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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF OLIGOSACCHARIDES, POLYSACCHARIDE AND POLYPHENOLS FROM FIVE NIGERIAN LICHENS

Erute Magdalene Adongbede[1*] and Grant Obinna Ojinkeya[2]
Department of Botany, University of Lagos, Akoka, Yaba, Lagos, Nigeria
Email: [1]eadongbede@unilag.edu.ng; [2] obinnagranto15@gmail.com

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Abstract The aim of the current study was to evaluate the antioxidant and antibacterial activity of polyphenol, oligosaccharide, and polysaccharide extracts of Nigerian lichens~ *Ochrolechia subpallescens* Vers., *Lecanora pinguis* Tuck., *Parmelia saxatilis* (L.) Ach., *Graphis scripta* (L.) Ach., *Verrucaria viridula* (Schrad.) Ach. The polyphenol and polysaccharides were extracted with acidified methanol and hot water respectively, lyophilized, purified, and quantified. Oligosaccharides were extracted with hot water, treated sequentially with neutral and acid detergent solutions, and finally hydrolyzed with sulphuric acid solution, lyophilized, purified, and quantified. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and Trolox equivalent antioxidant capacity were used to establish antioxidant potentials of lichen metabolites. The minimum inhibitory concentration of the metabolites was established to determine their antibacterial effects using the broth microdilution assay against gram-positive and gram-negative bacteria. The lichens~ *O. subpallescens*, *L. pinguis*, and *G. scripta* had the highest concentrations of oligosaccharide, polysaccharides, and polyphenols respectively. *O. subpallescens* oligosaccharide extracts showed the strongest antioxidant activity. There was positive correlation between the antioxidant activity and total phenolic content of the lichens. *G. scripta* oligosaccharide had the strongest antibacterial activity among all lichen species evaluated and inhibited the growth of all the test bacteria. *P. saxatilis* polyphenol extracts exhibited broad spectrum antibacterial activity inhibiting both gram-positive and gram-negative bacteria effectively. The oligosaccharides are low molecular compounds and were understandably more reactive than polysaccharides, having higher antioxidant and antibacterial activity. All tested lichen oligosaccharides extracts and polysaccharides of *O. subpallescens and L. pinguis* can be used as natural sources of antioxidants and antibacterial agents.

Keywords Antimicrobial, Oxidative stress, Metabolites, Drug resistance, Drug discovery, Functional oligosaccharide

Introduction

The known medicinal attributes of lichens are linked to their primary and secondary metabolites, which could be polysaccharides (Feuerer and Hawksworth 2007), low molecular weight compounds like oligosaccharides and phenolic compounds (Crawford, 2019). Lichen polysaccharides have scores of biological activities including but not limited to ~ antitumor, immunomodulating, antiviral, antimicrobial, and memory enhancing activities (Ullah *et al.*, 2019; Olafsdottir and Ingòlfsdottir 2001; Zambare and Christopher 2012). The polyphenols from fungi and lichens such as xanthones are bioactive and used for production of very reactive and effective drugs (Sieminńska-Kuczer et *al.*, 2022). Oligosaccharides are low molecular weight carbohydrates made up of three to ten monosaccharides and have anticancer, antiaging, anti-obesity and antioxidant activities (Zhao, 2020). Lichens are dual organisms that consist of a fungus and an alga in an intricately woven and successful association (Kranner *et al.*, 2008). Lichens inhabit all ecosystems and even the most extreme places like hot deserts, arctic tundra, icebergs, and toxic heaps (Thadhani and Karunaratne, 2017). The association is a beneficial one to both and ensures that they thrive under extremely harsh environmental conditions like extreme temperatures and moisture. Lichens therefore produce incredibly unique and characteristic compounds that help them survive extreme environmental conditions (Kranner *et al.*, 2008). There are over 13,500 lichen species across the world and a third of their population have been investigated for their metabolites and aromatic polyketides (Elkhateeb *et al.*, 2020). Over 200 secondary metabolites found in lichen association are useful bioactive compounds in the medical, pharmaceutical, and biotechnological industries. Scores of polysaccharides have been extracted from lichens and identified, and are mostly α-glucans, ß-glucans, and galactomannans (Elkhateeb *et al.*, 2020). The yield of polysaccharides in lichens like *Cetraria islandica* used in traditional medical practice is up to 57 %, indicating the high concentration of polysaccharides in the lichen tissues (Olafsdottir and Ingòlfsdottir 2001). Records show that polysaccharides from plants have remarkably high free radical scavenging and antimicrobial activities, and lichens are no different (Courtois, 2009; Kou *et al.*, 2016). The medicinal or pharmacological importance of oligosaccharides from lichen remains

unknown, hence the current study. However, the importance of functional oligosaccharides in modern medicine and pharmaceutical practice is key to production of new nutraceuticals (Lai *et al*., 2017). Functional oligosaccharides have been used to prevent inflammation, diabetes, obesity, and cardiovascular risks (Lai *et al*., 2017). They have also been used in the fight against cancer, and infection from pathogenic microorganisms (Lai *et al*., 2017). Oligosaccharides and their conjugates like glycoprotein and glycolipids are particularly important signaling compounds, which are important in drug delivery in the cellular environment (Seeberger and Werz, 2007). Therefore research into functional oligosaccharides in lichens and their use for health benefits attributed to these group of unique compounds is particularly important.

