**Research Paper**

**In vitro** determination of the inhibition of free radical activity and carbohydrate-hydrolysing enzymes by extracts and phytochemical fractions of *Cymbopogon citratus*

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**ABSTRACT**

Oxidative stress plays a major role in the pathogenesis and chronic complications of diabetes mellitus. The study evaluated the in vitro antidiabetic and antioxidant effects of extracts and isolated phytochemicals of *Cymbopogon citratus* (lemongrass) leaves. Free radical scavenging activities of the extracts (aqueous, and ethanol) and fractions (saponins, flavonoids, and tannins) of *C. citratus* were tested using metal chelating, 1,1-diphenyl-2-picryl hydrazyl (DPPH), nitric oxide, 2,2-azinobis (3-ethylbenzothiazoline-6) sulphonic acid (ABTS), hydroxyl radical, and reducing power assays; while the antidiabetic activities were assessed using the inhibition of carbohydrate-hydrolysing enzymes α-amylase and α-glucosidase. The aqueous extract showed the best DPPH scavenging activity (0.556 ± 0.02 mg/mL), tannins showed the best nitric oxide and hydroxyl radical scavenging activities while, saponins showed the best ABTS radical scavenging activities (0.925 ± 0.04 mg/mL). The metal chelating ability (0.584 ± 0.04 mg/mL), and reducing power of the ethanol extract was better than the ascorbic acid standard. Similarly, the aqueous extracts and saponins fractions produced better α-amylase and α-glucosidase inhibitory potentials respectively compared to acarbose. Thus, *C. citratus* is a viable candidate for further exploration of its antidiabetic and antioxidant potentials.

**Key words:** Cymbopogon citratus, antidiabetic, antioxidant, phytochemicals, α-amylase, α-glucosidase.

**INTRODUCTION**

Diabetes is characteristically known as a disease that occurs either when no insulin is produced by the pancreas or when the body cannot effectively use the insulin it produces (WHO, 2014). It has been described as one of the major contributors to ill health and premature death across the globe (WHO, 2013). In the year 1985, about 30 million people were reported to be diabetic worldwide compared to around 171 million cases in 2010 and almost 377 million estimated in 2030 (Wild et al., 2004). More than two types of diabetes mellitus are universally known. Diabetes mellitus type 1 (DMT 1) (Rother, 2007), diabetes mellitus type 2 (DT2) (Lee et al., 2010), and gestational diabetes mellitus (GDM) (Willi et al., 2007).

Oxidative stress arises from imbalances between the production of reactive oxygen species (ROS) or free radicals and the biological system's ability to readily detoxify the ROS produced (Valko et al., 2007). Chronic hyperglycaemia results in increases in the production of ROS in patients with type 2 diabetes (Patel et al., 2013). These excess ROS/free radicals attack most cellular macromolecules like proteins (enzymes), lipids, and DNA leading to tissue damaging oxidative stress (Valko et al., 2007).

Reports have shown that about 400 plant species as well as 700 recipes and compounds have been scientifically evaluated for treatment of diabetes (Singh et al., 2011). *C. citratus*, (lemongrass) which is a tropical plant from Southeast Asia, has been reported to have antidiabetic properties (Rauber et al., 2005). *C. citratus* contains z-citrall, borneol, estragole, methylenegol, and geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate); it exhibits binding activities towards proteins and other substances with appreciable antioxidant activities (Okuda et al., 2011).
Research has shown that *C. citratus* is rich in phytochemicals like saponins, flavonoids, and tannins, which has been reported to act as immunostimulants, and to have hypocholesterolaemic and anticarcinogenic properties (Rao, 2003). Additionally, they act as antioxidants, aid protein digestion, uptake of vitamins and minerals in the gut, induction of hypoglycaemia, and finally, as antifungal and antiviral agents (Balsundram et al., 2006).

Studies have shown that increase in reactive oxygen and nitrogen species (ROS/RNS) levels has been linked with non-enzymatic glycation of proteins and oxidation of glucose which exacerbates oxidative stress and contributes toward long-term diabetes mellitus complications. The antioxidant properties of medicinal plants may ameliorate the effect of oxidative stress on diabetes (Pham-Huy et al., 2008; Moussa, 2008).

This study therefore investigated the *in vitro* antioxidant and antidiabetic properties of *C. citratus* in order to assess its potential in combating diabetic complications.

