



## Phytochemical and anti-nutrients evaluation of some wild fruiting polypore macrofungi

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### ABSTRACT

Phytochemical and antinutrients analyses of *Trametes betulina*, *T. cingulata*, *Meripilus sumstinei* and *Oxyporus populinus* fruiting bodies were carried out. The phytochemical screening result shows the presence of sterols and triterpenes in large amounts, except in *M. sumstinei*. The quantity of alkaloids, saponins, flavonoids and carbohydrates varied in all samples. The antinutrients analysis revealed the presence of tannins ( $10.14 \pm 0.15 - 55.88 \pm 0.25$  mg/100g), phytates ( $4.12 \pm 0.20 - 27.00 \pm 0.03$  mg/100g), hydrogen cyanide ( $6.13 \pm 0.03 - 13.54 \pm 0.10$  mg/100g), soluble oxalates ( $7.04 \pm 0.09 - 70.4 \pm 0.02$  mg/100g), insoluble oxalates ( $52.70 \pm 0.09 - 98.56 \pm 0.25$  mg/100g) and total oxalates ( $105.60 \pm 0.31 - 158.40 \pm 0.44$  mg/100g). These corky and woody polypores may be inedible due to their fibrous nature; however, the permissible antinutrient levels and inherent phytochemicals may find medicinal and industrial relevance.

**Key words:** Basidiomycetes, mushroom, polypore, phytochemicals, antinutrients.

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### INTRODUCTION

Mushrooms are referred to as the fruiting bodies of macrofungi. They include both edible/medicinal and poisonous species. Polypores are aphyllorphorales with corky, woody, leathery, and papery basidiocarps [1]. Aphyllorphorales are mushrooms in the class basidiomycetes with holobasidia but usually lack gills [2], they include *Ganoderma* species, *Trametes* species, *Daedalea* species and other basidiomycetes. Polypores also cause wood rot of timber and are of great economic importance in ornamentals and forest trees in the tropics and temperate parts of the world [3]. Medicinal basidiomycetes represent unlimited source of phytochemical such as primary and secondary metabolites [4]. Medicinal importance of *Ganoderma* has been well researched and confirmed in Asia (Zjawiony, 2004), and *Ganoderma lucidum*, *Trametes cingulata*, *T. mariana*, *T. saejiara* and *Daedalea quercina* from Nigeria have also been reported to be active against *Bacillus subtilis* and *Pseudomonas syringae* which were plant pathogens [5, 6]. Morphological and several chemical tests are traditionally employed as criteria in the characterization of polypores [7, 8]. Most polypores are edible or at least non-toxic, however one genus of polypores has members that are very poisonous. Polypores from the *Hapalopilus* genus have caused poisoning in several people with effects including kidney dysfunction and deregulation of central nervous system functions [9]. However, the study of some chemical properties and constituents of polypores may suggest other useful alternative applications. A large body of evidence shows that *Trametes* is among the most versatile of white-rotters with ongoing intensive research into applications in bioremediation, effluent treatment, the pulp and paper industry, the food industry, synthetic chemistry, biofuels, cosmetics, biosensors and the textile industry, amongst others [10]. Also, the nutritional importance of a given food

depends on the nutrients or anti-nutrients composition [11, 12]; hence, the need to ascertain the edibility and phytochemical status of these mushrooms.

## EXPERIMENTAL SECTION

### *Samples collection and treatment*

Basidiocarps of species of polypores (*Trametes betulina*, *T. cingulata*, *Meripilus sumstinei* and *Oxyporus popolinus*) were collected from the stump and dead logs in the vicinity of the forest reserved area of the University of Uyo, Nigeria. Specimen identifications and authentication were done by a mycologist, Dr. Joseph Essien and the voucher specimens were deposited in the School of Pharmacy Herbarium, University of Uyo. The polypores were identified by their corky, woody and leathery basidiocarps and other characteristics [2, 7]. The samples were carefully cleaned manually to remove any extraneous materials, cut, sun-dried and oven-dried (Gallenkamp, DV 333) at 45°C for 48 h to constant weight. Dried samples were pulverized using an agate homogenizer, and stored in pre-cleaned polyethylene bottles, prior to analyses. All reagents were of analytical grade, except otherwise stated.

