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Nutraceutical potentials of Turkey Tail medicinal mushroom, *Trametes versicolor* (*Coriolus versicolor*, yun zhi, kawaratake) (Agaricomycetes) from Nigeria.

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Abstract: Fresh fruit bodies (3.5 kg) of Turkey tail medicinal mushroom, *Trametes versicolor* (*Coriolus versicolor*, yun zhi, kawaratake) collected from its natural environment was washed, air dried and ground into a fine smooth powder. From the analysis of its mineral elements by the AOAC method of 1970, potassium, calcium and magnesium had the highest concentrations amongst the major minerals with values of 75.98 $\pm$ 2.06 mg/kg, 48.36 $\pm$ 2.01 mg/kg and 45.38 $\pm$ 1.27 mg/kg, while manganese, zinc and copper were the highest trace elements with values of 5.16 $\pm$ 0.35 mg/kg, 2.59 $\pm$ 0.24 mg/kg and 0.39 $\pm$ 0.06 mg/kg respectively. Arginine was the highest amino acid detected in this mushroom using the 1989 method of Benitez with a value of 4.34 $\pm$ 0.13 mg/kg followed by aspartic acid and valine with values of 4.20 $\pm$ 0.16 mg/kg and 4.02$\pm$ 0.17 mg/kg respectively. The five predominant oils identified in this mushroom using an AgilentTech HP 6890 GC and MS model 5973 fitted with a HP-5 MS capillary column are 9,12-Octadecadienoic acid (Z,Z), methyl esters with R_T of 21.698 min. and 21.858 min. and percentage total of 56.355 and 2.382, n-Hexadecanoic acid with R_T of 20.187 min. and a percentage total of 16.936, while 1,2-Benzenedicarboxylic acid, diisooctyl ester, Pentadecanoic acid,14-methyl-ester, Octadecane, 1-chloro- had R_T of 26.052 min., 19.758 min. and 11.850 min. and percentage totals of 7.744, 3.887 and 3.023 respectively. These five major oils represent 90.327 % of the total constituent oil in *T. versicolor.* Sequel to these nutracuetical components, this mushroom may be beneficial as anti-inflammatory, anti-arthritic, anti-coronary, cancer preventive and hepatoprotective agent.

Key words: Mushroom, medicinal properties, amino acids, mineral elements and volatile oil.
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INTRODUCTION

The use of mushrooms in folk medicine has been a common practice by most cultures around the world. Though most mushrooms may not be considered edible, the act of consuming mushrooms dates back to Paleolithic period. Although the first reliable evidence of mushroom consumption dates to several hundred years BC in China, edible mushroom species have been found in association with 13,000-year-old archaeological sites in Chile [1]. While edible mushrooms are mainly consumed for their nutritional value, their consumption may also be occasioned by their supposed medicinal properties[2.] The concept of medicinal mushroom has a history spanning millennia in parts of Asia, mainly in traditional Chinese medicine[3]. Preliminary research on some mushrooms extracts such as polysaccharide-K (PSK),[4] polysaccharide peptide or lentinan[5.] revealed anti-disease properties. Some of these mushroom extracts have widespread use in Japan, Korea and China, as potential adjuvants to radiation treatments and chemotherapy[6,7]. One notable mushroom, whose extracts has shown anti-disease properties is the Turkey tail mushroom.

Turkey tail mushroom, (also known as *Trametes versicolor*, *Coriolus versicolor*, yun zhi or kawaratake) (L.) is a common edible but unpalatable polypore found throughout the world. Although available scientific evidence on this mushroom does not support claims that the raw mushroom is an effective anti-cancer agent in humans, there are scientific evidence that extracts from this mushroom may be useful in the treatment and management of cancer[8]. PSK extracted from this mushroom has been reported to show anticancer activity in both *in vitro*[9], and *in vivo*[10] laboratory studies and also in preliminary human research[11]. This polysaccharide has the potential to reduce mutagen, radiation, and even spontaneously induced development of experimental cancer cell preparations[12]. It has also been reported to reduce cancer recurrence when use as an adjuvant in the treatment of gastric, esophageal, colorectal, breast and lung cancers[13]. Though the *in vitro* inhibition of certain human cancer cell lines by extracts of this mushroom has been reported [14-16] a nutraceutical blend of PSK, lentinan and other fungal extracts has also inhibited cancer cell proliferation under laboratory conditions[17]. The aim of this study is to evaluate and link the medicinal potentials of this mushroom to its nutritive properties.

