Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(6): 24-30
Received: 10-07-2021
Accepted: 19-08-2021

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Lipids composition and antioxidant properties of selected microalgae isolated from freshwater and soil samples

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Abstract
Sourcing for natural antioxidants for nutraceutical and pharmaceutical industries is receiving global attention. In view of this, aqueous and methanolic extracts of *Chlorococcus* sp., *Chlamydomonas* sp. and *Chlorella* sp. isolated from Ogunpa river, Uren river and Oil palm plantation respectively were screened for their total phenolic, flavonoids and radical scavenging activity against the stable radical 1,1-diphenyl-2-picrylhydrazyl to establish their antioxidant potential. The culture samples using Bold's Basal Medium were analysed for lipids contents and fatty acid analysis was carried out with a Gas Chromatography Mass Spectrometer system fitted with an HP-5MS (5% Helium) column. For lipid content, *Chlorella* sp. recorded twenty-two fatty acids with Undecanoic acid (methyl ester) as most abundant, as compared with *Chlamydomonas* sp. and *Chlorococcus* sp. Methanolic extracts of *Chlorella* sp. exhibited the highest antioxidant activity (63.24µg/100ml), probably due to high contents of total phenols (33.02µg/100ml) and flavonoids (14.35 µg/100ml) while methanolic extract *of Chlorococcus* sp. recorded higher radical scavenging activity. However, *Chlamydomonas* showed higher percentage radical inhibition (72.48%) at 100µg/ml.

Keywords: Microalgae, lipids, antioxidants, GC-MS, total phenolic content, flavonoids

Introduction
Microalgae are photosynthetic organisms that are exposed to high oxygen and radical stresses and thus have developed several effective defensive systems against reactive oxygen species and free radicals (Pulz and Gross, 2004) [18]. Microalgae represent an almost untapped resource of natural antioxidants. Their enormous biodiversity and their significance as a source of natural antioxidants is further improved by the relative ease of purification of bioactive compounds from them (Li *et al.*, 2001) [16]. The use of synthetic antioxidants has decreased due to their suspected activity as promoters of carcinogenesis as well as a general consumer rejection of synthetic food additives (Namiki,1990) [29]. Meanwhile, Pratt, (1992) [28] as cited by Miranda *et al.*, (1998) observed that there is a current worldwide interest in finding new and safe antioxidants from natural sources such as plant material to prevent oxidative deterioration of food and to minimize oxidative damage to living cells. However, not all groups of microalgae can be used as natural sources of antioxidants, due to their widely varied contents of target products, growth rate or yields, ease of cultivation, and/or other factors.

Short note on greens
Reports on the antioxidant activities of microalgae are inadequate, especially concerning the relationship between their phenolic content and antioxidant activity (Li *et al.*, 2007) [17]. Pandey and Rizvi (2009) and Dai and Mumper (2010) reported that phenolic compounds are secondary metabolites widely distributed in plants with well-known health benefits and these compounds are described as radical scavengers because they are donors of hydrogen atoms or electrons, producing stable radical intermediates. Antioxidants, scavengers of ROS (reactive oxygen species), are substances that are able to protect, scavenge, and repair oxidative damage, thereby protecting target structures or molecules from oxidative injuries (Halliwell, 2007). In protecting against ROS, antioxidants help optimize human physiological functions, thus helping to maintain a healthy state and protect against diseases. Despite the reported antioxidant properties of the phenolic compounds and the potential of microalgae and cyanobacteria as sources of these compounds (Chac´on-Lee *et al.*, 2010) [15], few studies have focused on their identification and quantification in microalgae (Cirulis *et al.*, 2013; Safafar *et al.*, 2015) as well as on the role played by phenolics in microalgae defense mechanisms against high ROS levels (Heller *et al.*, 2016; L´opez *et al.*, 2015). Reports on the evaluation of antioxidant activity of microalgae and cyanobacteria extracts (L´opez *et al.*, 2007) have also

concluded that a high number of microalgal species produce a wide range of antioxidants, including carotenoids, polyunsaturated fatty acids, polysaccharides, or mycosporine-like amino acids (MAAs). Natrah *et al.* (2007) also worked on fourteen indigenous microalgal samples from Malaysia for their ability to be natural sources of antioxidants and their result showed that six microalgal methanolic crude extracts were active in inhibiting the lipid peroxidation of linoleic acid. The finding evidently corroborates previous assertions, but it is still far from the enormity of untapped resources in the abundance of algae, more research efforts should be deployed at elucidating more antioxidant compounds from these florals.

