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Estimation of total phenolic, flavonoid contents and free radical scavenging activity of a wild macrofungus, *Lenzites quercina* (L.) P. Karsten

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Abstract

Extracts obtained from raw and fermented *Lenzites quercina* were assessed for total phenol, flavonoid contents and antioxidant properties. The scavenging ability of *Lenzites quercina* extracts was tested against free radicals namely; 1, 1-diphenyl-2 picrylhydrazyl (DPPH), hydroxyl (OH-), nitric oxide (NO) and iron (Fe2+). Ethyl acetate extract from fermented *Lenzites quercina* (FEA) possessed higher phenolic content of 67.6 mg Gallic Acid Equivalent (GAE)/g extract, while an extract of ethyl acetate from raw *Lenzites quercina* (REA) have the highest flavonoid of 51.4 mg Quercetin Equivalent (QE)/g extract. Antioxidant property measured by FeCl3 reducing power was within 18.1 to 127.6 mg Ascorbic Acid Equivalent (AAE)/g extract for the extracts obtained from the raw and fermented *Lenzites quercina*. The scavenging properties of FEA were well pronounced against nitric oxide and ferrous ion radicals. FEA also exhibited better inhibition on thiobarbituric acid reactive species (TBARS) with highest inhibitory effect of 109.3%. Some of the extracts displayed inhibition concentration (IC50) that are lower than the positive control butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA). The results from this study suggest that the high total phenol and flavonoid found in *Lenzites quercina* extracts could make it serve as a good antioxidant agent; this may be exploited as an alternative therapy in health care delivery.

Key words – antioxidants – fermentation – medicinal mushroom – nutraceuticals

Introduction

Free radicals are molecular fragments that contain one or more unpaired electrons on their outermost atomic or molecular orbital and therefore, exist as unstable molecules (Halliwell & Gutteridge 2007). Free radicals are formed in the human body during cellular metabolisms and produced as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Sen et al. (2010) had attributed the production of free radicals in the body system to modern civilization, toxicity of different chemicals, pesticides, ionizing radiation, pollutants, cigarette smoke, UV light and intake of some synthetic medicines. Effects of free radical on proteins, nucleic acids, carbohydrates and membrane lipids have led to degenerative diseases (Ferreira et al. 2009). Excessive production of

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free radicals in the body and decrease in antioxidant level create oxidative stress, which affect macromolecules, damage of cellular components, disruption of homeostasis and thus, lead to disorder in physiological effectiveness (Valko et al. 2007).

Cells in the human body possess some enzymes like superoxide dismutase, catalase and glutathione peroxidase that inhibit reactive oxygen species and maintain redox homeostasis in the cells by scavenging free radicals (Halliwell & Gutteridge 2007, Valko et al. 2007). This mechanism of antioxidant in the body may become imbalance as a result of endogenous production; hence, antioxidant supplements could be used to reduce the damage caused by free radicals and thus, prevent injuries to cells in the body (Halliwell 1996). Antioxidants possess the ability to scavenge free radicals by inhibiting the initiation step of lipid oxidation and slowing the rate of oxidation. It also reduces the risk of chronic diseases that are caused as result of free radicals generated (Lobo et al. 2010). The synthetic antioxidant drugs cause various disorders and some undesirable side effects during the scavenging activity on free radicals. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) exhibit potent free radical scavenging effects but also exert toxicological, mutagenic effects, carcinogenic effects, apart from their inconsiderable cost of production (Saito et al. 2003). This has brought an upsurge in the search for and identification of effective antioxidant compounds from vegetables, fruits and medicinal mushrooms that are pharmacologically potent in scavenging free radicals with no side effects.

Wild macrofungi utilize diverse substrates such as lignin, cellulose, pectin and others by the use of degrading enzymes. This, therefore, enables them to produce wide range of secondary metabolites such as alkaloids, tannins, polyketides, terpenes, steroids and flavonoids. Hence, wild medicinal macrofungi have gained more importance due to the larger array of biological actions (DeSilva et al. 2013). A large number of common species of wild medicinal macrofungi that are prolific resources in drug production resides in Nigeria and most of them are grossly under-studied and utilized for both medicinal and commercial benefits. Oyetayo (2011) supported this fact that the ethnomedicinal uses of some of these wild macrofungi had been hushed up by traditional herbalists to acquire more consultancies. Therefore, many of these wild macrofungi have not been adequately utilized for medicinal purposes due to the dearth of information and as a result of its non-palatability. One of the underutilized macrofungi is a *L. quercina*, which had been reported to possess antimicrobial properties (Ogidi et al. 2015), but little is known about its antioxidant activity. This study was, therefore undertaken to assess the phenolic and flavonoid contents as well as the free radical scavenging properties of extracts of obtained from raw (unfermented) and fermented *L. quercina*.

