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Original article

Chemical Composition of the Lumpy Bracket Mushroom (*Trametes gibbosa*)

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Abstract

Background and objectives: *Trametes* species have been used for centuries in traditional medicine of Asian countries. Recently, some of the bioactive compound have been isolated and evaluated for therapeutic purposes from these species. The aim of this study was to report the isolation and structure elucidation of major sterols from fruiting bodies of *Trametes gibbosa*. Volatile compounds and antioxidant activities of the different mushroom extracts of mushroom were also examined. **Methods:** The fruiting bodies of *T. gibbosa* were extracted with n-hexane, methanol, and hot water, respectively. For isolation of sterols, the n-hexane extract was subjected to column chromatography and fractionated by step gradient of n-hexane: ethyl acetate. The volatile oil was prepared by hydrodistillation and analyzed by GC-MS. For evaluation of the antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power assays were used. Moreover, the phenolic and carbohydrate contents were assessed using spectrophotometry methods. **Results:** The column chromatography of the n-hexane extract led to the isolation of three sterols. These compounds were identified for the first time in *T. gibbosa* as follows: ergosta-5,7,22-trien-3β-ol (ergosterol); 5,8-epidioxy-ergosta-6,22-dien-3-ol; 5,9-epidioxy-8,14-epoxy-ergosta-6,22-dien-3-ol. The most abundant volatile compounds were identified as aldehydes (29.01%), fatty acids (21.2%) and alcohols (12.07%). Based on antioxidant results, methanol and hot water extracts showed the highest activities in DPPH (EC50=588.56±36.37 µg/mL) and FRAP (432±6.6 mmol Fe^{2+}/g DW) methods, respectively. **Conclusion:** *Trametes gibbosa* is a valuable source of mycochemicals such as sterols, carbohydrate and phenolics. Further investigations are required for evaluation of the therapeutic potentials of the isolated compounds.

Keywords: antioxidant; ergosterol; ergosterol peroxide; *Trametes gibbosa*

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Introduction

Mushrooms have been used in different countries as food and medicine. They have been cultivated for hundreds of years in Asian countries, like China and Japan for traditional therapeutic purposes. The mushrooms fruiting bodies or mycelia are rich in several compounds such as proteins, carbohydrates, minerals and vitamins. Different studies have been conducted to evaluate the bioactive properties of different extracts from mushroom fruiting bodies as well as their

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secondary metabolites [1]. Various significant bioactive compounds such as polysaccharides, terpenoids, phenolic acids and fatty acids have been reported as the main bioactive metabolites in mushroom sources [2]. The genus *Trametes* (Basidiomycetes: Polyporaceae), includes nearly 60 species which are widely distributed throughout the world and are recorded to be used as food and medicine [3]. These species has attracted special medical attention in recent years due to multiple pharmacological activities [4]. They are widely used in traditional and folk medicine of the world for treatment of various diseases including cancer, hepatitis, rheumatoid arthritis, and infections of the respiratory, urinary and digestive tracts, which have been confirmed by pharmacological studies [3-5]. Many of the recent investigation are attributed to the immunomodulatory activities of *Trametes* species and the related active constituents such as polysaccharides [6].

Trametes gibbosa, (Pers.) Fr (basidiomycete), commonly known as 'lumpy bracket' is a polypore mushroom which grows on dead woods [5]. It is widely distributed in Europe and Asia, with different therapeutic potential [7]. This mushroom has been used in traditional Chinese medicine for different therapeutic activities [8]. Recent studies have revealed the potential immunomodulatory, antiviral, anti-tumor, anti-oxidant, anti-inflammatory, antimicrobial and neuroprotective activities of *T. gibbosa* [3,9-11]. A vast spectrum of these biological activities is associated with the components such as polysaccharides, steroids and phenolics [3,5,6,11]. The aim of this study was isolation and structure elucidation of major sterols from fruiting bodies of *T. gibbosa* by chromatographic and spectroscopic methods. Moreover, the volatile oil composition, antioxidant activity, total phenol and carbohydrate contents were also determined.

Material and Methods

Ethical consideration

This study was approved by ethic committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1397.67). All investigators have considered ethics of biosafety throughout the research.

Chemicals

n-Hexane, methanol, ethyl acetate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ), phenol, and vanillin were purchased from Sigma Aldrich (USA). Silica gel (35-70 and 230-400 Mesh) for column chromatography, TLC silica gel G F254 plates, and D-glucose were purchased from Merck (Germany). All other chemicals and solvents used in this work were of the highest grade commercially available.

