Amino Acid Profiles and Bioactive Compounds of Four Inedible Mushrooms from Oban Division of Cross River National Park (CRNP), Nigeria

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ABSTRACT

Inedible mushrooms are endowed with diverse nutritional and nutraceutical compounds. In this study, the amino acid profile, and phytochemical fingerprints of four selected wild inedible mushroom species from Oban Division of Cross River National Park (CRNP), Nigeria including *Crepidotus applanatus* (Pers.) P. Kumm, *Daldinia concentrica* (Bolton) Cesati & de Notaris, *Oxyporus populinus* (Schumacher) Donk and *Trametes versicolor* (L.) Lloyd were investigated. The fresh sporocarps of study mushroom were collected from the decaying wood during July 2018. Samples for amino acids profiling were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the cartridge of the Applied Biosystems PTH Amino Acid Analyzer and analyzed for 45 minutes. The samples for phytochemical analysis were subjected to methanol extraction using a Soxhlet extractor and rotatory evaporator and injected into the column of an Agilent gas chromatography mass spectrometer (GCMS). The results of amino acid analysis revealed the presence of ten essential and eight non-essential amino acids in varying quantities across the species. The total amino acid content was highest in *C. applanatus* followed by *D. concentrica*, *T. versicolor*, and *O. populinus* in decreasing order. Glutamic acid, Arginine, and Aspartic acid were present in the highest amounts while Cystine, Lysine, and Histidine were the least across the species. The phytochemical screening of the samples led to the identification of 24 different compounds in *D. concentrica*, six compounds each in *C. applanatus* and *O. populinus* and three compounds in *T. versicolor*. In terms of percentage composition, 4H-Imidazo(1,5-a)(1,4)benzodiazepine-3-carboxylic acid, 5,6-dihydro-5-methyl-6-oxo-, ethyl ester, 1-Methylbicyclo[3.2.1]octane, Acetamide, N-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan-1-yl)- and 9,19-Cyclolanost-24-en-3-ol, (3.β)-, TMS derivative were the most dominant compounds in *C. applanatus*, *D. concentrica* and *T. versicolor* respectively. The identified compound reportedly shows diverse bioactive activities, including antiviral, anticancer, antioxidant, hypocholesterolemic, anaphylactic, neurostimulant, pesticidal, insecticide and insecticidal activities. This data may represent the baseline information on the amino acid profiles and bioactive constituents of inedible mushroom species from Nigeria, as sources of noble compounds of substantial nutritional, medicinal and agricultural importance.

Keywords: Inedible mushroom, Amino acid, Bioactive compounds, GC-MS, Nutraceuticals.

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I. INTRODUCTION

Mushroom refers to the fruiting body of macrofungi (Basidiomycetes and Ascomycetes) [1]. They can be epigeous or hypogeous, large enough to be seen with the naked eyes, and can be picked by hand [2]. Approximately 140,000 species of mushroom are known worldwide [3], with about 2166 species known to be edible [4] and 700 species known to exhibit different biological activities, with great potentials in nutritional and nutraceutical industries [5]. Mushrooms have been recognized for their nutritional and nutraceutical properties due to their inherent

composition. Mushrooms are generally rich in essential nutrients including an adequate amount of proteins, carbohydrates, vitamins, minerals, fibers, and fats; the low amount of calories, sodium, fats, and cholesterol [6], [7], [8], and variety of bioactive components [9].

Because of the nutritional and medicinally important components, mushrooms are considered as functional foods worldwide [10], [11], and can be compared favorably with the food value of milk, egg, meat, and commonly consumed vegetables [12], [13]. Besides, mushroom proteins contain non-essential amino acids, such as alanine, arginine, glycine, glutamic acid, aspartic acid, proline, and serine

[14], and polysaccharides long-chain molecules with a wide range of bioactive properties [7].

