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Antioxidant and Antibacterial Activities of Secondary Metabolites from *Microporus xanthopus* (Fr.) Kuntze (Polypore) Collected from the Wild in Lagos, Nigeria

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ABSTRACT: The secondary metabolites in medicinal mushrooms are responsible for their activity against infectious diseases, cancer, diabetes, and diseases caused by presence of free radicals in the body. *Microporus xanthopus* a polypore medicinal mushroom was collected from the wild in Lagos Nigeria and identified using standard manuals and oligosaccharides, polysaccharide and polyphenols extracted from its tissues were investigated for antibacterial and antioxidant activities. *M. xanthopus* oligosaccharides were extracted with neutral and acid detergents and hydrolysis with concentrated H_2SO_4. Polysaccharides and polyphenols extracted with hot water and acidified methanol, respectively. Concentrations of oligosaccharides, polysaccharides and polyphenols were determined with the total carbohydrate and total phenolic quantification assay kits, respectively. The antioxidant activities of the extracts investigated using the DPPH Radical Scavenging Assay and Trolox Antioxidant Equivalent Capacity (TEAC) Assay in *In-Vitro* experiments in 96-well microtiter plates. The antibacterial effect of the extracts was determined with broth microdilution assay using human pathogenic bacteria (*Escherichia coli* (0157:H7) and *Staphylococcus aureus* ATCC®700698 (MRSA). Oligosaccharides showed the highest DPPH radical scavenging activity (86%) with half maximal effective (EC_{50}) of 16.46µg/mL. The highest TEAC value (1.18µM TE/g) was recorded in the oligosaccharide extract and the least TEAC value (0.39µM TE/g) was in the polyphenol extract. The most potent antimicrobial agent was the oligosaccharide extract with IC_{50} of 44.64 µg/mL and 40.08 µg/mL for *E. coli* and *S. aureus*, respectively. Oligosaccharide extracts were more active than the polyphenol and polysaccharide extracts. *M. xanthopus* oligosaccharides can be developed as potential new dietary supplements with antioxidant and antibacterial activities.

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Mushrooms are rich in nutrients and medicinal benefits and are classified as superfoods for these reasons (Priyamvada *et al*, 2017; Waktola and Temesgen, 2018). Mushrooms abound in the wild in the forest floors and most ecosystems in the world where they play key roles as nutrient recyclers. Nigeria has varieties of mushrooms species in the wild that are yet to be properly identified, classified, and utilized. There is need to identify the vast resources of mushrooms that abound in Nigeria and tap into the benefits they can provide. A typical example is the polypore *Microporus xanthopus* a mushroom found in the wild in Nigeria and other tropical and temperate Countries. It is a medicinal mushroom and used as food in Cameroon but not in Nigeria (Kinge *et al*., 2019). Mushrooms are macro-fungi that possess bioactive compounds (secondary metabolites) that make them effective therapeutic agents in treating infections and various diseases (Niego et al., 2021; Adongbede and Aduralere, 2019). Secondary metabolites are biologically active compounds naturally present in mushroom or plants, they protect their host against pathogens, pest, and help them respond to environmental stress (Iash, 2019). The efficacy of the secondary metabolites from medicinal mushrooms collected from the wild are yet to be fully explored, concentration of secondary metabolites is dependent on the type of mushrooms, extraction

