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In vitro Antiradical Activities of *Trametes elegans* Collected from Osengere, Ibadan, Nigeria

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ABSTRACT

Extracts of *Trametes elegans* were examined for their total phenolic and flavonoid contents and *in vitro* antiradical potentials. Varying concentrations of the extracts were assessed against 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Nitric Oxide (NO), hydrogen peroxide (H_2O_2) and hydroxyl (OH) radicals. The result of the total phenolic content expressed in mg gallic acid equivalents per gram of dried extract (mg GAE g^{-1}) revealed that the acetone extract had higher phenolic content (4.79 mg GAE g^{-1}) than the methanol extract. However, the methanol extract had higher total flavonoid (2.27 mg RE g^{-1}) compared to the acetone extract. The extracts displayed appreciable radical scavenging activity to DPPH, NO and H_2O_2 radicals. The scavenging activities of the extracts were, however, lower to that of the positive control (butylhydroxytoluene). On the other hand, the acetone extract displayed better scavenging activity than the positive control at a concentration of 2 mg mL^{-1}. Result from this study suggest that this underutilized indigenous macrofungus, *Trametes elegans,* can be exploited as a source of bioactive compounds with antioxidant potentials that can neutralize the damaging effects of free radicals.

Key words: Antiradical, extracts, butylhydroxytoluene, macrofungus, bioactives

INTRODUCTION

Free radicals are defined as molecules having an unpaired electron in the outer orbit and are generally unstable and very reactive (Gilbert, 2000; Fang *et al*., 2002). All aerobic life forms existing on earth are associated with oxidation processes, which are vital for their survival (Fang *et al*., 2002). For example, oxygen radicals exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells (Zheng and Storz, 2000). However, this vital mechanism may also lead to cell and tissue damages and mediate the pathogenesis of many chronic diseases (McCord, 2000; Thetsrimuang *et al*., 2011). In other word, there are "Two faces" of free radicals in biology in that they serve as signalling and regulatory molecules at physiologic levels but also serves as highly deleterious and cytotoxic oxidants at pathologic levels (Fridovich, 1999).

These damaging effects of these free radicals can be prevented by natural and synthetic antioxidants. Although every organism has natural endogenous defence mechanisms to eliminate free radicals, habitually excess production of Reactive Oxygen Species (ROS) overwhelms the system (Tibuhwa, 2012). The restriction in the use of synthetic antioxidants such as such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) due to their toxicity and

carcinogenicity has raised an increased interest towards natural antioxidant. Since nature is the master chemist, exploration of natural sources for novel bioactive agents may provide leads or solutions for drug discovery and development (Debbab *et al*., 2012).

Mushrooms have been reported to be prolific producers of several bioactive metabolites and have been used for centuries as medicine to treat or prevent different diseases (Wasser, 2011; De Silva *et al*., 2012). The antioxidant properties of wild mushrooms have been studied and their antioxidant activity, has been mainly related to their phenolic, tocopherols, ascorbic acid and carotenoids contents, which could be extracted for the purpose of being used as functional ingredients against chronic diseases related to oxidative stress (Mohsin *et al*., 2011). Mushrooms belonging to the genus *Trametes* are polyporoid white rot fungi widely distributed in various biotopes and have been the subject of many physiological and biochemical studies (Koroleva *et al*., 2002).

This study is aimed at providing information on the total phenol and flavonoid contents, as well as the antiradical activities of *Trametes elegans* collected from Ibadan, Nigeria using various *in vitro* methods.

