



Full Length Article

Bioactive Contents and Antioxidant Activities of Wild Mushrooms of Southeastern Nigeria

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Abstract

Mushrooms are underexploited and efforts to domesticate them are not yielding enough results as over 95% of consumed mushrooms in Africa and most of the world is still collected from the wild. Their utility has been hampered by incorrect morphological and biochemical identifications. Thus, this research was centered on investigating the bioactive contents and antioxidant activities of molecularly identified wild mushrooms from the southeast region of Nigeria. Fifty samples of wild-growing mushrooms were collected using opportunistic sampling method in 5 states of the country. Standard methods were used to analyze the bioactive contents and antioxidant activities. Significant ($P \leq 0.05$) variations were recorded in the bioactive compounds and antioxidant activities of the sampled mushrooms. The bioactive compounds expressed in mg 100g⁻¹ revealed that phenols (58.15 ± 0.030 to 1599.45 ± 0.027) were the highest, while lutein (0.00 ± 0.000 to 265 ± 0.029) was the least. However, flavonoids (15 ± 0.008 to 753 ± 0.017) and beta-carotene (19.66 ± 0.019 to 261.44 ± 0.017) were also detected in appreciable quantities. The antioxidant activities ranged from 0.00 ± 0.000 – 61.55 ± 0.041 (50 mg mL⁻¹), 0.00 ± 0.000 – 69.28 ± 0.029 (75 mg mL⁻¹) and 0.00 ± 0.000 – 74.62 ± 0.029 (100 mg mL⁻¹). The sampled wild mushrooms have proven excellent sources of bioactive compounds and antioxidant properties which can be beneficial to human health, while the use of molecular markers (ITS region) was effective in proper identifications of the wild mushrooms.

Keywords: Antioxidant properties; Bioactive contents; Molecular identifications; Wild mushrooms

Introduction

Mushrooms are an excellent source of essential nutrients and accumulation of secondary metabolites such as phenolic compounds and flavonoids in their sporocarps which have validated their role in bioactivity (Bajwa *et al.* 1999; Ao *et al.* 2019). Previous studies have demonstrated that mushrooms contain essential organic compounds with antioxidant properties such as phenolic compounds, carotenoids, indole compounds, and glutathione which are the strongest known antioxidants (Bains and Bains 2015; Podkova *et al.* 2021). Important phenolic compounds including phenolic acids, tannins, lignans, hydroxycinnamic acids, hydroxybenzoic acid, oxidized polyphenols and stilbenes have been reported in various mushroom species (Ma *et al.* 2018). These organic compounds perform antioxidant functions by counteracting the deleterious effect of reactive nitrogen and oxygen species (Podkova *et al.* 2021). Generally, mushrooms are very rich in phenolic

compounds which protect the body against degenerative disorders such as cardiovascular diseases, aging and brain dysfunction (Butkhup *et al.* 2018). However, several literatures have cited that the most significant biological activity of phenolic compounds is their antioxidant activity where they act as free radical inhibitors, peroxide decomposers, oxygen scavengers and metal inactivators (Ma *et al.* 2018; Podkova *et al.* 2021).

The antioxidant potential of mushrooms has been attributed partly to the identification of carotenoids in most species. Carotenoids are organic compounds that perform their antioxidant activities by forming adducts with free radicals and reducing Fe³⁺ to Fe²⁺ (Im *et al.* 2014). The actions of carotenoids as antioxidants are basically due to the existence of beta-carotene which has been found in most mushroom species (Jayaprakasha *et al.* 2001; Podkova *et al.* 2021). Carotenoids also protect the body against oxidative stress diseases such as cancers, diseases of the circulatory and nervous systems (Fiedor and Burda 2014).

Carotenoid compounds are typically orange or red in color and apart from β -carotene may include other compounds such as zeaxanthin, lycopene, phytoene, lutein and cryptoxanthin which do not degrade during baking or cooking processes (Khachik 2006; Podkowa *et al.* 2021). The composition of carotenoids in mushrooms and the bioactivity of carotenoid extracts from mushrooms have been reported by some authors (Ribeiro *et al.* 2011; Kozarski *et al.* 2015; Sharma and Gautam 2015).

Although some authors categorize flavonoids as representative of the phenolic compounds, their separate classification may appear reasonable due to their large number which is about 8000 (Tungmunthum *et al.* 2018). Some of the notable flavonoids include quercetin, catechin and hesperidin which just like the phenolic compounds exert antioxidant activities (Pietta 2000). There are contrasting reports concerning the presence of flavonoids in mushrooms (Podkowa *et al.* 2021). While Gil-Ramírez *et al.* (2016) did not record the presence of flavonoids in mushrooms; several authors have validated the presence of flavonoids and determined their concentrations in mushroom species (Kozarski *et al.* 2015; Butkhup *et al.* 2018). In addition, the identification of 7 core genes involved in the biosynthesis of flavonoids in *Sanghuangporus sanghuang* may have further confirmed the presence of flavonoids in mushrooms (Shao *et al.* 2020). However, Podkowa *et al.* (2021) noted that due to several studies confirming the presence and concentration of flavonoids in mushrooms, it should be assumed that flavonoids are present in many but not all fungal lineages. Several notable studies have been carried out on the identification and quantification of flavonoids in mushroom fruiting bodies (Barros *et al.* 2007; Kosanić *et al.* 2012; Butkhup *et al.* 2018).

Recent advancements in DNA technology have significantly improved traditional taxonomic methods, particularly in fungal identification (Shi *et al.* 2015; Khan *et al.* 2021). Molecular techniques are increasingly used to address species classification issues, with DNA markers being the foundation for identification (Khan and Javaid 2022, 2023). Reference species are often used for specific fungi classes. Public DNA sequence databases can provide information for tentative identifications, but authenticating source material is rare. Important molecular techniques include Southern blotting, PCR-RFLP, RAPD, PCR, DNA sequencing, and microarrays. DNA extraction and purification are crucial steps in these methods (Sarwar *et al.* 2019). Direct sequencing of PCR products is a significant technique for phylogenetic investigations (Khan and Javaid 2021).