Free radicals cause tissue damage and lead to serious diseases like cancer and oxidative stress related ailments (Liguori *et al*., 2018). To prevent damage to tissues due to oxidative stress the body system produces antioxidants that can interact with the free radicals preventing any associated damage, there are never enough and as such taking them orally is encouraged (Liguori *et al*., 2018). The synthetic antioxidants taken as nutraceuticals or supplements have serious side effects and so, alternative natural sources are sought after (Atta *et al*., 2017). Naturally occurring antioxidants can be found in foods and in organisms used in traditional medicine like mushrooms, plants, and lichens (White *et al*., 2014).

Lichens thrive in extreme environmental conditions and as such can produce secondary metabolites with hundreds of bioactive compounds (Kranner *et al*., 2008). The secondary metabolites from scores of lichens have been shown to be with antiviral, antibacterial, anti-tumour, anti-allergic and immunomodulating activities (Kranner *et al*., 2008). They have been used as alternative medicine sources by a host of cultures across the globe from ancient times (Feuerer and Hawksworth, 2007). The need for new antimicrobial agents stems from the fact that there is worrisome development of multidrug resistance among human pathogenic bacteria like *Staphylococcus aureus* (Terreni *et al*., 2021). There is need to study the diversity of dual organisms ~lichens in Nigeria and tap into the natural compounds that are inherently found in them for development of novel drugs against multidrug resistant strains of pathogenic organism and prevalence of diseases caused by reactive oxygen species in man. The current study seeks to evaluate the antioxidant and antibacterial activity of oligosaccharides, polysaccharides and polyphenol extracts of lichens collected from the wild in Nigeria in *in vitro* experiments. The lichens ~ *Ochrolechia subpallescens, Lecanora pinguis, Parmelia saxatilis, Graphis scripta* and *Verrucaria viridula* studied here had not been characterized and are under-utilized in Nigeria.

Materials and Methods

Collection and Identification of Lichen Species

Fresh lichens were collected from the Lekki Conservation Centre, Lagos, Nigeria, and GPS locations noted. Samples of the lichens~ *Ochrolechia, Lecanora, Parmelia, Graphis, Verrucaria Graphis scripta* and *Verrucaria viridula* were collected from the bark of trees in the Nigerian Conservation Foundation Centre (NCF) Lekki, Lagos State, Nigeria from these GPS locations respectively ~ 6.4373º N 3.5369º S, 6.4377º N 3.5368º S, 6.4369º N 3.5364º S, 6.4408º N 3.5364º S, 6.4359º N 3.5336º S. The lichens were carefully scrapped off from the tree surfaces (substrates) and collected in transparent cellophane bags. The samples were identified based on morphological, anatomical, and chemical tests (Dobson, 2000). The chemical spot tests for lichen identification were performed using ~ potassium hydroxide, calcium hypochlorite, and p-phenylenediamine (Santesson, 1973).

Extraction of polysaccharides and oligosaccharides

Polysaccharides were extracted from lichen tissues by adopting the methods of (Jahromi *et al*., 2016). Collected lichen tissues were dried in a food grade dehydrator for 24 h at 40^0C. The dried tissues were pulverized with Warburg blender, and 10 g of the pulverized tissues were extracted with 300 ml of distilled water at 80^0C for 2 h in water bath with a shaker (Precision Shaking Water Baths Fischer Thermo Scientific, Waltham, MA, USA). Extraction was conducted three times and the extracts pooled together and filtered with Whatman No. 1 filter paper with a Buchner funnel attached to a vacuum pump. The pooled extract filtrates were concentrated with a rotary evaporator under pressure at 60^0C. The concentrated pastes were frozen solid in a -80^0C freezer and lyophilized in a Labconco Freeze Dryer (Labconco Corporation Kansas City, MO, USA). The yield of the extract was extrapolated from the weight of the extract and weight of initial lichen tissues used for the extraction. The extract was first purified after dissolving in hot water and an 80% solution of ethanol was made with the extract solution by adding absolute ethanol (analytical grade Sigma-Aldrich, Germany). The polysaccharides were precipitated out by refrigerating the solution at -4^0C overnight. The precipitate was recovered by centrifuging at 10,000rpm. The precipitate was washed with distilled water and the left overnight to solidify at -80^0C and lyophilized in a freeze dryer. The lyophilized polysaccharide was weighed and taken into solution a

second time with hot water and purified with a Sephadex cellulose column. A stock solution of 100 mg/ml and serial dilutions was made for antioxidant and antimicrobial assays.