MATERIALS AND METHODS

Collection of macrofungus: Fresh fruit bodies of macrofungus suspected to be *Trametes* species were collected from rotten cocoa woods from farms in Osengere, Egbeda Local government area of Ibadan, Oyo State (Latitude: 7.39814N, Longitude: 4.00051E) in the month of August 2013. The fruiting 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. This was done 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) search revealed that the macrofungus was 98% related to *Trametes elegans* 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) with 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 in a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). A calibration curve was earlier plotted as standard gallic acid curve. Total phenolic content was expressed as mg 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 6minutes, 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 with 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}).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) 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. The DPPH radical inhibition was calculated using the equation:

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

where, Abs control is the absorbance of the control (DPPH without sample), Abs sample is the absorbance of the test sample (the sample test and DPPH solution) and 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 and control (butylatedhydroxytoluene) 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:

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

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

Scavenging effect of 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 $FeSO_4$ 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:

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

where, Abs reference is absorbance of the reference (reacting mixture without the test sample) and Abs sample is 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:

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

where, Abs reference is absorbance of the reference (reacting mixture without the test sample) and Abs sample is absorbance of reacting mixture with the test sample.

Statistical analysis: All experiments were carried out in triplicates. Data obtained was 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

Table 1 shows the result for the total phenol and total flavonoid contents of extracts. The acetone extract gave higher amount of total phenol and lower amount of total flavonoid, while the methanol extract produced higher total flavonoid and lower amount of total phenol.

Table 1: Total phenolic and flavonoid content of *Trametes elegans* extracts

Solvent	Total phenolic (mg GAE g^{-1})	Total flavonoid (mg RE g^{-1})
Acetone	4.79±0.06	0.97±0.00
Methanol	4.37±0.06	2.27±0.16

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

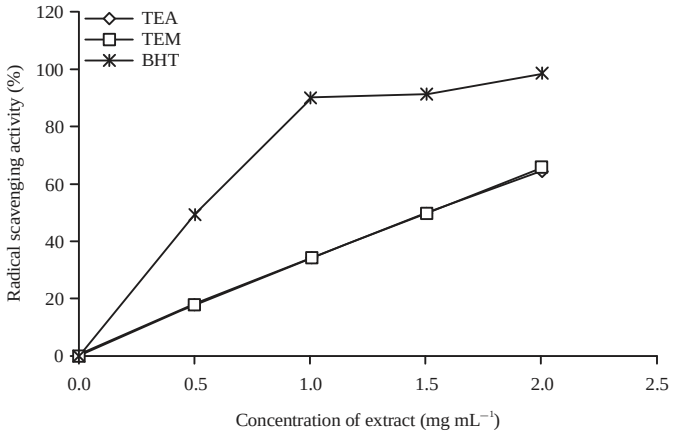

Fig. 1: Scavenging effect of *Trametes elegans* extracts on DPPH radicals. Each value is expressed as mean of triplicate results (n = 3), TEA: Acetone extract of *Trametes elegans*, TEM: Methanol extract of *Trametes elegans*, BHT: Butylatedhydroxy toluene

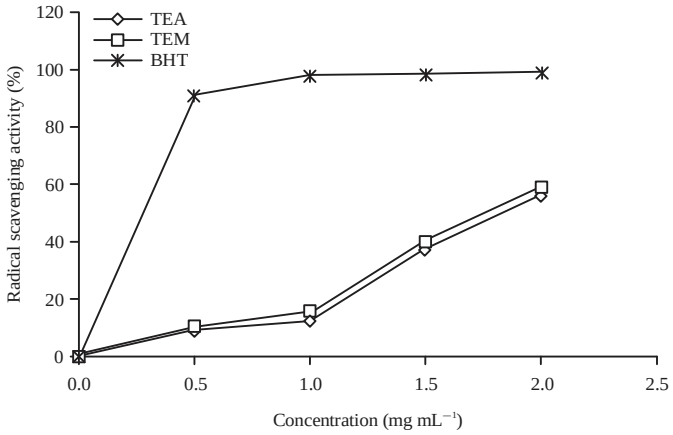

Fig. 2: Scavenging effect of *Trametes elegans* extracts on nitric oxide radicals. Each value is expressed as mean of triplicate results (n = 3), TEA: Acetone extract of *Trametes elegans*, TEM: Methanol extract of *Trametes elegans*, BHT: Butylatedhydroxy toluene

Figure 1 shows the result of the DPPH radical scavenging activity of the extracts of *Trametes elegans*. The extracts displayed concentration dependent free radical scavenging ability (0.5-2 mg mL^{-1}). At a concentration of 2 mg mL^{-1}, the acetone extract displayed an activity of 65%, while the methanol extract displayed 66.46% scavenging activity. The positive control (BHT) displayed activity that was higher (99.46%) and significantly different from that of the extracts.