Mushrooms may be edible, poisonous, or inedible and they can be collected wildy or cultivated [15]. While edible mushrooms are consumed for their nutritional and medicinal values, poisonous mushrooms also known as toadstools are considered unsafe for consumption due to the high concentrations of lethal compounds such as cyclopeptides, monomethylhydrazine, orelline, muscarine, ibotenic acid, muscimol, and psilocybin, that on ingestion result in mild to severe illness and even death [16]. In contrast, some species termed “inedible mushrooms” are not poisonous *per se*, but are ostracized due to their toughness, woody texture and not being tasty, they are unpalatable or indigestible in nature.

Like other mushrooms, inedible species are endowed with diverse nutritional and nutraceutical compounds. Studies have suggested that inedible mushrooms may have a diversity of chemicals ranging from bitter compounds that stimulate the digestive system, phenolic compounds for antioxidant and many other pharmacological properties, including antibacterial and antifungal, tannins that work as natural antibiotics, diuretic substances, and alkaloids [17]. Due to their high contents of primary and secondary metabolites with health-enhancing benefits, powders, extracts, and other products from inedible mushrooms can be applied as ingredients of dietary supplements, mushroom pharmaceuticals, or therapies against cancer, heart diseases, diabetes, and viral infections [6], [18]; as dietary foods and dietary supplements and as natural bio-control agents in plant protection with bactericidal, insecticidal, nematocidal, fungicidal, and herbicidal activities [19].

Several studies have evaluated mushrooms for their nutritional and nutraceutical profile including amino acids which are monomeric units of proteins and serve as antioxidant, energy metabolites, and precursors of many biologically important nitrogen-containing compounds [20], [21]. Also, studies leading to the discovery of various bioactive molecules known as secondary metabolites or phytochemicals from wild mushrooms proliferated within the last two decades [7], [22]. However, these studies are biased towards edible and to a fair extent, poisonous mushroom species. Consequently, there is a dearth of information on the nutritional and medicinal properties of inedible mushrooms despite their great potentials as sources of putative bioactive compounds. The premium step in resolving this challenge is to conduct repetitive screening of inedible mushroom species for putative bioactive compounds [7]. Such information would be helpful not only in the discovery of new therapeutic agents but also as cradles for the discovery of noble economic materials with diverse industrial applications. Therefore, the current study seeks to evaluate the Amino acids and phytochemical profiles of some inedible mushroom species growing naturally in Calabar, Southern Nigeria. The output would be useful in guiding future researches on the industrial application of inedible mushrooms.

II. MATERIALS AND METHOD

A. Survey Area and Collection

The fresh sporocarps of *C. applanatus*, *D. concentrica*, *O. populinus*, and *T. versicolor* were collected from the decaying wood at the Oban Division of CRNP during July 2018. The park lies within longitude 8°20' E and 8°55' E and latitudes 5°00' N and 6°00' N. The total area is about 3,000 km² and it shares a boundary with Korup National Park of Cameroon in the east. The terrain is rugged, and the elevation rises from the river valleys to over 1,000 m in mountainous areas. The area has a rainy season of at least nine months (March-November) and receives over 3,500 mm of precipitation annually [23]. The vegetation is a characteristically moist tropical rainforest. In the less accessible areas, the forest has had little interference, but elsewhere the vegetation has been much influenced by human activities. Exploitation in the buffer zone has resulted in secondary regrowth. Tree height reaches 50 m to about 65 m and sometimes more [24].

B. Identification and Preparation of Mushroom Material

The collected mushrooms were taxonomically studied and identified morphologically. Initial identification was done based on macroscopic features according to the published descriptions and manuals. Morphological characters such as color, size, texture shape, and margin of the fruit body, other features such as odor, stipe and stipe length, pileus length, gill attachment, and spacing were considered as previously used by Roy and Krishnappa [25]. The nomenclature was based on the Index Fungorum and Mycobank.

After proper identification, the materials were air-dried under room temperature (27 °C) for ten days, grind into powder with the aid of an electronic blinder order to increase the surface area [26].

C. Determination of Amino Acids Content

The Amino acid profile of the samples was determined using a well-known standard procedure described by Benitez [27]. The samples were defatted in chloroform and methanol solution in the ratio of 2:1 after 4 grams of the sample was placed in soxhlet extraction thimble and refluxed with gentle heating for 15 hours [26]. Exactly 0.2 mg of the defatted sample was weighed into glass ampoule, 7 mL of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105±5 °C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan which was destroyed by 6NHCL was recovered using alkaline hydrolysis method with 4.2 M sodium hydroxide [28]. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml to acetate buffer (pH 8.0) and stored in plastic specimen bottles kept in the freezer [28].

