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Phytochemical and antioxidant properties of *Trametes* species collected three districts of Ondo state Nigeria

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ABSTRACT

Trametes species (L) collected from Akure, Akoko and Ore districts of Ondo State, Nigeria were assessed for their phytochemical and antioxidant properties. Three extracting solvents viz: ethanol, ethyl acetate and hexane were used in extracting of bioactive components from the fungus. Extract yield was higher in ethanol extract of *Trametes* species when compared to yield from other extracting solvents. Qualitative analysis revealed the presence of flavonoids; tannins, terpenoid, saponnin, steroid and cardiac glycosides in the extracts. Antioxidant property of the extracts was assessed using scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH). Ethanol extracts exhibited better antioxidant property with values as high as 84.88%, 80.72% and 66.91% for extract of *Trametes* species from Akure (TETA), extract of *Trametes* species. from Ore (TETO) and extract of *Trametes* species from Ikare Akoko (TETAKK) respectively at 2mg/ml compared to control butylated hydroxytoluene (BHT) with the scavenging effect of 86.97%. The extracts displayed concentration dependent DPPH scavenging activity. The study suggests that this wild macrofungus could be source of effective antioxidant.

KEYWORDS

Trametes species;
Macrofungus;
Extracts phytochemical;
Antioxidant.

INTRODUCTION

Macrofungi have long been used as valuable food source and as traditional medicines around the world[31]. Macrofungi are known to produce large and diverse variety of secondary metabolites[17]. These secondary metabolites have health promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects[3,22,19]. Bioactive compounds such as glycolipids, compounds derived from shikimic acid, aromatic phenols, fatty acid derivatives, polyacetylamine, polyketides, nucleosides, sesterterpenes, and many other substances of different origins have been isolated from these macrofungi[17, 18, 22, 32].

Out of the 140,000 estimated to be on earth only 14,000 (10%) had been identified[13]. The pharmacological potential of about 90% of these macrofungi is yet to be explored. There are no data on these macrofungi and their medicinal potentials. The search for safe and effective pharmacological substances had increased of recent. Bioactive compounds obtained from macrofungi maybe the answer to these novel pharmacological agents. The antioxidative and free radical scavenging properties of mushroom have been reported[7,19,20]. Chinese Shiitake mushroom (*Lentinus edodes*) has also been reported to possess both anti-tumour and antimicrobial proper-

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ties[15]. Natural products have been the source of most of the active ingredients of medicines[15]. Natural substances provide a large reservoir for screening of anti-HIV-1 agents with novel structure[17]. The present study seeks therefore to assess the phytochemical properties and antioxidant effects of hexane, ethyl acetate and ethanol extracts obtained from three wild non-edible macrofungi *Trametes* species (Pers), collected from Akure, Akoko Ondo and Ore, representing the three senatorial district of Ondo State, Nigeria.

MATERIALS AND METHODS

Collection of macrofungi

Trametes species (L) were collected in the wild between June and October, 2010 in the three senatorial districts represented by Akure in the central, Akoko in the north and Ore in the South of Ondo State, Nigeria. The morphological and ecological characteristics of these macrofungi were recorded in their natural habitats. Dried samples of the macrofungi were numbered and kept in polythene bags. The collected macrofungi were identified based on their macroscopic and microscopic characteristics and the related literature[23,30]. Voucher specimens of dried macrofungi were deposited in the herbarium of Department of Microbiology, Federal University of Technology, Akure, Nigeria.

Preparation of macrofungi extracts

The method described by Oyetayo *et al*.[24] was adopted with slight modification. Briefly, dried samples of *Trametes* species were ground into fine powder with an electric mill. The bioactive components were sequentially extracted from non-polar to polar solvents using hexane, ethylacetate and ethanol. The extraction by the solvent was performed in Erlenmeyer flask at room temperature for 48 h. The extracts obtained were dried to constant weight in a laboratory hood overnight (12 hours). The extracts were designated THEA (hexane extract of *Trametes* species obtained from Akure), TEAKR (ethyl acetate extract of *Trametes* species obtained from Akure), TETA (ethanol extract of *Trametes* species obtained from Akure), THEAK (hexane extract of *Trametes* species obtained from Akoko), TEAKK (ethyl acetate extract of *Trametes* species obtained from Akoko), TETAKK (ethanol extract of *Trametes* species obtained from Akoko), THEO (hexane extract of *Trametes* species obtained from Ore), TEAO (ethyl acetate extract of *Trametes* species obtained from Ore), TETO (ethanol extract of *Trametes* species obtained from Ore).

