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Assessment of Free Radical Scavenging Potentials of Extracts of *Trametes lactinea* Collected from Akure

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ABSTRACT

The total phenolic and flavonoid contents, as well as the *in vitro* radical scavenging potentials of extracts of *Trametes lactinea* collected from forest near the Teaching and Research farm of the Federal University of Technology, Akure were assessed. The acetone extract possessed higher total phenolic content (3.21 mg GAE g^{-1}), whereas, the methanol extract had higher total flavonoid content (1.46 mg RE g^{-1}). All the extracts were able to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), hydrogen peroxide (H_2O_2) and hydroxyl (OH) radicals. At a concentration of 2 mg L^{-1} the extracts produced activities greater than 50%. The observed result indicates that this wild macrofungus, *Trametes lactinea* could be a valuable source of natural antioxidants that can protect against free radicals mediated damages.

Key words: Antioxidants, radicals, phenolics, flavonoid, macrofungus

INTRODUCTION

Free radicals are unstable molecules that can get stabilized by reacting with structural and functional cell components including cellular lipids, proteins and DNA, affecting normal function and leading to various detrimental effects in the long term (Fang *et al*., 2002; Thetsrimuang *et al*., 2011). These detrimental effects to cells and tissues are recognized as one of the major underlying mechanistic bases of aging and development of pathologies such as diabetes, cardiovascular diseases, neurodegenerative diseases, Alzheimer's disease and cancers (Alfadda and Sallam, 2012). In a normal healthy human body, the generation of free radicals in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are effectively kept in check by the various levels of antioxidant defence mechanisms such as superoxide dismutase (SOD), catalase, glutathione, glutathione peroxidases and reductase, vitamin E (tocopherols and tocotrienols) and vitamin C, apart from many dietary components (Devasagayam *et al*., 2004). However, when the body gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, cigarette smoking, ultraviolet rays, radiation, toxic chemicals, over-nutrition and advanced glycation end products (AGEs) in diabetes, this delicately maintained balance is shifted in favour of these reactive species resulting in 'Oxidative stress'. This has been implicated in the etiology of several of human diseases and in the process of ageing (Devasagayam *et al*., 2004).

A way of preventing the damaging effect of free radicals is by improving the body antioxidant status (Lobo *et al*., 2010). An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. They function by terminating the oxidation chain reactions through the removal of free

radical intermediates and inhibition of other oxidation reactions (Halliwell, 2012). The use of synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) due to their toxicity and carcinogenicity (Lobo *et al*., 2010), has raised the interest of both the clinical and scientific communities to explore natural sources for antioxidants.

Wild macrofungi are less intensively investigated and are attracting attention in the global exploration of natural sources for novel bioactive compounds. Pharmaceutical industries have explored several fungal sources for novel bioactive metabolites and this has led to the production of some the most successful drugs (Cragg and Newman, 2005). Macrofungi have been reported to be prolific producers of a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids (Turkoglu *et al*., 2007; Wasser, 2011).

Several studies carried out on wild mushrooms revealed potent antioxidant properties due to their components such as phenolics, vitamic C, tocopherols which are potent antioxidant compounds (Reis *et al.,* 2011; Akata *et al*., 2012). Phenolics are secondary metabolites of plants and macrofungi that have received several attentions in recent years due to their antioxidant capacity (Oboh *et al*., 2013; Yildiz *et al*., 2015). The antioxidant activities of phenolics have been attributed to their chemical structure (Scalbert and Williamson, 2000). The present study is aimed at evaluating the total phenolic content, total flavonoid content and the free radical scavenging potential of extracts of a wild macrofungus, *Trametes lactinea* collected from the forest around the Teaching and research farm of the Federal University of Technology, Akure.