### *Phytochemical Screening*

Standard methods for phytochemical screening (alkaloids, flavonoids, saponins, tannins, carbohydrates, phlobatannins, sterols and triterpenes) were employed. Alkaloids determination was done using Mayer's and Dragendoff's reagents following the methods of Kapoor *et al.* [13] and Odebiyi and Sofowora [14]; tannins and phlobatannins [14]. The methods described by Kapoor *et al.* [13] were used for determining flavonoids. The persistent frothing test as described by Kapoor *et al.* [13] and Odebiyi and Sofowora [14] were used for saponins. Carbohydrates determination was done using Fehling's reagent following the method described by Harbone [15]. Sterols and triterpenes were determined following the Eiebemmann-Burchard test as described by Odebiyi and Sofowora [14] and Harbone [15].

### *Determination of anti-nutritional factors*

#### *Phytate determination*

Extraction and precipitation of phytate were done through phytic acid determination using the procedure described by Lucas and Markaka [16]. This entails the weighing of sample (2g) into a 250 mL conical flask. 2% conc. HCl (100 mL) was used to soak the samples in the conical flask for 3 h and then filtered through a double layer filter paper. Sample filtrate (50 mL) was placed in a 250 mL beaker and distilled water (107 mL) added to give/ improve proper acidity. 0.3% ammoniumthiocyanate solution (10 mL) was added to each sample solution as indicator and titrated with standard iron chloride solution which contained 0.00195 g iron/mL and the end point was signified by brownish-yellow colouration that persisted for 5 min. The percentage phytic acid was calculated.

#### *Tannins determination*

Tannin values were obtained by adopting the method of Jaffe [17]. Each sample (1g) was dissolved in distilled water (10 mL) and agitated, left to stand for 30 min. at room temperature. The samples were centrifuged and the extracts recovered; the supernatant (2.5 mL each) were dispersed into 50 mL volumetric flask. Similarly, standard tann acid solution (2.5 mL) was dispersed into separate 50 mL flasks. Folin-dennis reagent (1.0 mL) was measured into each flask followed by the addition of saturated Na<sub>2</sub>CO<sub>3</sub> solution (2.5 mL). The mixture was diluted to 50 mL in the flask and incubated for 90 min at room temperature. The absorbance of each sample was measured at 250 nm with the reagent blank at zero. The % tannin was calculated.

#### *Cyanogenic glycoside determination*

The method used was alkaline picrate method of Onwuka [18]. The samples (5 g each) in conical flasks were added distilled water (50 mL) and allowed to stand overnight. Alkaline picrate (4 mL) was added to sample filtrate (1 mL) in a corked test tube and incubated in a water bath for 5 min. A colour change from yellow to reddish brown after incubation for 5 min in a water bath indicated the presence of cyanides. The absorbance of the samples was taken at 490 nm and that of a blank containing distilled water (1 mL) and alkaline picrate solution (4 mL) before the preparation of cyanide standard curve.

#### *Oxalates determination*

The oxalates content of the samples was determined using titration method. The samples (2 g each) were placed in a 250 mL volumetric flask suspended in distilled water (190 mL) for soluble oxalate determination; 6 M HCl solution (190 mL) was added to each of the samples (2 g each) for total oxalate determination. The suspensions were

digested at 100°C for 1h. The samples were then cooled and made up to 250 mL mark of the flask. The samples were filtered, triplicate portions of the filtrate (50 mL) were measured into beaker and four drops of methyl red indicator was added, followed by the addition of concentrated NH<sub>4</sub>OH solution (drop wise) until the solution changed from pink to yellow colour. Each portion was then heated to 90°C, cooled and filtered to remove the precipitate containing ferrous ion. The filtrates were again heated to 90°C and 5% CaCl<sub>2</sub> (10 mL) solution was added to each of the samples with consistent stirring. After cooling, the samples were left overnight. The solutions were then centrifuged at 2500 rpm for 5 min. The supernatant were decanted and the precipitates completely dissolved in 20% H<sub>2</sub>SO<sub>4</sub> (10 mL). The total filtrates resulting from digestion of the samples (2 g each) were made up to 200 mL. Aliquots of the filtrate (125 mL) were heated until near boiling and then titrated against 0.05 M standardized KMnO<sub>4</sub> solution to a pink colour which persisted for 30 sec. The oxalate contents of each sample were calculated. Insoluble oxalate, presumed to be primarily calcium oxalate, was computed as the difference between total and soluble oxalate [19]. All determinations were performed in triplicates and presented in mg/100g.