Extraction of Oligosaccharides

The oligosaccharides were extracted with hot water with modifications of methods by Szambelan and Nowak (2006). Ten grams of lichen tissues were extracted with distilled water in a water bath with shaker at 80^0C for 2 h. The tissues were extracted three times and extracts pooled together, filtered with Whatman No. 1 paper, and concentrated with a rotary evaporator at 60^0C. The concentrated extracts were lyophilized and taken into solution with hot water. The Neutral Detergent Solution (NDS) and the Acid Detergent Solution (ADS) were prepared with methods outlined in the Official Methods of Analysis (AOAC) International (AOAC 1990).

The extracted lichen oligosaccharides were dissolved in 100 mL of NDS and ADS in 250 mL Schot bottles and left in the water bath shaker for another 1 h to separate the insoluble fractions. The insoluble fractions were removed by centrifugation at 15, 0000 g for 10 minutes at room temperature. The extract was further processed to extract the oligosaccharides by shaking in neutral acid detergent solution at 60^0C in a water bath with a shaker for 1 h. The extract was further treated with 1 M H_2SO_4 and shaken for another 1 h at 60^0C. The supernatant was filtered with Whatman No. 1 filter paper and the extracted lichen oligosaccharides were further hydrolyzed with 10 mL of H_2SO_4 solution (pH 1) at 100^0C in the water bath shaker for 6 h. The extracted oligosaccharide solutions were sterilized with a 0.45 µm nylon micropore syringe filters (Pall German Laboratory, USA) concentrated and lyophilized. Solutions of known concentrations were made from lyophilized extracts before use. The concentration of polysaccharides and oligosaccharides was determined with phenol-sulphuric acid method following standard methods and expressed as mg/g glucose equivalent (AOAC, 1990).

Extraction of Polyphenols

A modification of methods by Kapasakalidis *et al*. (2006), was adopted for extraction of polyphenols from lichen tissues. Pulverized dried lichen tissues (10 g) were extracted with 200 mL of acidified methanol (pH 3) by stirring overnight on a magnetic stirrer at 250rpm. Extraction was conducted three times and the pooled extracts were concentrated and evaporated to dryness in a rotary evaporator at 40^0C. The lichen polyphenols were taken into solution with hot water, cooled down, solidified at -80^0C freezer and lyophilized. Lyophilized extracts were dissolved in 70% methanol to a 100 mg/ml working solution and stored in a 4^0C refrigerator until needed. The total phenolic content of the extracts was determined with the Folin-Ciocalteu method following standard methods and gallic acid used as standard with the results expressed as mg/ GAE/g of dry extract (AOAC, 1990).

DPPH Radical Scavenging Assay and Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox Equivalent Antioxidant Capacity (TEAC) of polysaccharide, Oligosaccharide and polyphenol lichen extracts was determined following modification of methods by Re *et al*. (1999). The ABTS radical cation decolorization assay was used, the ABTS $^+$cation radical was produced by mixing 7 mM of ABTS aqueous solution with 2.45 mM of potassium persulphate (1:1) and stored in the dark for 16hrs before use. The lichen extracts (5µl) was added to to 3.995ml of ABTS $^+$ solution and incubated in the dark for 30mins and absorbance read at 734nm with a UV-Vis Spectrophotometer (Shimadzu). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard and different concentrations of the standard and the extracts was used to plot a scatter plot graph that was used to for standard curve values. The Trolox equivalent antioxidant capacity was measured in mM/Trolox equivalent.

DPPH radicals scavenging activity of polysaccharide, oligosaccharide, and polyphenol extracts of five test lichens were estimated following modification methods by Brand-Williams *et al*. (1995). Test samples of different concentrations (20, 40, 60, 80 and 100mg/ml of oligosaccharides, polysaccharides and polyphenols was added to 2 ml of 0.1mM of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical solution. The reaction mix was left to incubate in the dark at room temperature for 30 minutes after shaking vigorously and absorbance measured at 517 nm (UV-Vis Spectrophotometer-Shimadzu). The scavenging activity was calculated using the formula

$$\text{Scavenging Activity (\%)} = \left[\text{A(control)} - \frac{\text{A(sample)}}{\text{A(control)}}\right] \text{x } 100$$

A(sample)- absorbance of the test samples at different concentrations, A(control) -absorbance of the control containing all the reaction reagents except the test samples.