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds such as alkaloids, tannins, saponins, anthraquinones, flavonoids, steroids, terpenoids, cardiac glycosides and phlobatinins using standards methods of Sofawara[28], Trease and Evans[29] and Edeoga[5].

Scavenging effect of extracts on DPPH radicals

The method of Blois[4] was used in determine the effect of extracts of *Trametes* species obtained from akoko, Akure and Ore on DPPH• radicals with some modifications. A solution of DPPH (0.5mmol/L) in ethanol and in 0.05 mol/L acetate buffer (pH 5.5) was prepared. Extract in solution (0.1mL of 2mg/mL) was mixed with 2mL of acetate buffer, 1.9mL of absolute ethanol and 1mL DPPH solution. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in dark for 30 min. The decrease in absorbance at 517nm was measured using Ultra Microplate Reader (Elx 808, BIO TEK Instruments Inc). BHT was used as positive control and the sample solution without DPPH was used as blank. The radical scavenging activity was measured as a decrease in absorbance of DPPH.

RESULTS AND DISCUSSION

Higher fungi are a major source of biological active natural substances among many diverse organisms which provide a rich variety of active metabolites[17]. There are potentially many bioactivities and novel compounds still to be discovered in higher fungi since until now only a few numbers of higher fungi have been biologically and chemically investigated[17,25]. Nigeria is extraordinarily rich in higher fungi. However, there are few data on the medicinal uses of these fungi The current study reports the phytochemical screening and antioxidant

effects of extracts of three wild mushrooms, *Trametes* species collected from Ondo State, Nigeria. *Trametes* species were collected and sequentially extracted using Hexane, Ethyl acetate and ethanol. The percentage yield show that ethanol was a better extracting solvent (TABLE 1).

The results of the comparative qualitative and quantitative phytochemical analyses of *Trametes* species collected from Ore, Akure and Akoko in ethanol, ethyl acetate and n-hexane extracts are presented in TABLE (2 and 3) reveals the presence of flavonoids; tannins, terpenoid, saponnin, steroid and cardiac glycosides in all the three solvent extracts of *Trametes* species except alkanoid, phlobatannin and anthraquinones. These results are in correlation with the previous work on mushrooms[8] and other leafy vegetables[1]. Also, Egwim *et al.*[6] had earlier reported the presence of flavonoids, saponnin and tannins in wild edible Nigerian mushroom. High Flavonoids level may help provide protection against oxidative stress induced diseases by contributing along with other antioxidant vitamins, and enzyme to the total antioxidative defense system of the human body. Many studies have attributed that antioxidant properties are due to the presence of flavonoids[12]. This may be the reason for the high lipid peroxidation inhibition found in certain species of the studied mushrooms. The medicinal values of mushroom therefore may be attributed to the presence of these phytochemicals.

Tannins and flavonoids are some of the most important bioactive components from plants[14]. Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to

TABLE 1 : The percentage yield (%) of *Trametes* species extract

Location	Ethanol	Ethyl acetate	N-hexane
Akure	5.42	5.28	4.20
Akoko	5.38	4.56	4.38
Ore	6.34	5.28	4.08

TABLE 2 : Qualitative analysis of the various phyto-chemical on the ethyl acetate, ethanol and n-hexane extracts of *Trametes* species from Akoko, Akure and Ore

Phytochemical	EAO	EAA	EAAK	ETO	ETA	ETAK	NHO	NHA	NHAK
ALKALOID	-	-	-	-	-	-	-	-	-
SAPONIN	+	+	+	+	+	+	+	+	+
TANNIN	+	+	+	+	+	+	-	-	-
FLAVONOID	+	+	+	+	+	+	+	+	+
STERIOD	+	+	+	+	+	+	+	+	+
TERPENOID	+	+	+	+	+	+	+	+	+
PHLOBATANIN	-	-	-	-	-	-	-	-	-
ANTRAQUINONE	-	-	-	-	-	-	-	-	-
CARDIAC GLYCOSIDES	+	+	+	+	+	+	+	+	+

