., McGarvey, D., Mulroy, L., Truscott, T. G. (1998). Relative one-electron reduction potentials of carotenoid radical cations and the interactions of carotenoids with the vitamin E radical cation. *Journal of the American Chemical Society*, 120(17), 4087-4090.

El Sheikha, A. F. (2022). Nutritional profile and health benefits of *Ganoderma lucidum* "*Lingzhi*, *Reishi*, or *Mannentake*" as functional foods: Current scenario and future perspectives. *Foods*, 11(7), 1030.

Farooq, A., Hussain, S. Z., Bhat, T. A., Naseer, B., Shafi, F. (2023). Walnut fruit: Impact of ethylene assisted hulling on *in vitro* antioxidant activity, polyphenols, PUFAs, amino acids and sensory attributes. *Food Chemistry*, 404, 134763.

Gharib, M. A., Radwan, H., Elhassaneen, Y. A. (2022). Nutrients and nutraceuticals content and *in vitro* biological activities of reishi mushroom (*Ganoderma lucidum*) fruiting bodies. *Alexandria Science Exchange Journal*, 43(2), 301-316.

Hoving, L. R., Heijink, M., van Harmelen, V., van Dijk, K. W., Giera, M. (2018). GC-MS analysis of medium-and long-chain fatty acids in blood samples. *Clinical Metabolomics*, 1730, 257-265.

Khoo, H. E., Azlan, A., Tang, S. T., Lim, S. M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, 61(1), 1361779.

Khoo, H. E., Ismail, A., Mohd-Esa, N., Idris, S. (2008). Carotenoid content of underutilized tropical fruits. *Plant Foods for Human Nutrition*, 63, 170-175.

Khoo, H. E., Prasad, K. N., Kong, K. W., Jiang, Y., Ismail, A. (2011). Carotenoids and their isomers: Color pigments in fruits and vegetables. *Molecules*, 16(2), 1710-1738.

Kozłowska, M., Gruczyńska, E., Ścibisz, I., Rudzińska, M. (2016). Fatty acids and sterols composition, and antioxidant activity of oils extracted from plant seeds. *Food Chemistry*, 213, 450-456.

Li, Z., Zhou, J., Lin, Z. (2019). Development and innovation of Ganoderma industry and products in China. In: Lin, Z., Yang, B. (Eds.), Ganoderma and Health. Advances in Experimental Medicine and Biology. Springer, Singapore, pp. 187-204.

Lin, Z., Deng, A. (2019). Antioxidative and free radical scavenging activity of *Ganoderma* (lingzhi). In: Lin, Z., Yang, B. (Eds.), Ganoderma and Health. Advances in Experimental Medicine and Biology. Springer, Singapore, pp. 271-297.

Lv, G. P., Zhao, J., Duan, J. A., Tang, Y. P., Li, S. P. (2012). Comparison of sterols and fatty acids in two species of *Ganoderma*. *Chemistry Central Journal*, 6, 10.

May, C. Y. (1994). Palm oil carotenoids. *Food and Nutrition Bulletin*, 15(2), 1-8.

Mota, J. C., Almeida, P. P., Freitas, M. Q., Stockler-Pinto, M. B., Guimarães, J. T. (2023). Far from being a simple question: The complexity between *in vitro* and *in vivo* responses from nutrients and bioactive compounds with antioxidant potential. *Food Chemistry*, 402, 134351.

Rodriguez-Amaya, D. B. (2010). Quantitative analysis, *in vitro* assessment of bioavailability and antioxidant activity of food carotenoids−A review. *Journal of Food Composition and Analysis*, 23(7), 726-740.

Salvatore, M. M., Elvetico, A., Gallo, M., Salvatore, F., DellaGreca, M., Naviglio, D., Andolfi, A. (2020). Fatty acids from *Ganoderma lucidum* spores: Extraction, identification and quantification. *Applied Sciences*, 10(11), 3907.

Sayahi, M., Shirali, S. (2017). The antidiabetic and antioxidant effects of carotenoids: A review. *Asian Journal of Pharmaceutical Research and Health Care*, 9(4), 186-191.

Stojković, D. S., Barros, L., Calhelha, R. C., Glamočlija, J., Ćirić, A., van Griensven, L. J. L. D., Soković, M., Ferreira, I. C. F. R. (2014). A detailed comparative study between chemical and bioactive properties of *Ganoderma lucidum* from different origins. *International Journal of Food Sciences and Nutrition*, 65(1), 42-47.

Tvrzicka, E., Kremmyda, L. S., Stankova, B., Zak, A. (2011). Fatty acids as biocompounds: their role in human metabolism, health and disease-a review. part 1: classification, dietary sources and biological functions. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 155(2), 117-130.

Upchurch, R. G. (2008). Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology Letters*, 30, 967-977.

Xu, Y., Yu, J. (2021). The trend of *Ganoderma lucidum* research (1936–2019). In: The Lingzhi Mushroom Genome. Springer, Cham, pp. 27-45.

Yao, X., Hou, H., Liang, H., Chen, K., Chen, X. (2019). Raman spectroscopy study of phosphorites combined with PCA-HCA and OPLS-DA models. *Minerals*, 9(10), 578.

Zhang, Y., Cai, H., Tao, Z., Yuan, C., Jiang, Z., Liu, J., Kurihara, H., Xu, W. (2021). *Ganoderma lucidum* spore oil (GLSO), a novel antioxidant, extends the average life span in *Drosophila melanogaster*. *Food Science and Human Wellness*, 10(1), 38-44.

Ziegenbein, F. C., Hanssen, H. P., König, W. A. (2006). Secondary metabolites from Ganoderma lucidum and Spongiporus leucomallellus. *Phytochemistry*, 67(2), 202-211.

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