Sixty (60) micro litres of the hydrolysate was loaded into the PTH Amino acid analyzer model 120A (USA). This was dispensed into the cartridge of the analyzer which had been designed to separate the free acidic, neutral and basic amino acids of the hydrolysate concentration of the amino acids to

produce a profile [26]. The results were obtained in g/100 gm.

D. Determination of Phytochemical Components

The method of Handa et al. [29] was used. Five (5) g of the mushroom material was packed into a thimble of a Soxhlet apparatus and extracted with 50ml of methanol. The sample was refluxed three times and the extract was transferred into the Rotary Evaporator and heated at 30-40 °C to rid the sample of any trace of the solvent and the extract was concentrated to 2 ml. This was transferred further into a Teflon screw-cap vial and cleaned up with 200 mm mesh silica gel and 3 g of anhydrous sodium sulfate in a well-packed column to obtain a clean extract for GC-MS screening.

The screening was with the aid of Agilent 6890N gas chromatography equipped with an autosampler connected to an Agilent Mass Spectrophotometric Detector was used. One (1) µl of the sample was injected in the pulsed spitless mode onto a 30 m × 0.25 mm id DB 5MS coated fused silica column with a film thickness of 0.15 micrometer. Helium gas was used as the carrier gas and the column head pressure was maintained at 20 psi to give a constant rate of 1 ml/min. Other operating conditions were preset. The column temperature was initially held at 55 °C for 0.4 minutes, increased to 200 °C at a rate of 25 °C/mins, then to 280 °C at a rate of 8 °C/mins and to the final temperature of 300 °C at a rate of 25 °C/mins, held for 2 minutes. The identification time was based on the retention time of the volatile and semi-volatile components in the column. The relative composition of each compound in the extract was expressed as a percentage with peak area normalization [30].

The identification of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with that stored chemical Library of National Institute for Standard and Technology (NIST) library version 2.4 as previously adopted by Jerome Jeyakumar et al. [31]

The prediction of the bioactive properties of the identified compounds was based on Dr. Duke's Phytochemical and Ethnobotanical Databases as adopted by Oni et al. [7]

III. RESULT

A. Amino Acid Composition

The amino acid profile of these mushrooms is shown in Table 5. The composition of analyzed amino acids revealed the presence of ten (10) essential and eight (8) non-essential amino acids in varying quantities across the species. The total amino acid content was highest in *C. applanatus* followed by *D. concentrica*, *T. versicolor*, and *O. populinus* in decreasing order. Glutamic acid, Arginine, and Aspartic acid were present in the highest amounts while Cystine, Lysine, and Histidine were the least across the species. Similarly, a higher total non-essential amino acid (100.91 g/100 g) compared to total essential amino acid (80.06 g/100 g) was recorded across the study species. Amongst the non-essential amino acids, Glutamic acid and Aspartic acid were observed as the most abundant across the species while Arginine was the abundant essential amino acids detected across the mushroom species.

TABLE 1: AMINO ACID CONTENT OF *CREPIDOTUS APPLANATUS*, *DALDINIA CONCENTRICA*, *OXYPORUS POPULINUS* AND *TRAMETES VERSICOLOR*