MATERIALS AND METHODS

Sample collection and preparation

Fresh fruit bodies of *T. versicolor* was collected from its natural environment within the premises of University of Port Harcourt in Nigeria. A quantity of the mushroom (3.5 kg) was thoroughly washed and air dried for one (1) week in a clean dust free environment. The dried samples were ground into a fine smooth powder using Thomas Scientific, (Model 4) Wiley's mill.

Determination mineral elements and amino acid content

Major minerals and trace elements content of *T. versicolor* was determined by the Association of Official Analytical Chemists (A.O.A.C.)[18] method, where 1.0 g of ground mushroom sample was digested at a temperature of 500 ^{0}C for 4 hours. After cooling, the ash was dissolved in 5.0 ml of lithium carbonate buffer and the solution was transferred to a Teflon boat and analysed on a direct reading Spectrolab S-OES arc-spark emission spectrogram. Nineteen of the twenty amino acids and were determined by the method of Benitez[19], where 200 mg of defatted ground mushroom sample was hydrolysed using 7.0 ml of 6 N HCl and evaporated in a rotary evaporator. The hydrolysed sample was loaded into a Technicon sequential multi-sample amino acid analyser. Tryptophan was determined by the method of Robel[20], where 100 mg of defatted ground mushroom was hydrolysed using 10.0 ml of 4.2 M NaOH. The sample was also evaporated and loaded into a Technicon sequential multi-sample amino acid analyser.

GC-MS analysis of mushroom oil

A hundred gram of the ground mushroom powder was added to 3 dm^3 of distilled water and the oil obtained by hydro-distillation was collected into hexane and the solution was concentrated by evaporation at room temperature. The oil was analysed using a combined gas chromatograph model HP 6890 and mass spectrometer model 5973 (AgilentTech.) fitted with a capillary column HP-5 MS (5% phenylmethylsiloxane) 30.0 m x 250µm x 0.25µm, using Helium as a carrier gas at initial column temperature 120°C for 5 minutes. Thereafter, the column temperature was increased at 5°C per minutes to 320°C and held for 5 minutes. Electron impact ionization for mass spectroscopy was done at ionization energy of 70eV. The oil was diluted with 98% hexane and 2µl of the diluted sample was automatically injected into AgilentTech model 5973 mass spectrometer. The constituent compounds were identified using the Chem-Office software attached to the MS library. The names molecular formula and molecular weights of the component oils were ascertained using the database of National Institute of Standard and Technology (NIST).

STATISTICAL ANALYSIS

Data are presented as mean $\pm$ standard deviation of triplicate determinations. Mean values were obtained using 2010 Microsoft Excel software program.

RESULTS

Concentrations of major minerals and trace element

Potassium, calcium and magnesium had the highest concentrations amongst the major minerals observed in this mushroom with values of 75.98 $\pm$ 2.06 mg/kg, 48.36 $\pm$ 2.01 mg/kg and 45.38 $\pm$ 1.27 mg/kg respectively (fig.1).

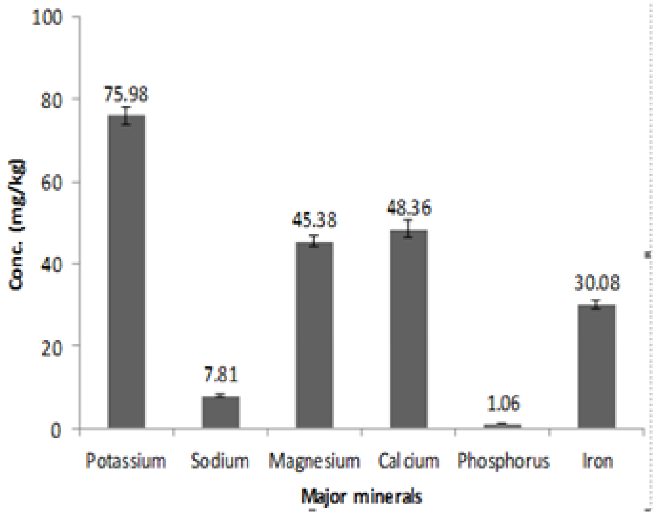

Fig.1: Major mineral concentration (mg/kg) in T. versicolor. Values plotted are means ± standard deviation of triplicate determination