General experimental procedures

The ^{1}H nuclear magnetic resonance (NMR) (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 500 DRX (Karlsruhe, Germany) spectrometer using tetramethylsilane as internal standard. Chemical shifts were reported in parts per million (ppm) scale and coupling constants (J) were expressed in Hertz, (s, singlet; d, doublet; dd, double doublet; m, multiplet). The multiplicity of ^{13}C NMR was determined as DEPT.

Fungal material

The *T. gibbosa* fruiting bodies were collected in 2019 from the Caspian forest located in North of Iran, Mazandaran province. The mushroom was identified by Saeed Ali Mousazadeh (Agriculture and Resource Research Center, Mazandaran, Iran). The voucher specimen (MAZ-B3-0101-001) was deposited at the Herbarium of Mazandaran University of medical sciences.

Extraction

The mushrooms were dried at 40 °C for 48 hours, and smashed into small particles. The materials were extracted by maceration method with n-hexane, and methanol at room temperature sequentially. The remaining residue was submitted to hot water extraction at 90 °C for 6 h. After filtration, the extracts were concentrated under reduced pressure with rotary evaporator.

Isolation of sterols

The n-hexane extract was subjected to silica gel column chromatography (5×15 cm, 35-70 mesh) with mobile phases consisting of n-hexane: EtOAc (10:0 to 0:10) to give 11 fractions.

Subsequently, the fractions number 7 and 9 were submitted to silica gel (1×40 cm, 230-400 mesh) column with n-hexane: EtOAc (10:0 to 7:3) as the mobile phase. Finally, the pure compounds 1, 2, and 3 were isolated. For detection of sterols in the collected fractions, the vanillin indicator was

used. Each fraction was checked by TLC plates precoated with silica gel F254 (vanillin as an indicator, n-hexane: EtOAc (7:3) as the solvent reagent).

Determination of total carbohydrates

The total carbohydrate content of polysaccharide fractions were determined by phenol-sulfuric acid method [12]. To 50 µL of the sample (100 µg/mL) and glucose solution (5-100 µg/mL) as standard, the amount of 150 µL of 96% sulfuric acid was added. Then, 30 µL of 5% phenol in water was added immediately. Finally, the reaction mixture was incubated in water bath (90 °C) for 5 min. The absorbance of the test samples was measured at 490 nm. Calibration curve was prepared by standard concentrations of D-glucose.

Determination of total phenolics content

The total phenolics content of the extracts were determined by Folin-Ciocalteu methods. The calibration curve was plotted using different concentrations of gallic acid (6.25-200 µg/mL). The total phenolic contents were expressed as milligrams of gallic acid equivalents (GAEs) per gram of dried extract [13].

Determination of Antioxidant Capacity

DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a common method to evaluate the radical-scavenging activity of chemicals. The dark purple color of DPPH (in the stable form) will be lost when it is reduced by antioxidants.

The hydrogen donating or radical scavenging ability of the n-hexane, methanol and hot water extracts of *T. gibbosa* fruiting bodies was measured as follow:

The amount of 1.5 mL of 0.15 mM DPPH in methanol was added to 1.5 mL of different concentration of the extract and ascorbic acid as the standard compound. Then the mixtures were incubated at room temperature in the dark for 30 min. Finally, the absorbance was measured at 517 nm. The percentage of inhibition of DPPH radical was calculated according to the following formula:

$$\text{Scavenging rate} = ((A_0 - A_s)/A_0) \times 100$$

Where A_0 and A_s are the absorbance of DPPH solution without sample and the absorbance of sample with DPPH, respectively. Finally, the EC_{50} value and the concentration of the sample required to scavenge 50% of DPPH free radical were calculated according to the standard curve [14].

FRAP assay

The determination of total antioxidant activity was done by FRAP assay (ferric reducing antioxidant power), with modification of Benzie and Strain method [15]. The FRAP colorimetric method is based on reduction of Fe (III). Reduction of colorless ferric-tripyridyltriazine produces blue-colored complex (ferrous-tripyridyltriazine). The working FRAP reagent was prepared daily by mixing 10 volume of 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, with 1 volume of 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and with 1 volume of 20 mM $FeCl_3.6H_2O$ solution. Plant extracts (150 µL) were allowed to react with 2850 µL of the FRAP solution for 30 min at 37 °C in the dark condition. The absorbance was measured at 593 nm. The results were expressed as mM Fe (II)/g dry mass and ascorbic acid was used as the standard [16].