Amino acid type	The amino acid content of different species (g/100 g of protein)			
	<i>C. applanatus</i>	<i>D. concentrica</i>	<i>O. populinus</i>	<i>T. versicolor</i>
Alanine	2.58±0.01 ^b	3.49±0.02 ^d	2.05±0.02 ^a	3.03±0.02 ^c
Arginine *	5.16±0.02 ^d	4.99±0.04 ^c	3.18±0.02 ^a	3.96±0.04 ^b
Aspartic acid	5.33±0.02 ^d	4.28±0.02 ^b	3.22±0.03 ^a	4.53±0.02 ^c
Cystine	0.30±0.01 ^a	0.36±0.01 ^b	0.36±0.01 ^b	0.48±0.01 ^c
Glutamic acid	12.56±0.05 ^d	10.6±0.03 ^b	6.66±0.02 ^a	11.00±0.05 ^c
Glycine	2.56±0.02 ^d	1.80±0.02 ^a	1.52±0.01 ^b	1.61±0.01 ^c
Histidine *	1.09±0.02 ^a	1.02±0.01 ^c	0.7±0.01 ^a	0.96±0.01 ^b
Isoleucine *	2.62±0.02 ^c	1.9±0.01 ^d	1.24±0.01 ^a	1.51±0.02 ^b
Leucine*	3.62±0.02 ^c	3.62±0.03 ^c	2.63±0.02 ^a	3.38±0.02 ^b
Lysine*	3.07±0.02 ^a	1.38±0.01 ^d	2.01±0.01 ^c	1.43±0.01 ^b
Methionine*	0.80±0.01 ^b	0.75±0.01 ^c	0.64±0.01 ^a	0.81±0.01 ^c
Norleucine	ND	ND	ND	ND
Phenylalanine*	2.84±0.02 ^c	2.30±0.02 ^b	1.86±0.01 ^a	2.30±0.01 ^b
Proline	2.03±0.02 ^c	1.83±0.01 ^a	2.30±0.02 ^b	2.30±0.01 ^c
Serine	2.57±0.02 ^d	1.89±0.01 ^b	1.3±0.01 ^a	2.00±0.01 ^c
Threonine*	1.94±0.02 ^a	2.16±0.02 ^b	2.22±0.02 ^c	2.27±0.02 ^d
Tryptophan*	1.38±0.02 ^c	1.31±0.01 ^b	1.1±0.01 ^a	1.42±0.01 ^d
Tyrosine	1.89±0.02 ^c	1.55±0.01 ^b	1.38±0.01 ^a	1.55±0.02 ^b
Valine*	3.04±0.01 ^d	1.29±0.02 ^b	1.99±0.01 ^c	1.17±0.01 ^a
The total amino acid content	55.38±0.35	46.52±0.31	36.36±0.26	45.71±0.31
Total essential amino acids	25.56±0.18	20.72±0.18	17.57±0.13	19.21±0.16
Total non-essential amino acids	29.82±0.15	25.8±0.12	18.79±0.12	26.5±0.13

Each value is an average of three replicate, Values are mean ± standard deviation, Means not sharing a similar letter in a row are significantly different, ND: Not Detected.

*Essential amino acid.

B. Phytochemical Components

The chromatograms of *C. applanatus*, *D. concentrica*, *O. populinus*, and *T. versicolor* are presented in Fig. 1–4 respectively while the identified compounds with their retention time, molecular formula, molecular weight, peak

area (%), and bioactivities are given in Tables 2-5 respectively. The screening led to the identification of 24 different compounds in *D. concentrica*, six (6) compounds each in *C. applanatus* and *O. populinus* and three (3) compounds in *T. versicolor*.

In terms of percentage composition, 4H-Imidazo(1,5-a)(1,4)benzodiazepine-3-carboxylic acid, 5,6-dihydro-5-methyl-6-oxo-, ethyl ester (71.13%) followed by trans-Sesquisabinene hydrate (9.48%) were the highest in *Crepidotus applanatus*, just as 1-Methylbicyclo[3.2.1]octane (24.88) and 9,12-Octadecadienoic acid (Z,Z)- were highest in *D. concentrica*. Others were Acetamide, N-(6-acetylaminothiazol-2-yl)-2-(adamantan-1-yl)- (41.78%), [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 7-amino-, ethyl Ester (23.52%) and 1H-1,2,4-Triazole-5(4H)-thione, 4- allyl-3-(3-furyl)- (23.37%) in *O. populinus*, and 9,19-Cyclolanost-24-en-3-ol, (3.β.)-,TMS derivative (89.09) in *T. versicolor*.