MATERIALS AND METHODS

Collection of macrofungus: Fresh fruit bodies of macrofungus suspected to be *Trametes* species were collected from rotten woods of *Gmelina arborea* in forest near the Teaching and Research farm of the Federal University of Technology, Akure (FUTA), Ondo State (Latitude: 7.3064 N, Longitude: 5.12227 E) in the month of June 2013. The fruit bodies were kept dry by wrapping in tissue paper and kept in a polythene paper containing silica gel. The polythene bags containing the samples were well labelled for easy identification and taken to the Department of Microbiology Laboratory of the Federal University of Technology, Akure for further examination. The molecular identification of the macrofungus was carried out at the Key Laboratory of Mycology and Lichenology, Institute of Microbiology, Beijing, by amplifying and sequencing the Internal Transcribed Spacer (ITS 4 and ITS 5) of the nuclear ribosomal DNA (nrDNA). Basic Local Alignment Search Tool (BLAST) analysis revealed that the macrofungus was 99% closely related to *Trametes lactinea* from the National Center for Biotechnology Information (NCBI) GenBank.

Preparation of mushrooms extracts: The powdered mushroom sample (100 g) was extracted with 2000 mL of 95% acetone and methanol separately in an Erlenmeyer flask. The flasks were covered with aluminium foil and allowed to stand for 3 days for extraction with occasional stirring. The extracts were then filtered through Whatman filter paper (0.45 μm) using vacuum pump. The filtrates were evaporated to dryness at 50°C in a rotary evaporator (RE-52A; Union Laboratory, England) at 90 rpm under reduced pressure. The obtained concentrated extracts were stored in dark at 4°C until further analysis.

Determination of total phenolic content: The total phenolic content of the extracts was determined by the method of Singleton *et al*. (1999). About 0.2 mL of each of the extracts was mixed

with 2.5 mL of 10% Folin ciocalteau's reagent and 2 mL of 7.5% sodium carbonate (Na_2CO_3). The reaction mixture was incubated at 45°C for 40 min and the absorbance was measured at 700 nm with a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). A calibration curve was plotted for the standard of gallic acid. Total phenolic content was expressed as milligram gallic acid equivalents per gram of dried extract (mg GAE g^{-1}) using the linear equation obtained from standard gallic acid calibration curve.

Determination of total flavonoid content: The total flavonoid content of the extracts was determined using a colorimeter assay developed by Bao *et al*. (2005). The extract (0.2 mL) was added to 0.3 mL of 5% sodium nitrate ($NaNO_3$) at zero time. After 5 min, 0.6 mL of 10% aluminium chloride ($AlCl_3$) was added and after 6 min, 2 mL of 1 M NaOH (sodium hydroxide) was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read using a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) at 510 nm against the reagent blank and the calibration curve was prepared by using rutin methanolic solutions at concentrations of 12.5-100 μg mL^{-1}. Total flavonoid was expressed as mg rutin equivalents per gram of dried extract (mg RE g^{-1}).

2, 2-diphenyl-1-picrylhydrazyl DPPH scavenging activity: The DPPH radical scavenging ability of the extracts was determined by the method of Gyamfi *et al*. (1999), with slight modification (Awah *et al*., 2010). Briefly, a 2.0 mL solution of the extract at different concentrations diluted two-fold in methanol was mixed with 1.0 mL of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 mL) and 1.0 mL of methanol while the negative control was 1.0 mL of 0.3 mM DPPH solution plus 2.0 mL of methanol. Butylatedhydroxytoluene (BHT) was used as positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with a UV-visible spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). Test was carried out in triplicate. DPPH radical inhibition was calculated using the equation:

$$\text{Inhibition}\,(\%) = \frac{\text{Abs control} - (\text{Abs sample} - \text{Abs blank})}{\text{Abs control}} \times 100$$

where, the Abs control is the absorbance of the control (DPPH without sample), the Abs sample is the absorbance of the test sample (the sample test and DPPH solution) and the Abs blank is the absorbance of the sample blank (Sample without the DPPH solution).

Nitric oxide scavenging activity: The method of Ebrahimzadeh *et al*. (2008) was used to determine the antiradical activity of the mushroom extracts against nitric oxide radical. A volume of 2 mL of sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of mushroom extract/butylatedhydroxytoluene (BHT) at various concentrations (0.5-2.0 mg mL^{-1}). The mixture was incubated at 25°C for 150 min. An aliquot of 0.5 mL of the solution was added to 0.5 mL of Griess reagents [(1.0 mL of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthyethylenediamine chloride (0.1% w/v)]. The mixture was incubated at room temperature (26±2°C) for 30 min. The

absorbance was then measured at 540 nm with a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). The amount of nitric oxide radical scavenging ability was calculated using the equation:

$$\text{NO radical scavening activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

where, Abs control is the absorbance of NO radical+methanol, Abs sample is the absorbance of NO radical+sample extract or standard.