Volatile compounds extraction and chemical analysis

The fruiting bodies of the *T. gibbosa* were frozen at -20 °C. After thawing, they were cut into small pieces. The volatile compounds were obtained by hydrodistillation using a Clevenger type apparatus for 4 h with 2 mL of pentane for trapping. After hydrodistillation, the volatile fraction was dried over anhydrous sodium sulfate and concentrated to a small volume for GC-MS analysis [17].

The GC-MS analysis was carried out on an Agilent 6890 with a BPX-5 MS capillary column (30 m×0.25 mm i.d., 0.25 mm). The temperature gradient started at 50 °C, held for 5 min, and programmed to 240 °C at 3 °C/min, then elevated to 300 °C at 15°C/min, held for 3 min; the sample injection temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 0.5 mL/min in split mode (1:35). MS interface temperature: 220 °C; MS mode: EI; detector voltage: 70 eV; mass range: 40 to 500 u.

Results and Discussion

The concentrated extracts obtained from n-hexane yielded 18 g (1.48%). The chromatography of the n-hexane extract from *T.*

gibbosa led to the isolation of ergosta-5,7,22-trien-3β-ol (ergosterol, compound 1); 5,8-epidioxy-ergosta-6,22-dien-3-ol (ergosterol peroxide, compound 2); 5,9-epidioxy-8,14-epoxy-ergosta-6,22-dien-3-ol (compound 3). The pure compounds 1, 2, and 3 weighted 71, 3.8, and 1.4 mg, respectively. The chemical structures are shown in Figure 1.

Compound 1, ergosterol: The isolated compound was a white crystalline substance. It was not visible on TLC plates under UV light (λ= 254 and 365 nm) and became visible after exposure to vanillin-sulfuric acid spray reagent (black spot). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.67 (3H, s, H18), 0.83 (3H, s, H19), 0.86 (3H, d, *J* = 6.4 Hz, H26), 0.87 (3H, d, *J* = 6.4 Hz, H27), 0.92 (3H, d, *J*=6.4 Hz, H28), 1.07 (3H, d, *J*= 6.8, H21), 3.67 (1H, m, H3), 5.22 (1H, dd, H22), 5.25 (1H, dd, H23), 5.42 (1H, d, *J*=8.4, H7), 5.60 (1H, d, *J*=8.4, H6). ^{13}C NMR (100 MHz, CDCl3**)** is shown in Table l.

Compound 2, 5,8-epidioxy-ergosta-6,22-dien-3-ol: the isolated compound was a white crystalline substance. It was not visible on TLC plates under UV light (λ= 254 and 365 nm) and was visible after exposure to vanillin-sulfuric acid spray reagent (black spot). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.81 (3H, d, *J* = 6.8 Hz, H26), 0.83 (1H, s, H18), 0.84 (3H, d, *J* = 6.8 Hz, H27), 0.87 (1H, s, H19), 0.95 (3H, d, *J*= 6.8 Hz, H28), 1.0 (3H, d, *J*=6.8, H21), 4.0 (1H, m, H3), 5.18 (1H, dd, *J*=15.6, H22), 5.24 (1H, dd, *J*=15.6, H23), 6.27 (1H, d, *J*=8.4, H7), 6.53 (1H, d, *J*=8.4, H6). ^{13}C NMR (100 MHz, CDCl3**)** is shown in Table l.

Compound 3; 5,9-epidioxy-8,14-epoxy-ergosta-6,22-dien-3-ol: the isolated compound was a white crystalline substance. It was not visible on TLC plates under UV light (λ= 254 and 365 nm) and was visible after exposure to vanillin-sulfuric acid spray reagent (black spot). ^{1}H-NMR (400 MHz, CDCl3) δ: 0.81 (3H, d, *J* = 6.8 Hz, H26), 0.84 (3H, d, *J* = 6.8 Hz, H27), 0.94 (3H, d, *J*= 6.8 Hz, H28), 0.92 (1H, s, H18), 1.0 (3H, d, *J*=6.8, H21), 1.19 (1H, s, H19), 4.05 (1H, m, H3), 5.16 (1H, dd, *J*=15.2, H22), 5.25 (1H, dd, *J*=15.2, H23), 5.58 (1H, d, *J*=9.6, H6), 5.92 (1H, d, *J*=9.6, H7). ^{13}C NMR (100 MHz, CDCl3**)** is shown in Table l.