**MATERIALS AND METHODS**

**Plant Collection and preparation**

*Cymbopogon citratus* was harvested from a farm in Akure and authenticated in the Department of Plant Science and Biotechnology, University of Benin. Herbarium specimen with voucher number UBH-C451, was deposited in the University of Benin herbarium.

**Extraction of Cymbopogon citratus**

A slight modification on the method of Onoagbe and Esekheigbe (1999) was used to prepare the extract. The powdered leaves of the plant were used. One kilogram each of the powdered leaves was soaked in distilled water (aqueous extract), and absolute (ethanol extract), with continuous stirring for 72 hours in a glass container and covered with cheesecloth. At the end of the third day, it was filtered through two layers of cheesecloth and later with a filter paper to completely remove residues. The filtrate was concentrated by a rotary evaporator, and then evaporated to dryness by a freeze dryer. The dried extract was weighed and stored in an air-tight container and kept in the freezer until use.

**Isolation of phytochemicals**

Solvent extraction of dried powder (500g) of *C. citratus* was done for flavonoids by the method described by Subramanian and Nagarjan (1969); saponins by Woo et al. (1980); and tannins by Wall et al. (1996).

**In vitro antioxidant assays**

Antioxidant activity of the extracts of *C. citratus* was determined by measuring their ability to decolorize the purple-coloured methanol solution of DPPH, according to the method described by Turkoglu et al. (2007); nitric oxide radical by Garrat (1964); metal chelation ability following the procedure of Dinis et al. (1994); reducing property according to the method of Oyaizu (1986); 2, 2-azinobis (3-ethylbenzothiazoline-6-) sulfonic acid (ABTS) radical scavenging ability according to the procedure of Re et al. (1999); and hydroxyl radical scavenging potential using the modified method of Oboh et al. (2007).

**In vitro antidiabetic assays**

**α-Amylase and α-glucosidase inhibitory assays:** The α-amylase and α-glucosidase inhibitory activities were determined separately according to the method of Elsnoussi et al. (2012).

**Calculation of half-maximal (50%) Inhibitory Concentration (IC₅₀):** The concentration of the plant extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by the expression: I % = (Aₓ - Aₖ)/Aₓ X 100 (Shai et al., 2010).

Where “Aₓ” is the absorbance of the control and “Aₖ” is the absorbance of the sample. The concentration of *C. citratus* extracts causing 50% inhibition (IC₅₀) was calculated from the standard calibration curve (Balogun and Ashafa, 2016b).

**Statistical analysis**

Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Bonferroni test. Results were expressed as mean ± standard error of mean (SEM) of triplicate determinations. Statistical significance of the mean values was considered at *p* < 0.05. The data was analyzed statistically using Tukey-Kramer post Hoc test. https://astatsa.com/OneWay_Anova_with_TukeyHSD

**RESULTS**

The results obtained in this study are presented below using Tables.

**DISCUSSION**

Plants are excellent sources of various therapeutic agents with little or no deleterious side effects commonly
associated with other allopathic drugs and they are readily available and relatively affordable. This study investigated the ability of *C. citratus* to serve as effective antioxidant and antidiabetic agents. Several methods have been engaged in the extraction and isolation of the various bioactive compounds of medicinal plants using different solvents. The relative high yield of aqueous and ethanol extracts could reflect the high content of bioactive chemicals in *C. citratus* (Table 4). In terms of isolated phytochemical content from 500 g of *C. citratus*, flavonoid had the highest yield (0.099 %) followed by saponins (0.039 %) with tannins (0.037%) having the least yield (Table 4).

It is widely known that oxygen is the major component of aerobic life. However, in oxidative stress, oxygen may be a killer of cells when it generates reactive species that cause cell damage and ultimately cell death (Weseler and Bast, 2010). Oxygen is a highly reactive species that has the ability to become part of potentially harmful and damaging molecules (free radicals) (Johansen et al., 2005). Oxidative stress causes healthy cells and molecules of the body to lose their structure and function. It is when the antioxidant capacity of the body is limited that this damage can become debilitating and cumulative (Ozougwu et al., 2013).