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solvents and even the substrate they grow on (Manan *et al.,* 2021). Over one hundred mushrooms have been used globally for medicinal purposes especially by Asians and it is believed they have anticancer, antioxidant, antimicrobial, antiviral, anti-obesity, antihypertension, antidiabetic, anti-inflammatory properties (Gaylan *et al.,* 2018; Ma *et al.,* 2018; Waktola and Temesgen, 2018; Gebreyohannes *et al.,* 2019; Zeb and Lee, 2021). Globally pathogenic microbes are becoming resistant to antibiotics and thus a major threat to the health sector especially in developing countries like Nigeria (Reta *et al.,* 2019). There are cases of severe illness caused by pathogenic bacteria such as methicillin resistant *S. aureus* and has fatal consequences as they have no cure (Verghese *et al.,* 2017; Puvača and Frutos *et al,* 2021; Varela *et al,* 2021). The failure of antibiotics has led to search for novel therapeutic alternatives from natural source (plant or mushrooms) that are more effective, cheaper with minimal or no side effects and little chance of allowing super bugs develop (Manan *et al.,* 2021). *Escherichia coli* (O157:H7) is a Shiga toxin producing foodborne bacterium, which causes severe illness and high death rates in human because of high degree of resistance to standard antibiotics (Tadese *et al.,* 2021). *Staphylococcus aureus* (ATCC 700698) is a methicillin resistant bacterium which causes serious infections in human because of drug resistance also (Ismail *et al.,* 2021; Rani *et al.,* 2021). Drug delivery and bioavailability of active constituents is particularly important in treating diseases effectively. The size and reactivity of the molecules or drugs is put in into consideration in formulating new drugs (Carecho *et al*., 2020). Low molecular weight compounds like oligosaccharides therefore make more efficient drug delivery agents in the human system (Higashi *et al*., 2016). There are distinct types of oligosaccharides in various species of mushrooms that are very potent compounds not yet exploited. The Oligosaccharides, polysaccharides, and polyphenols compounds extracted from mushrooms have antioxidant, anticancer and immunomodulating abilities (Attarat and Phermathai, 2015). Oligosaccharides and polysaccharides are fundamentally important biomolecules used for effective drug delivery in the pharmaceutical industries (Higashi *et al*., 2016). The low molecular weight compounds are faster and readily bioavailable in the system compared to the high molecular weight compounds because of their size and reactivity (Carecho *et al*., 2020). The high molecular weight compounds are however more stable than the low molecular weight compounds particularly those of fungal origin. The low molecular weight compounds can be polyphenols and oligosaccharides (Carecho et al., 2020). This study evaluated the *In vitro* antioxidant and antibacterial activities of oligosaccharides, polysaccharides and polyphenols extracted from *M. xanthopus* a medicinal and potentially edible macro-fungi.

MATERIALS AND METHODS

Collection and Identification of Mushroom Specimens: Microporus xanthopus specimens, were collected from Nigerian Conservation Centre (NCF) Lekki, Lagos. Fresh specimens of *M. xanthopus* collected from on dead logs of wood on the forest floor at a GPS coordinate $006^{O}43'64"$ N $003^{O}53'55"$ E. The collected specimens were cleaned with soft brush and rinsed with distilled water to remove dirt attached to the surface and identified morphologically using microscopic, macroscopic, and anatomical features with standard manuals (Largent *et al*., 1977; Largent, 1986; Largent *et al*., 1988). The test polypore was a funnel shaped mushroom that is concentrically zoned with different shades of brown, the cap had a smooth surface and smooth cap margin, the gill was white in colour with tiny pores, the stipe was white and sinuated with pileus having an average height of 5.65±0.21cm and width of 3.48±0.43cm. The fresh tissues of *M. xanthopus* were dried in the dehydrator at 40^{0}C for 24hours and freeze dried (Labconco Freezone 7960030 model). The dried lyophilized samples were ground into fine powder using high-speed multifunctional grinder and stored at 4^{0}C. *M. xanthopus* (Collection No. NCF/M 045) was deposited at the Department of Botany, University of Lagos herbarium.

Extraction of Oligosaccharides and polysaccharides: Oligosaccharides were extracted from the pulverized tissues of *M. xanthopus* using methods described by Ahmad *et al*., (2015) with little modifications. Hot water extract of pulverized tissue was conducted in a water bath with a shaker at 80^{0}C for 1 h and the extraction process was repeated three times. The extracts were pooled together after filtering with Whatman No 1 filter paper. The filtrate was concentrated in a rotary evaporator under pressure at 60^{0}C. The filtrate was further processed for oligosaccharides by shaking in neutral acid detergent solution at 60^{0}C in a water bath with shaker for 1h. The solution was transferred to an acid detergent solution and shaken for another 1h. The final acid hydrolysis was done by adding 1 M H_2SO_4 and shaken for another 1h at 60^{0}C. The acids were prepared by adopting methods in the Official Methods of Analysis (AOAC, 1990). The total yield of the individual extracts got by weighing and stored at -20°C in freezer.

