Excessive production of reactive oxygen species, a condition known as oxidative stress, has been linked with pathogenesis of chronic and degenerative diseases, such as; atherosclerosis, ageing, diabetes, hypertension, malignant tumours, inflammation, neurodegenerative and immune disorders (Halliwell and Gutteridge, 1984; Harman, 1998).

Antioxidants are substances that delay or prevent the formation of reactive oxygen species and are thus important in the prevention and treatment of oxidative stress-related diseases. Green vegetables are a rich source of antioxidants, and their consumption is recommended as part of a healthy diet. The phenolic and antioxidant profiles of five green vegetables (African spinach, bitter leaf, jute leaf, scent leaf, and water leaf) grown in southern Nigeria were investigated in this study.

**Methods.** The total phenolic content (GAE mg/100 g) of the fresh vegetables ranges from 738.70 ± 7.90 in African spinach to 1464.63 ± 7.60 in scent leaf. A higher flavonoid content (QE mg/100 g) was found in water leaf (512.07 ± 1.47) and jute leaf (510.13 ± 2.22) compared to the other vegetables examined; scent leaf (496.51 ± 1.31), African spinach (457.40 ± 1.50), bitter leaf (371.89 ± 0.44). Scent leaf possesses the highest DPPH radical scavenging activity with an EC50 [μg/mL] of 74.64 ± 7.73. The other vegetables show a lower EC50 [μg/mL]; jute leaf (125.86 ± 11.34) > water leaf (132.43 ± 9.43) > bitter leaf (156.02 ± 5.43) > African spinach (213.44 ± 9.29). The hydroxyl radical scavenging activity (EC50, μg/mL) of the vegetables in order of decreasing activity, was bitter leaf (50.38 ± 7.17) > water leaf (103.41 ± 6.27) > African spinach (110.54 ± 8.15) > jute leaf (144.39 ± 9.37) > scent leaf (217.51 ± 6.90). The total antioxidant capacity (EC50, μg/mL) of the vegetables increases in the following order; African spinach (227.39 ± 7.25) < bitter leaf (169.96 ± 6.86) < water leaf (106.31 ± 7.66) < scent leaf (65.47 ± 14.43) < jute leaf (27.52 ± 4.14).

**Conclusions.** The green vegetables possess appreciable phenolic and antioxidant potentials, which underscore their regular consumption as part of healthy Nigerian diet. Principal component analysis (PCA) buttresses the correlations and variations of the aforementioned potential among vegetable species.

**Key words:** flavonoid, Nigeria, phenolic, vegetable, oxidative stress, south
oxidation from radicals, even at low concentration, compared to that of oxidized substrates (Yan and Liu, 2006). Because of the multifaceted mechanisms of combating oxidative species (Yan and Liu, 2006), regular intake of dietary antioxidants is recommended to synergize the action of endogenous antioxidants.

A diet rich in green vegetables has been linked to reduced incidence of chronic diseases due to their high content of antioxidant compounds (Lu and Foo, 2000). Green leafy vegetables are relatively affordable, are present in traditional Nigerian cooking and are readily available, as they grow as weeds around households, gardens and farms in Nigeria. Green leafy vegetables indigenous to Africa have been reported to have health-promoting properties (Opabode and Adebooye, 2005). They are sources of dietary fibre, proteins, minerals, vitamins and essential amino acids, and are low in calories (Muhammad and Shinkafi, 2014). They have been documented to provide 80% of vitamin A in diets (Kwenin et al., 2011). An increased intake of vegetables has been reported to be an effective strategy to reduce calorie intake and improve health-related quality of life (Blatt et al., 2011).

**Material and Methods**

**Chemicals**

Gallic acid, Quercertin, Ammonium phosphomolybdate, Folin reagent, and DPPH were obtained from Sigma Aldrich, USA. Ascorbic acid, Sodium carbonate, and Aluminium Chloride were from BDH, India. Other chemicals and reagents were of analytical grade.