Antibacterial Assay

The antibacterial activity of lichen polysaccharide, oligosaccharide, and polyphenol extracts against the bacteria ~ *Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyrogenes, Escherichia coli,*

Pseudomonas aeruginosa, and *Klebsiella pneumoniae* was conducted in 96 well microtiter plates with the broth microdilution assay following standard methods (CLSI, 2012). The test bacterial were got from the Nigerian Medical Research Institute (NMRI), Yaba, Lagos, Nigeria. A concentrated cell suspension was made using Tryptic Soy Broth as the growth medium at a 0.5 McFarland standard and incubated overnight. Serial dilutions were made in the first two wells of the 96 well plate (four-fold dilutions). A 200 µL broth composed of Tryptic soy broth, extract and 0.5 McFarland test bacterial cell suspension, controls were not seeded. The plates were incubated overnight at 37ºC and after incubation, the plates read with a micro-plate reader at a wavelength of ~625nm. The minimum inhibitory concentrations of the extracts were done by making series of dilution in the 96well plates for each extract, starting from 0.5 mg/ml to 20 mg/ml. The control had no extract, and the absorbance of the treated/extract wells were blanked with the extract only control. The MIC used here was the lowest concentration of the lichen extracts which inhibited the growth of the bacterium after incubation period.

Results and Discussion

The antioxidant and antibacterial activities of oligosaccharides, polysaccharides, and polyphenol extracts of the Nigerian lichens *Ochrolechia subpallescens, Lecanora pinguis, Parmelia saxatilis, Graphis scripta,* and *Verrucaria viridula* are evaluated for the first time. The metabolites in lichens that abound in the wild in Nigeria and their activities are yet to discovered or harnessed, hence the current study. The current study characterized the lichens collected from the wild at the Lekki Conservation Centre, Lagos, Nigeria and established that the different Nigerian lichen metabolites or extracts showed promising degrees of antioxidant activity and antibacterial activities. The yield of the three metabolites from the lichen tissues varied with the different species. The yield of lichen metabolite extracts varied from 2.81 % with polyphenol extracts of *V. viridula* to 44.13 % in the oligosaccharide extract of *L. pinguis* (Table 1). The polysaccharide content of different lichens species in the wild vary depending on the inherent nature of the species, environment, host, and other factors. In previous research, polysaccharides content of the lichen *Cetraria islandica* was up to 57 %, indicating the presence of high concentrations of polysaccharides in lichen tissues consistent with results in the current study (Olafsdottir and Ingòlfsdottir 2001).

The lichen *O. subpallescens* recorded the highest concentration of oligosaccharides followed by *L. pinguis* and the least concentration was noted in *P. saxatilis* (Table 2). *Graphis scripta* had the highest concentration of polysaccharides while *O. subpallescens* recorded the least polysaccharide content (Table 2). *Graphis scripta* had the highest concentration of polyphenols and the least total phenolic content was recorded by *O. subpallescens*. All the lichens species had more oligosaccharides and polysaccharides than polyphenols and this is because the carbohydrates are mostly primary metabolites while the polyphenols are secondary metabolites (Table 2) (Olafsdottir and Ingòlfsdottir 2001). The results here are consistent with literature as there is evidence that lichens have a wide array of polysaccharides that have been associated with immunomodulatory and antiiflammatory activities (Shrestha *et al*., 2015).

Antioxidant Activities of Lichen Metabolites

The antioxidant activity of the lichen extracts was assessed with the DPPH radical scavenging activity and the Trolox Equivalent Antioxidant Capacity. The polyphenol extracts of *O. subpallescens, L. pinguis* and *V. viridula* showed strong DPPH radical scavenging activity (75.78%, 72.49% and 68.55% respectively (Table 2) but not outperforming the lichen oligosaccharides. The higher antioxidant capacity displayed by lichen oligosaccharide is attributed to their lower molecular weights and ease of solubility in water because of shorter chains and more free groups attached to the D-glucosamine units compared to polysaccharides (Yuan *et al*., 2009). The polyphenol extracts of *P. saxatilis* and *G. scripta* showed moderate antioxidant activity (49.69% and 48.61 percentage reduction of DPPH radical activity respectively). The TEAC of the lichen polyphenols varied from 0.161 mM/Trolox equivalent in *O. subpallescens* to 0.072 mM/Trolox equivalent in *G. scripta* (Table 2).

The polysaccharide fraction of *O. subpallescens, L. pinguis* and *P. saxatilis* all recorded more than 50 % DPPH radical scavenging activity but the *G. scripta* and *V. viridula* polysaccharide reactive oxygen reducing capacities were 65.42 % and 68.55 % respectively (Table 3). The Trolox Equivalent Antioxidant Capacity of polysaccharides varied from 0.010 mM/Trolox equivalent in *O. subpallescens* to 0.091 mM/ Trolox equivalent in G. *scripta* (Table 3). The strong to moderate antioxidant activity of lichen polysaccharide displayed in this study is consistent with literature (Courtois, 2009; Kou, *et al*., 2016). The oligosaccharide fraction of the lichen extracts was the most reactive of all three metabolites. There is paucity of information on lichen oligosaccharide but records on oligosaccharides from plant indicate that they are very reactive compounds in cellular systems and make

strong antioxidant agents (Lai *et al*., 2017). However, lichen oligosaccharides play very important roles in the control of Type 2 Diabetes Mellitus (Kershengolts et al., 2015) Oligosaccharides from the plant *Panax ginseng* showed stronger antioxidant activity than other fractions from the plant in reports by Zhao *et al*. (2020) as reported here for the test lichen species.