while manganese, zinc and copper were the highest amongst the trace elements with values of 5.16 $\pm$ 0.35 mg/kg, 2.59 $\pm$ 0.24 mg/kg and 0.39 $\pm$ 0.06 mg/kg respectively (fig.2).

Amino acid Concentration

Arginine was the highest amino acid detected in this study with a value of 4.34 + 0.13 mg/kg (see fig. 3), followed by aspartic acid and valine with values of 4.20 + 0.16 mg/kg and 4.02+ 0.17

mg/kg respectively. Although glutamine and asparagine were not detected, cysteine and methionine had the lowest concentrations with values of 0.25 + 0.03 mg/kg and 0.53 + 0.02 mg/kg respectively.

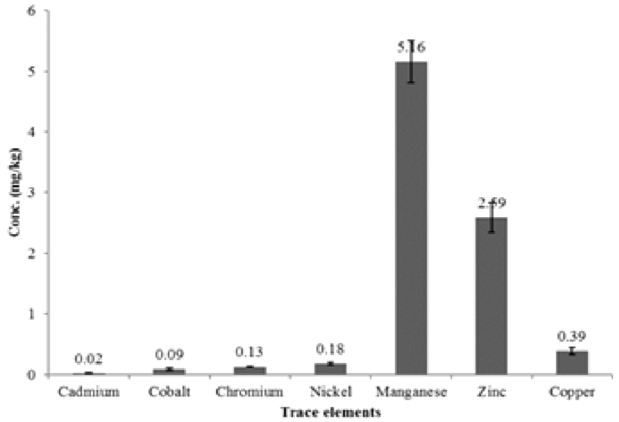

Fig. 2: Trace elements concentration (mg/kg) in *T. versicolor*. Values plotted are means ± standard deviation of triplicate determination.

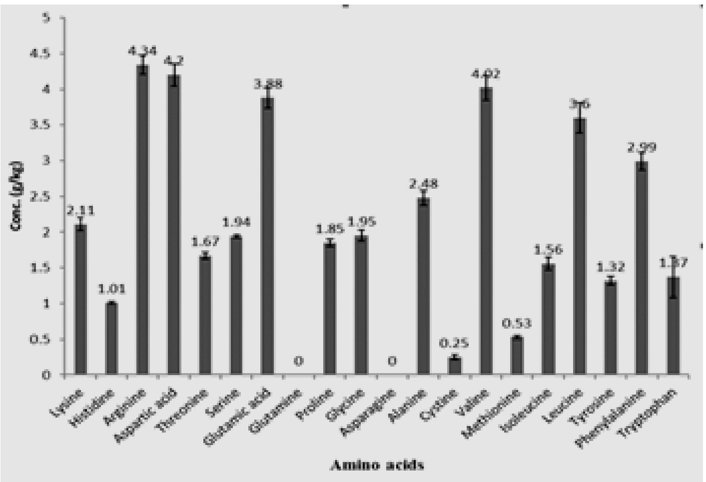

Fig. 3: Amino-acid concentration (g/kg) in T. versicolor. Values plotted are means ± standard deviation of triplicate determination.