Despite the enormous bioactive contents and antioxidant properties of mushrooms, their utilization for food and medicine has suffered serious setbacks due to incorrect morphological identifications. However, the advent and use of biotechnological tools in species identifications have shown promise in bypassing the bottlenecks of morphological identifications. Thus, this study aimed to investigate the bioactive contents and

antioxidant activities of molecularly identified wild mushrooms from the southeast region of Nigeria.

Materials and Methods

Study location

The study was conducted within the Southeast region, which is one of the six geopolitical zones of Nigeria comprising Enugu, Anambra, Imo, Abia and Ebonyi states (Anejionu *et al.* 2013). The region is located within latitudes 5°N and 6°N and longitudes 6°E to 8°E (Osugiri *et al.* 2019), with an estimated population of 16,395,554 and a land mass of 10,952,400 hectares (Ibe *et al.* 2022). Southeast Nigeria predominantly belongs to the tropical rainforest zone, although the vegetation stretches from the derived savannah in the interior to the mangrove swamp on the coast (Okorafor *et al.* 2017). It is difficult to distinguish between the rainy and dry seasons, even though the rainy and dry seasons are more pronounced between May to October and November to April, respectively. The region has an annual mean temperature of 28°C and annual rainfall of 2000 to 3000 mm (Osugiri *et al.* 2019). People of southeast Nigeria are mostly the Igbo-speaking tribe with unique culture and practice subsistence level of agriculture under rain-fed conditions.

Sample collection

The mushrooms were sampled from the five states of Southeast Nigeria using the opportunistic method of sampling macrofungi or mushroom biodiversity (Lodge *et al.* 2004; Prayudi *et al.* 2019). A total of 50 samples of wild mushrooms were obtained from different locations in Southeastern Nigeria. They were harvested and put into separate paper bags and labeled properly. All the collected mushroom samples were oven-dried at 50°C for 48 h.

Morphological and molecular identifications

All the mushroom samples were identified using mushroom identification keys by a trained mycologist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka before proceeding with molecular identifications. A total of 41 mushrooms samples were successfully identified using molecular methods, while molecular methods failed to identify 9 samples (S3, S11, S13, S19, S29, S34, S45, S48 and S49). The DNA of the mushroom samples was extracted using a Zymo Research Quick-DNA Plant/Seed Miniprep kit (cat. D6020) following the manufacturer's procedures. The internal transcribed spacer (ITS) regions (~600 bp) of rRNA gene were amplified with ITS-5 forward (5'- GGA AGT AAA AGT CGT AAC AAG G - 3') and ITS-4 reverse (5'- TCC TCC GCT TAT TGA TAT GC - 3') primers according to White *et al.* (1990) and Adedokun *et al.* (2016). The PCR products

were sequenced at Inqaba Biotec Company, Pretoria, South Africa to obtain the nucleotide sequence of the DNA barcodes of the different mushroom samples. Each of the nucleotide sequences was identified and compared with the nucleotide sequence in the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST).

Determination of bioactive contents and antioxidant activity

Bioactive compounds (total phenolic compounds, total flavonoids and β -carotene) were determined according to the methods adopted by Hussein *et al.* (2015), lutein was determined by methods of Hajare *et al.* (2013), while the antioxidant activities of the mushroom samples were determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay.

Total phenolic compounds

Folin-Ciocalteu assay was used to determine the total phenolic compounds in each of the mushroom species (Hussein *et al.* 2015). Each mushroom extract (1 g) was dissolved in 5 mL methanol and 200 μ L of the mushroom extracts were transferred to a test tube and 1 mL of Folin-Ciocalteu reagent was added before thorough mixing. Sodium carbonate (0.8 mL of 7.5% w/v) was added to the mixture after 3 min. The mixture was shaken for 30 min in the dark before centrifugation at 3300 g for a period of 5 min. The absorbance of mushroom extracts and the blank were measured at 515 nm using a spectrophotometer. Different concentrations of the gallic acid standard were used in the calibration of the curve and each result was determined as gallic acid equivalents in mg 100 g⁻¹ of each extract.

Total flavonoids

The total flavonoid concentration of the mushroom samples will be determined using the AlCl₃ assay method as was also adopted by Hussein *et al.* (2015). One milliliter of each mushroom extract was diluted in aqueous ethanol (4.3 mL of 80%) which also contained 0.1 mL of 1 M aqueous potassium acetate and 0.1 mL of 10% aluminum nitrate. The mixture was incubated at room temperature for 40 min before calorimetric determination of the absorbance in 515 nm against a blank using a spectrophotometer. Total flavonoid was then computed using the calibration curve of the different concentrations of quercetin standard and results were reported as quercetin equivalent per 100 g of the mushroom extracts.

β -carotene contents

β -carotene contents were determined by the methodology of Nagata and Yamashita (1992) as was also adopted by

Hussein *et al.* (2015). Hundred (100 mg) of the mushroom extracts were mixed with 10 mL of Acetone-hexane mixture (92:3) shaken for 1 min and filtered with a filter paper. The filtrate was evaluated for absorbance at 453, 505 and 663 nm and the β -carotene contents were computed as follows:

$$\beta\text{-carotene (\%)} = (0.216 \times A_{663}) - (1.22 \times A_{645}) - (0.304 \times A_{505}) + (0.452 \times A_{453})$$

Lutein contents

The mushroom samples (10 g) were ground and mixed with 40 mL solvents (Acetone, ethanol and butanol). The solutions were filtered using no 1 Whatman filter paper and then the filtrate was centrifuged at 10000 rpm for a period of 10 min. The aqueous solution was stored at 4°C until measurement. A spectrophotometer was used to measure the absorbance at 446 nm at intervals of 3 h for a total of 9 h for both the samples and standards (Hajare *et al.* 2013). Lutein concentration was then calculated using the following formula:

$$\text{Lutein contents mg g}^{-1} = \frac{A \times V \text{ (mL)} \times \text{dilution factor}}{\epsilon \times W \text{ (g)}}$$

Where; A = 446 nm absorbance, V = extract volume in mL, ϵ = coefficient of absorption (2589), W = sample dry weight.