Polysaccharides were extracted using 10g of pulverized *M. xanthopus* tissues with 300ml of

distilled water, by boiling for 2 hours at 100°C in a water bath with a shaker. The extract was filtered with thermo scientific vacuum pump, and the residues were re-soaked and extraction process repeated two more times. The three filtrates were pooled together and concentrated with a rotary evaporator under pressure at 60°C. The concentrated paste was solidified by freezing in a -80 freezer and immediately transferred to a freeze dryer. The lyophilized extract was crushed to fine powder after weighing. The lyophilized extract was taken into solution with hot water and analytical grade absolute ethanol was added to get an 80% ethanol solution. The solution was precipitated overnight in refrigerator at 4°C and precipitate was recovered by centrifuging at 10,000rpm for 10minutes in refrigerated centrifuge. The recovered precipitate was left in the -80 freezer overnight after decanting into falcon tubes centrifuged tubes and freeze dried.

Extraction of polyphenols: Absolute analytical grade (98%) methanol was acidified with one molar solution of Hydrochloric acid (1M HCl) to pH 3. Polyphenol was extracted from *M. xanthopus* using method described by Selvakumar and Sankar, (2015) with little modifications. Ten grams (10g) of *M. xanthopus* fine powdered was dissolved in 200ml of acidified methanol wrapped with aluminum foil and stirred overnight using magnetic stirrer at 250rpm, then filtered with thermo scientific vacuum pump, the residues were re-soaked in 200mL of acidified methanol and stirred for another 1 hour at 250rpm. The residues were re-soaked with 200mL of acidified methanol and extraction repeated two more times. The three filtrates were then evaporated to dryness using rotary evaporator at 40°C. The extract was taken into solution with 5 mL of distilled water and left overnight in a -80 freezer and freeze dried. The yield of the extract was got by weighing the dried extract, and the dried extracts were dissolved in 70% methanol to make a 10mg/mL working solution and stored at -20°C.

Determination of total phenolic content The total phenolic content of *M. xanthopus* was found using Folin-Ciocalteu phenolic content quantification assay kit obtained from BioQuoChem (Parque Tecnológico de Asturias, CEEI 33428 Llanera- Asturias, Spain) and expressed as Gallic acid equivalent (GAE), following manufacturer's instruction, the absorbance of the standards, blanks and the extracts were read in a microplate reader at 700 nm (T=37^{o}C) after 40minutes incubation at room temperature in the dark, the total phenolic contents were expressed as gallic acid equivalents (mg GAE/g of dry extract.

Determination of Total Polysaccharides and Total Oligosaccharides Contents Total high molecular weight polysaccharides and low molecular weight compounds contents of *M. xanthopus* were figured out using Total Carbohydrate Assay Kit-Quantification (ABCAM) and expressed as glucose equivalent, based on manufacture procedures and with absorbance measured at 490nm.

DPPH Free Radical Scavenging Activity: M. xanthopus extracts free radical scavenging activity was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity according to methods by Ting *et al.,* (2017) with little modifications. Quickly, DPPH powder was dissolved in absolute methanol to attain concentration of 6 x 10 mol/L, 270 µl of the DPPH solution was added to 30 µl of different concentrations of the mushroom extracts (0, 25, 50, 75, 100 µg/ml) in quadruplicates. The mixture was allowed to incubate for 40 min in the dark, and the DPPH radical scavenging percentage and the IC_{50} value was determined using Graph Pad prism 8 with the determined absorbance values and concentration of standard. Formula for calculating DPPH radical scavenging:

$$RSA = (A_{DPPH} - AS)x\frac{100}{A_{DPPH}}$$

AS = absorbance of the DPPH solution and the *M. xanthopus* extracts: A_{DPPH} = absorbance of the DPPH solution

Trolox Equivalent Antioxidant Capacity (TEAC): The free radical scavenging capacity of antioxidants of extracts was determined using ABTS (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox)). Trolox standard was incubated for with the extracts for 30 minutes at room temperature and absorbance read at 734 for the various solutions and standards. The Trolox equivalent values of *M. xanthopus* extracts were determined from calibration curve of Trolox absorbance against known concentrations.