Materials & Methods

Chemicals Used

Folin-Ciocalteu's-reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, ascorbic acid; butylated hydroxytoluene (BHT), gallic acid, quercetin were products of Sigma-Aldrich (Steinheim, Germany); 2-thiobarbituric acid (TBA), sodium acetate trihydrate, acetic acid, hydrogen peroxide (H2O2) were from Acros Organics (Geel, Belgium); ethylenediaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were from Amresco (Ohio, USA). All the reagents used during this study were of analytical grade.

Collection of Macrofungus and its Identification

Sample of macrofungus was collected from farmland, a nearby forest at The Federal University of Technology Akure (FUTA), Nigeria (Lat 07^{o} 14^{l}N Long 05^{o} 11IE). The sample was collected in November, 2012. The sample of the fruit body was morphologically identified and confirmed by molecular tools using the internal transcribed spacer (ITS) region of the rDNA (Ogidi et al. 2017). Voucher specimen was deposited in the Department of Microbiology, FUTA.

Preparation of *Lenzites quercina* extracts

Collected samples of *L. quercina* were divided into two portions after being dried; raw and the other portion was solidly-fermented for four days at room temperature (26±2 ^{o}C) and later dried in an oven (DHG9053-A) at 28^{o}C. The dried samples were ground into powder using a mill machine (Retsch GmbH 5657 HAAN). The raw and fermented powder of *L. quercina* were sequentially extracted with different solvents of the same volume and concentration (500 ml of 95% v/v) of petroleum ether, followed by ethyl acetate and ethanol. Ground samples (100 g each) of raw and fermented mushrooms were soaked for 72 hours and filtered with Whatman filter paper No 1. The filtrates obtained were concentrated in rotary evaporator (RE-52A, UNION Laboratories, England), lyophilized and maintained in refrigerator at 4^{o}C. The extracts obtained were coded as RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol. The studied extracts of *L. quercina* were reconstituted in the (0.1 % v/v) dimethyl sulfoxide (DMSO) to obtain varying concentration of 0.125 mg/ml, 0.250 mg/ml, 0.500 mg/ml, 1.000 and 2.000 mg/ml.

Determination of Total Phenol Content

The total phenolic content of extracts of *L. quercina* was determined according to the method described by Singleton et al. (1999) with little modification. Briefly, each extract (0.5 ml) was taken in a 10 ml glass tube and 2.5 ml of 10% Folin -Ciocalteu's reagent was added. After 2 min, 2 ml of 7.5% sodium carbonate was added. The preparation above was incubated for 1 hour at 25 ^{o}C and the absorbance was measured at 765 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents (mg Gallic Acid Equivalent (GAE) /g extract).

Determination of Total Flavonoid Content

The total flavonoid content of the mushroom extracts was determined using the method of Meda et al. (2005). The volume (0.5 ml) of samples (extract/quercetin) was mixed with 0.5 ml of methanol, 0.1 ml of 10% aluminium trichloride (AlCl3), 0.5 ml of potassium acetate (1.0 M) and 1.4 ml of distilled water and incubated at room temperature (27±1 ^{o}C) for 30 min. The absorbance was measured at 415 nm in a spectrophotometer. Quercetin was used as a positive control. The concentration of total flavonoid content was calculated as equivalent of quercetin (mg Quercetin (QE)/g extract).

Determination of Reducing Property

The reducing power of the extracts of *L. quercina* was determined by assessing the ability of the extracts to reduce FeCl3 solution as described by Oyaizu (1986). Each extract (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 min. After that, 2.5 ml 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. The supernatants were collected and mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. Ascorbic acid at various concentrations was used as the standard and reducing property of extracts was expressed in mg Ascorbic Acid Equivalent (AAE) /g of extract.

DPPH Scavenging Effect

The free radical scavenging ability of the extracts on DPPH (1, 1- diphenyl-2-picryhydrazyl) was determined using the methods of Gyamfi et al. (1999). Extract (0.1 ml) of varying concentration was mixed with 1.0 ml of 0.4 mM DPPH prepared in methanol. The mixture was incubated at room temperature for 30 min in dark. The control contained only DPPH solution in methanol instead of sample, while methanol served as the blank. Absorbance was noted at 517 nm

by using a UV-visible spectrophotometer (Buck 201 VGP). The capacity of free radical scavenging was calculated as:

$$\text{Scavenging activity (\%)} = [Ab - (As-Abs)]/ Ab \times 100$$

Where Ab is absorbance of blank, Abs is absorbance of sample blank and As is absorbance of the sample