Concentration of constituent oils

The gas chromatogram and the eleven constituent oils from *T. versicolor* are shown in Fig. 4 and Table 1 respectively. The five predominant oils identified are 9,12-Octadecadienoic acid (Z,Z),methyl esters with R_T of 21.698 min. and 21.858 min. and percentage total of 56.355 and 2.382 respectively, n-Hexadecanoic acid with R_T of 20.187 min. and a percentage total of 16.936, 1,2-Benzenedicarboxylic acid, diisooctyl ester with R_T of 26.052 min. and a percentage total of 7.744, Pentadecanoic acid,14-methyl-ester with R_T of 19.758 min. and a percentage total of 3.887 and Octadecane, 1-chloro- , with R_T of 11.850 min. and a percentage total of 3.023. These five major oils represent 90.327 % of the total constituent oil in *T. versicolor*. The six minor oils identified are Trans-13-Octadecenoic acid, methyl-ester with R_T of 21.303 min. and a percentage total of 2.598, Hexadecane with R_T of 16.050 min. and a percentage total of 2.408, Methyl 11,14-octadecadienoate with R_T of 21.252 min. and a percentage total of 2.012, Heptadecanoic acid, 16-methyl-ester with R_T of 21.509 min. and a percentage total of 1.240, 10-Methylnonadecane with R_T of 18.494 min. and a percentage total of 0.958 and Decane, 1,1'-oxybis- with R_T of 20.416 min. and a percentage total of 0.457. These six minor oils represent 9.673 % of the total constituent oil in *T. versicolor*.

Table 1: Constituent oils of *T. versicolor*

S/N	Compound	Retention Time (min)	Percentage of the total	Peak height	Molecular formula	Molecular weight
1.	**Octadecane, 1-chloro-**	**11.850**	**3.023**	**39288**	$C_{18}H_{37}Cl$	**288.939**
2.	**Hexadecane**	**16.050**	**2.408**	**77146**	$C_{16}H_{34}$	**226.441**
3.	**10-Methylnonadecane**	**18.494**	**0.958**	**39049**	$C_{20}H_{42}$	**282.548**
4.	**Pentadecanoic acid, 14-methyl-ester**	**19.758**	**3.887**	**145616**	$C_{17}H_{34}O_2$	**270.451**
5.	**n-Hexadecanoic acid**	**20.187**	**16.936**	**334332**	$C_{16}H_{32}O_2$	**256.424**
6.	**Decane, 1,1'-oxybis-**	**20.416**	**0.457**	**17296**	$C_{20}H_{42}O$	**298.547**
7	**Methyl 11,14-octadecadienoate**	**21.252**	**2.012**	**76314**	$C_{19}H_{34}O_2$	**294.472**
8	**Trans-13-Octadecenoic acid, methyl-ester**	**21.303**	**2.598**	**95314**	$C_{19}H_{36}O_2$	**296.488**
9	**Heptadecanoic acid, 16-methyl-ester**	**21.509**	**1.240**	**38686**	$C_{19}H_{38}O_2$	**298.504**
10	**9,12-Octadecadienoic acid (Z,Z)-**	**21.698**	**56.355**	**647229**	$C_{18}H_{32}O_2$	**280.446**
11	**9,12-Octadecadienoic acid (Z,Z)-**	**21.858**	**2.382**	**42455**	$C_{18}H_{32}O_2$	**280.446**
12	**1,2-Benzenedicarboxylic**	**26.052**	**7.744**	**140279**	$C_{24}H_{38}O_4$	**390.556**

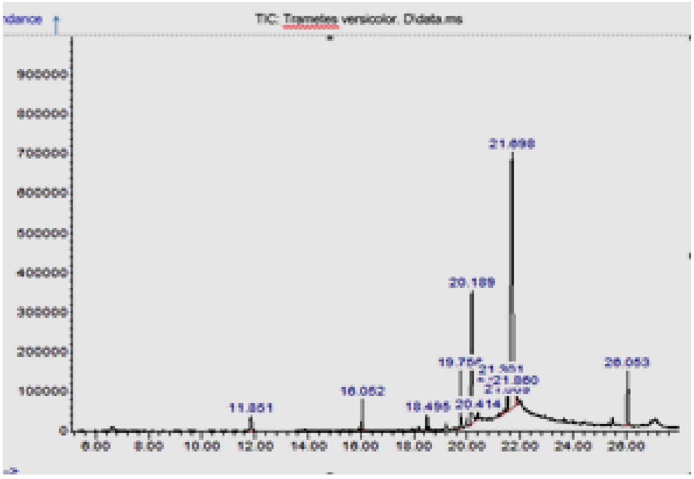

Fig. 4: Gas chromatogram of the constituent oils of T. versicolor.