In terms of bioactive properties, the various compounds detected in *C. applanatus* reportedly showed antiviral, anticancer, antitumor, antioxidant, antidote, hypocholesterolemic, nematocide, pesticide, lubricant, antiandrogenic, neuro-stimulant, helicicide, hemopoietic, herbicide, herpetifuge, hirudicide, hydrocholeretic and abortifacient properties while *D. concentrica* reported have Hallucinogenic, antiviral, antidote, hematopoietic, herbicide, herpetifuge, diuretic, larvicide, lactafuge, laxative, lubricant, bactericide, antioxidant, hypocholesterolemic, anaphylactic, nematocide, pesticide, and herbicidal properties. Others are acidulant, antioxidant, anaphylactic, antitumor, natriuretic, nematocide, neuro stimulant, and Neurosedative activities reported for compounds isolated from *O. populinus*, and oligosaccharide provider, endocrinactive, enterocontractant, and enterostimulant properties reported reviewed for compounds isolated from *T. versicolor*.

Fig. 1. GC-MS chromatogram of methanolic extract of *Crepidotus applanatus*.

Fig. 2. GC-MS chromatogram of methanolic extract of *Daldinia concentrica*.

Fig. 3. GC-MS chromatogram of methanolic extract of *Oxyporus populinus*.

Fig. 4. GC-MS chromatogram of methanolic extract of *Trametes versicolor*.

TABLE 2: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *CREPIDOTUS APPLANATUS*

SN	RT (min.)	Compound	Molecular formula	Molecular weight g/mol	Peak Area %	Activity*
1	18.030	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	5.13	Antioxidant, antitumor, hypocholesterolemic, anaphylactic, nematocide, pesticide, lubricant, antiandrogenic, neuro-stimulant, anaphylactic
2	20.319	9,12-Octadecadien-1-ol, (Z,Z)-	C ₁₈ H ₃₄ O	266.5	3.14	Oligosaccharide provider, the bioavailability of zink
3	20.977	Linoelaidic acid	C ₁₈ H ₃₂ O ₂	280.4	3.32	Antioxidant, acidulant
4	30.007	trans-Sesquisabinene hydrate	C ₁₅ H ₂₆ O	222.37	9.48	Increase Glutathione-S-Transferase (GST) activity, transdermal, antioxidant activities
5	32.547	Lanost-8-en-3-ol, (3.β.)-	C ₃₂ H ₅₄ O ₂	470.8	7.80	Endoanesthetic, oligosaccharide provider, beta-andrenergic-agents, beta-galactosidase-inhibitor, enterostimulant, fertility-enhancer, memory-enhancer, antioxidant
6	32.747	4H-Imidazo(1,5-a)(1,4)benzodiazepine-3-carboxylic acid, 5,6-dihydro-5-methyl-6-oxo-, ethyl ester	C ₁₅ H ₁₅ N ₃ O ₃	285.3	71.13	Acidulant, antiviral, antidote (against heavy metal, hydrazine, hypoglycin-a), helicicide, hemopoietic, herbicide, herpetifuge, hirudicide, hydrocholeretic, hypercholesterolemic, abortifacient, antioxidant

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases (Online database)

TABLE 3: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *DALDINIA CONCENTRICA*