A total of 30 chemical compounds were characterized with GC–MS analysis of *T. gibbosa* volatile fraction which represened 82.84% of the total volatiles. The volatile compounds were categorized into five major chemical classes of aldehydes, alcohols, ketones, terpenoids and fatty acids. The most abundant compounds were identified as aldehydes (29.01%) and fatty acids (21.2%) (Table 2).

Table 1. ^{13}C NMR spectral data of compounds 1-3 from *Trametes gibbosa* at 100MHz in $CDCl_3$

Position of C	Compound 1	Compound 2	Compound 3
1	38.41	34.71	27.65
2	31.96	30.12	30.80
3	70.45	66.46	66.03
4	40.83	36.94	33.65
5	139.82	82.20	85.83
6	119.63	135.45	135.62
7	116.32	130.76	128.71
8	141.39	79.46	63.81
9	46.28	51.08	86.92
10	36.98	36.97	51.58
11	22.73	20.65	19.78
12	39.11	39.36	33.35
13	44.58	44.58	40.28
14	54.59	51.70	75.23
15	23.02	23.42	27.22
16	28.33	28.67	26.43
17	55.76	56.20	55.67
18	12.08	12.90	15.53
19	16.32	18.21	15.62
20	40.47	39.77	39.19
21	17.64	20.90	21.01
22	132.00	135.23	134.72
23	135.60	132.32	132.86
24	42.86	42.79	42.89
25	33.12	33.09	33.0
26	19.99	19.67	19.60
27	19.68	19.98	19.90
28	21.14	17.59	17.67

For determination of the total sugar content, the standard curve of glucose was obtained according to phenol-sulfuric acid method. Based on the standard curve, the total sugar content of hot water extract was obtained to be 90.43±2.1g/100 g dry weight.

The concentration of phenolics in the extracts, expressed as mg of GAEs per mg of extract, was dependent on the solvent used in the extraction, as shown in Table 3. Phenolic compounds were not observed in n-hexane extract of *T. gibbosa*. The hot water extract showed a higher phenolic content compared to the methanol extract.

Ferric reducing ability of different extracts from *T. gibbosa* is illustrated in Table 3. The antioxidant activities were expressed as mmol Fe(II) equivalent/g sample in dry weight.

The DPPH free radical scavenging activity of the extracts is shown in Table 3. The methanol extract showed the highest DPPH free radical activity (lowest EC_{50}) while the n-hexane extract demonstrated the lowest activity (highest EC_{50}). EC_{50} value of ascorbic acid (as standard) was 14.3±1.08 µg/mL.

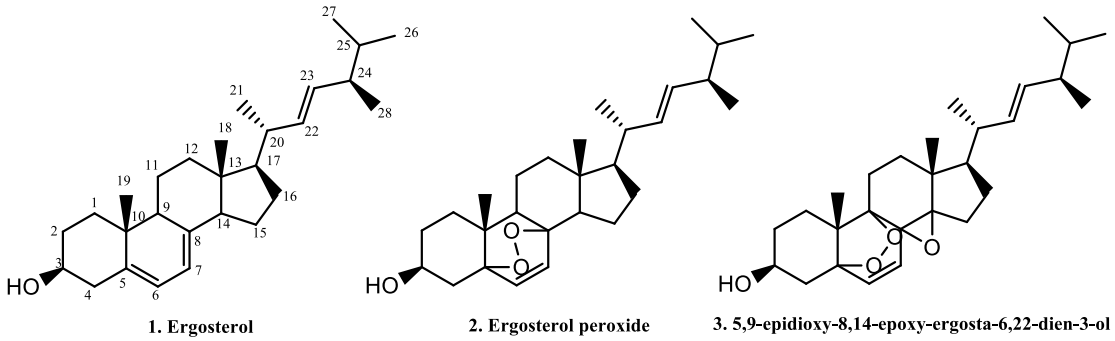


Figure 1. The chemical structures of the compounds isolated from *Trametes gibbosa*