Estimation of antioxidant activities

The antioxidant activities of the mushroom samples were determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay according to procedures described by Tibuhwa (2014) and Hussein *et al.* (2015). Methanol extracts of the mushroom samples (0.006 - 0.3 mg/mL) were prepared and ascorbic acid was used as the standard control. Stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical containing 1 mL of 0.4 mmol⁻¹ methanolic solutions was mixed with 1 mL of the mushroom extract. The mixture was agitated vigorously and kept in a dark room for 30 min before being evaluated at 515 nm using a spectrophotometer. The radical scavenging activity of each mushroom sample was determined as follows:

$$\text{DPPH radical scavenging activity} = \frac{A_0 - (A_1 - A_s)}{A_0} \times 100$$

Where;

A₀ = absorbance of the control solution containing only DPPH.

A₁ = absorbance in the presence of mushroom extract in DPPH solution

A_s = the absorbance of the sample extract solution without DPPH.

Statistical analysis

All analyses were done in triplicates and data collected from the proximate analyses were subjected to Analysis of Variance (ANOVA) using the GENSTAT statistical software (edition 23.1) and the significant means were separated using the least significant difference (LSD) at $P \leq 0.05$ level of significance.

Results

Bioactive contents of the sampled mushrooms

Significant variations were recorded in the analyzed bioactive contents of the mushroom samples (Table 2). The total phenolic content of the sampled mushrooms was significantly highest in S43 (1599.45 ± 0.027) and was followed closely by S1 (1459.63 ± 0.029), S39 (1432.16 ± 0.037), S12 (1295.00 ± 0.000) and S4 (948.03 ± 0.000) in that order. The lowest concentration of phenols was recorded in S22 (58.15 ± 0.030). However, other mushrooms samples such as S2 (675.00 ± 0.000), S6 (401.97 ± 0.000), S9 (558.99 ± 0.034), S13 (417.06 ± 0.000) and S44 (604.01 ± 0.029) had comparatively high amounts ($> 400 \text{ mg } 100 \text{ g}^{-1}$) of phenols, while mushroom samples such as S4 (94.80 ± 0.016), S8 (182.17 ± 0.039), S23 (84.92 ± 0.033), S27 (143.75 ± 0.027), S32 (148.53 ± 0.022), S34 (118.82 ± 0.023), S35 (103.88 ± 0.019), S40 (147.47 ± 0.018), S41 (92.28 ± 0.027), S46 (103.65 ± 0.038), S47 (152.95 ± 0.017) and S48 (120.08 ± 0.025) were comparatively low in phenols ($< 200 \text{ mg } 100 \text{ g}^{-1}$).

The flavonoid composition of the mushroom samples was significantly highest in S2 (753 ± 0.017) and was trailed by S3 (194 ± 0.024) and S21 (193 ± 0.028) which did not differ significantly from each other. They were followed by other mushroom samples such as S8 (177 ± 0.020) and then S25 (158 ± 0.029) and S24 (156 ± 0.013) which did not differ significantly. However, flavonoid composition was significantly lower in S32 (15 ± 0.008). Generally, the flavonoid contents of the mushroom samples were comparatively low except in very few samples that showed substantial amounts above $100 \text{ mg } 100 \text{ g}^{-1}$ (Table 1 and 2).

Lutein concentration in the sampled mushrooms varied significantly and ranged from 0.00 ± 0.000 recorded in S11, S14 and S16 to 265 ± 0.029 recorded in S21 (Table 2). However, S49 which had the second largest amounts of lutein (178 ± 0.029) was followed closely in a sequential order by S15 (159 ± 0.029), S37 (126 ± 0.028), S42 (115 ± 0.034) and S25 (106 ± 0.026). Generally, most of the sampled mushrooms are low in lutein, because apart from the few listed samples that had lutein concentration above $100 \text{ mg}/100 \text{ g}$, every other sampled mushroom had a lutein concentration $< 100 \text{ mg } 100 \text{ g}^{-1}$ (Table 2).

Beta-carotene concentrations measured in $\text{mg } 100 \text{ g}^{-1}$ in the sampled mushrooms (Table 2) were significantly highest in S31 (261.44 ± 0.017) and was followed sequentially by S49 (243.36 ± 0.029) and then S18 (229.65 ± 0.028) and S33 (228.92 ± 0.032) which did not differ significantly from each other. However, S45 (19.66 ± 0.019) had significantly the least beta-carotene. Over 50% of the sampled mushrooms had beta-carotene above $100 \text{ mg } 100 \text{ g}^{-1}$, while the remaining percentage recorded lesser ($< 100 \text{ mg } 100 \text{ g}^{-1}$) of beta-carotene.

Antioxidant activities of sampled mushrooms

The antioxidant capacity of the sampled mushrooms as measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) showed significant variations (Table 3). Sample 21 at a DPPH of 50 mg/mL (61.55 ± 0.041), 75 mg/mL (69.28 ± 0.029) and 100 mg/mL (74.62 ± 0.029) had significantly the highest antioxidant activity among the sampled mushrooms and were significantly lesser than the antioxidant activity of the vitamin C standard used. This was followed by S24 (58.74 ± 0.032 , 66.71 ± 0.029 and 71.40 ± 0.029), S2 (50.22 ± 0.021 , 56.71 ± 0.029 and 67.02 ± 0.000), S32 (49.92 ± 0.028 , 54.01 ± 0.000 and 65.49 ± 0.029) and S8 (39.97 ± 0.000 , 48.17 ± 0.029 and 56.51 ± 0.029) at all the DPPH concentrations viz. 50 , 75 and 100 mg mL^{-1} , respectively.

Mushroom samples such as S3, S9, S16, S17, S18, S22, S23, S27 and S45 did not record any antioxidant activity at 50 , 75 and 100 mg mL^{-1} (Table 3). However, samples such as S1 and S19 recorded slight antioxidant activity at DPPH concentrations of 100 mg/mL but did not have any antioxidant activity at 50 and 75 mg mL^{-1} . More so, mushroom samples such as S30, S31, S34, S35, S36, S44, S46 and S50 did not record any antioxidant activity at a DPPH concentration of 50 mg/mL , had slight antioxidant activity at 75 mg/mL and 100 mg mL^{-1} . Antioxidant activity increased consistently with the increase in DPPH concentration ($100 > 75 > 50 \text{ mg mL}^{-1}$). Compared to the antioxidant activity of the vitamin C standard, most of the sampled mushrooms had very slight antioxidant activity.