Although many microalgae species have been analysed for their fatty acid compositions, and some have been determined for certain lipid classes such as polar lipids 2,3,5−8 or sterols,9−15 few studies have presented the complete characterization of lipid components (both simple and complex lipids) of a particular microalga.

Information on complete lipid characterization of microalgae is essential for successful strain selection, biomass extraction, and utilization for the production of biofuels and high-value compounds. The availability and sensitivity of modern instrumentations such as GC-MS and GC-FID provide powerful tools to gain in-depth knowledge of the lipid composition of microalgae. Since there is dearth of information on the level of abundance of phenolic compounds, fatty acids, and their antioxidant potential in *Chlorella* sp., *Chlamydomonaas* sp., and *Chloroccocus* sp. (Chlorophyta). The main objective of this work was to determine the lipids content and antioxidant activity of aqueous and methanolic extracts of *Chlorella* sp., *Chlamydomonas* sp. and *Chlorococcus* sp. (Chlorophyta).

Materials and Methods

Description of study site, collection and culturing of sample

Surface water samples were collected from Ogunpa River (3052ºE and 3056ºE and 7022ºN 7026ºN) in Ibadan and Uren River (N6 52'02'.6 E3 43'09.2) North of Ikenne which flows along Odogbolu-Aiyepe road while soil sample (top soil 0-15cm) was collected from Oil palm plantation (N06.34796°, E005. 39289) within Okomu National Park, Ovia South-West Local Government Area of Edo State in Nigeria. The sampling locations (Ogun and Edo States) represent two geopolitical zones (South-West and South-South) in Nigeria. Surface water samples for biological and physiochemical analysis from both rivers were collected in clean labelled plastic bottles while soil sample was collected using a Stainless Dutch soil Auger. The collected soil sample was sealed in labelled airtight polythene bag. In the laboratory, 250ml of distilled water was added to 10g of soil sample after which decantation method was used, also 250ml of surface water samples from each river were poured in conical flasks and all environmental samples were cultured using Bold Basal Medium. All conical flasks were kept on a shaker to prevent clumping and placed close to the window for sunlight. Growth of microalgae were observed and monoculture of each of *Chlamydomonas, Chlorella* and *Chlorococcus* were reached through subculturing (Batch method).

Preparation of Microalgae Extracts for Antioxidant Activity Determination

Dry samples were prepared by using 25ml of each culture sample of freshwater and soil in beakers and kept in the oven at 40^0C to dry. To Oven-dried microalgae (*Chlorococcus* sp., *Chlamydomonas* sp. and *Chlorella* sp.) biomass (56.99g, 50.12g and 71.24g) respectively, 20mL of solvent (methanol and water; 89.2) were added separately, and stirred using a magnetic stirrer, the samples were allowed to stand for 40 minutes at room temperature, then centrifuged at 3000rpm for 10minutes, after which the supernatant was collected and stored in specimen bottle for further analysis.

Analysis of Physical Parameters

Surface water samples were analysed for pH, conductivity, temperature and salinity *in-situ* using HANNA Combo pH and EC Multi Meter Hi 98129, Mercury-in-glass thermometer and refractometer, respectively. The nutrient composition and pH of the soil were also measured.

Lipids Extraction of Microalgae

Gradient method was used for both soil and water samples; Twenty-five millilitres of each microalgae monoculture was extracted using 50ml of hexane using separating funnel. This procedure was repeated with chloroform. The hexane and chloroform extracts of each microalga were left to form oil through evaporation of solvents. Anhydrous $NaHSO_4$ was added to each extract to absorb excess water and aid formation of oil. The oil formed was subjected to esterification reaction which leads to the production of esters and water. The ester was then transferred into a sample amber bottle for characterization. The lipids were characterized using Gas Chromatography Mass Spectrophotometry (GC-MS) (Adesalu *et al*., 2017).