**Plant collection**

Fresh vegetables; African spinach (*Amaranthus hybridus*), jute leaves (*Corchorus olitorius*), scent leaves (*Ocimum gratissimum*), water leaves (*Talinum triangulare*), and bitter leaves (*Vernonia amygdalina*) were harvested in the month of August from a garden in Ibadan, Oyo State, Nigeria. The leaves were botanically authenticated in Federal College of Forestry, Ibadan, Oyo State, Nigeria by Mr. Ogele E. O.

**Sample extract preparation**

The leaves were washed, air dried within three hours, and blended freshly with Nakkai MX320. The blended fresh leaves (0.5 grams) were extracted by soaking in 100 mL of 80% Methanol for 48 hours. The solutions were centrifuged at 5000 rpm for 15 minutes and the supernatants were stored at 4°C prior to *in vitro* antioxidant assays.

**Determination of total phenolic content**

The total phenolic content (TPC) was determined using the spectrophotometric method (Singleton et al., 1999) with the use of Folin-Ciocalteu’s phenol reagent at 750 nm. Total phenolic content was determined using gallic acid as standard. The TPC was expressed as gallic acid equivalents (GAE) [mg/100 g of fresh sample].

**Estimation of total flavonoid content**

The amount of total flavonoid content (TFC) was determined using the aluminium chloride spectropho-
metric method at 415 nm (Chang et al., 2002). The flavonoid content was expressed in terms of quercetin equivalent [mg/100 g of fresh sample].

### Anthocyanin contents

The anthocyanin content (ACC) was evaluated using the pH differential method (Lim et al., 2012). The results were calculated as cyanidin-3-glucoside equivalents [CGE mg/100 g] of sample fresh weight (FW).

### Phosphomolybdate assay (total antioxidant capacity)

The total antioxidant capacity was determined using the phosphomolybdate method, using ascorbic acid as a standard at 765 nm. (Umamaheswari and Chatterjee, 2008). Then, the EC\textsubscript{50} of antioxidant capacity was extrapolated from an activity [%] vs. concentration graph.

### DPPH (2,2’-diphenyl-1-picrylhydrazyl) radical scavenging profile

DPPH radical scavenging assay was performed using the spectrophotometric method at 517 nm as described by Brand-Williams et al. (1995). The positive control used was ascorbic acid at 50–500 μg/mL. The EC\textsubscript{50} of DPPH scavenging activity was extrapolated from an activity [%] vs. concentration graph.

### Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activities of the samples were determined using the spectrophotometric method at 510 nm (Smirnoff and Cumbes, 1989). Ascorbic acid was used as a positive control. The EC\textsubscript{50} of hydroxyl radical scavenging activity was extrapolated from an activity [%] vs. concentration graph.

### Statistical analysis

All experiments were performed in triplicate. Statistical analyses and graphs were plotted using Graphpad prism 6.04 (GraphPad Software Inc) and SPSS 22.0 (IBM corporation). Significant differences between samples were analysed using One-way ANOVA at \( p < 0.05 \). Phytochemical and antioxidant parameters obtained were subjected to principal component analysis (PCA) to explore the relationship between different variables and samples. Eigen value (Loadings) > |0.6| indicates a significant correlation between the original variables and extracted components. One-way ANOVA and PCA were carried out using SPSS 22.0.

### RESULTS AND DISCUSSION

Table 1 presents the total phenolic (TPC), total flavonoid (TFC) and total anthocyanin (ACC) content of the vegetables. Scent leaf had the highest TPC (1464.63 ±7.90 GAE mg/100 g), exceeding the values in water leaf, jute leaf, bitter leaf, and African spinach by 5.38%, 24.37%, 41.60% and 49.56% respectively. Phenolic compounds are considered secondary metabolites and these phytochemical compounds, derived from phenylalanine and tyrosine, occur ubiquitously in plants. The phenolic content of jute leaf (\textit{C. olitorius}) observed in this study corroborates with those