Table 2. The volatile constituents of *Trametes gibbosa* fruiting bodies

NO.	Compounds	Reported KI	Calculated KI*	Percentage	Chemical class
1	Hexanal	808	800	9.42	Aldehyde
2	Furfural	843	836	0.34	Heteroaromatic aldehyde
3	1-Hexanol	874	867	3.00	Alcohol
4	2-Heptanone	896	888	0.75	Ketone
5	Heptanal	910	903	0.36	Aldehyde
6	Benzaldehyde	976	969	1.52	Aldehyde
7	1-Octene-3-one	985	978	0.36	Ketone
8	1-Octene-3-ol	988	980	7.92	Alcohol
9	2-Pentylfuran	995	988	11.33	Heteroaromatic
10	Octanal	1012	1000	0.65	Aldehyde
11	3-Ethyl-2-methyl-1,3-hexadiene	1042	1035	1.11	Hydrocarbon
12	Trans-2-Octenal	1069	1062	3.00	Aldehyde
13	1-Octanol	1079	1072	1.15	Alcohol
14	Nonanal	1114	1106	1.87	Aldehyde
15	Trans-2-Nonenal	1172	1163	1.04	Aldehyde
16	Decanal	1216	1207	0.50	Aldehyde
17	2,4-Trans,trans-Nonadienal	1231	1222	0.98	Aldehyde
18	2E-Decanal	1274	1265	1.16	Aldehyde
19	Nonanoic acid	1283	1274	0.68	Fatty acid
20	2-Undecanone	1301	1292	4.62	Ketone
21	2E, 4Z-Decadienal	1308	1298	0.72	Aldehyde
22	Undecanal	1317	1308	0.34	Aldehyde
23	2E, 4E-Decadienal	1333	1324	5.10	Aldehyde
24	2-Undecenal	1376	1367	1.44	Aldehyde
25	Tetradecanal	1623	1611	0.57	Aldehyde
26	Cadalene	1691	1680	0.35	Terpenoid
27	n-Pentadecanoic acid	1878	1865	8.55	Fatty acid
28	Farnesyl acetone	1915	1902	2.04	Terpenoid
29	Palmitic acid	1976	1966	11.37	Fatty acid
30	Palmitic acid ethyl ester	2000	1986	0.60	Fatty acid ethyl ester
	Total identified			82.84	

*: Kovats index

Table 3. The total phenolics content and antioxidant activity of different extracts from *Trametes gibbosa*

Samples	Total phenolics content (mg GAEs/g of dry weight)	Ferric reducing antioxidant power assay (mmol Fe^{2+} equivalent/ g dry weight)	DPPH radical scavenging assay (EC_{50} value, µg/mL)
n-Hexane extract	n. d.	208.5±17.7	2505.0±40.1
Methanol extract	44.78±1.48	317.1±32.1	588.5±36.37
Hot water extract	96.16±0.68	432.0±6.6	702.9±39.6
Ascorbic acid	-	1542.0±8.3	14.3±1.08

Each value is expressed as means ± standard deviation (n = 3); n. d.= not detected

The genus *Trametes* contains diverse chemical compounds which it them rather interesting to evaluate the related therapeutic effects and to perform pharmacological studies. The major chemical compounds such as steroids, polysaccharides, fatty acids and phenolics are detected in *Trametes* species [4,18,19].

Ergosterol is one of the major chemical components in medicinal mushroom and one of the primary compounds for synthesizing vitamin D [20].

The fungal steroids are mainly in the form of sterol-type compounds such as ergosterol, ergosterol peroxide, sitosterol and campesterol [21].

In the present study, chemical investigation of the n-hexane extract from the fruiting bodies of *T. gibossa*, led to the isolation of ergosterol and two ergosterol derivatives (5,8-epidioxy-ergosta-6,22-dien-3-ol and 5,9-epidioxy-8,14-epoxy-ergosta-6,22-dien-3-ol). The structures were identified by the analysis of the ^{1}H-NMR and ^{13}C-NMR spectra which were confirmed by the data published earlier [18, 22-24].

Recently, several investigations have been done for identification and isolation of *Trametes* chemical compounds especially their sterol constituents. Different types of ergostanes and lanostanes, the common sterols in *Trametes* species, are detected in the related genus. Based on our previous study, column chromatography of the nonpolar extracts (n-hexane, chloroform) from *T. versicolor* fruiting bodies, yielded different ergosterol derivatives including ergosterol peroxide [18]. Similarly, Borlagdan et al. isolated several sterols such as ergosterol, ergosterol peroxide and a mixture of stellasterol in the dichloromethane fraction of *T. versicolor* by chromatographic method [25]. The ergosterol peroxide, 7,22-ergostadien-3β-ol and trametenolic acid B were also detected in n-hexane fraction of *T. versicolor* [26]. In another study, Mayaka et al., detected several types of ergosterol from *T. elegans* fruiting bodies. The compounds were isolated from the ethyl acetate fraction of the mushroom and identified based on NMR spectroscopic data [27].