Antibacterial activity of the polypore mushroom ~M. xanthopus: A 96-well microbroth dilution bioassay was used according to Bala *et al.,* (2011) and Teh *et al.,* (2017) with modifications. Different concentrations were used for the antibacterial activity with each well having 50 µl of the bacterial culture and 50 µl of the mushroom extracts of concentrations (0, 25, 50, 75 and 100%) and 100 µl of tryptic soy broth. The bacterial culture (100 µl) and 100 µl of tryptic soy broth served as negative control, while the two last well which contained 200µl of the tryptic soy was used

as the blank, the antibiotic ~Ceftazidime was used as positive control.

RESULTS AND DISCUSSION

Microporus xanthopus is a funnel shaped polypore mushroom with peculiar concentrically zoned shades of brown, the cap had a smooth surface and margin, the underside of the pileus was white in colour with tiny pores, the stipe white and sinuated, found on decayed log (Figure 1). The morphometric characteristics are shown in table 1.

Fig 1: *Microporus xanthopus* (Fr.) Kuntze

Table 1: Morphometric Characteristics of Wild *Microporous xanthopus*

Morphometric Characteristics	(cm) Mean ± SE
Pileus diameter	5.75±0.65
Pileus height	2.74±0.09
Stipe height	4.28 ±0.28
Stipe girth	1.62±0.04

Table 2: Total Oligosaccharide, Polysaccharide and Polyphenol Content of *Microporus xanthopus*

Extracts	Extract Concentration
Oligosaccharides	1229±10.00 mg/g glucose equivalent of dry extract
Polysaccharides	269±5.02 mg/g glucose equivalent of dry extract
Polyphenols	100.02±2.51 mg GAE/ g of dry extract

The yield of polysaccharide, oligosaccharides, and polyphenol extracts of *M. xanthopus* was 19 mg/g, 8 mg/g, and 7 mg/g of Dry tissue, respectively. SEM statistics. The polypore mushroom had higher concentrations of oligosaccharides and polysaccharides (Table 2). The concentration of polyphenol was comparatively higher (100.02mg/ GAE/ g of extract) than that of previous records for the test polypore *M. xanthopus* (38.82 mg GAE/g) (Orango-Bourdette, *et al.*, 2018). The higher concentrations can be attributed to environment and substrate from which the polypore grew. Antibacterial, anticancer, antiangiogenic, and anthelmintic activities had been reported for *M. xanthopus* by other researchers justifying its being termed medicinal polypore mushroom (Chittaragi and Meghalatha, 2014; Orango-Bourdette *et al.*, 2018). Wild Polypore mushrooms in Nigeria can serve as huge reservoir therapeutic compounds that can be used to combat multidrug resistance of pathogenic bacteria and potentially could be cheaper, more accessible, safer, and more effective than the standard synthetic ones. The Oligosaccharides, polysaccharide, and polyphenol compounds from mushrooms have been reported to be important therapeutic compounds that are effective in treating infectious diseases and could be potential replacements of antibiotics used for the treatment of developing superbugs (Hu *et al.*, 2018). The DPPH scavenging activity and Trolox equivalent capacity of oligosaccharides, polysaccharides, and polyphenols fractions of *M. xanthopus* exhibited a dose-dependent response (Figure 2). The highest scavenging activity among the test extracts was recorded with the oligosaccharide extract of *M. xanthopus* (86% at 100 µg/ml and an EC_{50} value of 16.46 µg/ml) (Figure 2; Table 3). The lowest antioxidant activity was observed in polyphenol fraction of the mushroom (43% at 100 µg/ml and EC_{50} value of 88.02 µg/ml) (Figure 2; Table 3).