Scavenging effect on hydroxyl radical: The ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared extract (0-100 μL) was added to a reaction mixture 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 and the volume was made to 800 μL with distilled water. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 0.5 mL of 2.8% trichloroacetic (TCA). This was then followed by the addition of 0.4 mL of 0.6% thiobarbituric acid (TBA) solution. The tubes were incubated in boiling water for 20 min and the absorbance measured at 532 nm with a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). The amount of hydroxyl radical scavenging ability was calculated using the equation:

$$\text{Hydroxyl radical scavening activity (\%)} = \frac{\text{Abs reference} - \text{Abs sample}}{\text{Abs references}} \times 100$$

Where:
Abs reference = Absorbance of the reference (reacting mixture without the test sample)
Abs sample = Absorbance of reacting mixture with the test sample

Hydrogen peroxide scavenging assay: Hydrogen peroxide scavenging potential of the mushrooms extract was determined using the method described by Jayaprakasha *et al*. (2004). A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Different concentrations of the extract (0.5-2 mg mL^{-1}) in ethanol (1 mL) were added to 2 mL of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 230 nm against a blank solution that contained hydrogen peroxide solution without the extract. The percentage of H_2O_2 scavenging of the mushroom extract was calculated as follows:

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{\text{Abs reference} - \text{Abs sample}}{\text{Abs reference}} \times 100$$

Where:
Abs reference = Absorbance of the reference (reacting mixture without the test sample)
Abs sample = Absorbance of reacting mixture with the test sample

Statistical analysis: All experiments were carried out in triplicates. Data obtained were analyzed by One way analysis of variance (ANOVA) and means were compared by new Duncan's Multiple Range Test (SPSS version 16). Differences were considered significant at p = 0.05.

RESULTS

The total phenol and total flavonoid contents of the extracts is displayed in Table 1. The total phenolic content was seen to be higher in the acetone extract (3.21 mg GAE g^{-1}), whereas the total flavonoid content was higher in the methanol extract (1.46 mg RE g^{-1}).

Figure 1 shows the scavenging effects of different concentrations (0.5-2.0 mg mL^{-1}) of acetone and methanol extracts of *Trametes lactinea* on DPPH radical. The extracts produced moderate activity which was concentration dependent (0.5-2.0 mg mL^{-1}) and steadily increased with increasing concentration. The acetone extract displayed better scavenging effect than the methanol extract at all given concentrations. However, the positive control (BHT) displayed better radical scavenging effect than the extracts.

Figure 2 depicts the nitric oxide radical scavenging activities of acetone and methanol extracts of *Trametes lactinea* at varying concentrations (0.5-2.0 mg mL^{-1}). The extracts produced activities below 50% at concentration of 0.5-1.5 mg mL^{-1}. However, at a concentration of 2 mg mL^{-1} their activity was above 50%, with the methanol extract producing slightly better scavenging effect (59.59%). In comparison, the positive control (BHT) produced better activity DPPH radical effect (above 90%) than the extracts at all tested concentrations.

Figure 3 shows the hydroxyl radical scavenging effect of the extracts of *Trametes lactinea* at varying concentrations (0.5-2.0 mg mL^{-1}). The radical scavenging effect of the extracts was dose dependent. The acetone extract displayed the highest activity of 69.47% at a concentration of 2 mg mL^{-1} in comparison, the positive (BHT) had a better hydroxyl radical scavenging effect at all tested concentrations.