The DPPH radical scavenging activity was highest with the oligosaccharide fraction of *O. subpallescens* (90.75%) and least with *P. saxatilis* (71.15%) (Table 3). The TEAC activity of the oligosaccharide fraction of the lichen species also followed the same pattern as the reduction of the DPPH radicals. The TEAC of the lichen species' oligosaccharide varied from 0.124 mM/Trolox equivalent in *O. subpallescens* to 0.094 mM/Trolox equivalent in *G. scripta*.

Literature show that the lichen association has a huge deposit of secondary metabolites that account for their antimicrobial, antioxidant, antiviral, anticancer, anti-inflammatory, analgesics, and antipyretic activities (Ranković & Kosanić, 2019; Gupta *et al*., 2020). The strong antioxidant effects of lichen polyphenol extracts of *O. subpallescens* (75.78%) and *L. pinguis* (72.49%) recorded here is consistent with reports of other researchers (Aslan, et al., 2006) (Ranković, et al., 2010). The high antioxidant activity recorded by the polyphenols can be attributed to the type of phenolic compounds found in them. Interesting compounds responsible for antioxidant activity of lichen polyphenols include salazinic acid, gyrophoric acid and divaricatic acid (Thadhani and Karunaratne, 2017). Antioxidant activity of test lichens showed with high polyphenol concentrations are linked to stronger antioxidant activity (Table 3), and this is confirmed by the positive correlation (~ R^2 = 0.952) between total phenolic content and DPPH radical scavenging activity (Figure 1). The conclusion drawn here agrees with reports by Behera *et al*., (2009) but disagrees with Odabasolglu *et al*. (2004) with the conclusion that there is no correlation between total phenolic content of lichen extract and antioxidant activity. This implies that the antioxidant activity of lichen extracts may be multifaceted with other non-phenolic compounds playing a role. The polysaccharide and oligosaccharide extracts showed moderate to strong antioxidant activities buttressing the fact that other non-phenolic components of the lichen tissues can account for their antioxidant activity.

The half maximal effective concentration (EC_{50}) of the lichen extracts for the DPPH radical scavenging activity varied from 26.11 mg/ml in oligosaccharide extracts of *O. subpallescens* to 107.19 mg/ml in polysaccharide extract of *P. saxatilis* (Table 4), showing the superior antioxidant effect of oligosaccharide fraction.

The antimicrobial activity of the lichens *O. subpallescens, L. pinguis, P. saxatilis, G. scripta, and V. viridula* was assessed against six pathogenic bacteria (*Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyrogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*). The antibacterial activity of lichen polyphenols varied with the different lichen and bacterial species, inhibiting both gram negative and gram-positive bacteria. Polyphenol extracts of *O. subpallescens, P. saxatilis, V. viridula* and *G. scripta* showed very strong inhibitions against *Pseudomonas aeruginosa* (94.52%, 96.08%, 90.7%) and *Escherichia coli* (90.55%) respectively (Table 5). The polyphenol extracts of *O. subpallescens* and *P. saxatilis* showed stronger antibacterial activity outperformed the reference antibiotic ciprofloxacin (Table 5). This result is consistent with reports of (Bate, et al., 2020) for the Cameroonian lichen *Usnea articulata* that showed broad spectrum activity against multidrug resistant bacteria strains. The polyphenol fraction performed better than the polysaccharide fraction of the lichen metabolites against the growth of the test bacteria. Some of the phenolic compounds in lichens that have been associated with their antimicrobial activity include depsides, depsidones and dibenzofurans (Ranković et al., 2008). The Nigerian lichen *Ramalina farina* had hydroquinone depside and 2, 3-dihydroxy-4-methoxy-6-pentylbenzoic acid as phenolic compounds responsible for its antibacterial activity (Lai et., 2013).