It is believed that oxidative stress plays an important role in the development of vascular complications in diabetes especially type 2 diabetes (Pham-Huy, 2008). Elevation in the level of reactive oxygen species in diabetes may be due to decrease in the production of enzymatic and non-enzymatic antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications (Lipinski, 2001). According to epidemiological studies, diabetic mortalities can be explained notably by an increase in vascular diseases other than hyperglycemia (Pham-Huy, 2008). The extracts and phytochemicals of *C. citratus* showed significant antioxidant activities. In DPPH antioxidant assays, the aqueous extract, and saponins fraction of *C. citratus* showed the lowest IC$_{50}$ values (0.556 ± 0.02 and 0.917 ± 0.02 mg/mL respectively) in comparison with other test samples, which implies they have the strongest radical scavenging activity (Table 1). The aqueous extract displayed the strongest DPPH scavenging ability compared with other extracts and isolated phytochemicals and the positive control, ascorbic acid; even though the differences are not statistically significant. On the other hand, the IC$_{50}$ for flavonoids and tannins DPPH scavenging activities were significantly higher than ascorbic acid and other extracts and fractions; implying lesser ability of these fractions to scavenge DPPH. Moyo et al. (2010) had reported stronger DPPH radical scavenging activity (IC$_{50}$ = 5.02 µg/mL) for methanol extracts of *Sclerocarya birrea*, than the positive control, ascorbic acid. Meanwhile Alimi and Ashafa (2017) and Adewusi and Steennkamp (2011) stated that the ethanol extract of *Sutherlandia montana* elicited the most potent antioxidant capacity in scavenging DPPH, NO, ABTS and OH radicals. As the extracts compared favorably well with ascorbic acid in annihilating ABTS and OH radicals, it is noteworthy that it had significantly ($p < 0.05$) better effect than ascorbic acid against DPPH, NO and OH radicals. Muleya et al. (2015) reported high activity of *Pentanisia prunelloides* acetone root extract in DPPH radical scavenging assay. Results obtained from the current study revealed that the water extract had the highest activity in scavenging DPPH. This is similar with the report of Auwal and Islam (2014), which states that the active components in the water extract are capable of donating hydrogen to a free radical to remove electron which is responsible for the radical’s reactivity.

In biological tissues, nitric oxide synthase metabolizes arginine to citrulline with the generation of nitric oxide radical (NO), via a five electron oxidative reaction (Boora et al., 2014). During the *in vitro* studies, the compound sodium nitroprusside decomposes in aqueous solution at physiological pH (7.2) producing NO, making it an ideal assay to mimic the body’s system in scavenging the free radical (Pacher et al., 2007). This study also measured the capacity of the extracts and phytochemicals of *C. citratus* to scavenge ABTS$^+$ radical cation. ABTS scavenging activity measures the reduction of the blue–green chromophore ABTS$^+$ (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to colowexssdfaaAAAAAAurless ABTS by an antioxidant. *C. citratus* aqueous, ethanol and tannin fractions showed no significance difference in ABTS scavenging ability compared to ascorbic acid. The IC$_{50}$ values for saponin and flavonoid fractions were however lower than the standard (Table 1). This implies that the saponin and flavonoid fractions of *C. citratus* exhibits better ABTS scavenging ability than ascorbic acid. Adewusi and Steennkamp (2011) stated that the ethanol extract of *Sutherlandia montana* elicited the most potent antioxidant capacity in scavenging DPPH, NO, ABTS, and OH radicals. As the extracts compared favourably well with ascorbic acid in inhibiting ABTS and OH radicals, it is noteworthy that it had better effect than ascorbic acid against DPPH, NO and OH radicals. In this study, the results show that the aqueous extract, flavonoids and tannins fractions of *C. citratus* have the capability of mopping up this radical (Table 1).

As noted by Kazeem and Ashafa (2015), hydroxyl radicals are highly reactive in causing enormous biological damage to living cells, but this untoward effect may be mitigated by the ethanol extract of *Pentanisia prunelloides*, which is comparable to the results of this study, as it is seen that the aqueous and ethanol extracts, saponins and tannins possessed the lower IC$_{50}$ values for hydroxyl radicals compared to ascorbic acid (Table 1). Similarly, the IC$_{50}$ values obtained for the metal chelating effect of the extracts and fractions of *C. citratus* were lower when compared with the standard ascorbic acid (Table 1). The strong metal chelating effect displayed by all the extracts and isolated phytochemicals in this study may be linked with the active constituents present in the extracts. Alimi and Ashafa (2017) had earlier reported that the strongest metal...
Table 1: IC₅₀ (mg/mL) values for the antioxidant properties of Cymbopogon citratus leaves.