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC, GAE mg/100 g</th>
<th>TFC, QE mg/100 g</th>
<th>ACC, CGE mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>African spinach</td>
<td>738.70 ±7.90</td>
<td>457.40 ±1.50</td>
<td>217.09 ±3.91</td>
</tr>
<tr>
<td>Jute leaf (\textit{Corchorus olitorius})</td>
<td>1 107.68 ±4.90</td>
<td>510.13 ±2.22</td>
<td>116.89 ±4.11</td>
</tr>
<tr>
<td>Scent leaf (\textit{Ocimum gratissinum})</td>
<td>1 464.63 ±7.90</td>
<td>496.51 ±1.31</td>
<td>100.96 ±6.28</td>
</tr>
<tr>
<td>Water leaf (\textit{Talinum triangulare})</td>
<td>1 385.88 ±33.65</td>
<td>512.07 ±1.47</td>
<td>166.98 ±3.20</td>
</tr>
<tr>
<td>Bitter leaf (\textit{Vernonia amygdalina})</td>
<td>855.33 ±21.29</td>
<td>371.89 ±0.44</td>
<td>103.53 ±5.12</td>
</tr>
</tbody>
</table>

Values were presented as mean ±SD (\( n = 3 \)).

* Differences are significant when compared with One-way ANOVA at \( p < 0.05 \).

GAE – gallic acid equivalent. QE – quercetin equivalent. CGE – cyanidin-3-glucoside equivalent.

TPC – total phenolic content. TFC – total flavonoid content. ACC – anthocyanin content.

Flavonoids and their derivatives are a group of polyphenols that are present naturally in food and medicinal plants, and are known for their antioxidant or free radical scavenging properties (Kar, 2007; Nunes et al., 2012). Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavonoids present in nearly 70% of plants (Kar, 2007). The total flavonoid content (TFC) content was found to be high in water leaf, then followed respectively by; jute leaf > scent leaf > African spinach > bitter leaf. Total anthocyanin content (ACC) decreased in the following order; African spinach > water leaf > jute leaf > bitter leaf > scent leaf.

Scent leaf has high DPPH radical scavenging activity (74.64 ±7.73 μg/mL) compared to L-ascorbic acid (89.03 ±14.82 μg/mL), while the others, in decreasing order of activity, are; jute leaf > water leaf > bitter leaf > African spinach (Table 2, Fig. 1). The DPPH scavenging assays explain the possible ways by which antioxidants could help to reduce biological free radicals. Oil extracted from scent leaf has been reported to have potent antioxidant activity (Chanda et al., 2011). The EC_{50} of the DPPH scavenging effect of scent leaf (Ocimum gratissimum) is lower, which contrasts to the high DPPH radical scavenging profile obtained in the essential oil of Ocimum canum (Selvi et al., 2015).

This substantiates claims of some studies that crude plant extracts possesses greater antioxidant properties compared to fractionated extract of the same plant.

Hydroxyl radicals react with polyunsaturated fatty acids in the cell membrane (phospholipids) and consequently causes damage to the cell (Halliwell and Gutteridge, 1981). The hydroxyl radical scavenging activity of bitter leaf, water leaf, African spinach, and jute leaf respectively were good compared to L-ascorbic acid, while scent leaf had the least hydroxyl radical scavenging activity (Table 2, Fig. 2).

Table 2. The EC_{50} [μg/mL fresh weight] of total antioxidant capacity (TAC), 2,2’-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (OH) radical scavenging activity of the vegetable extracts

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH</th>
<th>OH</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-ascorbic acids</td>
<td>89.03±14.82</td>
<td>178.23±4.13</td>
<td>188.29±7.48</td>
</tr>
<tr>
<td>African spinach</td>
<td>213.44±9.29a</td>
<td>110.54±8.15a</td>
<td>227.39±7.25a</td>
</tr>
<tr>
<td>Jute leaf</td>
<td>125.86±11.34a</td>
<td>144.39±9.37a</td>
<td>27.52±4.14a</td>
</tr>
<tr>
<td>Scent leaf</td>
<td>74.64±7.73a</td>
<td>217.52±6.90a</td>
<td>65.47±14.43a</td>
</tr>
<tr>
<td>Water leaf</td>
<td>132.43±9.43a</td>
<td>103.41±6.27a</td>
<td>106.31±7.66a</td>
</tr>
<tr>
<td>Bitter leaf</td>
<td>156.02±5.43a</td>
<td>50.38±7.17a</td>
<td>169.96±6.86a</td>
</tr>
</tbody>
</table>

Values were presented as mean ±SD (n = 3).