Table 1: Total phenolic and flavonoid contents of *Trametes lactinea* extracts

Solvents	Total phenolic (mg GAE g^{-1})	Total flavonoid (mg RE g^{-1})
Acetone	3.21±0.00	0.97±0.00
Methanol	3.09±0.02	1.46±0.00

Each value is expressed as Mean±standard error (n = 3), GAE: Gallic acid equivalent and RE: Rutin equivalent

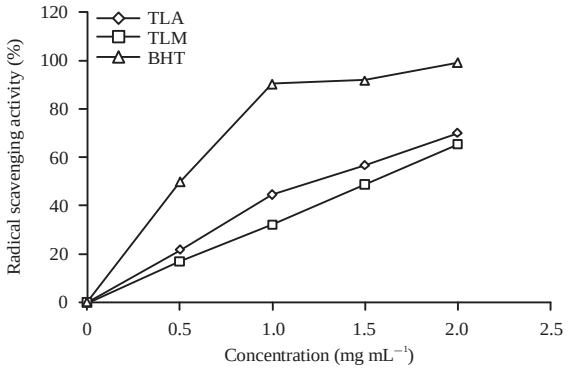

Fig. 1: Scavenging effect of *Trametes lactinea* extracts on DPPH radicals, Each value is expressed as mean of triplicate results (n = 3), TLA: Acetone extract of *Trametes lactinea*, TLM: Methanol extract of *Trametes lactinea* and BHT: Butylatedhydroxytoluene

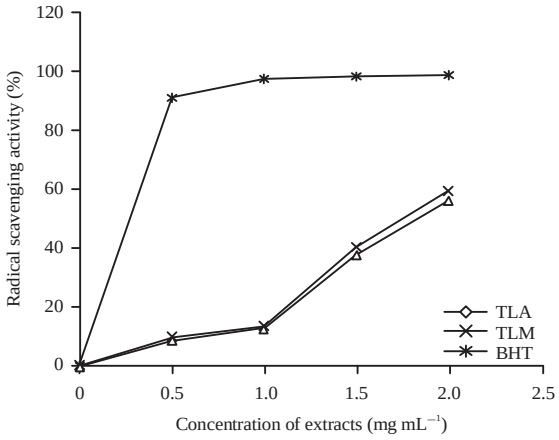

Fig. 2: Scavenging effect of *Trametes lactinea* extracts on nitric oxide radicals. Each value is expressed as mean of triplicate results (n = 3), TLA: Acetone extract of *Trametes lactinea*, TLM: Methanol extract of *Trametes lactinea* and BHT: Butylatedhydroxytoluene

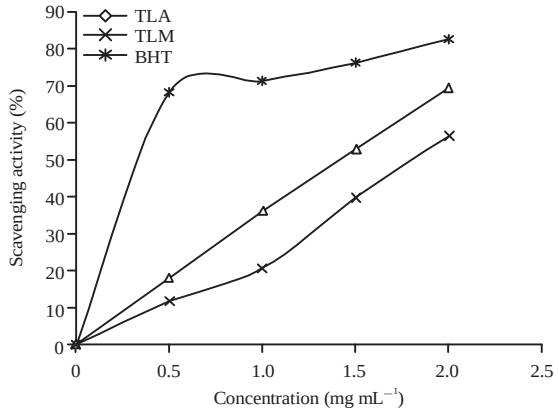

Fig. 3: Scavenging effect of *Trametes lactinea* extracts on hydroxyl radicals. Each value is expressed as mean of triplicate results (n = 3), TLA: Acetone extract of *Trametes lactinea*, TLM: Methanol extract of *Trametes lactinea* and BHT: Butylatedhydroxytoluene

Figure 4 illustrates the scavenging effects of different concentrations (0.5-2.0 mg mL^{-1}) of acetone and methanol extracts of *Trametes lactinea* on hydrogen peroxide radical. The scavenging effects of the extracts steadily increased with increasing concentration while that of the positive control (BHT) was not dose dependent. The acetone extract exhibited better scavenging effect at all test concentrations. However, the positive control displayed higher radical scavenging effect than the extracts.

DISCUSSION

Several wild macrofungi have been reported to be potential sources of natural antioxidants (Keles *et al*., 2011; Yildiz *et al*., 2015). The present study reports the free radical scavenging potentials of extracts of *Trametes lactinea* collected from the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria.