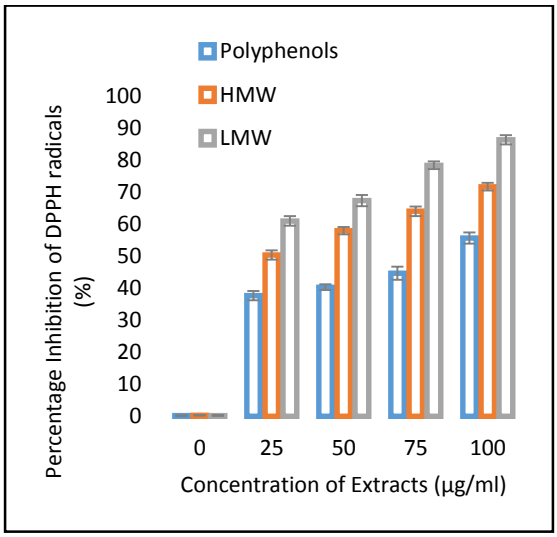

Fig 2: DPPH (2, 2-diphenyl-1-picryhydrazyl) radical scavenging activity of *Microporus xanthopus*
**Values are Mean ±SEM; HMW- polysaccharide; LMW- Oligosaccharide*

Table 3: Half maximal effective concentration EC_{50} values of DPPH radical scavenging extracts of *Microporus xanthopus*

Extracts	Half maximal effective concentration EC_{50} (mg/ml)
Low molecular weight	16.46±0.11
High molecular weight	26.31±0.04
Polyphenols	88.02±0.06

The oligosaccharides exhibited the highest Trolox Equivalent Antioxidant Capacity (TEAC) of 1.18 µM TE/g at 100 µg/ml while polyphenol showed the least TEAC (Figure 3). The oligosaccharide fraction of other edible mushrooms like *Pleurotus eryngii* recorded very remarkably high antioxidant activities and hence the interest in this fraction of extracts (Wu and Chen, 2016). Strong radical scavenging activity was reported for aqueous, aqueous-ethanol and ethanolic extracts of *M. xanthopus* by previous researchers (Orango-Bourdette *et al.*, 2018; Gaylan *et al.*, 2018). Liew *et al.*, (2015) however reported low DPPH radical scavenging activity for ethanolic extracts of *M. xanthopus* in their research. The antioxidant activity of the extracts therefore depends on the reactivity of the bioactive compounds and the type of compound.

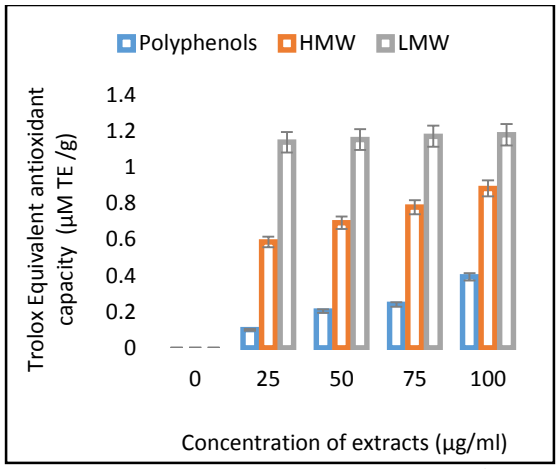

Fig 3: Trolox equivalent antioxidant capacity (TEAC) of extracts of *Microporus xanthopus*
**Values are Mean ±SE; HMW- Polysaccharide; LMW- Oligosaccharide*