The Total Antioxidant Capacity (TAC) was analysed by a slight modification of the method described by Prieto *et al*. (1999). Three millilitres of the TAC reagent which composed of 4mM Ammonium Molybdate, 0.6M H_2SO_4 and 28mM Sodium Hydrogen Phosphate, was mixed with 1ml of the methanolic extract of each algal extract samples in capped bottles and boiled at 95ºC for 90 minutes. It was then allowed to cool at room temperature and absorbance of each solution was measured at 695nm wavelength. The total antioxidant capacity was expressed as milligram ascorbic acid equivalent per gram of extract (mg AAE/g).

Analysis of total phenolics.

Total phenolic concentration was evaluated by spectrophotometric analysis, using the Folin-Ciocalteau method (Prieto *et al*., 1999). To 0.5ml of methanolic extract of the algal, was added 0.5ml of Folin Ciocalteau's reagent before incubation for 20 minutes. After this, 2ml of Na_2CO_3 was added and the mixture was allowed to incubate for 30 minutes. The absorbance was measured at 760nm with a spectrophotometer (Tecan Spectra Classic). A standard curve was generated using different concentrations of gallic acid and the total phenol content was extrapolated from the generated calibration curve. The phenolic concentration was expressed as the gallic acid equivalent (GAE) in mg/100 g of dried microalgae sample.

Analysis of Total Flavonoids

Total flavonoids were quantified according to a slightly modified colorimetric aluminium chloride method as earlier described by Do *et al*., (2014). To 1ml of varied concentrations of quercetin (0-100 µg/mg), 3ml of 5% Sodium Nitrite and 3ml of 10% Aluminium Chloride were added and incubated for 6 minutes at room temperature. 2ml

of 1M NaOH was then added to the mixture and topped up with distilled water to make the total volume of 10ml. Furthermore, to 0.5ml of methanolic extract of sample, 1ml of 10% $AlCl_3$ was added, then, 1ml of Sodium Nitrite was added and left for 2 minutes at room temperature. Readings of absorbance for both samples were taken at 415nm wavelength, Ascorbic acid was used as standard and the total flavonoid content calculated.

DPPH (1-diphenyl-2 picrylhydrazyl) radical Scavenging Activity

The free radical scavenging activity of the extract was estimated based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the procedure described by Cuendet *et al.* (1997) and Burits and Bucar (2000). Different concentrations (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) of each algal extract in 1ml of 80% methanol) were added respectively to 2ml of 0.135mM DPPH in methanol. The mixtures were each incubated in the dark for 30 minutes. The reduction in absorbance was thereafter measured using spectrophotometer at 517nm wavelength. The control tube contained 1.0ml of methanol and 2ml of DPPH. The verying concentrations of ascorbic acid (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) served as standard control (reference compound). All the tests were run in triplicates. Percentage of inhibition was calculated using the equation below:

$$\frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of Control}} \times 100$$

Results

Physico-chemical parameters

Salinity value, 0‰ of the surface water and the pH 6.3, slightly acidic were recorded while conductivity was 92.0µS/cm. For the soil, pH of 5.0 was recorded, while the values for Calcium, Magnesium, Potassium, Sodium, Sulphur, Phosphorus and Nitrogen were 0.251mg/kg, 35.75mg/kg, 0.56mg/kg, 0.48 mg/kg, 0.1666mg/kg, 15.83mg/kg and 0.38mg/kg, respectively.

Biological Analysis

Gas Chromatography Mass Spectrum Analysis

Lipids extracted using Chloroform for both freshwater microalgae (*Chlorococcus* sp. and *Chlamydomonas*) and Soil alga *Chlorella* sp. were as presented in Table 1. Soil alga *Chlorella* sp. had a total of twenty-two compounds with most of the compound recording percentage composition of 55% or above while fifteen and six compounds were recorded for *Chlamydomonas* sp. and *Chlorococcus* sp. respectively with Caproic acid being the most abundant fatty acid for both microalgae (Table 1). Comparing results of lipids extract of *Chlorococcus* sp. using hexane and chloroform, the hexane extract yielded more lipids, recorded higher percentage composition and quality of lipids than Chloroform extract (Table 2).

Table 1: GC-MS analysis of lipids extracted from freshwater microalgae *(Chlorococcus* sp. and *Chlamydomonas* sp.) and soil *alga (Chlorella* sp.) using Chloroform.