<table>
<thead>
<tr>
<th>Assay</th>
<th>DPPH</th>
<th>Nitric oxide</th>
<th>ABTS</th>
<th>Hydroxyl Radical</th>
<th>Metal chelating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic</td>
<td>0.562 ± 0.05</td>
<td>4.501 ± 0.67</td>
<td>4.150 ± 0.49</td>
<td>1.259 ± 0.18</td>
<td>4.758 ± 0.64</td>
</tr>
<tr>
<td>Aqueous</td>
<td>0.556 ± 0.02</td>
<td>4.238 ± 0.13</td>
<td>4.010 ± 0.82</td>
<td>0.717 ± 0.18</td>
<td>0.675 ± 0.02</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.738 ± 0.07</td>
<td>7.390 ± 0.31</td>
<td>4.477 ± 0.45</td>
<td>0.653 ± 0.04</td>
<td>0.584 ± 0.01</td>
</tr>
<tr>
<td>Saponins</td>
<td>0.917 ± 0.02</td>
<td>7.214 ± 0.04</td>
<td>0.925 ± 0.02</td>
<td>1.018 ± 0.16</td>
<td>1.185 ± 0.08</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>4.106 ± 0.42</td>
<td>4.000 ± 0.11</td>
<td>1.256 ± 0.56</td>
<td>1.416 ± 0.02</td>
<td>1.182 ± 0.06</td>
</tr>
<tr>
<td>Tannins</td>
<td>2.307 ± 0.17</td>
<td>0.605 ± 0.04</td>
<td>2.827 ± 0.60</td>
<td>0.259 ± 0.02</td>
<td>0.778 ± 0.08</td>
</tr>
</tbody>
</table>

The values are presented as means ± standard error of mean (SEM) of triplicate determinations. Means along the same column not sharing a common superscript for each parameter are significantly different (p < 0.05). DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid.

Table 2: Reducing power potential of Cymbopogon citratus extracts and phytochemicals.

<table>
<thead>
<tr>
<th>Absorbance (nm)</th>
<th>Conc. (mg/ml)</th>
<th>Ascorbic acid</th>
<th>Aqueous</th>
<th>Ethanol</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.065</td>
<td>0.46 ± 0.01</td>
<td>0.37 ± 0.03</td>
<td>0.54 ± 0.02</td>
<td>1.30 ± 0.02</td>
<td>0.58 ± 0.01</td>
<td>1.13 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>0.125</td>
<td>0.52 ± 0.02</td>
<td>0.49 ± 0.01</td>
<td>0.59 ± 0.01</td>
<td>1.39 ± 0.01</td>
<td>0.67 ± 0.01</td>
<td>1.24 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.59 ± 0.01</td>
<td>0.64 ± 0.02</td>
<td>0.65 ± 0.03</td>
<td>1.46 ± 0.01</td>
<td>0.73 ± 0.02</td>
<td>1.26 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.74 ± 0.02</td>
<td>0.81 ± 0.02</td>
<td>0.77 ± 0.02</td>
<td>1.59 ± 0.03</td>
<td>1.01 ± 0.05</td>
<td>1.67 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1.15 ± 0.01</td>
<td>0.99 ± 0.01</td>
<td>0.83 ± 0.02</td>
<td>1.89 ± 0.01</td>
<td>1.37 ± 0.01</td>
<td>1.93 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

The values are presented as means ± standard error of mean (SEM) of triplicate determinations. Means along the same column not sharing a common superscript for each parameter are significantly different (p < 0.05).

Table 3: IC₅₀ (mg/mL) values for the inhibitory activities of Cymbopogon citratus extracts and phytochemicals on the activities of α-amylase and α-glucosidase.

<table>
<thead>
<tr>
<th>Samples</th>
<th>α- amylase</th>
<th>α- glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarbose</td>
<td>1.855 ± 0.45</td>
<td>2.507 ± 0.91</td>
</tr>
<tr>
<td>Aqueous</td>
<td>0.555 ± 0.01</td>
<td>2.326 ± 0.04</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.179 ± 0.80</td>
<td>1.352 ± 0.12</td>
</tr>
<tr>
<td>Saponins</td>
<td>2.058 ± 0.25</td>
<td>0.312 ± 0.01</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>3.558 ± 0.39</td>
<td>0.595 ± 0.09</td>
</tr>
<tr>
<td>Tannins</td>
<td>1.818 ± 0.35</td>
<td>1.684 ± 0.94</td>
</tr>
</tbody>
</table>

The values are expressed as means ± standard error of mean (SEM) of triplicate determinations. Means along vertical columns not sharing a common superscript are significantly different (p < 0.05) from each other. Acarbose is the standard α-amylase and α-glucosidase inhibitor.