EAO : Ethyl acetate extract of *Trametes* species from Ore; EAA: Ethyl acetate extract of *Trametes* species from Akure; EAAK: Ethyl acetate extract of *Trametes* species from Akoko; ETO: Ethanol extract of *Trametes* species; ETA: Ethanol extract of *Trametes* species from Akure; EAKK: Ethanol extract of *Trametes* species from Akoko; NHO: n-hexane extract of *Trametes* species from Ore; NHA: n-hexane extract of *Trametes* species from Akure and NHAK: n-hexane extract of *Trametes* species from Akoko.

TABLE 3 : Quantitative analysis of the various phyto-chemical present in extracts of *Trametes* species

P Phytochemical	EAO	EAA	EAAK	ETO	ETA	ETAK	NHO	NHA	NHAK
TaT Tannin	1.46 ± 0.12^{f}	1.4 ± 0.02^{e}	1.28 ± 0.20^{d}	1.98 ± 0.50^{h}	2.03 ± 0.01^{f}	1.55 ± 0.11^{g}	0.86 ± 0.09^{b}	0.98 ± 0.10^{c}	0.48 ± 0.02^{a}
Fl Flavonoid	1.25 ± 0.33^{g}	1.15 ± 0.05^{f}	1.04 ± 0.02^{d}	1.80 ± 0.10^{h}	1.81 ± 0.23^{h}	1.10 ± 0.00^{e}	0.76 ± 0.02^{b}	0.85 ± 0.05^{c}	0.51 ± 0.05^{a}
Ster Steroid	0.33 ± 0.03^{d}	0.28 ± 0.20^{d}	0.29 ± 0.10^{d}	0.42 ± 0.02^{d}	0.39 ± 0.33^{e}	0.36 ± 0.02^{e}	0.18 ± 0.04^{c}	0.19 ± 0.07^{b}	0.12 ± 0.11^{a}
Te Terpenoid	0.30 ± 0.33^{c}	0.27 ± 0.33^{c}	0.26±0.50c	0.40 ± 0.02^{e}	0.37 ± 0.03^{e}	0.31 ± 0.03^{cd}	0.18 ± 0.02^{b}	0.32 ± 0.02^{cd}	0.11 ± 0.01^{a}
Cg Cardiac glycosides	0.25 ± 0.50^{c}	0.20 ± 0.00^{b}	0.16 ± 0.22^{ab}	0.37 ± 0.33^{d}	0.35 ± 0.03^{d}	0.29 ± 0.03^{cd}	0.13 ± 0.33^{a}	0.14 ± 0.02^{a}	0.10 ± 0.00^{a}
S Saponin	1.10 ± 0.02^{d}	1.13 ± 0.33^{d}	1.01 ± 0.01^{c}	1.40 ± 0.02^{e}	1.43 ± 0.31^{e}	1.11 ± 0.05^{d}	0.39 ± 0.03^{b}	0.41 ± 0.13^{b}	0.27 ± 0.09^{a}

Values with the same superscript across a row are not significant different at P<0.05