Discussion

Bioactive compositions (total phenols, total flavonoids, lutein and beta-carotene) were observed to vary widely amongst the sampled mushrooms. Due to differences in the genetic backgrounds, substrates and climatic conditions of the sampled mushrooms, it will be reasonable to suggest that the variations in bioactive compositions are related to both the genotypic and environmental influences. Phenols and flavonoids are regarded as non-essential dietary components that are beneficial to human health (Aboubakr *et al.* 2018). They possess free radical scavenging activities and chain-breaking antioxidant properties due to the presence of the hydroxyl group (Liu *et al.* 2013).

Although total phenols varied widely across the sampled mushrooms, most of the samples had high concentrations which were greater than $3.76\text{--}21.13 \text{ mg } 100 \text{ g}^{-1}$ reported in cultivated mushrooms of Thailand (Srikrum and Supapvanich 2016), $0.015\text{--}0.075 \text{ mg } 100 \text{ g}^{-1}$ in wild Malaysian mushrooms (Azieana *et al.* 2017), and 15.24 and $16.21 \text{ mg } 100 \text{ g}^{-1}$ in white button and oyster mushrooms, respectively (Sahu *et al.* 2018). Interestingly, they compared favorably with 13 mg/g , 9.61 mg/g , and 6.22 mg/g , recorded in wild samples of *A. bisporus* in Turkey, Iran and Libya respectively (Elmastas *et al.* 2007; Tajalli *et al.* 2015; Aboubakr *et al.* 2018). Likewise, the range of total phenols

Table 1: Lists of samples and their habitats, date of collection, town, local government and state of collection

Samples	Date of collection	Town	LGA	State	Substrate/Habitat
S1	23/5/2023	Imilike-Agu	Udenu	Enugu	Soil
S2	23/5/2023	Botanic Garden UNN	Nsukka	Enugu	Dead Tree Stump
S3	25/5/2023	Isuochi	Umunneochi	Abia	Soil
S4	25/5/2023	Isuochi	Umunneochi	Abia	Soil
S5	25/5/2023	Iheakpu-Awka	Igbo-Eze South	Enugu	Soil
S6	25/5/2023	Uhunowerre	Igbo-Eze South	Enugu	Dead Tree Stump
S7	30/5/2023	Arondiokoroji	Okigwe	Imo	Dead Tree Stump
S8	30/5/2023	Arondiokoroji	Okigwe	Imo	Soil
S9	30/5/2023	Isuochi	Umunneochi	Abia	Dead Wood
S10	30/5/2023	Arondiokoroji	Okigwe	Imo	Soil
S11	1/6/2023	Uvuru	Uzo-Uwani	Enugu	Soil
S12	1/6/2023	Amube	Igbo-Eze North	Enugu	Dead Palm Tree
S13	3/6/2023	Okoji Ula-Ekwulobia	Aguata	Anambra	Dead Wood
S14	3/6/2023	Okoji Ula-Ekwulobia	Aguata	Anambra	Dead Wood
S15	3/6/2023	Nkwelle -Ezunaka	Oyi	Anambra	Dead Wood
S16	3/6/2023	Nkwelle -Ezunaka	Oyi	Anambra	Soil
S17	3/6/2023	Nkwelle -Ezunaka	Oyi	Anambra	Soil
S18	5/6/2023	Unadu	Igbo-Eze South	Enugu	Dead wood
S19	5/6/2023	Iwollo	Ezeagu	Enugu	Soil
S20	6/6/2023	Oboro	Ikwo	Abia	Soil
S21	6/6/2023	Oboro	Ikwo	Abia	Soil
S22	6/6/2023	Okoji Ula-Ekwulobia	Aguata	Anambra	Dead Wood
S23	8/6/2023	Umuduru	Isiala Mbano	Imo	Soil
S24	8/6/2023	Ibagwa-Aka	Igbo-Eze South	Enugu	Dead Tree Stump
S25	9/6/2023	Uturu	Isuikwuato	Abia	Dead Wood
S26	9/6/2023	Uturu	Isuikwuato	Abia	Dead Wood
S27	4/7/2023	Aguleri	Anambra North	Anambra	Soil
S28	5/7/2023	Umuduru	Isiala Mbano	Imo	Dead Wood
S29	5/7/2023	Umuduru	Isiala Mbano	Imo	Dead Wood
S30	5/7/2023	Awba-Ofemili	Awka North	Anambra	Decaying Wood
S31	12/7/2023	Uturu	Isuikwuato	Abia	Dead Tree Branch
S32	12/7/2023	Obollo-Afor	Udenu	Enugu	Soil
S33	12/7/2023	Aguleri	Anambra North	Anambra	Dead Tree Stump
S34	12/7/2023	Aguleri	Anambra North	Anambra	Dead Palm Log
S35	18/7/2023	Amufie	Igbo-Eze North	Enugu	Tree Stump
S36	18/7/2023	Umuduru	Isiala Mbano	Imo	Dead Wood
S37	23/7/2023	PG Hostel UNN	Nsukka	Enugu	Soil
S38	23/7/2023	Okata	Igbo-Eze North	Enugu	Soil
S39	23/7/2023	Obukpa	Nsukka	Enugu	Dead Mango Wood
S40	28/7/2023	Oboro	Ikwo	Abia	Dead Wood
S41	29/7/2023	Awba-Ofemili	Awka North	Anambra	Dead Palm Wood
S42	29/7/2023	Ndufu-Aliki Ikwo	Ikwo	Ebonyi	Dead Tree Trunk
S43	29/7/2023	Akokwa	Ideato North	Imo	Dead Wood
S44	29/7/2023	Akokwa	Ideato North	Imo	Dead Wood
S45	29/7/2023	PG Hostel UNN	Nsukka	Enugu	Soil
S46	7/8/2023	Aguleri	Anambra North	Anambra	Dead Wood
S47	7/8/2023	Awba-Ofemili	Awka-North	Anambra	Soil
S48	7/8/2023	Iheakpu-Awka	Igbo-Eze South	Enugu	Soil
S49	7/8/2023	Iheakpu-Awka	Igbo-Eze South	Enugu	Soil
S50	7/8/2023	Awba-Ofemili	Awka-North	Anambra	Dead Wood

in this study was considerably similar to the ranges of 2.83–6.29 mg g⁻¹, 10.66–567.65 mg 100 g⁻¹, 120.30–1277.5 mg 100 g⁻¹ and 0.80–51.40 mg g⁻¹ reported in some wild mushrooms (Srikum and Supapvanich 2016; Nagy *et al.* 2017; Rugolo *et al.* 2022). However, the average total phenols recorded in this study are lower than the ranges of 420–12775.56 mg kg⁻¹ reported in wild mushrooms by Keles *et al.* (2011) and 97.16–223.11 mg g⁻¹ recorded in wild edible mushrooms of Nigeria (Unekwu *et al.* 2014). Mushrooms sampled in this study exhibited high phenol concentrations that could compare favorably with those reported in other parts of the world.