S/N	NAME OF COMPOUND	COMPOUND NATURE	CHEMICAL FORMULA	PERCENTAGE COMPOSITION (%)			QUALITY		
				Chloro coccus	*Chlo rella*	*Chlamydo monas*	*Chloro coccus*	*Chlo rella*	*Chlamydom onas*
1	Caproic acid methyl ester	Fatty acid	$C_6H_{12}O_2$	74	74	15.28	48	13	66
2	Caprylic acid methyl ester	Fatty acid	$C_8H_{16}O_2$	74	74	4.17	1	45	18
3	Capric acid methyl ester	Fatty acid	$C_{10}H_{20}O_2$	74	74	8.79	39	46	38
4	Undecanoic acid methyl ester	Fatty acid	$C_{11}H_{22}O_2$	74	74	7.87	1	50	34
5	Lauric acid methyl ester	Fatty acid	$C_{12}H_{24}O_2$	74	74	7.4	32	39	32
6	Tridecanoic acid methyl ester	Fatty acid	$C_{13}H_{26}O_2$	0	74	7.18	0	41	31
7	Cyclopropanonanoic acid methyl ester	Fatty acid	$C_9H_{18}O_2$	0	55	0	0	11	0
8	Tridecanoic acid 12- methyl ester	Fatty acid	$C_{13}H_{26}O_2$	0	74	7.18	0	12	31
9	Pentadecanoic acid methyl ester	Fatty acid	$C_{15}H_{30}O_2$	0	74	6.71	0	42	29
10	Palmitoleic (7-Hexadecenoic) acid methyl ester	Fatty acid	$C_{16}H_{30}O_2$	0	55	0	0	27	0
11	Palmitic (Hexadecanoic) acid methyl ester	Fatty acid	$C_{16}H_{32}O_2$	0	74	6.94	0	32	30
12	Hexadecanoic acid methyl ester	Fatty acid	$C_{16}H_{32}O_2$	0	55	0	0	47	0
13	Heptadecanoic acid methyl ester	Fatty acid	$C_{17}H_{34}O_2$	0	74	6.02	0	1	26
14	Stearic (10-Octadecenoic) acid methyl ester	Fatty acid	$C_{18}H_{36}O_2$	0	55	0	0	31	0
15	Trans-13-octadecanoic acid methyl ester	Fatty acid	$C_{17}H_{34}O_2$	0	55	0	0	39	0
16	Stearic acid methyl ester	Fatty acid	$C_{18}H_{36}O_2$	0	74	6.48	0	28	28
17	Cis-10-heptadecenoic acid methyl ester	Fatty acid	$C_{17}H_{32}O_2$	0	55	0	0	68	0
18	Arachidic (Eicosanoic) acid methyl ester	Fatty acid	$C_{20}H_4O_2$	0	74	1.85	0	8	8
19	Heneicosanoic acid methyl ester	Fatty acid	$C_{21}H_4O_2$	0	74	4.63	0	20	20
20	11-Hexadecanoic acid methyl ester	Fatty acid	$C_{16}H_{32}O_3$	0	55	0	0	20	0
21	Behenic (Docosanoic) acid methyl ester	Fatty acid	$C_{22}H_{44}O_2$	0	74	4.86	0	22	21
22	Tricisanoic acid methyl ester	Fatty acid	$C_{23}H_{46}O_2$	74	74	4.63	1	1	20

Table 2: Comparison of chloroform and hexane extract lipid components of *Chlorococcus us* sp.