The lichen oligosaccharide extracts inhibited both gram-positive and gram-negative test bacteria in varying degrees, showing broad-spectrum activity. The oligosaccharide extract of *O. subpallescens* however, exhibited the strongest inhibitory effects against *Staphylococcus aureus* and *Klebsiella pneumoniae* with 94.02% and 90.81% inhibition respectively (Table 6). On the other hand, *P. saxatilis* and *G. scripta* oligosaccharides showed the strongest inhibition against *K. pneumoniae* (92.25%) and *S. aureus* (98.15%) respectively (Table 6). The oligosaccharide extracts of *O. subpallescens, G. scripta* and *V. viridula* also outperformed the reference antibiotic with inhibition of *S. aureus* growth (Table 6). The reference antibiotic outperformed all lichen oligosaccharides in inhibiting the growth of *P. aeruginosa* and *Streptococcus pyrogenes* (Table 6). The MIC of oligosaccharide fraction varied from 0.5 mg/ml 20 mg/ml with the very active species recording the least MIC (Table 6). The very strong antibacterial activity of oligosaccharide fraction in this study is supported by the reports of Qian *et al*. (2014) with the oligosaccharides from dandelion (*Taraxacum officinale*) inhibiting the growth of *E. coli* and *S. aureus* strongly.

V. viridula polysaccharide extract exhibited the strongest antimicrobial activity (92.64 % inhibition) compared to the polysaccharide extracts of other lichen species with regards to *S. aureus* (Table 7). The *O. subpallescens* polysaccharide extract recorded moderate to very weak inhibitory activity against all test bacteria varying from *59.41%* with *S. aureus* to 15.33% with *E. coli* (Table 7). On the other hand, the polysaccharide extract of *L. pinguis* showed strong activity (73.85% inhibition) against *S. aureus* and very weak activity (18.58% inhibition) against *Pseudomonas aeruginosa*. The polysaccharide fraction of the five lichens showed their strongest effect against *Staphylococcus aureus* a gram-positive bacterium (Table 7). This result is consistent with those of Bisht *et al*. (2014) with the lichens~ *Peltigera* sp and *Cladonia* sp with polysaccharide extracts showing the strongest antibacterial activity against *S. aureus*. The inhibitory effects of *P. saxatilis* polysaccharide also varied with the bacteria species ranging from 70.22% inhibition against *K. pneumoniae* to 24.79% inhibition with *Streptococcus pyrogenes* (Table 7). *Graphis scripta* polysaccharide recorded antibacterial activity that varied from 68.89% inhibition with *S. aureus* to 25.50% inhibition against *E. coli*. The reference antibiotic ~ciprofloxacin outperformed the lichen polysaccharide extracts in inhibiting the growth of *Enterococcus faecalis*, *S. pyrogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

The MIC values of lichen polysaccharide against test bacteria varied from 0.5 mg/ml in *O. subpallescens* with *S. aureus* to 20 mg/ml in *L. pinguis* (*E. coli*), and *V. viridula* (*S. pyrogenes*) respectively (Table 7). The polysaccharide fraction of the five lichens showed their strongest effect against *Staphylococcus aureus* a gram-positive bacterium. This result is consistent with those of (Bisht *et al*., 2014) with the lichens~ *Peltigera* sp and *Cladonia* sp with polysaccharide extracts showing the strongest antibacterial activity against *S. aureus*.

The lichen polysaccharides showed some degree of specificity for the species of bacteria they inhibited their growth. More research must be conducted to find out exactly which pathogenic organism, the different lichen species are able to inhibit their growth.

The specificity shown by the lichen extracts could be because of differences in cell wall composition of bacteria cells. The cell wall of gram-positive bacteria is made of peptidoglycans and teichoic acid and those of gram-negative bacteria of peptidoglycans, lipopolysaccharides, and lipoproteins (Heijenoort 2001). The differences in cell wall composition can therefore affect the nature of compounds they can be susceptible to and hence the differences in degree of inhibition shown with the different metabolites or extracts.

In nature, lichens are unique organisms, forming one of the most successful associations on earth (Zambare and Christopher 2012). This uniqueness makes it possible for them to synthesize hundreds of very extraordinary secondary metabolites that find use in pharmaceutical and medical practice.

Lichens in the wild in Nigeria display a vast array of activity as shown by those of the foliose lichen *Dirinaria picta* with antimalaria potentials (Afieroho *et al*., 2018).

The oligosaccharide extract of *G. scripta* had the strongest antibacterial activity among all species evaluated and inhibited the growth of all the test bacteria.

The oligosaccharides were more reactive than the polysaccharides and this can be attributed to the molecular weights of both. It is expected that compounds with less molecular weight can be more reactive and effective in drug delivery. The low molecular weight compounds from lichens are therefore of interest during investigation for drugs especially as anticancer agents, and brain tumour related diseases where precision of drug delivery is important (Li and Kang 2020). The extracts of these Nigerian lichens acted against both on gram positive and gram-negative bacteria.

The lichen extracts with strong activity against certain species of bacteria can find application in the food and pharmaceutical industry against the specific pathogenic bacteria. Lichens species like *Parmotrema tinctorum* and *Usnea bismolliuscula* from India are already being used and sold as nutraceuticals (Muthu *et al*., 2021).