The extracts of *Trametes elegans* displayed moderate and concentration dependent nitric oxide (NO) scavenging ability as shown in Fig. 2. The ability of the extracts to scavenge NO radicals were 56.18 and 58.87% for acetone and methanol extracts respectively at 2 mg mL^{-1}. The positive control (BHT) exhibited a higher and significantly different ($p<0.05$) NO scavenging effect when compared to the extracts (Fig. 2).

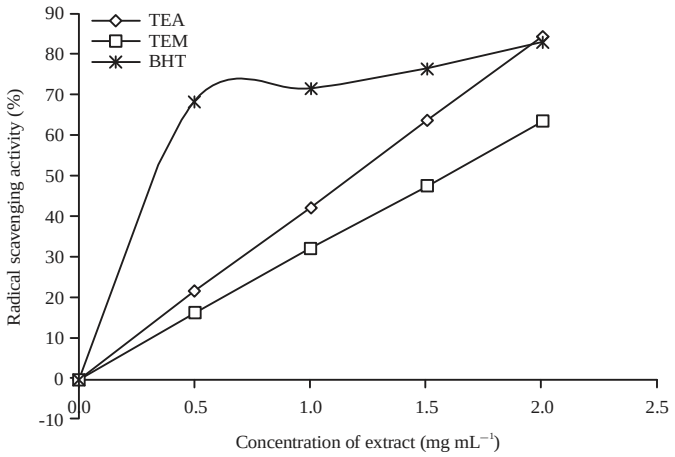

Fig. 3: Scavenging effect of *Trametes elegans* extracts on hydroxyl radicals. Each value is expressed as mean of triplicate results (n = 3), TEA: Acetone extract of *Trametes elegans*, TEM: Methanol extract of *Trametes elegans*, BHT: Butylatedhydroxy toluene

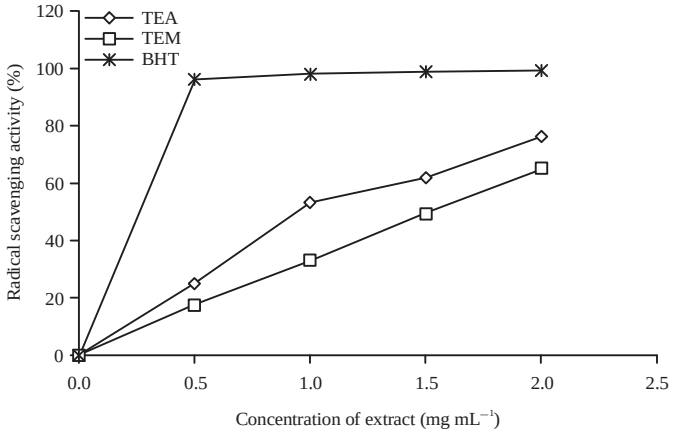

Fig. 4: Scavenging effect of *Trametes elegans* extracts on hydrogen peroxide radicals. Each value is expressed as mean of triplicate results (n = 3), TEA: Acetone extract of *Trametes elegans*, TEM: Methanol extract of *Trametes elegans*, BHT: Butylatedhydroxy toluene

The hydroxyl radical scavenging effect of the extract of *Trametes elegans* is shown in Fig. 3. The scavenging effects of extracts at 2 mg mL^{-1} were above 50% and well pronounced. Acetone extract of *Trametes elegans* produced an effect of 84.18% that was slightly higher than that of the positive control (BHT) at a concentration of 2 mg mL^{-1}.