S/N	NAME OF COMPOUND	COMPOUND NATURE	CHEMICAL FORMULA	RETENTION TIME (mins)		PERCENTAGE COMPOSITION (%)		QUALITY	
				Chloroform	Hexane	Chloroform	Hexane	Chloroform	Hexane
1	Caproic acid methyl ester	Fatty acid	$C_6H_{12}O_2$	3.327	3.293	74	74	48	1
2	Caprylic acid methyl ester	Fatty acid	$C_8H_{16}O_2$	4.609	4.712	74	74	1	9
3	Capric acid methyl ester	Fatty acid	$C_{10}H_{20}O_2$	5.771	5.891	74	74	39	39
4	Undecanoic acid methyl ester	Fatty acid	$C_{11}H_{22}O_2$	5.988	5.891	74	74	1	35
5	Lauric acid methyl ester	Fatty acid	$C_{12}H_{24}O_2$	6.892	6.921	74	74	32	32
7	Cyclopropanonanoic acid methyl ester	Fatty acid	$C_9H_{18}O_2$	0	8.717	0	55	0	23
8	Tridecanoic acid 12- methyl ester	Fatty acid	$C_{13}H_{26}O_2$	0	8.826	0	74	0	1
9	Palmitoleic (7-Hexadecenoic) acid methyl ester	Fatty acid	$C_{16}H_{30}O_2$	0	11.31	0	55	0	1
10	Palmitic (Hexadecanoic) acid methyl ester	Fatty acid	$C_{16}H_{32}O_2$	0	11.32	0	74	0	72
11	Hexadecanoic acid methyl ester	Fatty acid	$C_{16}H_{32}O_2$	0	12.49	0	55	0	37
12	Heptadecanoic acid methyl ester	Fatty acid	$C_{17}H_{34}O_2$	0	12.72	0	74	0	1
13	Stearic (10-Octadecenoic) acid methyl ester	Fatty acid	$C_{18}H_{36}O_2$	0	14.15	0	55	0	42
14	Trans-13-octadecanoic acid methyl ester	Fatty acid	$C_{17}H_{34}O_2$	0	14.15	0	55	0	26
15	Stearic acid methyl ester	Fatty acid	$C_{18}H_{36}O_2$	0	14.39	0	74	0	1
16	Cis-10-heptadecenoic acid methyl ester	Fatty acid	$C_{17}H_{32}O_2$	0	14.46	0	55	0	36
17	Heneicosanoic acid methyl ester	Fatty acid	$C_{21}H_4O_2$	0	18.31	0	74	0	35
18	11-Hexadecanoic acid methyl ester	Fatty acid	$C_{16}H_{32}O_3$	0	19.9	0	55	0	17
19	Behenic (Docosanoic) acid methyl ester	Fatty acid	$C_{22}H_{44}O_2$	0	21.59	0	74	0	21
20	Tricisanoic acid methyl ester	Fatty acid	$C_{23}H_{46}O_2$	24.132	0	74	0	1	0

Phenols and flavonoids Content

In the aqueous extract, *Chlorella* sp. showed a high content of total phenol and flavonoid with values of 39.4µg/100ml and 26.4µg/100ml, respectively followed by *Chlamydomonas* sp. with 21.62µg/100ml and 5.63µg/100ml while *Chlorococcus* sp. had values 3.28µg/100ml and 5.63µg/ml respectively (Figure 1). For methanolic extract of the three microalgae, *Chlorella* sp. recorded high concentrations of phenols (33.02µg/ml) and flavonoids (14.35µg/ml) as compared with *Chlamydomonas*, 18.42µg/ml phenols and 10.17µg/ml flavonoid whereas *Chlorococcus* sp. contained 21.94µg/ml and 12.99µg/ml of total phenols and flavonoids respectively (Figure 2). In addition, the methanolic extract of *Chlorella* sp. exhibited a very high total antioxidant capacity with value of 63.24 µg/ml followed by *Chlamydomonas* sp. having 32.11 µg/ml then *Chlorococcus* sp. having 26.88 µg/ml (Figure 2).