Conclusion

The oligosaccharide fraction of *Ochrolechia subpallescens*, *Lecanora pinguis*, *Parmelia saxatilis*, *Graphis scripta*, and *Verrucaria viridula* proved to be potent antioxidant agents and good alternatives to synthetic antioxidant supplements. The evaluated lichen extracts showed promising biological effects supporting their use as natural products with new pharmacologically important values.

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Conflict of Interest

The authors declare no conflict of interest and did not receive funds from any public or private individual.

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Table 1: Yield Oligosaccharide, Polysaccharides and Polyphenols from the lichens~Ochrolechia subpallescens, Lecanora pinguis, Parmelia saxatilis, Graphis scripta and Verrucaria viridula.

Test Lichens	Oligosaccharides	Polysaccharide	Polyphenols
Ochrolechia subpallescens	33.15±2.06[a]	12.29±0.82[bc]	5.13±0.62[c]
Lecanora pinguis	44.13±1.91[a]	6.75±0.68[c]	4.52±0.81[c]
Parmelia saxatilis	17.95±1.08[b]	5.87±0.71[c]	3.75±0.35[c]
Graphis scripta	15.07±2.02[b]	3.48±0.49[c]	2.94±0.55[c]
Verrucaria viridula	17.05±1.85[b]	2.95±0.44[c]	2.81±0.27[c]

*Superscripts with same letters are not significantly different with the Duncan's Multiple Range Test at 0.05% Level of Significance

Table 2: Concentration of Metabolites Extracted from Ochrolechia subpallescens, Lecanora pinguis, Parmelia saxatilis, Graphis scripta and Verrucaria viridula

Lichen species	Oligosaccharide (mg/g of glucose equivalent)	Polysaccharide (mg/g of glucose equivalent)	Polyphenols (mg/GAE/ g)
Ochrolechia subpallescens	1955.92±10.15	80.14±2.61	39.04±6.01
Lecanora pinguis	1108.25±12.02	180.25±8.84	64.19±5.51
Parmelia saxatilis	892.15±9.65	106.15±7.27	50.10±3.18
Graphis scripta	551.62±8.46	113.06±6.62	78.22±5.27
Verrucaria viridula	609.11±11.75	159.04±5.88	55.61±4.45

Table 3: DPPH radical scavenging activity and Trolox Equivalent Antioxidant capacity of Lichen species

Test Lichens	DPPH radicals Inhibition (%)			TEAC (mM/Trolox equivalent)		
	Polysaccharide	Oligosaccharide	Polyphenol	Polysaccharide	Oligosaccharide	Polyphenol
Ochrolechia subpallescens	47.91	90.75	75.78	0.010	0.124	0.161
Lecanora pinguis	41.65	82.68	72.49	0.035	0.114	0.108
Parmelia saxatilis	37.92	71.15	49.69	0.029	0.116	0.078
Graphis scripta	65.42	80.62	48.61	0.091	0.095	0.072
Verrucaria viridula	68.55	85.66	68.55	0.059	0.118	0.089

Table 4: Half maximal effective concentration (EC_{50}) of Metabolites from Ochrolechia subpallescens, Lecanora pinguis, Parmelia saxatilis, Graphis scripta and Verrucaria viridula

Lichen Species	DPPH radicals Inhibition EC_{50} (mg/ml)		
	Polysaccharides	Oligosaccharides	Polyphenols
Ochrolechia subpallescens	84.38	26.11	52.23
Lecanora pinguis	96.04	47.24	55.18
Parmelia saxatilis	107.19	45.29	80.49
Graphis scripta	61.14	49.61	82.28

Verrucaria viridula	58.35	40.69	58.35

Table 5: Antibacterial Activity of Lichen Polyphenol Extract

Bacteria Species	Polyphenol Bacterial Inhibitory Effects											
	Ochrolechia subpallescens		*Lecanora pinguis*		*Parmelia saxatilis*		*Graphis scripta*		*Verrucaria viridula*		Ciprofloxacin	
	PI	MIC	PI	MIC	PI	MIC	PI	MIC	PI	MIC	PI	MIC
Staphylococcus aureus.	76.08	5.75	88.66	2.50	78.15	4.50	79.12	3.75	85.75	2.50	75.84	5.00
Enterococcus faecalis	88.82	1.75	36.55	20.00	51.65	7.50	70.05	5.75	56.19	7.50	60.88	8.25
Streptococcus pyrogenes	85.11	0.50	61.94	7.50	75.28	3.75	58.61	10.00	60.15	7.50	65.92	5.75
Escherichia coli	52.75	15.00	28.26	20.00	85.76	1.75	90.55	1.25	35.94	15.00	45.71	15.00
Pseudomonas aeruginosa	94.52	0.5	58.44	4.75	96.08	0.50	61.02	10.00	90.71	0.50	92.66	0.50
Klebsiella pneumoniae	68.20	2.75	35.61	15.00	85.02	2.50	50.11	10.00	74.25	3.50	80.54	1.25