Figure 4 shows the scavenging effect of the extracts of *Trametes* elegans on hydrogen peroxide radicals. The extracts displayed concentration dependent activities (0.5-2 mg mL^{-1}). At a concentration of 2 mg mL^{-1}, acetone extract of *Trametes elegans* exhibited higher activity of 76.03%. The activity of the positive control (BHT) was not concentration dependent and it produced higher scavenging effect than the extracts at all tested concentrations.

DISCUSSION

Several studies have reported the antioxidative potency of wild mushrooms (Kosanic *et al*., 2013; Pennerman *et al*., 2015). These antioxidant activities have been attributed to the presence of various bioactives (Chowdhury *et al*., 2015). The present study investigated the *in vitro* antiradical activities of extracts obtained from *Trametes elegans* from Osengere, Ibadan, Nigeria. Varying amount of total phenol and total flavonoids were found in the extracts. This observation might be connected with the solvent and the chemical nature of the bioactives. It has been reported that the chemical nature of phytochemicals, extraction method, sample particle size, solvent used, as well as the presence of interfering substances affects extraction efficiency (Stalikas, 2007).

The presence of phenolics and flavonoids in wild mushrooms has been reported by many authors (Chowdhury *et al*., 2015; Pennerman *et al*., 2015). Phenolic compounds present in mushrooms possess strong antioxidants properties, with a strong positive correlation between the phenolic content and antioxidant activity (Reis *et al*., 2011). They are capable of playing a protective role in preventing radical related disease, cancer and cardiovascular disease (Ferreira *et al*., 2010). Flavonoids are a large group of polyphenolic compounds having a benzo-γ-pyrone structure (Kumar and Pandey, 2013). The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations and degree of polymerization (Heim *et al*., 2002). They are capable of scavenging free radicals and chelating metal ions (Kumar *et al*., 2013). The presence of these phenolic compounds in extracts of *Trametes elegans* used in this study, suggest that the macrofungus could be a potential source of natural antioxidants.

The result from this study shows that the extracts of *Trametes elegans* were able to scavenge for DPPH (Fig. 1). Several authors have also reported the DPPH radical scavenging activities of wild mushrooms (Reis *et al*., 2011; Vamanu and Nita, 2013). When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, it gives rise to the reduced form of the DPPH compound, leading to the reduction of the violet colour. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Ebrahimzadeh *et al*., 2010). This implies that *Trametes elegans* extracts contain bioactive compounds capable of donating hydrogen atom to DPPH.

It has been found that phenolics, flavonoids and tocopherols reduce the DPPH radicals by their hydrogen donating ability (Zhao *et al*., 2006). Results from several studies have shown positive correlation between Total Phenol Content (TPC) assay and DPPH radical scavenging activity assay (Luther *et al*., 2007; Silva *et al*., 2007). It was however noted from this study that though the acetone extract had higher TPC value than the methanol extract, yet it displayed lower radical scavenging activity. It is therefore suggested that other biologically active substances could be present and this might have contributed to the higher radical scavenging activity displayed by the methanol extract of *T. elegans*. Similar observation was reported by Othman *et al*. (2011) while carrying out a comparative study on the antioxidant activity of red onion and garlic. From their study, it was observed that there was a negative correlation between the result of TPC assay and DPPH radical scavenging assay. Khamsiah *et al*. (2006) suggested that free radical scavenging activity is not due to the phenolics only because they found that the antioxidant activity of methanol extract of *Orthosiphon stamineus* was not solely caused by phenolic compounds.

The extracts of *T. elegans* were capable of scavenging nitric oxide (Fig. 2). The nitric oxide scavenging effect of various wild mushrooms has also been reported (Menaga *et al*., 2013; Vamanu and Nita, 2013). Nitric Oxide (NO) is classified as a free radical because of its unpaired

electron and displays important reactivity with certain types of proteins and other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion ($ONOO^-$) (Nagmoti *et al*., 2012).