Despite the wide variations, flavonoid was recorded in all the sampled mushrooms, suggesting that mushrooms could be fountains of important bioactive compounds such as flavonoids. On average, flavonoid concentration in the sampled mushrooms are considerably high and surpasses the range of 0.53–205.26 mg 100 g⁻¹ reported in wild and cultivated edible mushrooms in Thailand (Srikum and Supapvanich 2016), 0.025–0.131 mg g⁻¹ reported in wild mushrooms of Malaysia (Azieana *et al.* 2017) and 3.46–5.41 mg 100 g⁻¹ reported by Sahu *et al.* (2018). However, the sampled mushrooms were low in flavonoids compared to the ranges of 6.41–42.63 mg g⁻¹ reported in wild

Table 2: Bioactive contents of the sampled mushrooms

Identifications	Phenol (mg 100 g ⁻¹)	Flavonoid (mg 100 g ⁻¹)	Lutein (mg/100 g)	B-carotene (mg 100 g ⁻¹)
S1 <i>Amanita naseosa</i>	1459.63 ± 0.029b	125 ± 0.021g	11 ± 0.013y	36.16 ± 0.023w
S2 <i>Ganoderma mbrekobenum</i>	675.00 ± 0.000g	753 ± 0.017a	62 ± 0.021kl	64.53 ± 2. 673rs
S3 <i>Agaricus</i> sp.	333.71 ± 0.023o	194 ± 0.024b	53 ± 0.018mn	106.38 ± 0.045lm
S4 <i>Leucocoprinus cepistipes</i>	948.03 ± 0.000e	49 ± 0.028t-v	51 ± 0.018m-o	116.67 ± 0.035kl
S5 <i>Marasmiellus</i> sp.	299.57 ± 0.020u	133 ± 0.022fg	22 ± 0.011v-x	88.67 ± 0.029op
S6 <i>Neonothopanus</i> sp.	401.97 ± 0.000k	63 ± 0.026rs	39 ± 0.015q-s	57.88 ± 0.029stu
S7 <i>Lentinus squarrosulus</i>	310.00 ± 0.000s	69 ± 0.016qr	40 ± 0.012q-s	47.28 ± 0.019uv
S8 <i>Neonothopanus hygrophanus</i>	182.17 ± 0.039J	177 ± 0.020c	91 ± 0.016g	121.64 ± 0.023jk
S9 <i>Auricularia polytricha</i>	558.99 ± 0.034i	83 ± 0.033m-o	62 ± 0.014kl	113.48 ± 0.028kl
S10 <i>Marasmius corrugatiformis</i>	94.80 ± 0.016K	88 ± 0.023lm	81 ± 0.021h	164.63 ± 0.033f
S11 <i>Micropsalliota</i> sp.	169.38 ± 0.073JK	94 ± 0.029kl	0.00 ± 0.000z	99.71 ± 0.023mn
S12 <i>Leucocoprinus cretaceus</i>	1295.00 ± 0.000d	106 ± 0.038hi	21 ± 0.015v-x	83.24 ± 0.029pq
S13 <i>Leucocoprinus</i> sp.	417.06 ± 0.000j	127 ± 0.034fg	42 ± 0.022p-r	67.98 ± 0.020rs
S14 <i>Trametes polyzona</i>	317.28 ± 0.023q	113 ± 0.039h	0.00 ± 0.000z	108.91 ± 0.044lm
S15 <i>Lentinus squarrosulus</i>	308.58 ± 0.022t	132 ± 0.029fg	159 ± 0.029c	219.72 ± 0.029cd
S16 <i>Ganoderma lucidum</i>	364.04 ± 0.028m	106 ± 0.016hi	0.00 ± 0.000z	106.35 ± 0.022lm
S17 <i>Chlorophyllum palaeotropicum</i>	279.09 ± 0.018y	27 ± 0.011wx	41 ± 0.019p-s	86.44 ± 0.025op
S18 <i>Lentinus squarrosulus</i>	266.71 ± 0.015A	51 ± 0.013tu	16 ± 0.008xy	229.65 ± 0.028c
S19 <i>Agaricus</i> sp.	249.02 ± 0.000D	86 ± 0.018mn	58 ± 0.020lm	134.75 ± 0.033hi
S20 <i>Micropsalliota</i> sp.	256.56 ± 0.027B	20 ± 0.011xy	49 ± 0.018n-p	195.74 ± 0.018e
S21 <i>Ganoderma</i> sp.	231.53 ± 0.029F	193 ± 0.028b	265 ± 0.029a	39.32 ± 0.021vw