Scavenging Ability on Hydroxyl Radicals

The ability of *L. quercina* extracts to prevent Fe2+/H2O2 induced decomposition of deoxyribose was assessed using the method of Halliwell (1987). Aliquot of each extracts (0.1 ml) at varying concentrations was added to a solution containing 120 µl of 20 mM deoxyribose, 400 µl of 0.1 M phosphate buffer (pH 7.4), 40 µl of 20 mM hydrogen peroxide and 40 µl of 500 µM FeSO4. The volume was made up to 800 µl with distilled water. The reaction mixture was incubated at 37OC for 30min and the reaction was stopped by the addition of 0.5 ml of 2.8% Trichloro acetic acid (TCA). This was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid solution (TBA). The mixture in tube was boiled for 20 min, cooled in ice and absorbance measured at 532 nm in spectrophotometer. Butylated hydroxytoluene (BHT) was used as a positive control. In blank, distilled water was used instead of extracts or BHT and the sample without adding deoxyribose served as sample blank.

$$\text{Scavenging activity (\%)} = [Ab - (As-Abs)]/ Ab \times 100.$$

Where Ab is absorbance of blank, Abs is absorbance of sample blank and As is absorbance of the sample at 532 nm.

Nitric Oxide Radical Scavenging Assay

Nitric oxide scavenging activity of the mushroom extracts was performed according to the method described by Jagetia & Baliga (2004) with little modification. A volume of 1.0 ml of sodium nitroprusside (10 mM) prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with different concentrations of extract (100 µl) and vortexed. The mixture was incubated at 25oC for 150 min. Thereafter, 1.0 mL of previous solution was mixed with 1.0 ml of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthylethylenediamine hydrochloride) and incubated at room temperature for 30 min. The absorbance was recorded at 546 nm. Scavenging activity was calculated using the following formula:

$$\text{NO Scavenging activity (\%)} = [(Ab - Abs)/ Ab] \times 100$$

Where Ab is absorbance of blank and Abs is absorbance of reacting mixture with the tested samples. Half minimum (50%) inhibitory concentration (IC50) was calculated for each extract from the curve of the percentage inhibition against the concentrations (mg/ml) of the mushroom extracts.

Iron Chelation Assay

The ability of the extracts to chelate iron (II) sulphate (FeSO4) was determined using methods of Puntel et al. (2005) with little modification. Briefly, 150 mM FeSO4 was added to a reaction mixture containing 168 µl of 0.1M Tris-HCl pH 7.4, 218 µl saline (0.9% NaCl) and extract (0.1 mL) of different concentrations. The reaction mixture was incubated for 5min, before the addition of 13 µl of 0.25% (w/v) 1, 10-phenantroline and the absorbance was subsequently measured at 510 nm in the spectrophotometer. Chelating activity of extracts on iron radical was calculated using this equation:

$$\text{Chelating effect (\%)} = ((Ab - As)/Ab) \times 100$$

Where Ab is the absorbance of the blank without the sample, while As is the absorbance in the presence of extract or EDTA. Extracts concentration causing 50 % inhibition (IC50) was calculated from the graph, plotting the % inhibition against extract concentrations

Lipid Peroxidation and Thiobarbituric Acid Reactions

The mice used in this study have adequate access to food and water. The animals' weights were within 47 to 55 g. They were kept in the cage for 7 days to acclimate them to the environmental conditions at 12 h light–dark periods and temperature of 25±2 oC. The procedures used in this assays were approved by research committee, Microbiology Department in accordance with international standard of animal welfare described by the National Research Council (NRC 2011).

Thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxidation according to the method of Ohkawa et al. (1979) as described by Puntel et al. (2005). Rats were decapitated under mild ether anesthesia and the hepatic (liver) tissues were rapidly dissected, placed on ice and weighed. One gram (1.0 g) of tissues was homogenized in 10 ml of cold saline with up and down stroke in a Teflon homogenizer. The homogenates were centrifuged at 4000 rpm for 10 min to yield a pellet that was discarded and the supernatant (S) was used for the assay. The supernatant (S) 100 µl with 50 µl of freshly prepared prooxidant or without prooxidant; Iron (II) sulphate was mixed with 50 µl of 0.1 M pH 7.4 Tris–HCl buffer, different concentrations of the *L. quercina* extracts (100 µl) and appropriate volume of distilled water, which gave a total volume of 300 µl and were incubated at 37oC for 1 h. The colour reaction was carried out by adding 300 µl of 8.1% sodium dodecyl sulphate (SDS) to the solution (S), followed by sequential addition of 1.33 M acetic acid (pH 3.4) and 0.6% TBA respectively. The reaction mixtures were incubated at 95o C for 1hour. The absorbance was read after cooling at a wavelength of 532 nm in a UV-visible spectrophotometer, the TBARS produced were measured and percentage inhibition of TBARS was calculated as:

$$\text{TBARS Inhibition (\%)} = [(Ao - A)/Ao] \text{ X } 100.$$

Where Ao is absorbance of the control sample and A is absorbance in the presence of the antioxidant compounds at various concentrations. The inhibitory concentration (IC50) values were calculated from data obtained by graphically plotting the % inhibition against extract concentrations. Butylated hydroxytoluene (BHT) was used as positive control.

Statistical Analysis of Data

Each experiment was replicated three times. Data obtained were subjected to analysis of variance (ANOVA) using SPSS version 21. The means were separated using Duncan's Range Multiple Test at ($P = 0.05$).

Results and Discussion

Wild Mushroom as a Source of Antioxidant Compounds

Metabolic processes in humans are known to generate free radicals. There is, therefore, a need to create stable reactions between the free radicals and antioxidant defense mechanisms. This will bring essential conditions for the support of normal physiological functions. Antioxidant compounds had been sourced from medicinal mushrooms as a better choice and means of scavenging free radicals without negative side effects (Ferreira et al. 2009). Macrofungi had been reported to be a rich source of antioxidants such as phenol, flavonoid, vitamin C, phytosterol and caroetenoids (Barros et al. 2008). The phenolic contents of extracts obtained from raw and fermented *L. quercina* ranged from 22.3 to 67.6 mg GAE/g extract, while the flavonoid ranged from 3.98 to 51.4 mg QE/g extract (Table 1). Phenolic compounds were highly present in some studied medicinal mushrooms such as Pleurotus osteartus, Agaricus bisporus, Copinus comatus, Volvareilla volvacea, Amanita porphyria, Piptoporus betulinis, Hebeloma sinapizans and Russula emetica (Reis et al. 2013, Parihar et al. 2014). These are the medicinal mushrooms with their pharmacological importance being attributed to the accumulated secondary metabolites.

Fermentation and Phenolic contents

Ethyl acetate extract from fermented *L. quercina* possessed highest phenolic content of 67.6 mg GAE/g extract. Huynh et al. (2014) had revealed that microbial fermentation enhanced the release of bound phenolic compounds and new metabolites through the process of glycosylation, deglycosylation, ring cleavage, methylation, glucuronidation and sulfate conjugation. The phenolic and flavonoid contents obtained for the extracts of *Lenzites quercina* are more than the content in some edible mushrooms such as a white button mushroom known as Agaricus bisporus from Malaysia; a Brazilian mushroom called A. brasiliensis (Gan et al. 2013); and a white variety of Auricularia fuscosuccinea from Taiwan (Lin et al. 2013). The disparity in the phenolic content may due to the extraction methods and species of mushrooms. Kim et al. (2008) had earlier reported that the variation in the level of phenolic compounds in mushrooms is much dependent on the following factors; location, stress conditions and substrates that are involved in the production of the fruiting body. The variability in phenolic and flavonoid contents of the examined macrofungus could be due to the solubility of phytochemicals in used solvent. De Bruijn et al. (2009) had associated the extract yield, antioxidants activity, phenolic and flavonoid contents of Grifola gargal extracts to the choice of solvent.

Table 1 Phenolic and flavonoid contents of *Lenzites quercina* extracts

Extracts	Phenol (mg GAE/g extract)	Flavonoids (mg QE/g extract)
RPP	31.0 d ±1.0	41.0 b ±1.0
REA	53.0 c ±1.0	51.4 a ±0.8
RET	59.0 b ±1.0	40.0 b ±0.0
FPP	22.3 e ±0.5	15.3 d ±0.5
FEA	67.6 a ±0.5	36.6 c ±0.15
FET	30.0 d ±0.02	3.98 e ±0.52

Values are mean±S.D of replicates (n=3),
Values with different alphabets along column are significantly different from each other (P= 0.05). GAE: Gallic Acid Equivalent, QE: Quercetin, RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol.