In this study, the methanol and acetone extracts of *T. elegans* at different concentrations were assessed for their nitrite free radical scavenging activity in an *in vitro* model. In this method, nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The nitrite ions diazotize with sulphanilamide acid. This couples with naphthylethylenediamine to form a pink colour which is measured at 540 nm. As antioxidants donate protons to the nitrite radical, the absorbance is decreased. The decrease in absorbance is used to measure the extent of nitrite radical scavenging (Turkoglu *et al*., 2007).

The ability of the extracts to scavenge nitric oxide suggests that the extracts contain substances that can directly compete with oxygen in the reaction with nitric oxide. It was observed that the methanol extracts of *T. elegans* when compared to the acetone extracts of both mushrooms exhibited higher scavenging ability. Boora *et al*. (2014) attributed the higher NO scavenging potency of *Parinari curatellifolia* as compared to that of *Combretum zeyheri* and *Combretum platypetalum* to the presence of flavonoids and saponins. The higher scavenging ability of NO by the methanol extract of *T. elegans* when compared to the acetone extract might be attributed to the higher flavonoids content. *Trametes elegans*, can therefore be a useful source of natural antioxidant that can help combat the detrimental effect of nitric oxide generated in the body.

Potent hydroxyl radical scavenging effect was observed in the extracts of *T. elegans* (Fig. 3). Several authors have reported the hydroxyl radical scavenging effect of various mushrooms (Oyetayo, 2009; Khatua *et al*., 2015). Hydroxyl radical is an extremely reactive oxygen species that can propagate a chain reaction, wreaking havoc on many biological molecules such as DNA and lipids (Uttara *et al*., 2009; Kumar *et al*., 2011). The removal of hydroxyl radicals may be due to the presence of substances capable of donating hydrogen or electron. Phenolic compounds possess the ability to scavenge free radicals to the presence of hydroxyl groups that can give up its hydrogen atom to the hydroxyl group and form a stable phenoxyl radicals (Ozgen *et al*., 2010). Several studies have reported strong correlation between the total phenolic contents and hydroxyl radical scavenging activity (Olabinri *et al*., 2010; Aksoy *et al*., 2013). Higher OH radical scavenging effect of the acetone extract of *T. elegans* might be related to its higher total phenolic contents. Results from this study suggest that extracts of *T. elegans* could be promising candidates in the prevention of DNA damage.

The extracts were also able to scavenge for hydrogen peroxide (Fig. 4). The ability of the extracts to scavenge hydrogen peroxide may be attributed to the presence of phenolics, which can donate electron to H_2O_2, thus converting it to water. It was observed that the acetone extract of *T. elegans* which displayed the higher TPC, also exhibited higher scavenging ability at all concentrations. Kim *et al*. (2013) in his finding observed a strong linear correlation between the TPC and scavenging of H_2O_2 while evaluating the antioxidant activity of the water and ethanol extract of dried citrus fruit peel (*Citrus unshiu*).

Hydrogen peroxide (H_2O_2) is not a highly reactive oxygen species. Its low reactivity gives it enough time to penetrate biological membranes. In the presence of transition metals, it might form highly reactive hydroxyl radical which might wreak havoc on macromolecules such as lipids and DNA (Sahreen *et al*., 2011). The removal of H_2O_2 in biological and food system is of outmost importance for antioxidant defence.

CONCLUSION

Extracts of *Trametes elegans* displayed effective *in vitro* radical scavenging activity against DPPH, NO and H_2O_2 radicals. Natural antioxidants that can neutralize the damaging effects of free radicals can therefore be sourced for from this macrofungus. However, further studies are needed to establish the pharmacological effects of these natural extracts in the treatment or prevention of radical and age associated diseases.

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