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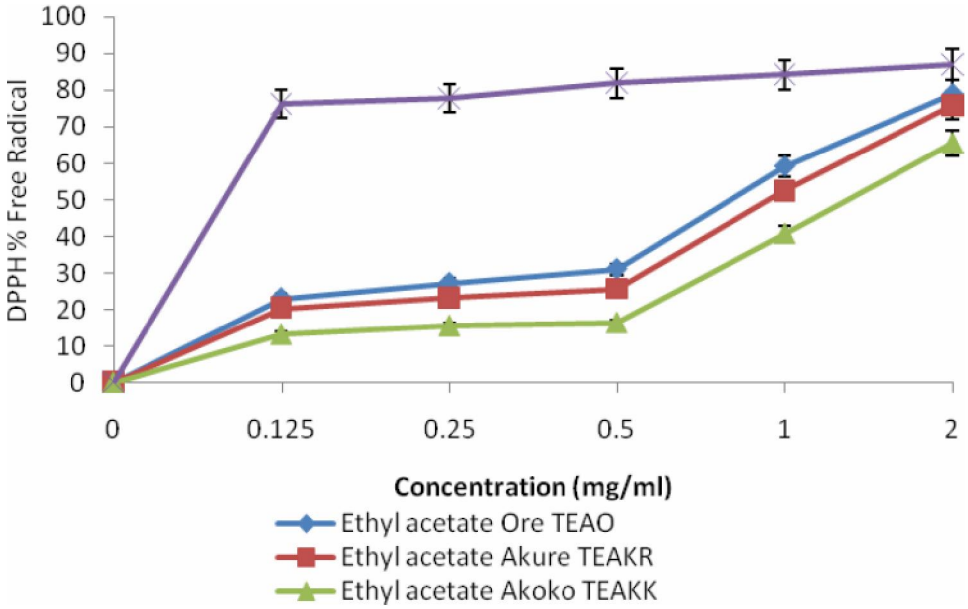

Figure 1 : Scavenging activity of ethyl acetate extracts of *Trametes* species species on DPPH radicals. Each value is means ± standard deviation (n=3)

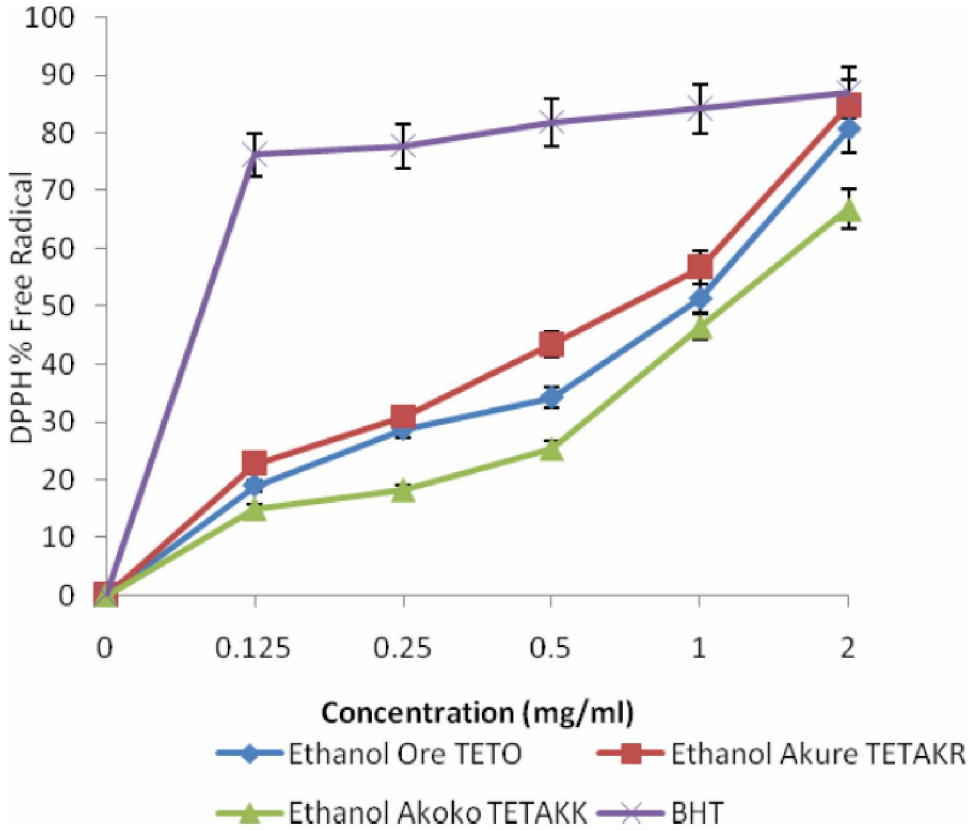

Figure 2 : Scavenging activity of ethanol extracts of *Trametes* species on DPPH radicals. Each value is means ± standard deviation (n=3)

form a protective covering[9]. They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and antidote[2]. Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents[27]. The presence of steroids, saponins, cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance