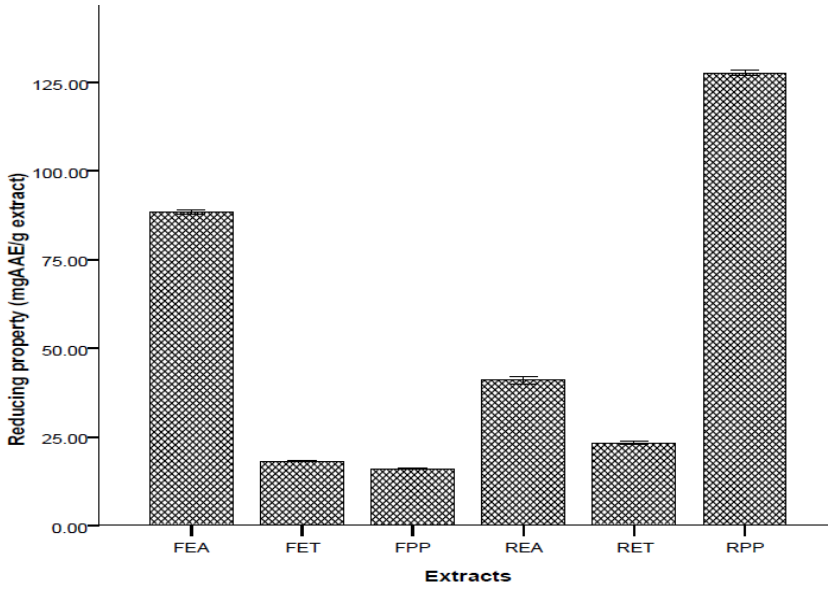

Fig. 1 – Ferric reducing property of *Lenzites quercina* extracts. RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol, BHT: Butylated hydroxytoluene

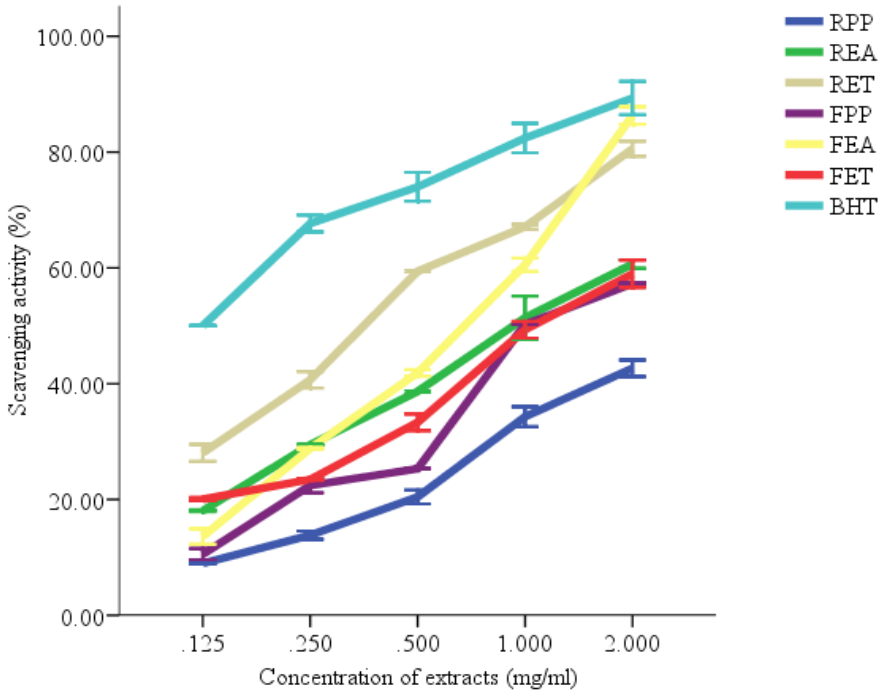

Fig. 2 – Scavenging activity of *Lenzites quercina* extracts on DPPH. RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol, BHT: Butylated hydroxytoluene