Considering the recent studies, ergosterol derivatives possess significant antitumor, antioxidant, anti-inflammatory [28] and immunomodulatory activities [20]. It has been reported that ergosterol peroxide inhibits TPA-induced inflammation and tumor promotion in mice [29]. Takei et. al, reported that two ergosterol peroxide (5a,8a-epidioxy-22E-ergosta-6, 22-dien-3b-ol), inhibited the growth of some cancer cells and induced apoptosis in HL60 human leukaemia cells [30]. Based on Zhang et al., ergosterol isolated from *Leucocalocybe mongolica* mushroom, decreased the growth of HeLa, MCF-7 and HepG2 cell lines via increasing BAX protein expression and a decrease in VEGF and Bcl-2 protein expression [31].

It has been reported that the isolated ergosterol from *Grifola frondosa* exhibited antioxidant activity and inhibited the cyclooxygenase enzymes COX-1 and COX-2 [32]. Based on Mei et al., sterols isolated from the fruiting bodies of *Ganoderma sinense* exhibited inhibitory activities against NO production [33].

It has been demonstrated that two sterols from *Leucocalocybe mongolica* mushroom could effectively enhanced the immunity in H22 tumor infected mice via increasing the serum cytokines including TNF-α, IFN-γ, IL-2 and IL-6 [20].

Based on the GC-MS results of the present study, a range of volatile compounds, including aldehydes, alcohols, ketones, terpenoids and fatty acids were found in *T. gibbosa* fruiting bodies. The alcoholic compound, 1-octene-3-ol, was detected dominantly in the essential oil composition of this mushroom. This compound was also found in high proportion in *T. gibbosa* fruiting bodies that were collected from Gottingen, Germany, some oyster mushrooms and other fungal species such as *Schizophyllum commune* and *Phlebia radiate* [34].

The substantial component of mushroom volatiles includes a series of C_8 compounds, especially 1-octen-3-ol which is one of the important factors in mushroom flavor. This compound could be considered as biologically active with considerable antioxidant activity [35].

In the present study, a numbers of acidic compounds were also detected in a large amount in the volatiles of *T. gibbosa* which was consistent with the result of Ma et al., whom investigated the volatile oil compositions of *Trametes suaveolens* with the same method [36].

The present results indicated that the hot water extract from *T. gibbosa* fruiting bodies possess high carbohydrate content. Edible mushrooms contain considerable amount of carbohydrate

constituents which are mainly in the form of polysaccharides. These compounds have been widely used in medicine due to their bioactivities especially the anticancer and immunomodulatory properties [37].

In the present study, the spectrophotometric analysis of different extract from *T. gibbosa* fruiting bodies, revealed the high content of phenolics in the hot water extract with the total amount of 96.16±0.68 mg gallic acid equivalents per gram of dried extract. Moreover, this extract showed more considerable reducing activity (FRAP), but it was observed that the radical scavenging activity (DPPH) was higher in methanol the extract. Based on the recent findings, antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently due to steric hindrance [38]. Similarly, Gan et. al, reported that there was a positive correlation between the presence of phenolics content and antioxidant activity in *Agaricus* mushroom different extracts (aqueous and ethanol). They also observed that the ethanol extract showed the lowest EC_{50} value [39]. This was also in accordance with the result of Cheung et al., who investigated the antioxidant activity and total phenolics of edible mushroom extracts of *Lentinus edodes* and *Volvariella volvacea* [40].

Conclusion

The study was the first attempt to isolate the chemical compounds from *T. gibbosa* fruiting bodies. Based on the results, *T. gibbosa* mushroom is a valuable source of mycochemicals such as sterols, carbohydrate and phenolics. Further investigations are required for evaluation of the therapeutic potentials of the isolated compounds.

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Author contributions

Emran Habibi and Ahmadreza Bekhradnia designed and supervised the study; Fatemeh Mirzaee prepared the manuscript; Hossein Bakhshi Jouybari performed the experiments and analyzed the data; Mohammad Hossein Hosseinzadeh performed the experiments.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

EtOAc: ethyl acetate; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TPTZ: 2, 4, 6-tripyridyl-s-triazine; TLC: thin layer chromatography; UV: ultra violet; GC-MS: gas chromatography- mass spectrometry; EC_{50}: half maximal effective concentration