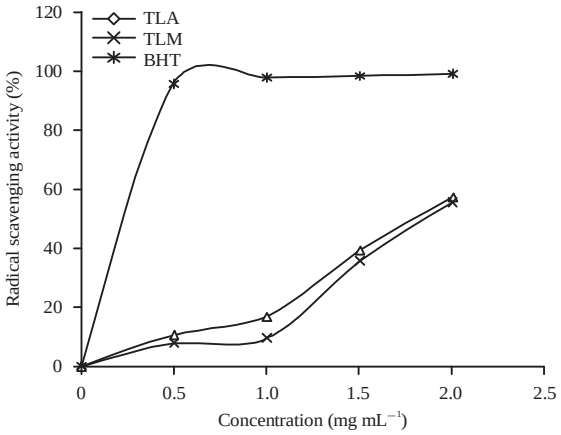

Fig. 4: Scavenging effect of *Trametes lactinea* extracts on hydrogen peroxide radicals. Each value is expressed as mean of triplicate results (n = 3), TLA: Acetone extract of *Trametes lactinea*, TLM: Methanol extract of *Trametes lactinea* and BHT: Butylatedhydroxytoluene

The acetone and methanol extracts of *T. lactinea* displayed varying total phenol and total flavonoid content. This could be due to the difference in the extracting solvents used. The observed result is in line with the findings of Nadhiya and Vijayalakshmi (2014), who reported variation in the amount of total phenolic and flavonoids contents of the ethanol, aqueous and ethylacetate extracts of *Benincasa hispida* fruit. It is known that the yield of chemical extraction is affected by factors such as; type of solvents, polarity of solvent, pH, extraction time and temperature, as well as on the chemical compositions of the sample (Hayouni *et al*., 2007; Brahmi *et al*., 2012).

Reports have shown phenolic compounds which are well known secondary metabolites found in plants and mushrooms, to have vital pharmacological functions including antioxidant activities (Oboh *et al*., 2013; Yildiz *et al*., 2015). They are known to exhibit strong antioxidant activities, through the removal of free radicals, chelation of metal ions, activation of antioxidant enzymes, reduction of *α*-tocopherol radicals and inhibition of oxidases (Amic *et al*., 2003).

Flavonoids are hydroxylated phenolics and are potent water-soluble antioxidants. They have been reported to possess strong antioxidant activities and capable of lowering cellular oxidative stress (Loots *et al*., 2007; Oboh *et al*., 2007). Their strong antioxidant activity has been attributed to the redox properties of their hydroxyl groups (Ozgen *et al*., 2010). The presence of phenolic compounds in extracts of *Trametes lactinea* suggest that this macrofungus could be valuable in the treatment of radical related and age associated disease.

The extracts of *Trametes lactinea* were capable of scavenging DPPH radicals effectively (Fig. 1). The scavenging activities of several wild mushrooms have also been reported by Jaszek *et al*. (2013) and Sadi *et al*. (2015). The DPPH radicals can be scavenged by substances capable of donating hydrogen atom, leading to a reduced form of DPPH. This manifests as decolourization of the violet colour of DPPH solution. The degree of the decolourization is an indicator of the radical scavenging potentials. Substances capable of performing this reaction can be considered as antioxidants and therefore radical scavengers (Ebrahimzadeh *et al*., 2010). This implies that extracts of *Trametes lactinea* contain substances that can donate hydrogen to DPPH. This observed result concurs with the findings of studies carried out on several mushrooms (Hasnat *et al*., 2014; Zhang *et al*., 2014).

The acetone extract of *Trametes lactinea* which had the higher total phenolics, also displayed the higher scavenging activity. This is in accordance with reports from several studies which have shown positive correlation between the Total Phenolic Content (TPC) and DPPH scavenging activity (Lim *et al*., 2006; Vamanu and Nita, 2013). For instance, Lim *et al*. (2006) described that high radical scavenging activity was contributed by the presence of high phenolic content in guava extracts.