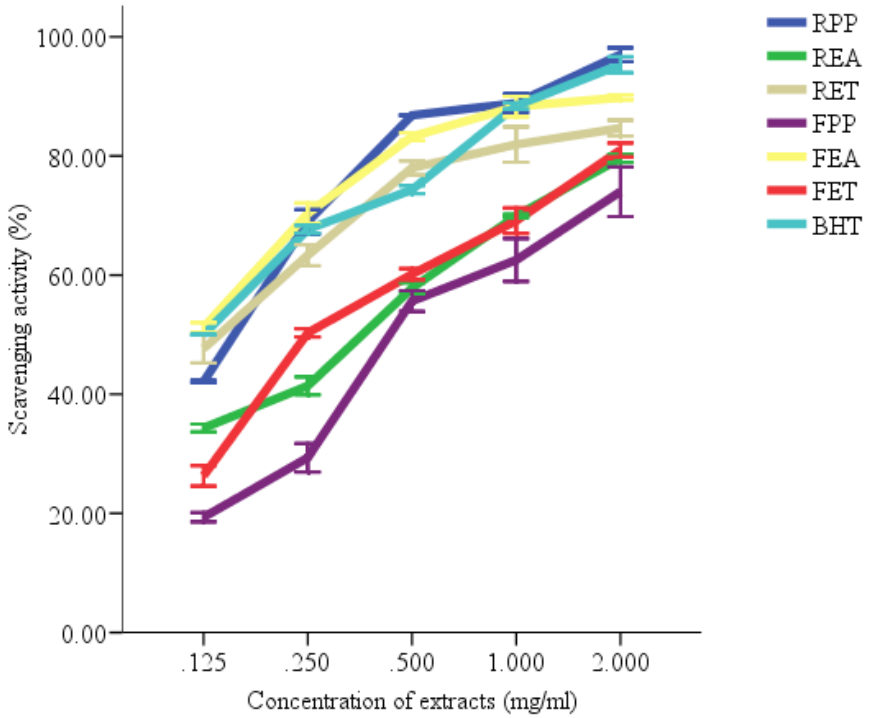

Fig. 3 – Scavenging activity of *Lenzites quercina* extracts on hydroxyl radicals. RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol, BHT: Butylated hydroxytoluene.

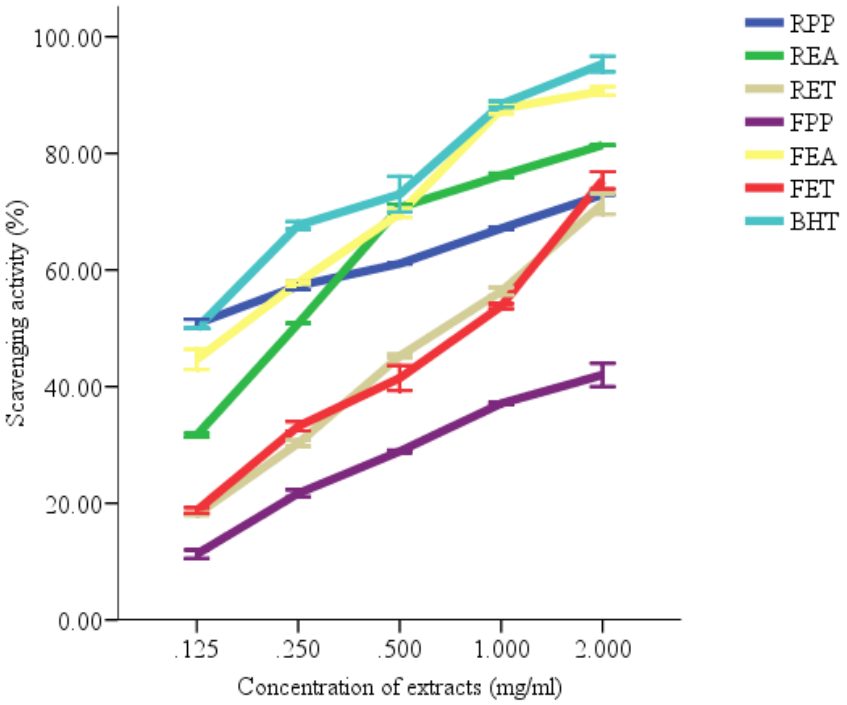

Fig. 4 – Scavenging activity of *Lenzites quercina* extracts on nitric oxides. RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol, BHT: Butylated hydroxytoluene