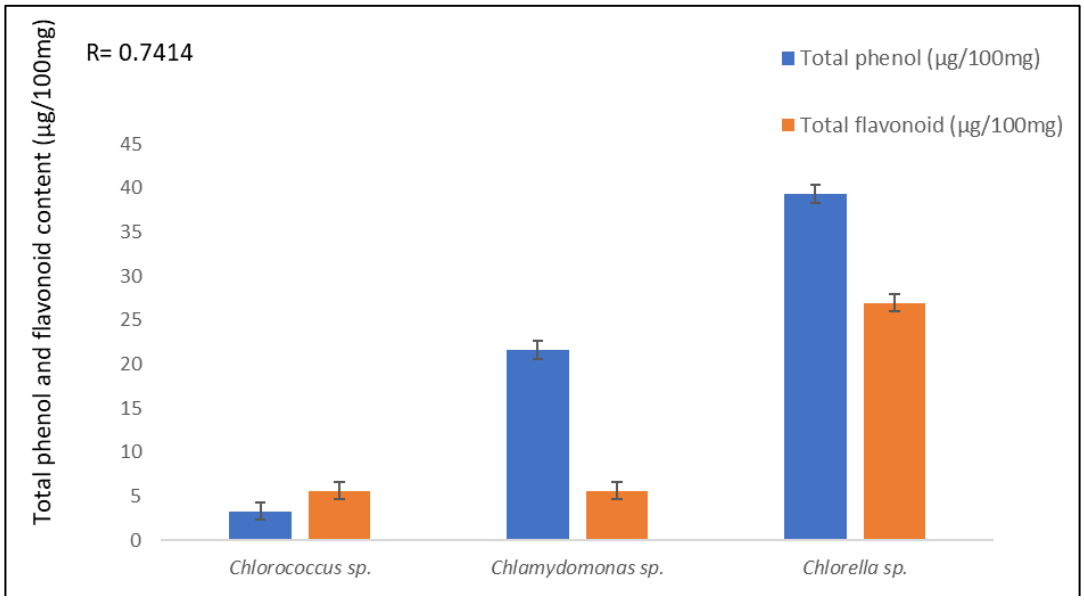

Fig 1: Total phenol and flavonoid content in aqueous extract of *Chlorococcus* sp., *Chlamydomonas* sp. and *Chlorella* sp.

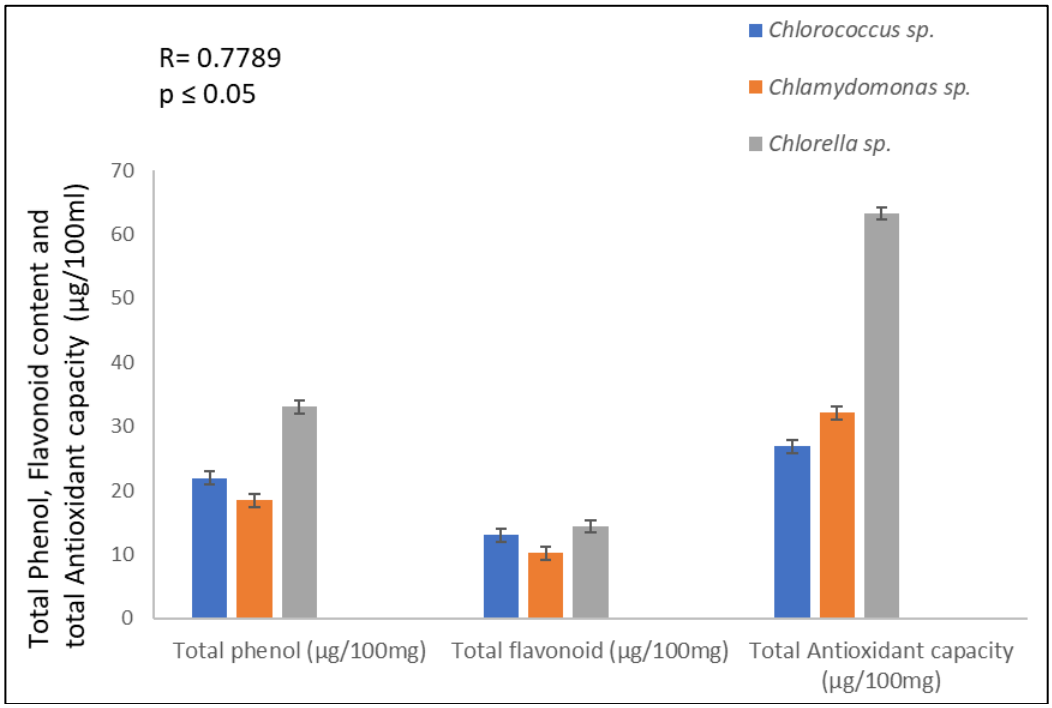

Fig 2: Total phenol and flavonoid content in methanolic extract of *Chlorococcus* sp., *Chlamydomonas* sp. and *Chlorella* sp.

DPPH radical scavenging activity

Figure 3 shows the 2,2-diphenyl-1 picrylhydrazyl (DPPH) radical scavenging activity of aqueous extract of *Chlorococcus* sp., *Chlamydomonas* and *Chlorella* sp. At different concentrations, (25µg/mg, 50µg/mg, 75µg/mg), the aqueous extract of *Chlorella* sp. showed the highest percentage of radical scavenging inhibition compared to *Chlorococcus* and *Chlamydomonas.* Although, lower than the inhibition by standard ascorbic acid-standard reference antioxidant, but the scavenging activity of *Chlamydomonas* sp. was highest (72.48%) at 100µm/mg (Figure 3). Furthermore, the methanolic extracts of *Chlorococcus* sp. elicited the highest radical scavenging activity in a concentration-dependent manner followed by *Chlamydomonas* and *Chlorella* spp respectively. There was no significant difference between inhibition by *Chlamydomonas* and *Chlorococcus* sp. at 100 µg/mg.