S22 <i>Neonothopanus hygrophanus</i>	58.15 ± 0.0302C	42 ± 0.016v	43 ± 0.021o-q	95.04 ± 0.019no
S23 <i>Lactifluus bicapillus</i>	84.92 ± 0.0332B	31 ± 0.009w	31 ± 0.021tu	59.67 ± 0.025st
S24 <i>Ganoderma mbrekobenum</i>	236.54 ± 0.025E	156 ± 0.013d	72 ± 0.020ij	154.61 ± 0.018fg
S25 <i>Leucocoprinus cepistipes</i>	276.83 ± 0.021z	158 ± 0.029d	106 ± 0.026f	143.23 ± 0.017gh
S26 <i>Trametes polyzona</i>	383.01 ± 0.032A	145 ± 0.029e	95 ± 0.017g	82.37 ± 0.017pq
S27 <i>Chlorophyllum globosum</i>	143.75 ± 0.0271E	97 ± 0.016i-k	59 ± 0.014lm	41.13 ± 0.016vw
S28 <i>Trametes polyzona</i>	208.57 ± 0.021G	87 ± 0.014m	113 ± 0.021ef	116.56 ± 0.025kl
S29 <i>Neonothopanus</i> sp.	286.94 ± 0.030x	134 ± 0.021f	12 ± 0.007y	131.91 ± 0.023ij
S30 <i>Neonothopanus hygrophanus</i>	331.64 ± 0.027p	43 ± 0.014uv	45 ± 0.021n-q	117.54 ± 0.021kl
S31 <i>Trametes parvispora</i>	205.11 ± 0.037H	103 ± 0.029ij	33 ± 0.018s-u	261.44 ± 0.017a
S32 <i>Agaricus</i> sp.	148.53 ± 0.0221C	15 ± 0.008y	21 ± 0.011v-x	86.94 ± 0.017op
S33 <i>Trametes polyzona</i>	203.51 ± 0.028I	44 ± 0.020uv	53 ± 0.012m	228.92 ± 0.032c
S34 <i>Marasmius</i> sp.	118.82 ± 0.0231G	57 ± 0.017st	34 ± 0.012r-u	99.71 ± 0.031mn
S35 <i>Trametes parvispora</i>	103.88 ± 0.019I	67 ± 0.020r	18 ± 0.013xy	158.69 ± 0.029f
S36 <i>Lentinus squarrosulus</i>	254.07 ± 0.016C	145 ± 0.029e	28 ± 0.012uv	144.63 ± 0.020gh
S37 <i>Chlorophyllum globosum</i>	923.63 ± 0.022f	153 ± 0.032de	126 ± 0.028d	48.23 ± 0.019t-v
S38 <i>Gymnopus aff. Brunneigracilis</i>	340.03 ± 0.029n	78 ± 0.017op	19 ± 0.017w-y	88.48 ± 0.025op
S39 <i>Daldinia eschscholtzii</i>	1432.16 ± 0.037c	146 ± 0.033e	108 ± 0.031ef	110.95 ± 0.000kl
S40 <i>Trametes parvispora</i>	147.47 ± 0.0181D	53 ± 0.021t	14 ± 0.015xy	83.44 ± 0.021pq
S41 <i>Marasmius palmivorus</i>	92.28 ± 0.0272A	78 ± 0.014op	80 ± 0.020hi	106.23 ± 0.036lm
S42 <i>Neonothopanus hygrophanus</i>	288.75 ± 0.020w	155 ± 0.026d	115 ± 0.034e	121.99 ± 0.000jk
S43 <i>Lentinus squarrosulus</i>	1599.45 ± 0.027a	79 ± 0.022n-p	96 ± 0.018g	92.83 ± 0.027no
S44 <i>Oudemansiella canarii</i>	604.01 ± 0.029h	85 ± 0.014m-o	27 ± 0.016u-w	87.61 ± 0.022op
S45 <i>Micropsalliota</i> sp.	340.03 ± 0.029n	74 ± 0.014pq	39 ± 0.017q-t	19.66 ± 0.019x
S46 <i>Lentinus squarrosulus</i>	103.65 ± 0.0381I	66 ± 0.021r	69 ± 0.016jk	65.36 ± 0.028rs
S47 <i>Agaricus</i> sp.	152.95 ± 0.0171B	95 ± 0.017jk	63 ± 0.021kl	201.42 ± 0.038de
S48 <i>Ramaria</i> sp.	120.08 ± 0.0251F	67 ± 0.015r	92 ± 0.018g	144.65 ± 0.036gh
S49 <i>Trametes suaveolens</i>	289.47 ± 0.036v	51 ± 0.023tu	178 ± 0.029b	243.36 ± 0.029b
S50 <i>Amyloporus campbellii</i>	313.48 ± 0.023r	113 ± 0.035h	47 ± 0.029n-q	72.31 ± 0.022qr
LSD	0.07511	8.117	7.853	10.978