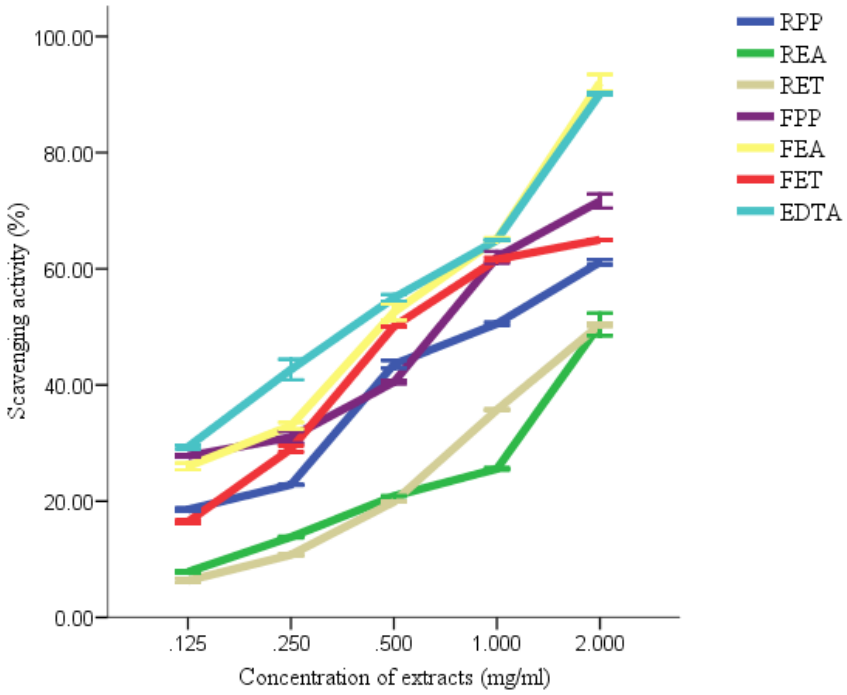

Fig. 5 – Iron II chelating effect of *Lenzites quercina* extracts. RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol, EDTA= ethylenediaminetetraacetic acid.

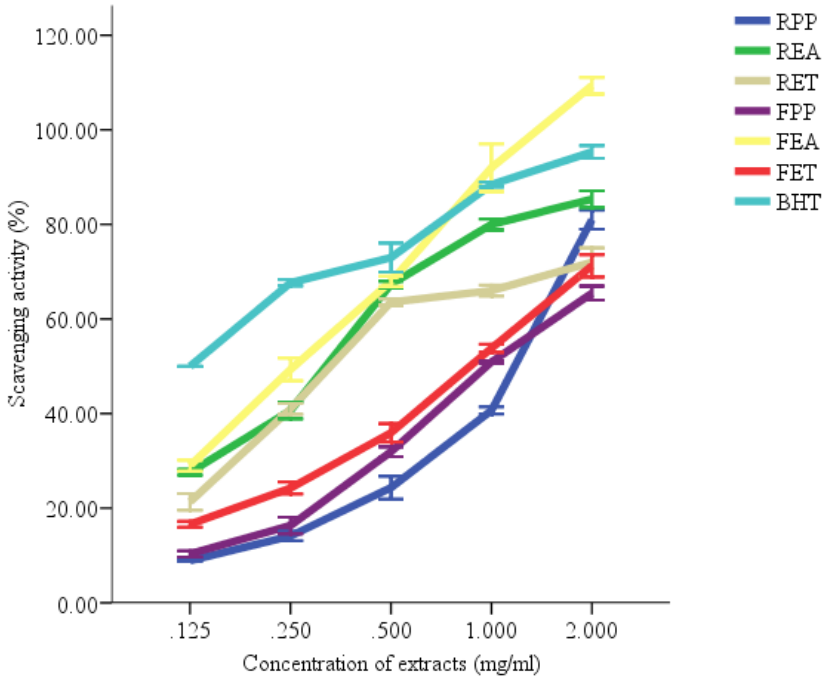

Fig. 6 – Inhibition of TBARS production by extracts of *Lenzites quercina*. RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol, BHT: Butylated hydroxytoluene.

Scavenging Activities of *L. quercina* against some free radicals

Mushrooms contain phenolic compounds that are able to transfer electrons to free radicals and thereby scavenge those free radicals. Hence, phenolic compounds have the potential to bind to metal ions due to their chemical structures and exhibit antioxidant activity through the chelation of metal ions (Zhao et al. 2008). Fig. 1 showed the ferric reducing power of *L. quercina* extracts, which ranged from 18.1 to 127.6 mg AAE/g extract. In Fig. 2, the percent scavenging activities of *L. quercina* extracts on DPPH (43.0 to 86.3%) was reported and in Table 2, its linear IC50 (0.56 to 1.80) mg/ml was lower when compared to the ethanolic extract of Ganoderma lucidum, which has IC50 of 7.03 mg/ml for DPPH (Celık et al. 2014). The lower IC50 obtained for extracts of *L. quercina* indicated better scavenging activity on DPPH radical. This observation is similar to the results obtained for the scavenging effect of Auricularia fuscosuccinea and Auricularia polytricha against DPPH radical (Lin et al. 2013).

This study shows that *L. quercina* extracts displayed good hydroxyl scavenging capacity (75-97%) as shown in Fig. 3. The fraction obtained from species of *Lenzites* displayed good scavenging ability on OH- radical generated via Fenton reaction when compared to ascorbic acid used as positive control (Liu et al. 2013). This is evidence that *Lenzites* species possess some natural antioxidant compounds; flavonoids, which are suitable for free radical-scavenging activities. The presence of functional hydroxyl groups in flavonoid mediates the antioxidant effect by scavenging free radicals and chelating metal ions, which is a crucial process in the prevention of radical generation and damage of target biomolecules (Kumar & Pandey 2013).