DISCUSSION

The high concentration of potassium, calcium and magnesium observed in this study agrees with the study of Falandysz and Borovička[21], which reported macro-fungi as potential accumulators of extremely high concentrations of metallic elements even when grown on a low metal containing medium. This shows that this mushroom may have absorbed and accumulate these mineral elements from its growth medium. Mallikarjuna *et al*.,[22] also reported high concentrations of potassium and magnesium in wild *Lentinus cladopus, and Pleurotus djamor* and cultivated *Lentinula edodes, Pleurotus florida* mushrooms. The high concentration of potassium and calcium indicates that the consumption of this mushroom may be helpful in the regulation of acid–base balance, nerve impulse transmission, muscle contraction and bone calcification[23]. Its high magnesium content reflects its importance in enzymatic reactions, protein synthesis, formation of cyclic AMP and neuromuscular transmission[24]. The fairly high concentration of manganese, zinc and copper observed in this mushroom. Manganese acts either as a cofactor activating a number of enzymes thereby forming metal-enzyme complexes or as an integral part of some metalloenzymes,[25] while zinc has been reported to increase the activities of more than 70 enzymes[26]. A decrease in DNA, RNA and protein synthesis and subsequent impairment in cellular division, growth and repair of worn-out tissues has also been reported in zinc deficient animals[27]. As an essential nutrient in animals, copper deficiency impairs the lysyl oxidase catalysed cross-linking of the connective tissue proteins, collagen and elastin[28]. Though the resultant effect of this impairment can be specie specific, it may result in bone disorders, defective cardiovascular system or an abnormal lung structure[28]. Although copper also plays a role in iron absorption and mobilization, the concentrations of two brain neurotransmitter (dopamine and norepinephrine) are decreased by copper deficiency[28].

Amongst the eighteen amino acids observed in this mushroom, arginine, aspartic acid, valine glutamic acid and lucine were of fairly high concentrations. This agrees with the findings of Sharma *et al*.,[29] where aspartic acid and arginine were found to be the predominant amino acids in five edible mushrooms species of the *Lentinus* genus. Though Oyetayo *et al*.,[30] reported leucine as the most abundant amino acid in both wild and cultivated varieties of *Pleurotus sajor-caju*, they also stated that amino acid concentration of a mushroom is a function of mushroom specie, growth medium and the specific part of the mushroom that was selected for analysis[30]. The high concentrations of these amino acids reflects the nutritive potentials and the relevance of this mushroom to human health.

Among the eleven different compounds detected in the GC-MS analysis of the component oil from this mushroom, 9, 12-Octadecadienoic acid (Z,Z), methyl esters, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid, diisooctyl ester and pentadecanoic acid,14-methyl ester were predominant. 9,12-Octadecadienoic acid (Z,Z), methyl ester has been reported to have anti-inflammatory and anti-arthritic properties[31]. Cancer preventive, hepatoprotective, anti-arthritic, and anti-coronary properties of 9,12-Octadecadienoic acid (Z,Z) and the methyl ester of n- hexadecanoic acid have also been reported.[32] Studies also revealed the antioxidant, hypochloesterolemic, nematicide, pesticide, insecticide, lubricant, antiandrogenic, haemolytic, and 5-Alpha reductase inhibitory properties of n-hexadecanoic acid[33]. In Indian traditional medicine, oils rich in n-hexadecanoic acid have been used for the treatment of rheumatic symptoms[33]. The success of n-hexadecanoic acid in the treatment of rheumatoid arthritis is attributed to its ability to inhibit phospholipase A_2[34]. As a phatalate, 1,2-Benzenedicarboxylic acid, diisooctyl ester is generally regarded as a plasticizer and potential carcinogen, Rajeswari *et al*.,[35] reported its antimicrobial and antifouling activities, while Vijisaral and Arumugam[33] reported both antioxidant and antimicrobial activities of pentadecanoic acid,14-methyl ester.

In conclusion, the presence of these nutraceutical components suggests the use of this mushroom as possible nutritional supplement, while the extraction of these components may be helpful in the production of pharmaceuticals for the treatment and management of various disease conditions.

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