SN	RT (min.)	Compound	Molecular formula	Molecular weight g/mol	Peak Area %	Activity*
1	5.459	(Z)-2-Heptene	C ₇ H ₁₄	98.19	0.84	Zinc Bioavailability
2	5.951	5H-1-Pyridine	C ₈ H ₉ N	119.16	1.93	Hallucinogenic, antiviral, antidote (against heavy metal, hydrazine, hypoglycin-a), hematopoietic, herbicide, herpetifuge,
3	6.117	Ethoxy(dimethyl)isopropylsilane	C ₇ H ₁₈ OSi	146.3	1.59	NA
4	7.204	6-Ethyl-5,6-dihydro-2H-pyran-2-one	C ₇ H ₁₀ O ₂	126.15	4.27	Hallucinogenic, antiviral (HIV), antidote (heavy metal, Hydrazine, Hypoglycin-A), Hematopoietic, Herbicide, Herpetifuge
5	8.549	Dianhydromannitol	C ₆ H ₁₀ O ₄	146.14	1.51	NA
6	8.829	(2R)-3-[[Tert-Butyl(dimethyl)silyl]oxy]propane-1,2-diol	C ₉ H ₂₂ O ₃ Si	206.35	0.76	Diuretic, antidote (diazepam, digoxin, tetrodotoxin, theophylline), antitumor (thyroid), termiticide, taenicide, testosteronegenic, thermogenic, tracheorelaxant, tranquilizer, trematodicide, trichomonicide, antioxidant
7	9.745	3-Oxetanol, 2,2,3-trimethyl-	C ₆ H ₁₂ O ₂	116.16	1.39	NA
8	11.450	Allyl(2-tetrahydrofurylmethoxy)dimethylsiloxane	C ₁₀ H ₂₀ O ₂ Si	200.00	0.98	NA
9	11.685	Tetrasiloxane, 3,5-diethoxy-1,1,1,7,7,7-hexamethyl-3,5-bis(trimethylsiloxy)-	C ₁₆ H ₄₆ O ₇ Si ₆	519.00	1.49	NA
10	13.813	6-Desoxy-1-gulitol	C ₆ H ₁₄ O ₅	166.17	1.97	Anticancer, antitumor, antidote (lead, lobelia), larvicide, lactafuge, laxative, lubricant
11	13.991	4,5,6,7-Tetrachloro-2a,8a-dihydro-2a-phenyl-2H-thiacyclobuta(b)(1,4)benzodioxin 1,1-dioxide	C ₁₆ H ₁₄ N ₂ S	266.40	0.90	Anticancer, antidote (heavy metal, hydrazine, hypoglycin-a, barbiturate, black widow), antitumor, antiviral, bactericide, antioxidant
12	14.076	2-Naphthalenol, 3-methoxy-	C ₁₁ H ₁₀ O ₂	174.20	2.73	NA
13	15.370	1-Tetradecene	C ₁₄ H ₂₈	196.37	8.19	NA
14	16.394	2-Butynedioic acid, di-2-propenyl			1.24	Acidulant, antidote (digoxin, diazepam), diuretic, antioxidant
15	18.042	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	11.12	Antioxidant, antitumor, hypocholesterolemic, anaphylactic, nematocide, pesticide, lubricant, Antiandrogenic, neuro-stimulant, anaphylactic
16	19.598	1-Methylbicyclo[3.2.1]octane	C ₉ H ₁₆	124.22	24.88	NA
17	19.816	1-Methylcycloheptene	C ₈ H ₁₄	110.2	0.98	NA
18	20.342	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₄ O	266.5	14.28	Oligosaccharide provider, the bioavailability of zinc
19	20.394	13-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	266.5	8.72	Bioavailability of zinc
20	20.645	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.48	1.44	Acidulant, antioxidant
21	21.235	(3-Methoxyphenyl)acetonitrile	C ₉ H ₉ NO	147.17	1.08	NA
22	23.689	Hexadecanal, 2-methyl-	C ₁₇ H ₃₄ O ₂	270.45	0.82	Antioxidant
23	26.814	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	C ₁₄ H ₂₄ O	208.34	1.80	Oligosaccharide Provider
24	28.353	5,6-Diphenyl-4,5-dihydro-1,2,4-triazin-3(2H)-one	C ₁₅ H ₁₃ N ₃ O	251.28	5.09	Hallucinogenic, antiviral (HIV), antidote (heavy metal, Hydrazine, hypoglycin-A), herbicide, hirudicide, hypocholesterolemic, antioxidant

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases (Online database).

TABLE 4: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *OXYPORUS POPULINUS*

SN	RT (min.)	Compound	Molecular formula	Molecular weight g/mol	Peak Area %	Activity*
1	17.334	1-Methylene-2-vinylcyclopentane	C ₈ H ₁₂	108.18	4.79	NA
2	23.399	Bromoxynil	C ₇ H ₃ Br ₂ N ₂ O	276.92	1.45	NA
3	32.459	Silicic acid, diethyl bis(trimethylsilyl) ester	C ₁₀ H ₂₈ O ₄ Si ₃	296.58	5.12	Acidulant, antioxidant
4	32.719	1H-1,2,4-Triazole-5(4H)-thione, 4-allyl-3-(3-furyl)-[1,2,4]Triazolo[1,5-a] pyrimidine-6-carboxylic acid, 7-amino-, ethyl Ester	C ₉ H ₉ N ₃ OS	207.25	23.37	NA
5	33.012		C ₁₀ H ₁₂ N ₄ O ₃ S	268.29	23.52	NA
6	35.143	Acetamide, N-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan-1-yl)-	C ₂₁ H ₂₅ N ₃ O ₂ S	383.5	41.78	Anaphylactic, antitumor, natriuretic, nematocide, neurostimulant, Neurosedative