Finding shows that extracts of *T. lactinea* scavenged nitric oxide radicals in a concentration dependent manner (Fig. 2). Several authors have documented the nitric oxide scavenging abilities of wild mushrooms (Vamanu and Nita, 2013; Sheikh *et al*., 2015). Nitric oxide reacts with oxygen forming nitrate and nitrite. These compounds, nitrite and nitrate, are known to have detrimental effect on the structures and functions of biomolecules such as protein, lipids and nucleic acids (Wang *et al*., 2005). Patel *et al*. (2010) while studying the antioxidant activities of some selected plants, reported NO to be directly scavenged by flavonoids. Similar observation was also observed by Boora *et al*. (2014). In their study, the higher NO scavenging potency of *Parinari curatellifolia* as compared to that of *Combretum zeyheri* and *Combretum platypetalum* is connected to the presence of flavonoids and saponins. Result from this study conforms to the aforementioned observations, as the methanol extract which has higher amount of total flavonoids displayed better scavenging ability. Based on the observed result, bioactives present in the extract of *T. lactinea* may help neutralize the damaging effect of NO, thus prevent the negative effect of immoderate production of NO in humans.

Extracts of *T. lactinea* were effective in scavenging hydroxyl radical (Fig. 3). Hydroxyl radical directly cause lipid peroxidation and is the most harmful among the Reactive Oxygen Species (ROS) to damage the cellular components (Lobo *et al*., 2010). The generation of hydroxyl radicals near nucleic acids leads to its reaction with purine and pyrimidine bases and 2-deoxyribose, leading to mutations which play an important role in carcinogenesis, as well as in neurodegenerative and cardiovascular diseases (Uttara *et al*., 2009). Hydroxyl radicals formed in free solution are measured by their ability to degrade deoxyribose into fragments (thiobarbituric acid reactive substances) and form a pink chromophore upon reaction with TBA (thiobarbituric acid) at low pH, which is measured at 532 nm. The addition of extracts of *T. lactinea* to the reaction mixture prevented the reaction forming the pink chromophore, which implies that the extracts were able to remove hydroxyl radical, thus preventing the degradation of deoxyribose. This indicates the potency of the *T. lactinea* extracts in preventing the joining of nucleosides in the DNA and possible breakage leading to carcinogenesis and cytotoxicity.

The OH scavenging ability of the extracts of *T. lactinea* might be due to the presence of bioactive substances such as phenolic compounds, which can donate hydrogen or electron (Yildiz *et al*., 2015). Phenolic compounds have been found to be potent free radicals scavengers due to their redox properties that allow them to act as reducing agents (Oyedemi *et al*., 2010).

Higher OH radical scavenging activity was observed in the acetone extract of *T. lactinea* and this might be connected to its higher total phenolic content. The observation corresponds with the findings of Onar *et al*. (2012) who observed high correlation between hydroxyl radical scavenging activity and phenolic compounds of aqueous extract of *Epilobium angustifolium*. Result from this study show that drugs for the prevention of OH radical induced DNA damage could be sourced from *Trametes lactinea*.

Extracts of *Trametes lactinea* displayed moderate hydrogen peroxide (H_2O_2) scavenging effect (Fig. 4). Reactivity of hydrogen peroxide is low but its ability to penetrate cell membranes

influences its cytotoxicity and clastogenesis (Schubert and Wilmer, 1991). Formation of highly reactive hydroxyl radicals occurs in the presence of transition metals such as iron, which initiates lipid peroxidation and cause DNA damage (Sahreen *et al*., 2011). It is therefore pertinent to control accumulation of hydrogen peroxide in biological systems.

Scavenging effects of the extracts might be attributed to the presence of hydrogen donors such as phenolic compounds that could donate hydrogen from their hydroxyl groups to hydrogen peroxide, converting it to water (Yildiz *et al*., 2015). Higher scavenging effect was observed for the acetone extract of *T. lactinea* and this might be connected to its higher TPC. A strong correlation between the H_2O_2 scavenging activity and the TPC of *Rosa canina* was reported by Kilicgun and Altiner (2010).

CONCLUSION

The present investigation reveals the free radical scavenging potential of extracts of *Trametes lactinea*. This implies that this wild macrofungus can be bioprospected for natural antioxidants which can be used for the treatment of radical and age related diseases. However, further study is required to identify the phenolic compounds present in this macrofungus and ascertain their pharmacological potentials.

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