Values with different letters in a column show significant difference ($P \leq 0.05$)

mushrooms of Nigeria (Unekwu et al. 2014). Despite the discrepancies in the flavonoid concentration recorded in this study and reports of other investigators, consumption of these wild mushrooms, especially the edible ones may be beneficial health-wise. This view may be correct as flavonoids contained in mushrooms are known to possess anti-inflammatory, antioxidant and anti-aging properties (Kumar et al. 2021).

Moderate concentrations and large variations of lutein among the sampled mushrooms could also be associated with the influence of genes, substrates and environments.

Our reports showed that lutein was detected in all the sampled mushrooms except in 3 samples. However, this could be a pointer that, although the sampled mushrooms contained lutein, their availability and concentrations may depend on genotype or substrates. Previous reports have shown that lutein is distributed mostly in dark green vegetables, fruits, maize, and egg yolk and can be biosynthesized in bacteria, algae, some fungi and plants (Fuad et al. 2020). Unfortunately, the quantification of lutein in mushrooms has been grossly neglected by researchers with more attention paid to beta-carotene and lycopene.

Table 3: Antioxidant capacity of sampled mushrooms

	Identifications	% DPPH at 50 $\mu\text{g mL}^{-1}$	% DPPH at 75 $\mu\text{g mL}^{-1}$	% DPPH at 100 $\mu\text{g mL}^{-1}$
S1	<i>Amanita nauseosa</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.06 $\pm$ 0.0001E
S2	<i>Ganoderma mbrekobenum</i>	50.22 $\pm$ 0.029d	56.71 $\pm$ 0.029d	67.02 $\pm$ 0.000d
S3	<i>Agaricus</i> sp.	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S4	<i>Leucocoprinus cepistipes</i>	0.64 $\pm$ 0.029y	4.63 $\pm$ 0.032C	9.88 $\pm$ 0.029B
S5	<i>Marasmiellus</i> sp.	0.56 $\pm$ 0.029y	3.22 $\pm$ 0.029E	7.54 $\pm$ 0.029D
S6	<i>Neonothopanus</i> sp.	22.54 $\pm$ 0.029h	28.64 $\pm$ 0.029h	36.90 $\pm$ 0.029h
S7	<i>Lentinus squarrosulus</i>	10.27 $\pm$ 0.029o	17.93 $\pm$ 0.029n	27.60 $\pm$ 0.029m
S8	<i>Neonothopanus hygrophanus</i>	39.97 $\pm$ 0.000f	48.17 $\pm$ 0.029f	56.51 $\pm$ 0.029f
S9	<i>Auricularia polytricha</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.00013	0.00 $\pm$ 0.0001E
S10	<i>Marasmius corrugatiformis</i>	5.51 $\pm$ 0.029r	10.10 $\pm$ 0.029u	17.60 $\pm$ 0.029t
S11	<i>Micropsalliota</i> sp.	1.01 $\pm$ 0.000x	4.24 $\pm$ 0.029D	6.61 $\pm$ 0.029F
S12	<i>Leucocoprinus cretaceus</i>	1.17 $\pm$ 0.029x	4.60 $\pm$ 0.029C	6.98 $\pm$ 0.000E
S13	<i>Leucocoprinus</i> sp.	15.66 $\pm$ 0.029j	21.77 $\pm$ 0.029k	30.82 $\pm$ 0.029k
S14	<i>Trametes polyzona</i>	12.23 $\pm$ 0.029m	18.79 $\pm$ 0.029A	28.83 $\pm$ 0.029A
S15	<i>Lentinus squarrosulus</i>	7.17 $\pm$ 0.029q	13.37 $\pm$ 0.029r	21.29 $\pm$ 0.029r
S16	<i>Ganoderma lucidum</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S17	<i>Chlorophyllum palaeotropicum</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S18	<i>Lentinus squarrosulus</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S19	<i>Agaricus</i> sp.	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	1.75 $\pm$ 0.0291E
S20	<i>Micropsalliota</i> sp.	10.71 $\pm$ 0.029n	16.66 $\pm$ 0.029p	24.09 $\pm$ 0.029p
S21	<i>Ganoderma</i> sp.	61.55 $\pm$ 0.029b	69.28 $\pm$ 0.029b	74.62 $\pm$ 0.029b
S22	<i>Neonothopanus hygrophanus</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S23	<i>Lactifluus bicapillus</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S24	<i>Ganoderma mbrekobenum</i>	58.74 $\pm$ 0.029c	66.71 $\pm$ 0.029c	71.40 $\pm$ 0.029c
S25	<i>Leucocoprinus cepistipes</i>	3.32 $\pm$ 0.029t	9.83 $\pm$ 0.029w	14.50 $\pm$ 0.029w
S26	<i>Trametes polyzona</i>	4.24 $\pm$ 0.029s	10.11 $\pm$ 0.029t	16.49 $\pm$ 0.029v
S27	<i>Chlorophyllum globosum</i>	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S28	<i>Trametes polyzona</i>	22.64 $\pm$ 0.029h	29.72 $\pm$ 0.029g	37.66 $\pm$ 0.029g
S29	<i>Neonothopanus</i> sp.	0.74 $\pm$ 0.029y	6.00 $\pm$ 0.000A	10.88 $\pm$ 0.029A
S30	<i>Neonothopanus hygrophanus</i>	0.00 $\pm$ 0.000z	1.32 $\pm$ 0.029H	5.33 $\pm$ 0.029H
S31	<i>Trametes parvispora</i>	0.00 $\pm$ 0.000z	1.56 $\pm$ 0.029G	5.61 $\pm$ 0.029G
S32	<i>Agaricus</i> sp.	49.92 $\pm$ 0.029e	54.01 $\pm$ 0.000e	65.49 $\pm$ 0.029e
S33	<i>Trametes polyzona</i>	2.02 $\pm$ 0.000v	7.32 $\pm$ 0.029y	11.58 $\pm$ 0.029y
S34	<i>Marasmius</i> sp.	0.00 $\pm$ 0.000z	0.56 $\pm$ 0.0291B	1.23 $\pm$ 0.0291D
S35	<i>Trametes parvispora</i>	0.00 $\pm$ 0.000z	1.07 $\pm$ 0.029J	3.86 $\pm$ 0.0291A
S36	<i>Lentinus squarrosulus</i>	0.00 $\pm$ 0.000z	1.64 $\pm$ 0.029F	3.98 $\pm$ 0.000J
S37	<i>Chlorophyllum globosum</i>	0.71 $\pm$ 0.029y	4.93 $\pm$ 0.029B	8.13 $\pm$ 0.029C
S38	<i>Gymnopus aff. Brunneigracilis</i>	7.22 $\pm$ 0.029q	13.29 $\pm$ 0.029s	21.17 $\pm$ 0.029s
S39	<i>Daldinia eschscholtzii</i>	10.23 $\pm$ 0.029o	16.97 $\pm$ 0.000o	24.77 $\pm$ 0.029o
S40	<i>Trametes parvispora</i>	2.91 $\pm$ 0.029u	9.74 $\pm$ 0.029x	14.33 $\pm$ 0.029x
S41	<i>Marasmius palmivorus</i>	1.51 $\pm$ 0.000w	7.22 $\pm$ 0.029z	11.17 $\pm$ 0.029z
S42	<i>Neonothopanus hygrophanus</i>	18.00 $\pm$ 0.000i	24.63 $\pm$ 0.029j	34.62 $\pm$ 0.029j
S43	<i>Lentinus squarrosulus</i>	7.82 $\pm$ 0.029p	13.97 $\pm$ 0.000q	22.92 $\pm$ 0.029q
S44	<i>Oudemansiella canarii</i>	0.00 $\pm$ 0.000z	0.80 $\pm$ 0.0291A	3.51 $\pm$ 0.0291B
S45	<i>Micropsalliota</i> sp.	0.00 $\pm$ 0.000z	0.00 $\pm$ 0.0001C	0.00 $\pm$ 0.0001E
S46	<i>Lentinus squarrosulus</i>	0.00 $\pm$ 0.000z	1.35 $\pm$ 0.044H	4.56 $\pm$ 0.029I
S47	<i>Agaricus</i> sp.	13.66 $\pm$ 0.029k	18.01 $\pm$ 0.029m	26.20 $\pm$ 0.029n
S48	<i>Ramaria</i> sp.	24.20 $\pm$ 0.029g	28.33 $\pm$ 0.029i	36.73 $\pm$ 0.029i
S49	<i>Trametes suaveolens</i>	13.48 $\pm$ 0.029A	10.01 $\pm$ 0.029v	17.25 $\pm$ 0.029u
S50	<i>Amylosporus campbellii</i>	0.00 $\pm$ 0.000z	1.44 $\pm$ 0.029G	4.55 $\pm$ 0.029I
Vitamin C		80.13 $\pm$ 0.456a	88.65 $\pm$ 0.073a	98.07 $\pm$ 0.186a
LSD		0.188	0.074	0.100