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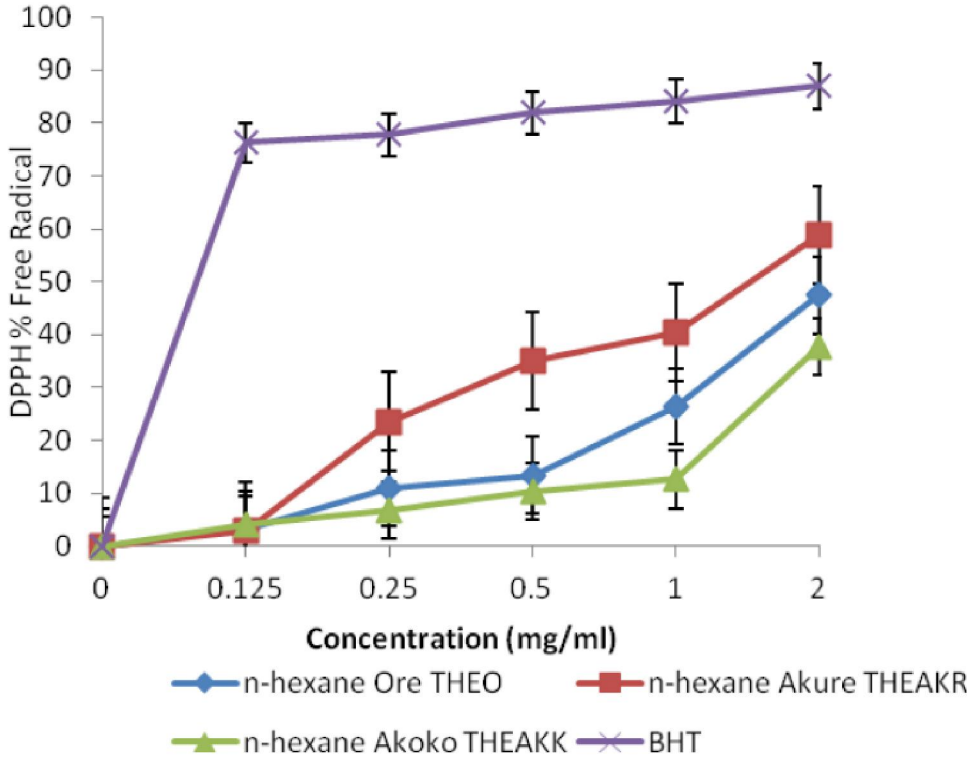

Figure 3 : Scavenging activity of n-hexane extracts *Trametes* species on DPPH radicals. Each value is means ± standard deviation (n=3)

because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D[26]. Generally, the phytochemical contents of ethanol extracts were higher than extracts obtained with hexane and ethyl acetate.

The ability of extracts to scavenge for DPPH radicals is presented in Figures 1, 2 and 3. The extracts displayed concentration dependent DPPH scavenging activity. Ethanol extracts (TETAKR, TETO and TETAKK) exhibited the strongest antioxidant activity on DPPH radical with values as high as 84.88%, 80.72% and 66.91% at 2mg/ml compared to control butylated hydroxytoluene (BHT) with the scavenging effect of (86.97) (Figure 2**)**. The scavenging activity of ethanol *Trametes* species (TETAKR) extract of 84.88% at 2mg/ml was higher when compared with the other extracts. This may be as a result of higher phenolic contents of ethanol extract. It had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds[33]. The antioxidant extracts of ethanol of *Trametes* species obtained from Akure and Ore was observed to be higher than the one obtained from Akoko. The lower values obtained from Akoko may be attributed to high laterized content of the soil. Laterized soils are known to be poor in nutrients and this can affect the chemical composition of mushroom growing on plant in such areas.

CONCLUSION

The results obtained from this research shows that ethanol extracts of *Trametes* species collected from Akure and Ore exhibited a better antioxidant properties than *Trametes* species collected from Akoko. Further works of isolation, purification, identification and bioassay of specific bioactives from these macrofungi will be the next focus of this research.

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