The percentage of scavenging activities of extracts obtained from *L. quercina* on nitric oxides at 2 mg/ml (Fig. 4) is similar to what reported for Macrolepiota mastiodea (FR.) Singer at 5mg/ml (Jose & Radhamany 2013). The scavenging activities of *L. quercina* extracts on nitric oxides were greater than what was reported for Pleurotus squarrosullus at 2 mg/ml (Pal et al. 2010). The results of iron chelating activity by extracts of raw and fermented *L. quercina* (Fig. 5) similar to the findings of Muruke (2014) who reported a higher chelating effects of 78-80% for oyster

mushrooms at a concentration of 1.5 mg/ml but deviate from Kalogeropoulos et al. (2013) who reported the iron chelating of some medicinal mushroom at 10 mg/ml. This could be due to appreciable quantity of phenolic and flavonoid contents in extracts of *L. quercina*. These phytochemicals are antioxidant compounds that terminate free radical chains (Vamanu & Nita 2013). Extracts of *L. quercina* show strong inhibition of lipid peroxidation (Fig. 6) with value ranging from 12.1 % to 125%. This supports the findings of Ferreira et al. (2009) who observed that the amount of flavonoid and phenolic compounds in an extract is responsible for chelation of redox active metal ions that are capable of catalyzing lipid peroxidation. Lipid peroxidation is a consequence of oxidative stress but flavonoids protect lipids against oxidative damage (Kumar & Pandey 2013). The phenolic compounds, polysaccharides and other low molecular compounds in medicinal mushrooms are significant indicators of their antioxidants. He et al. (2012) had reported that polysaccharides isolated from some edible mushrooms remarkably contributed to their antioxidant activity.

The scavenging potentials of *L. quercina* extracts and IC50 values of some extracts (RPP, REA, FEA, FET) towards DPPH, OH, NO, Fe radicals were lower than (< 0.56) of BHT and EDTA (Table 2) and thus, correlate with quantity of phenolic and flavonoid contents in the extracts. *L. quercina* extract (RPP) with low phenolic and flavonoid contents exhibited ferric reducing power and scavenging activity on OH radicals. This could be attributed to the presence of other phytochemicals like polyphenols, saponins, alkaloids, and steroids. These extracts possess a higher amount of saponin and steroids in our previous study (Ogidi et al. 2015). The estimated amount of phytochemicals in studied extracts of *L. quercina* signify it as a source of functional biomolecules that are capable of preventing cellular damage by trapping free radicals, reducing or breaking the bond of reactive oxygen species and chelating transition metal catalyst.

Effect of fermentation on the Scavenging Activities of *Lenzites quercina* Extracts

The fermented extracts of *L. quercina* displayed good scavenging activities like the raw extracts of *L. quercina*. This study shows that fermentation does not reduce the action of bioactive compounds for antioxidant activities. The findings of Martins et al. (2011) have stated that fermentation is a convectional technique to obtain quality extracts with potential bioactive compounds. Therefore, fermentation is a biotechnology technique that can be adopted to produce more and new metabolites from wild macrofungi.

Table 2 Inhibition Concentration (IC)50 values (mg/ml) of various extracts from *L. quercina*

Free radicals	**RPP**	**REA**	**RET**	**FPP**	**FEA**	**FET**	**BHT**	**EDTA**
DPPH	1.80	1.27	0.56	1.46	0.88	1.39	0.56	
OH-	0.43	0.38	0.57	0.84	0.90	0.46	0.46	
NO	0.34	0.21	0.90	1.80	0.12	0.97	0.42	
Fe2+	1.27	1.73	1.05	0.91	0.66	1.00	-	0.56
TBARS	1.20	0.40	0.64	1.29	0.27	1.10	0.27	

Values are mean of replicates (n=3)

DPPH: 1, 1-diphenyl-2-picrylhydrazyl radical, OH: hydroxyl radical, NO: nitric oxide radical, Fe2+: iron radical, TBARS: Thiobarbituric Acid Reactive Species, RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, FET: fermented *L. quercina* extracted with ethanol, BHT: Butylated hydroxytoluene, EDTA= ethylenediaminetetraacetic acid.

Conclusion

The extracts obtained from raw and fermented *L. quercina* scavenged free radicals. The antioxidant activity of bioactive extracts from studied macrofungus could be attributed to the presence of useful metabolites such as polyphenols, saponin, alkaloids and steriods in medicinal mushroom. Therefore, the bioactive compounds in the extracts of wild medicinal mushrooms could make them a good source of antioxidant compounds to prevent degenerative diseases such as cardiovascular illnesses, neurodegenerative disorders, rheumatoid arthritis and cancer that had been attributed to generated free radicals in the body.

Conflict of interest: None

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