*PI-Percentage Inhibition (%); MIC extracts-Minimum Inhibitory Concentration- (mg/ml); MIC antibiotics-µg/ml

Table 6: Antibacterial Activity of Lichen Oligosaccharide Extract

Bacteria Species	Oligosaccharide Bacterial Inhibitory Effects											
	Ochrolechia subpallescens		*Lecanora pinguis*		*Parmelia saxatilis*		*Graphis scripta*		*Verrucaria viridula*		Ciprofloxacin	
	PI	MIC	PI	MIC	PI	MIC	PI	MIC	PI	MIC	PI	MIC
Staphylococcus aureus.	94.02	0.50	55.92	10.00	65.16	3.75	98.15	0.50	78.38	3.00	75.84	5.00
Enterococcus faecalis	85.24	2.75	62.19	7.50	78.66	2.75	48.91	7.50	72.04	2.50	60.88	8.25
Streptococcus pyrogenes	55.02	5.75	38.75	15.00	59.38	4.75	44.06	7.50	54.93	7.50	65.92	5.75
Escherichia coli	60.04	4.75	38.90	20.00	67.35	5.85	58.22	4.65	65.29	3.75	45.71	15.00
Pseudomonas aeruginosa	88.82	1.25	50.27	7.50	75.74	3.75	61.05	4.75	71.35	4.50	92.66	0.50
Klebsiella pneumoniae	90.81	1.25	82.95	2.50	92.25	1.25	71.18	3.25	62.08	3.75	80.54	1.25

*PI-Percentage Inhibition (%); MIC extracts-Minimum Inhibitory Concentration- (mg/ml); MIC antibiotics-µg/ml

Table 7: Antibacterial Activity of Lichen Polysaccharide Extract

Bacteria Species	Polysaccharide Bacterial Inhibitory Effects											
	Ochrolechia subpallescens		*Lecanora pinguis*		*Parmelia saxatilis*		*Graphis scripta*		*Verrucaria viridula*		Ciprofloxacin	
	PI	MIC	PI	MIC	PI	MIC	PI	MIC	PI	MIC	PI	MIC
Staphylococcus aureus.	59.41	6.75	73.85	5.25	51.28	8.50	68.89	6.50	92.64	0.50	75.84	5.00
Enterococcus faecalis	28.52	-	40.18	10.00	41.62	12.50	50.25	7.50	42.91	12.50	60.88	8.25
Streptococcus pyrogenes	20.66	-	38.15	18.25	24.79	-	41.82	12.50	35.70	20.00	65.92	5.75
Escherichia coli	15.33	-	35.26	20.00	40.05	18.25	25.50	-	30.55	18.25	45.71	15.00
Pseudomonas aeruginosa	45.81	12.50	18.58	-	61.75	5.25	50.92	6.50	41.77	18.25	92.66	0.50
Klebsiella pneumoniae	55.04	7.50	48.29	15.00	70.22	3.75	41.92	15.00	51.88	6.75	80.54	1.25

*PI-Percentage Inhibition (%); MIC extracts-Minimum Inhibitory Concentration- (mg/ml); MIC antibiotics-µg/ml

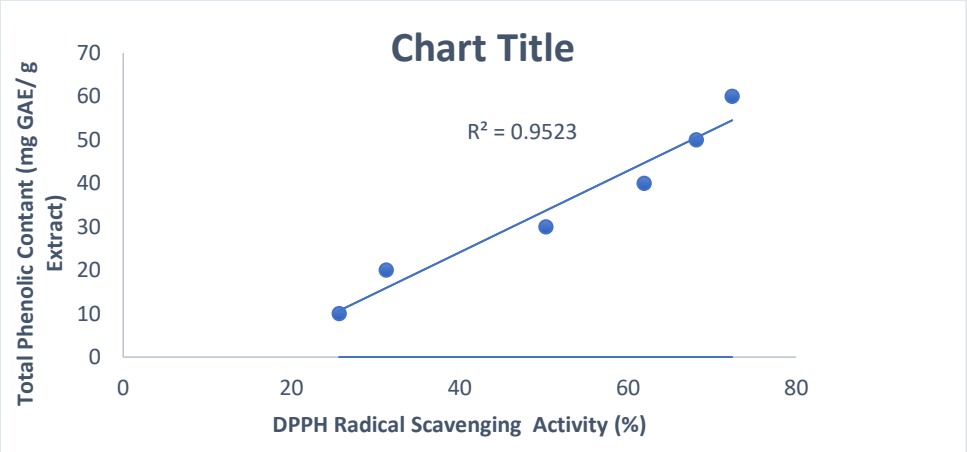

Figure 1: Correlation between Total Phenolic Content and EC_{50} of Lichen Extracts