Using methanolic extract, *Chlorococcus* sp. indicated a high activity at 25µg/mg, 50µg/mg, 75µg/mg and 100µg/mg compared to *Chlamydomonas* and *Chlorella* sp. against the standard ascorbic acid (Figure 4). In addition, the methanolic extract of *Chlorella* sp. exhibited a very high total antioxidant capacity with value of 63.24µg/ml followed by *Chlamydomonas* having 32.11µg/mg then *Chlorococcus* sp. having 26.88µg/mg.

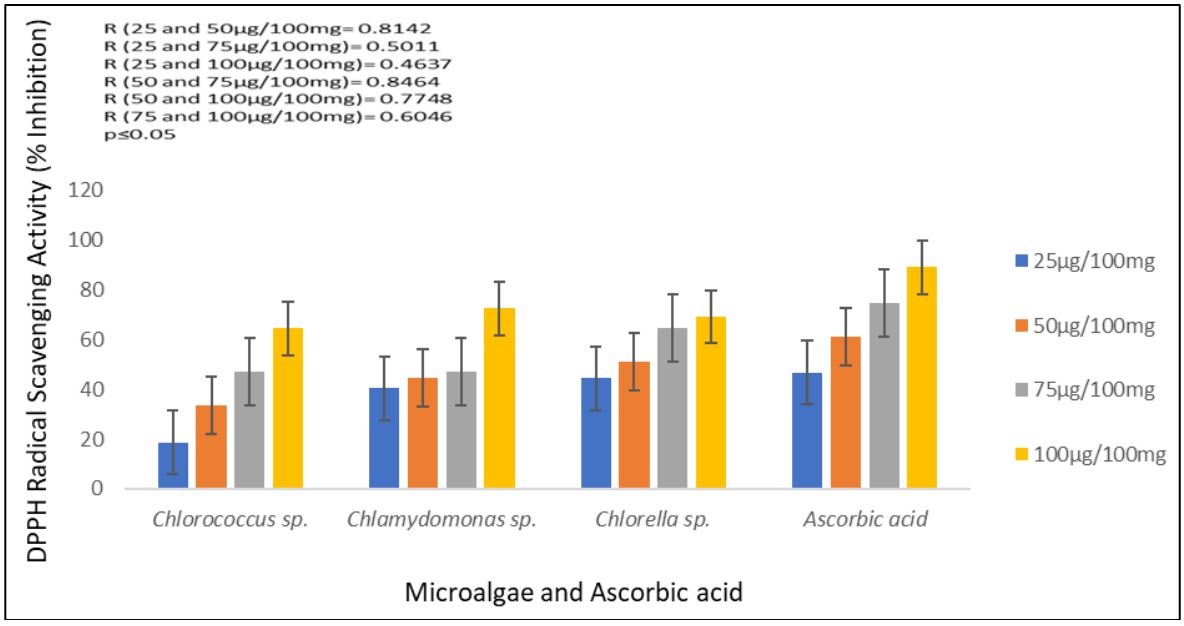

Fig 4: Scavengng activity of 2-2, diphenyl-1-picryhydrazyl (DPPH) in metganolic extract of *Chlorococcus* sp,. *Chlamydomonas* sp. and *Chlorella* sp. at different concentrations

Discussion

One of the major constituents of microalgal biomass is the fatty acid (Christi, 2007) and the quality of lipids is a function of its fatty acids (Weena *et al.*, 2013). Chloroform extract of *Chlorella* sp. recorded high percentage of saturated methyl esters. According to Lee *et al.* (2010) [15], palmitic and stearic

acid are known to be most common fatty acids contained in biodiesel and this, in line with the reports of Sara *et al*. (2010) [24] established the presence of saturated fatty acids with no unsaturated or polysaturated fatty acid in *Chlorella* sp. This is also in conformity with the result of this study in which *Chlorella* sp. (isolated from soil) had higher composition of saturated fatty acid.