## RESULTS AND DISCUSSION

The phytochemical analysis of the studied polypores is presented in Table 1. The result reveals the presence of sterols and triterpenes in large amounts, except in *M. sumstinei*. The quantity of alkaloids, saponins, flavonoids and carbohydrates varied in all samples. Tannins and phlobatannins were not detected using the method reported, except moderate amounts in *O. popolinus*. Macrofungi are known to produce large and diverse variety of secondary metabolites [20]. Ofodile *et al.* [5] demonstrated that *T. Marianna* and *T. cingulata* extracts which contained phenolics and similar profile of other compounds showed antimicrobial activity. Triterpenoids and other secondary metabolites were also identified in *T. cingulata* using HPLC [21]. He also concluded from his work that the age, locality, method of drying and season of collection influences secondary metabolites isolation and characterization. Some bioactive chemical compounds (such as saponins and tannins) are known to have therapeutic effects against microbes and parasites [22]. The quantitative determinations revealed the presence of the presence of tannins in all studied samples.

Table 1: Phytochemical evaluation of mushroom polypores

Phytochemicals	<i>Trametes betulina</i>	<i>Trametes cingulata</i>	<i>Meripilus sumstinei</i>	<i>Oxyporus popolinus</i>
Alkaloids	+	+++	+++	++
Flavonoids	++	-	-	+
Saponins	+	+++	+++	++
Sterols/triterpenes	+++	+++	-	+++
Tannins	-	-	-	-
Phlobatannins	-	-	-	++
Carbohydrates	++	++	++	+

- = not detected or present in negligible amount; + = present in trace amount; ++ = moderately present; +++ = present in high amount.

Table 2 indicates varying amounts of tannins, phytates, cyanide and oxalates in the studied mushrooms. The quantitative analysis shows that tannins (10.14±0.15 – 55.88±0.25mg/100g), phytates (4.12±0.20 – 27.00±0.03 mg/100g), hydrogen cyanide (6.13±0.03 – 13.54±0.10 mg/100g), soluble oxalates (7.04±0.09 – 70.4± 0.02 mg/100g), insoluble oxalates (52.70±0.09 – 98.56±0.25 mg/100g) and total oxalates (105.60±0.31 – 158.40±0.44 mg/100g) levels are within permissible dose [23, 24]. These values are comparable to results for some edible mushroom polypores, *G. lucidum* [25], *B. berkeleyi* and *G. lucidum* [26]. Many authors report soluble and insoluble oxalates as separately measurable components of the oxalate content of foods [27, 28]. In food, oxalic acid is typically found as either sodium or potassium oxalate, which are water soluble, or calcium oxalate, which is insoluble. Magnesium oxalate is also poorly soluble in water, although the contribution of this salt to the insoluble fraction of oxalate in food is unclear. Cyanide taken in the diet is detoxified in the body, resulting in the production of thiocyanate. Thiocyanate has the same molecular size as iodine and interferes with iodine uptake by the thyroid gland [29]. Therefore, some amounts of these anti-nutrients (phytate, oxalate and tannins) can be reduced by proper processing [30].

Table 2: Antinutrient factors in mushroom polypores

Toxins (mg/100g)	<i>Trametes betulina</i>	<i>Trametes cingulata</i>	<i>Meripilus sumstinei</i>	<i>Oxyporus popolinus</i>
Tannins	51.46±0.15	55.88±0.25	27.94±0.47	10.14±0.15
Phytate	7.40±0.10	4.12±0.20	27.00±0.03	22.89±0.18
Hydrogen cyanide	7.08±0.04	9.03±0.13	6.13±0.03	13.54±0.03
Soluble oxalates	61.60±0.18	70.4±0.88	7.04±0.09	61.60±0.09
Insoluble oxalates	52.70±0.09	88.00±0.10	98.56±0.25	79.20±0.10
Total oxalates	114.3±0.01	158.40±0.44	105.60±0.31	140.80±0.08

## CONCLUSION

The four mature mushroom polypores are described as corky, woody and inedible due to their fibrous nature. These fungi may be processed to meet desired needs since the antinutrients levels were below the permissible toxic limits. The phytochemicals contained in these macrofungi indicates potential applications in medicine for their bioactive constituents. Intensive research into the possible utilization of these mushrooms in bioremediation, effluent treatment, the pulp and paper industry, the food industry, synthetic chemistry, bio-fuels, cosmetics, biosensors and the textile industry would also be worthwhile.

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