 Values with different letters in a column show significant difference ($P \leq 0.05$)

Within the limits of our literature search, there are no reports on lutein quantifications in mushrooms, with a majority of the publications based on mere assumptions that since mushrooms contain carotenoids, they may likely contain lutein. In general terms, lutein might be inferior to other carotenoids such as beta-carotene and lycopene in antioxidant activities. However, they are reported to be more powerful than lycopene and carotene in fighting specific free radicals such as superoxide anion, hydroxyl, hypochlorous acid and peroxy groups (Wang *et al.* 2006; Li *et al.* 2013;

Sun *et al.* 2015). According to Fuad *et al.* (2020) lutein is beneficial in attenuating the effects of burn-induced multiple organ injury, optic nerve injury and severe traumatic brain injury. Unfortunately, the bioavailability of lutein is very low because it is a fat-soluble pigment that is difficult to absorb. But recently, a nanoparticle of lutein (poly lactic-coglycolic acid) has been formulated and utilized to boost the bioavailability of lutein (Bodoki *et al.* 2020).

Wide variations in beta-carotene contents of the sampled mushrooms could also be ascribed to differences in

genetic and environmental backgrounds. Although beta-carotene is found in different mushroom species, their concentrations may vary based on genotype and the ability of the species to bioaccumulate nutrients which may boost or hamper their biosynthesis (Nakalembe *et al.* 2015; Strapáč *et al.* 2017). The range of beta-carotene concentrations recorded in this study was visibly superior to the ranges reported by previous researchers elsewhere. They were higher compared to 1.88–2.97 $\mu\text{g/g}$ reported in Portuguese wild edible mushrooms (Barros *et al.* 2007), 9.88–13.70 $\mu\text{g/g}$ reported in wild *Cantharellus* in India (Kumari *et al.* 2011), 5.35–48.59 mg/100 g reported in wild and domesticated edible mushrooms of Tanzania (Hussein *et al.* 2015; Juma *et al.* 2016) and 12.60–17.70 $\mu\text{g/g}$ recorded in selected wild mushrooms of Uganda (Nakalembe *et al.* 2015). However, Chye *et al.* (2008) reported higher ranges of 0.37–2711 $\mu\text{g/g}$ in fresh mushrooms and suggested that drying of mushrooms could result in losing substantial amounts of biomolecules. Beta-carotene is an important antioxidant and acts as a pro-vitamin A and together with α -carotene and β -cryptoxanthin are vital in vitamin A synthesis (Kozarski *et al.* 2015). Mushroom samples analyzed in this study had considerable quantities of beta-carotene and thus may be an important source of cheap and readily accessible beta-carotene.

The majority of the sampled mushrooms exhibited antioxidant activities, except in only a few cases. As revealed by the DPPH radical scavenging assay, the antioxidant activities of the sampled mushrooms varied widely and this could be linked to differences in the interactions between genotype, substrates and environments, since the samples varied genetically and were found inhabiting various substrates. Apart from a few samples that had high antioxidant activity close to the activity of vitamin C standard, others had activities ranging from moderate to very low. Antioxidant activities recorded in this study compared favorably with the ranges of 5–22% reported in selected wild edible mushrooms of Nigeria (Ejelonu *et al.* 2013), 2.01–17.39% in wild and cultivated mushrooms of Ghana (Obodai *et al.* 2014) and 1.84–33% in six wild edible mushrooms (Dimitrijević *et al.* 2016). However, the antioxidant activities recorded in this study are lower than the ranges of 10.17–97.96 reported in wild edible mushrooms (Keles *et al.* 2011), 43.88–88.33% in mushroom extracts from Bosnian market (Alispahić *et al.* 2015), 46.53–93.33% in wild edible mushrooms of Tanzania (Hussein *et al.* 2015) and 6.20–84.25 in Syrian wild mushrooms (Hala *et al.* 2023). The inconsistencies recorded may be attributed to factors such as the type of assay and concentrations of the samples analyzed. For example, Ejelonu *et al.* (2013) reported variations in the antioxidant activities of selected Nigerian wild mushrooms using different antioxidant assays. The antioxidant activities of the mushroom samples increased with an increase in the concentration of the samples. This trend was also observed

by Ejelonu *et al.* (2013) where concentrations of 250 and 100 $\mu\text{g mL}^{-1}$ had more antioxidant activities than lesser concentrations of 50, 25 and 5 $\mu\text{g mL}^{-1}$. Likewise, there were increased antioxidant activities with an increase in the concentrations of wild edible mushrooms (Hussein *et al.* 2015; Juma *et al.* 2016).

Conclusion

Significant amounts of essential bioactive contents detected in the sampled mushrooms, especially in the edible species further validate them as excellent sources of health-promoting compounds. Although consumption of edible wild mushrooms may not deliver the recommended daily allowances of nutrients required for proper maintenance and functioning of the body, they could supply significant percentages which can augment bioactive content shortfalls. Substantial amounts of bioactive compounds found in the sampled mushrooms also certify them as important sources of health-promoting biomolecules. Even though the antioxidant activities of the sampled mushrooms were lower than the ascorbic acid standard, they exhibited some level of antioxidant activity which might be beneficial in fighting against free radicals.

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Author Contributions

OCV: Research design, samples collection, laboratory analysis, literature search, wrote first draft. ANE: Supervised the research, read and edited the draft. ECJ: literature search, read and edited the draft. OCN: research design, data management, read and edited the draft; NJC: Literature search, sample collection; read and edited the draft. AMC: literature search, read and edited the draft. CCI: Literature search, sample collection; read and edited the draft. OEO: Data analysis, wrote the first draft, literature search.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Data will be available on reasonable request through the corresponding author

Ethics Approval

Not applicable

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