The antibacterial activity of oligosaccharides, polysaccharides, and polyphenols extracts of *M. xanthopus* demonstrated a dose dependent response (Figure 4). Oligosaccharide extracts of *M. xanthopus* showed the highest antibacterial activity against test Shiga-toxin producing *E. coli* (67% and IC_{50} value of 44.64 µg/ml), while the lowest bacterial inhibitory activity was exhibited by the polysaccharides extracted from *M. xanthopus* (55% at 100 µg/ml and IC_{50} values of 77.64 µg/ml) (Figure 4). The test *E. coli* was more susceptible to the antibiotic Ceftazidime than the three extracts and had an IC_{50} values 9.68µg/ml (Figure 4; Table 4). The oligosaccharides extract showed the highest antibacterial activity against the test methicillin resistant *Staphylococcus aureus* (64% at 100 µg/ml and IC_{50} value of 40.08µg/ml) while the least inhibitory activity against the bacterium was with the polysaccharide extract (56% at 100 µg/ml and an IC_{50} value of 74.11µg/ml) (Figure 5). *S. aureus* was more susceptible to oligosaccharides extracted from *M. xanthopus* than to Ceftazidime the standard antibiotic (Figure 5). The reports of Liew *et al.*, for extract of *M. xanthopus* (2015) is consistent with current data on the antibacterial activity against *E. coli* and *Staphylococcus aureus*.

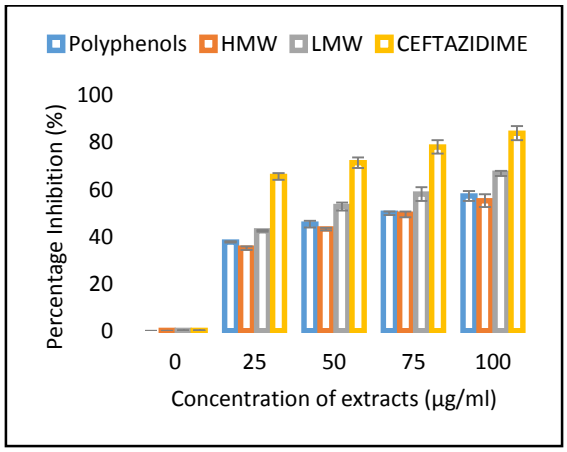

Fig 4: Antibacterial activity of *Microporus xanthopus* extracts against *E. coli*
**Values are Mean ±SE; HMW-Polysaccharide; LMW-Oligosaccharide*

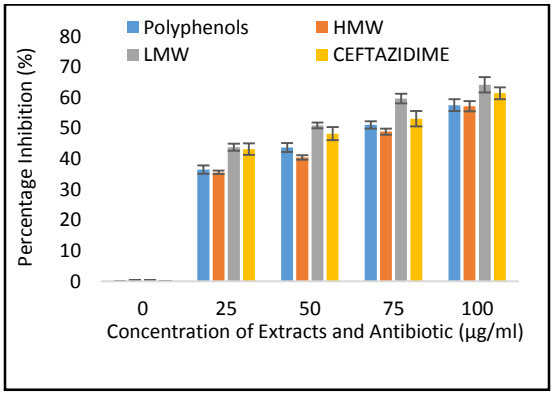

Fig 5: Inhibitory Effect of *Microporus xanthopus* extracts against *Staphylococcus aureus*
**Values are Mean ±SE; HMW- Polysaccharide; LMW- Oligosaccharide*

Table 4: Half maximal inhibitory concentration IC_{50} values of *Microporus xanthopus* extracts

Extracts	Half Maximal Inhibitory Concentration (IC_{50}) values (µg/ml)	
	Escherichia coli	*Staphylococcus aureus*
Low molecular weight	44.64± 2.11	40.08±1.93
High molecular weight	77.64±3.09	74.11±2.73
Polyphenols	66.80±1.18	67.39±2.20

**Values are mean± standard error (SEM) of four replicates (n= 4)*

The reactivity of oligosaccharide from the test polypore can be attributed to size and reactivity and

this can be especially useful and effective in treating infections of the brain crossing the blood-brain barrier (Carecho *et al*., 2021).

Conclusion: Microporus xanthopus oligosaccharides showed strong antioxidant activity with the DPPH Radical Scavenging and the Trolox Equivalent Antioxidant Capacity Assays. Oligosaccharides were more reactive because of their low molecular weights and nature of compound. The polysaccharides and polyphenols from mushrooms have been established to be effective antioxidant and antibacterial agents as recorded in the current study. The oligosaccharide had stronger antioxidant and antibacterial activity than the polysaccharides and polyphenols. The oligosaccharides outperformed ceftazidime with the *S. aureus* a methicillin resistant strain.

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