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases (Online database).

TABLE 5: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *TRAMETES VERSICOLOR*

SN	RT (min.)	Compound	Molecular formula	Molecular weight g/mol	Peak Area %	Activity*
1	30.194	Benz[e]azulene-3,8-dione, 3a,4,6a,7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-5-(hydroxymethyl)-2,10-dimethyl-, (3a.alpha.,6a.alpha.,10b.beta.,10a.beta.,10b.beta.)-(+)-	C ₁₇ H ₂₂ O ₅	306.4	3.02	NA
2	30.932	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone	C ₁₀ H ₁₃ N ₃ S	207.3	7.89	NA
3	33.365	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-, TMS derivative	C ₃₃ H ₅₈ OSi	498.90	89.09	Oligosaccharide provider, endocrinative, enterocontractant, enterostimulant

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases (Online database).

IV. DISCUSSION

Mushrooms are an important source of biologically active constituents with high nutritional and medicinal properties [32]. Several studies have evaluated mushrooms especially edible and poisonous species for their bioactive compounds including amino acids which are monomeric units of proteins, and secondary metabolites Kidd et al. [7], [20], [21], [22]. However, there is limited information on putative bioactive molecules of inedible, tough mushroom species. In the present study, we evaluate the amino acids and phytochemical profiles of four inedible mushroom species: *C. applanatus*, *D. concentrica*, *T. versicolor*, and *O. populinus*. From the result, the amino acid profile of *C. applanatus*, *D. concentrica*, *T. versicolor*, and *O. populinus* were similar to those previously reported for some edible mushrooms including *Lentinus sajor-caju* [13], *Pleurotus sajor-caju* [33], and *Russula lepida*, *R. mustelina*, and *R. delica* [34], [35].

The presence of 18 amino acids at different concentrations across the species was similar to those reported by Ribeiro et al. [36] and Beluhan and Ranogajec [37]. The highest total amino acids were recorded in *Crepidotus applanatus* whereas the least record was in *O. populinus*. These variations could be attributed to the variety of their growth substrates [38]. The results suggested that the studied mushrooms were rich in essential amino acids, similar to the findings of Atri [13] and Kayode [33]. The ratios of essential amino acids to total amino acids ranged between 0.42 and 0.48 across the studied mushrooms and may well meet the FAO's minimum daily requirements for dietary protein quality in human nutrition [39]. Kouassi et al. [34] and Jaures et al. [35] reported similar essential amino acids to total amino acids ratios (0.40 to 0.65) for *Russula lepida*, *R. mustelina*, and *R. delica*. Arginine was the highest essential amino acids recorded across the studied mushrooms. Arginine is necessary for the body to make proteins and is found in red meat, poultry, fish, and dairy products, suggesting the huge potential of these mushrooms as substitutes for animal-based protein. Also, arginine is used for chest pain, treatment of peripheral arterial disease, high blood pressure, erectile dysfunction, a pregnancy complication, pre-eclampsia, and necrotizing enterocolitis, suggesting the usefulness of the studied mushrooms in medicine.

The quality of a food protein depends largely on its amino acid content. The cells, in making their protein, need a full

array of amino acids from food. Cells can synthesize non-essential amino acids when they are unavailable from food, but essential amino acids can only be obtained from foods [40]-[42]. The high concentration of essential amino acids presents in these mushrooms implied that they have a high biological protein value. This is particularly important as there is an increasing need for novel protein sources owing to the increasing cost of conventional sources of protein in the third world [34]. Besides, the cereal-based diets common in developing countries could receive a boost with the inclusion of these mushrooms in their diet.