Table 4: Showing the yield of the extracts and isolated phytochemicals from Cymbopogon citratus.

<table>
<thead>
<tr>
<th>Extracts &amp; Phytochemicals</th>
<th>Total yield (g)</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>58.45</td>
<td>5.85</td>
</tr>
<tr>
<td>Ethanol</td>
<td>51.90</td>
<td>5.19</td>
</tr>
<tr>
<td>Saponins</td>
<td>0.195</td>
<td>0.039</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.247</td>
<td>0.099</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.184</td>
<td>0.037</td>
</tr>
</tbody>
</table>

The chelating effect of Sutherlandia montana was displayed by ethanol and decoction extracts of the plant. Reducing power is a quantitative assay which measures the ability of medicinal plants/natural products to reduce
ferric iron (Fe$^{3+}$) to its ferrous form (Fe$^{2+}$) by electron-donating antioxidants which are present in extracts and phytochemicals. According to Khled-Khodja et al. (2014), when this reduction occurs, the iron complex turns into a dark blue product, thus enhancing the evaluation of its capacity to donate an electron or hydrogen. From the present findings in Table 2, the reducing power of C. citratus was dose-dependent, with the aqueous and ethanol extracts, as well as the flavonoid fractions, comparing favourably with ascorbic acid in its reducing power ability.

In a bid to study the antidiabetic effects of C. citratus, α-amylase and α-glucosidase inhibitory parameters were determined. Alpha-amylase is an enzyme that hydrolyses alpha-bonds of alpha-linked polysaccharide such as starch to yield high levels of glucose and maltose. α-Amylase inhibitors bind to α-bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharides (Nair et al., 2013). The aqueous extract had the lowest IC$_{50}$ value for α-amylase compared to the reference drug, acarbose, and other extract and phytochemicals. The aqueous extract thus demonstrated the strongest inhibition on α-amylase, making it more active than the other extracts, fractions, and the standard drug. The strong inhibition shown by the aqueous extract may suggest that the active α-amylase inhibitory components are largely polar in nature (Table 3).

Saponins showed the strongest inhibition of α-glucosidase, thus making it the most active in comparison with other tested extracts, fractions and the standard. All extracts and isolated phytochemicals showed lower IC$_{50}$ values, with the values for saponins and flavonoids being significantly ($p < 0.05$) lower when compared with the standard, thus both exhibited better antidiabetic activities than acarbose. This shows that C. citratus contains bioactive compound(s) with α-glucosidase inhibitory activities (Table 3). The strong inhibition of α-glucosidase and α-amylase by these extracts (aqueous, and ethanol) and isolated phytochemicals agrees with the reports of Kwon et al. (2007) that natural α-glucosidase inhibitors from plants have shown strong inhibitory activity against α-glucosidase and therefore can be potentially used as an effective therapy for the management of postprandial hyperglycemia with minimal side effects. This result compliments the reports of Pallavi et al. (2015), which states that the inhibition of α-glucosidase and α-amylase enzymes by plant extracts provides a strong biochemical basis for the management of diabetes via the control of glucose absorption. These findings corroborate previous studies that reported extracts with strong inhibitory potential against α-glucosidase as an ideal antidiabetic agent both in vitro and in vivo (Ojewole, 2003; Aslan et al., 2010). It might be inferred that the synergistic relationship among most of the phytochemicals might be responsible for the overall medicinal effects of C. citratus, since the antioxidant and antidiabetic efficacies of medicinal plants has been linked to their high phenolic contents (Huang et al., 2010; Maisuthisakul, 2012). These activities may work together to protect diabetics from hyperglycaemia and other diabetic complications.

CONCLUSION

The overall results obtained from this study indicate that the aqueous and ethanol extracts as well as flavonoids, saponins, and tannins isolated from C. citratus possessed significant antioxidant and antidiabetic properties. Indeed, with respect to hydroxyl radical and metal chelating ability as well as α-amylase and α-glucosidase inhibitory activities; most of the extracts and fractions of C. citratus showed better activities than standards. C. citratus extracts and fractions thus have significant potential to be used as antioxidant and anti-diabetic agents.

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