In vitro evaluation of antioxidant properties of *Chlorella vulgaris* and its derivatives for use as antioxidant supplements in animal production

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Microalgae are biological resources with potential application in animal and human nutrition as supplements. *Chlorella vulgaris* is an example of such microalgae that is commercially available, but there is a need to quantify the extent of the antioxidants in the algae in order to fully explore its nutri-functional benefits for animal nutrition application, as these could eliminate high cost and improve efficiency of utilizing the algae (Kodner et al. 2009). The quantification can also enhance the use of the microalgae for its functional potential uses such as antioxidants, as anti-inflammatory, antiviral, antibacterial and general antimicrobial agents (Kluru et al. 2014).

Oxidative stress prevention and management in animals is important because it is a stress compromise which is responsible for reproductive failures and inefficiencies, generally limiting the productivity of animals. Specifically, oxidative stress is reported to be linked with reproductive failures such as abnormal reproductive cycles, embryonic or fetal loss, neonatal mortality, delayed attainment of puberty and poor sperm production, since they are all associated with metabolic imbalance which nutritional management could ameliorate through exogenous supply of antioxidant supplements (Miller and Brzezinska-Przybylska 1993). *Chlorella vulgaris* is a reported source of antioxidant including astaxanthin, lutein, fucoxanthin, and carotenoids which can serve as free radical scavengers for improving performance of breeding animals via oxidative stress attenuation. This position was agreed upon by authors including Fan Vonshak and Boussiba (1994), Harker Tsavalos and Young (1996), He Duncan and Barber (2007) and Desai (2016). Therefore, in this study, an *in vitro* evaluation of the microalgae and its derivatives was carried out to determine its potential for oxidative stress attenuation potentials.

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The microalgae biomass used in this study was a commercial microalgae product SeaLipro™ (Seagrass Tech Pvt. Ltd, India). Two approaches were used for the preparation of the microalgae derivatives, the first approach was preparation of the derivatives using whole raw unextracted biomass of the microalgae while the second approach was preparation of derivatives using hot water extracts of the *Chlorella vulgaris* biomass. This method of hot water extraction has been reported by Konishi et al. (1985), Hasegawa et al. (1990), Tatke and Jaiswal (2011), Flórez Conde and Domínguez (2015) and Sánchez Roque et al. (2018). The eight derivatives prepared were labelled T1–T8; the labelled derivatives T1–T4 were derivates prepared with raw unextracted biomass while T5–T8 were derivatives prepared with hot-water extracts of the *Chlorella vulgaris* biomass. These two forms of derivatives contained 20%, 40%, 60%, and 80% *Chlorella vulgaris* biomass and a corresponding balance of 80%, 60%, 40% and 20% whole wheat flour, respectively.

Each derivative was sampled in triplicates of 10 g each and dissolved in 100 ml methanol, then kept at room temperature for 24 h after which the mixtures were filtered through a Whatman filter paper while the filtrates were stored at 4°C for downstream analysis (Bhuvana et al. 2019). Total phenolic content of the samples was determined using the principles of Folin Cioalteau’s phenol reagent test while the Ferric Reducing Antioxidant Power (FRAP) of the samples were determined using FRAP chemical assay described by Vijayalakshmi and Ruckmamani (2016). The assay uses antioxidant as reductants in a redox-linked reaction where the reducing oxidant is iron (II) and the change in colour of ferric iron (III) from colourless to blue coloured iron (II) was used for the determination of antioxidant power of the samples through absorbance readings (Benzie and Strain 1996). The procedure described by Mohamed et al. (2014) was adopted for the determination of the hydrogen peroxide scavenging power of the microalgae and its derivatives using the absorbance of H₂O₂ at 230 nm. The total antioxidant composition of the samples was determined using the protocol with an assay...
principle based on reduction of iron (III) to iron (II) at low pH. The data analysis involved subjecting all data obtained to one-way analysis of variance, the significant differences in means were determined at p<0.05, while means separations was based on Duncan test and in addition, all the parameters evaluated were ranked with Kruskal-Wallis’s test for ranking them based on their antioxidant potentials using all the parameters.

The samples differed significantly in total antioxidant composition, ferric reducing antioxidant power, hydrogen peroxide scavenging capacity and total phenolic content (p<0.05). The mean total antioxidants concentration was 621.83±36.41 µmol/g ascorbic acid equivalent (p<0.001), while minimum and maximum total antioxidant concentrations were 280.00 and 962.18 µmol/g ascorbic acid equivalent, respectively. The average FRAP was 1.28±0.09, while the minimum and maximum antioxidant FRAP were 0.56 and 2.65, respectively (p<0.001).

The average Hydrogen peroxide scavenging potential capacity was 34.04±3.18%, while minimum and maximum hydrogen peroxide scavenging capacities were 4.66 and 59.91%, respectively (p<0.001). The average total phenolic content of the samples was 30.54 mg/g ellagic acid equivalent, while minimum and maximum total phenolic contents were 16.20 and 45.27 mg/g ellagic acid equivalent, respectively (p<0.001). These indicated that the samples differ in all the antioxidant indicators upon which they were assessed (Table 1). There was also a significant relational difference in the total antioxidant capacities, ferric reducing antioxidant power and total phenolic content (p<0.05) of the samples (Fig. 1).