Furthermore, the study led to the isolation of different secondary compounds, with diverse reported bioactive properties in methanolic extracts of *C. applanatus*, *D. concentrica*, *T. versicolor*, and *O. populinus*. The highest number of compounds was recorded in *D. concentrica* depicting its potential as sources of bioactive compounds [43]. The presence of n-Hexadecanoic acid and 9,12-Octadecadienoic acid (Z,Z)- in *C. applanatus* and *D. concentrica* has been reported in wild-edible mushrooms from Calabar, Nigeria by Oni et al. [7], indicating that inedible mushrooms share certain similarities in chemical profiles with the edible counterparts. Previous studies equally confirmed that mushroom fruiting bodies contain mixtures of biologically active compounds with putative health and industrial benefits including antimicrobial and antioxidant properties, and detriments [7], [44].

According to Mithöfer and Maffei [45] and Navarro et al. [46], bioactive compounds such as secondary metabolites protect mushrooms from microbes, insects, and other herbivores. This suggests that the compounds identified in the studied mushrooms may have great potential as sources of drugs and bio-control agents in medicine and agriculture respectively. Reportedly, the compounds identified in the studied mushrooms play diverse pharmaceutical and nutritive roles including antiviral, anticancer, antimicrobial, antioxidant, hypocholesterolemic, anaphylactic, narcotic, neuro stimulant, emollient, expectorant, laxative, and other prophylactic agents [47]. According to Kozarski et al. [48], Carmen [49] and Thu et al. [50], several mushroom compounds including n-Hexadecanoic acid; Undecanoic acid, 10-methyl-, methyl ester; 9-Octadecynoic acid, 2-Nonenoic acid; 2(3H)-Furanone and Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl] detected in the current study reacts with free radicals and neutralize them, thus helping stop or limit oxidative damage caused by these reactive species. This entails the potential of the studied

mushrooms or their products in food preservation, nutraceutical, and pharmaceutical industries [7].

Also, 6-Ethyl-5,6-dihydro-2H-pyran-2-one and 5,6-Diphenyl-4,5-dihydro-1,2,4-triazin-3(2H)-one detected in *D. concentrica* has been reported to possess antiviral activities against Human Immune Deficiency Virus (HIV) virus whereas all the study mushrooms except *T. versicolor*, synthesizes compounds with cancer-preventive, antitumor and anti-inflammatory activities. This suggests the potential of these mushrooms and their products in foiling infectious diseases caused by viruses [51]–[54], as well as an immunomodulator in cancer immunotherapy and anti-hyperplasia, and hypertrophic cell growth [55]–[57].

Similarly, the pesticidal, insecticidal, and nematocidal activities of the compounds detected in *C. applanatus*, *D. concentrica*, and *O. populinus* aligned with the reported potential of mushroom products in agriculture and pest management [58]. Studies had shown that mushroom-based agrochemicals are not toxic to humans, pollinators, fish, birds, or any other non-targeted animal as against synthetic chemicals [59]. Also, with the alarming global environmental and health problems arising from the use of synthetic chemicals, the search for novel nutrient and bioactive agents from biological sources is imperative. Extracts of *C. applanatus*, *D. concentrica*, *O. populinus*, and *T. versicolor* used in the present study led to the identification of essential and non-essential Amino Acids as well as various secondary compounds with reported antiviral, anticancer, antioxidant, hypocholesterolemic, anaphylactic, neurostimulator, pesticidal, and insecticidal activities, and could be considered as potential sources of natural protein and bioactive substances to improve health and food production.

V. CONCLUSION

This study has confirmed that the four species of inedible mushrooms are good sources of supplementary protein, thus providing baseline information on the nutritional composition of wild inedible mushroom species in Nigeria. The study also showed the phytochemical composition and bioactive potentials of the *C. applanatus*, *D. concentrica*, *O. populinus*, and *T. versicolor*, thus giving credence to the therapeutic and agricultural uses of these mushrooms in medicine and food security. Finding from this study is noble; however, variations may exist in the chemical compounds reported with similar species under different environmental conditions.

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