* Differences are significant when compared using One-way ANOVA (p < 0.05).

DPPH – EC_{50} of DPPH radical scavenging activity. OH – EC_{50} of hydroxyl radical scavenging activity. TAC – EC_{50} of total antioxidant capacity.

Fig. 1. DPPH scavenging activities of vegetable extracts: AA – ascorbic acid, AS – African spinach, JL – jute leaf, SL – scent leaf, WL – water leaf, BL – bitter leaf
The determination of total antioxidant capacity was based on the reduction of Mo (VI) to Mo (V) by the samples. African spinach has the least total antioxidant capacity (227.39 ±7.25 μg/mL), lower than that of L-ascorbic acid (188.29 ±7.48 μg/mL). However, the other vegetables assessed in this study have a higher total antioxidant capacity than L-ascorbic acid (Table 2, Fig. 3).

Principal component analysis (PCA) has been proved to provide an in-depth approach to study correlation and variation among species with respect to traits (variables) (Colonna et al., 2016). A two dimensional principal component analysis (PCA) was performed in order to identify and summarise behavioural clusters among phytochemical and antioxidant profiles across the vegetable species. The PC1 and PC2 explained 71.25% and 27.13% of the variance respectively. PC1 was positively and significantly correlated with DPPH, ACC, and TAC, while PC2 was positively and significantly correlated with TFC (Table 3). The loading plot (Fig. 4A) shows a relationship between phytochemicals and the antioxidant profiles of the vegetables. Variables with angle <90° between them are strongly correlated, while those with angle >90° are weakly correlated or strong in variance. For example, TPC was strongly correlated with TFC and OH. Likewise, ACC was strongly correlated with DPPH and TAC. However, TAC, DPPH and ACC were not correlated with TFC, TPC and OH (Fig. 4A). The component object score plots (Fig. 4B) discriminate the vegetable species into four quadrants. The positive side of PC1 in the lower right quadrant included African spinach and bitter leaf, which were characterised by low total antioxidant capacities and

Table 3. Eigen values, relative proportion of total variance and correlation coefficients of phytochemical and antioxidant components with respect to the two principal components

<table>
<thead>
<tr>
<th>Principal components</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigen values</td>
<td>4.28</td>
<td>1.63</td>
</tr>
<tr>
<td>Percentage of variance</td>
<td>71.25</td>
<td>27.13</td>
</tr>
<tr>
<td>Cumulative variance</td>
<td>71.25</td>
<td>98.38</td>
</tr>
<tr>
<td>Eigen vectors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPC</td>
<td>-0.975</td>
<td>0.202</td>
</tr>
<tr>
<td>TFC</td>
<td>-0.142</td>
<td>0.977</td>
</tr>
<tr>
<td>ACC</td>
<td>0.919</td>
<td>0.392</td>
</tr>
<tr>
<td>DPPH</td>
<td>0.997</td>
<td>0.074</td>
</tr>
<tr>
<td>OH</td>
<td>-0.913</td>
<td>-0.391</td>
</tr>
<tr>
<td>TAC</td>
<td>0.794</td>
<td>-0.566</td>
</tr>
</tbody>
</table>

Bold characters are markedly significant in the component. ACC – total anthocyanin content. DPPH – EC50 of DPPH radical scavenging activity. OH – EC50 of hydroxyl radical scavenging activity. TPC – total phenolic content. TFC – total flavonoid content. TAC – EC50 of total antioxidant capacity.
CONCLUSIONS

Water leaf and jute leaf possess high flavonoid content. The highest phenolic content was found in scent leaf, but it has a lower flavonoid content compared to those mentioned earlier. African spinach possesses the highest anthocyanin content. African spinach and bitter leaf have low DPPH radical and phosphomolybdate scavenging effects, which can be attributed to their low phenolic and flavonoid contents compared to the other vegetables. African spinach, bitter leaf, jute leaf and water leaf exhibit good hydroxyl radical scavenging properties. However, scent leaf possesses a low hydroxyl radical scavenging profile, despite a high phenolic content. This shows that some phenolic compounds in this vegetable may have low antioxidant potentials.
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REFERENCES