The study showed that *Chlorella vulgaris* and its derivatives highlighted above are potential antioxidant supplements for use in animal feeds and nutrition taking advantage bioactive antioxidant compounds they contained. The evaluations carried out in this study showed that the microalgae derivatives prepared with untreated algae biomass are better compared with the derivatives prepared with the hot-water extracts of the biomass. This observation specifically indicated that proportional addition of the microalgae in a non-antioxidant material such as wheat flour could serve as antioxidant supplements that are suitable as additives for improving animal productivity. This is in agreement with reports of Yan Lim and Kim (2012), which suggested that the microalgae *Chlorella vulgaris* is a suitable source of antioxidant additives for animal feeds production.

In agreement with the antioxidant capacities recorded in this study, *Chlorella vulgaris* was described as a feed resource that could be used for enhancement of physiological activities, growth promotion, antioxidant protection, immune modulation and protection of animals against pathogenic microorganisms in separate submissions by Guzmán et al. (2003), Lee et al. (2010), Kang et al. (2013) and Wan et al. (2019). Further to these assertions, the outcome of this present study also indicated that supplementing the microalgae in the forms evaluated

### Table 1. *In vitro* total antioxidant capacity, ferric reducing antioxidant power, hydrogen peroxide scavenging power and total phenolic composition of the microalgae *Chlorella vulgaris* biomass and its derivatives

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>SEM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total antioxidant capacity (µmol/g ascorbic acid)</td>
<td>663.03±36.41</td>
<td>601.21±13.48</td>
<td>670.20±25.67</td>
<td>760.00±29.81</td>
<td>298.18±20.41</td>
<td>412.12±13.48</td>
<td>545.82±25.67</td>
<td>707.39±29.81</td>
<td>36.41</td>
<td>0.001</td>
</tr>
<tr>
<td>Ferric reducing antioxidant power (%)</td>
<td>1.10±0.09</td>
<td>1.29±0.09</td>
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<td>1.29±0.09</td>
<td>0.09</td>
<td>0.001</td>
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<tr>
<td>Hydrogen peroxide scavenging power (%)</td>
<td>41.15±4.69</td>
<td>41.15±4.69</td>
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<td>41.15±4.69</td>
<td>4.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Total phenolic composition (mg/g ellagic acid)</td>
<td>43.53±0.09</td>
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<td>43.53±0.09</td>
<td>43.53±0.09</td>
<td>43.53±0.09</td>
<td>4.66</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Means with different superscripts along the same row are significantly different from each other (p<0.05). T1: Derivative prepared with 20% untreated *Chlorella vulgaris* biomass extract; T2: Derivative prepared with 20% hot water *Chlorella vulgaris* biomass extract; T3: Derivative prepared with 40% untreated *Chlorella vulgaris* biomass extract; T4: Derivative prepared with 40% hot water *Chlorella vulgaris* biomass extract; T5: Derivative prepared with 60% untreated *Chlorella vulgaris* biomass extract; T6: Derivative prepared with 60% hot water *Chlorella vulgaris* biomass extract; T7: Derivative prepared with 80% untreated *Chlorella vulgaris* biomass extract; T8: Derivative prepared with 80% hot water *Chlorella vulgaris* biomass extract.

*IN VITRO ASSESSMENT OF ANTIOXIDANT CAPACITY OF C. VULGARIS BIOMASS* January 2024
could attenuate oxidative stress in in vivo trials in pregnant rabbits (Sikiru et al. 2021). Therefore, the topmost ranked samples and whole unextracted Chlorella vulgaris biomass are recommended for in vivo exploration as antioxidant supplement in animals at different physiological states.

The above recommendations were made because the in vitro evaluation of the prepared derivatives and the microalgae biomass suggested that the samples investigated have higher antioxidant capabilities which could serve as a source of free radical scavengers when consumed by animals. These cannot be unconnected with other promising areas using these derivatives such as their uses for the improvement of animal physiological performances and products qualities. This is because both the physiological performances and products qualities improvements are closely linked to oxidative stress and usually associated with an imbalance between free radicals and antioxidants.

**SUMMARY**

Bioactive compounds with varied functional properties found in microalgae such as Chlorella vulgaris can serve as antioxidants supplement against free radicals causing oxidative stress in food animals, but there is a need to quantify these antioxidants in the algae for effective applications. Therefore, this study quantified antioxidants in Chlorella vulgaris and derivatives prepared with the microalgae biomass for enhancing its application in animal nutrition. Eight derivatives prepared using the Chlorella vulgaris biomass were subjected to in vitro antioxidant supplements in animals for attenuation of oxidative stress to improve performances and productivity because of their antioxidant potentials. Antioxidant assessments including ferric antioxidant power, hydrogen peroxide scavenging potential, total antioxidant capacity, and total phenolic content. The results showed that the mean total capacities of the preparations were 621.83±36.41 µmol/g ascorbic acid equivalent, ferric reducing antioxidant power (FRAP) was 1.28±0.09, while the hydrogen peroxide scavenging (HPS) potential was 34.04±3.18%, and the total phenolic content was 30.54 mg/g ellagic acid equivalent. It was concluded from these observations that the microalgae Chlorella vulgaris and its derivatives as prepared in this study could be used as antioxidant supplements in animals for attenuation of oxidative stress to improve performances and productivity because of their antioxidant potentials.

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