Stearic acid is widely used in organic synthesis, pharmaceuticals and perfumes, medicine, lubrication and grease, dye, rubber and latex, plastics and flavorings (Ashwani *et al.*, 2018). while Caproic acid and Capric acid were the most abundant of the fatty acids identified in the fresh water algae species (*Chlamydomonas* and *Chlorococcus* sp). These acids have been reported to have anticancer potentials (Amoolya *et al*., 2015). Sang and Kyung (2017) in their study established that capric acid treatment alleviated oxidative stress induced by cyclophosphamide in small intestinal epithelial cells in pigs.

Lipid extraction using chloroform and GC-MS analysis of *Chlorella* sp., *Chlorococcus* and *Chlamydomonas* sp., *Chlorella* sp. yielded more lipids with higher percentage composition and better quality. This is probably due to stress conditions of the environment the *Chlorella* sp. has been adapted to; this is in conformity with Chisti, (2007) and Bona *et al*. (2014) who reported that lipid production from algae are largely determined by their high productivity and broad environmental sustainability. The report of Mohammed *et al*., (2013) also confirmed the high production of lipids under stress condition by *Chlorella* sp. Furthermore, comparing the hexane and chloroform extract of *Chlorococcus* sp. for lipids components, the hexane extract yielded higher percentage and quality than the chloroform extract. This probably shows that hexane solvent is best used for this strain of *Chlorococcus* sp. and in agreement with report of Adesalu *et al*. (2016) [1] who determined the bioactive compounds in a West African strain of the green alga, *Oedogonium.* Also, the extraction of oil using different solvents on *Ulva compressa* (Kaluzny, 1985), revealed that the highest oil extraction yield of 7.33% was achieved from the *Ulva compressa* biomass using the n-hexane solvent. Therefore, this shows hexane as the most widely used solvent for the extraction of oil from micro and macro algae (Miao and Wu, 2006) [10].

Total phenol and flavonoid content in the aqueous and methanolic extract of *Chlorella* sp. was higher compared with that of *Chlamydomonas* and *Chlorococcus* sp. but the latter presented a high DPPH activity in methanolic extract. Despite the fact that water was a more efficient solvent to extract greater amount of extractable polar substances, this study showed that methanol was more efficient to extract a selected group of compounds especially polyphenolics with a higher antioxidant activity. The concentration-dependent increase (100µg/ml), in percentage of antioxidant property of *Chlamydomonas* sp. is in conformity with Jayshree *et al*. (2016) [7], who reported that *Chlorella* sp. had efficiently high amount of total phenol, flavonoid content and radical scavenging activity in aqueous extract when compared with *Chlamydomonas reinhardtii* but methanolic extract of *Chlamydomonas* showed higher inhibition than *Chlorella* sp. This confirms that *Chlorella* sp. may contain high amount of flavonoids and other secondary metabolites but show less inhibition (DPPH activity) when extracted with methanol. Also, Koen *et al*. (2012) [27] stated that *Chlorella* sp. showed very high antioxidant capacity when compared with other species of algae due to its high phenolic compounds as depicted in this study. Moreso, Idaira (2017) observed that several cyanobacteria and microalgae were effective as scavengers of free radicals and this activity might be related to the phenolics compounds detected in some of the strains they worked with. Highest antioxidant activity exhibited by aqueous and methanolic extracts of *Chlorella* sp. in this study agrees with Idaira (2017) who stated that it could be probably due to the presence of the high contents of phenolics which is known to contribute significantly to the antioxidant capacity of microalgae (Mannan *et al*., 2010; Koen Goirisa *et al.*, 2012) [27].

Conclusion

In this study, *Chlorella* sp. contained the highest amount of flavonoid, phenol and TAC irrespective of whether isolated from fresh water or extracted with methanol from soil. The results showed that different solvent extracts contained different amount of phenolics and flavonoids. Methanol extracts of *Chlorococcus* sp. and *Chlamydomonas* sp. elicited the highest radical scavenging activity which is attributable to their high flavonoid, phenolics and total antioxidant capacity. This work showed that phenolic compounds also contribute significantly to the antioxidant capacity of microalgae. Furthermore, the extract of extraction could determine the amount, number and quality of lipids and fatty acids obtainable from an extraction process. Further study on other antioxidant compounds from more microalgal strains is proposed to confirm new possibilities.

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