Nutritional, anti–nutritional composition and in vitro protein digestibility of tropical edible pulse bean ‘Dioclea reflexa’ – an under-utilized legume

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ABSTRACT: The nutritive composition of three tropical locally cultivated Dioclea reflexa, were investigated using standard methods in other to assess the numerous potentials of the pulse seeds. The proximate composition depicts high values of dry matter protein content within the range of 26.11 ± 0.18 – 33.75 ± 0.35%, crude fibre, ether extract, ash and Gross energy were; (0.92 ± 0.04 -1.28 ± 0.08 %), (5.23± 0.18 - 6.96 ± 0.23%), (7.79 ± 0.28 - 8.56 ± 0.19%), (1486.94 ± 1.97, 1497.97 ± 1.54 kcal/kg), respectively. The moisture content ranged between (7.75 ±0.37– 12.95±0.28 %), while In-vitro digestibility was determined having, 72.35 - 83.90%. The protein constituents were relatively soluble at both acidic and basic pH regions. The macro and micro elements; potassium, sodium, calcium, phosphorus and magnesium were respectively in ranges of (406.50 ± 6.39 - 446.61 ± 5.49 mg/100g; 174.56 ± 3.46 – 223.51±4.80 mg/100g; 197.30 ± 1.99 – 182.31 ± 1.37 mg/100g; 110.64 ± 2.1 – 156.14 ± 1.06 mg/100g; 264.10 ± 5.65 – 335.74 ± 3.1 mg/100g). While copper showed the least value of all the micro-elements, but was plausibly comparable to that of common pulse seeds, anti-nutritional compounds were determined; tannin, oxalate and Phytic acid were in the range of 327.43 ±0.40 - 360.27 ± 1.30 mg/100g; 93.77±0.14 – 115.34 ± 0.11mg/100g; 40.35 ± 0.35 – 80.35 ± 0.38 mg/100g, respectively. The study confirmed that in terms of both quantity and quality, Dioclea reflexa could serve as a good source of protein and also revealed the potentials of different types of cultivar to produce essential and bio-available protein.

Keywords: Anti-nutrient; Cultivars; In-vitro digestibility; Under-utilized; Pulse seed;

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INTRODUCTION

Increasing demand for plant protein in lieu of expensive animal protein has been emphasized previously by several authors on the development of legumes that are less known but are potential sources of cheap protein in developing country (Dzudie and Hardy, 1996; Chel – Guerroero et al., 2002; Adebowale and Lawal., 2006)

Legumes are target crops in this regards because they offer high and abundant sources of protein. Many legumes have protein contents between 20% and 60% and a few ranged between 40% and 60% (Aykroyd and Doughty, 1948). Dioclea reflexa ranks among the under-utilized legumes that could ameliorate protein deficiency in human nutrition.

Dioclea reflexa seeds are also called ‘sea pulse’ as the English name and sea beans as the common name or marble vine or horse eye. The seeds are called Agba-aarin by the Yorubas in the south-western part of Nigeria and also called Ukpo or Ebba by the Igbo in the South-eastern part of Nigeria. It is a drift seed from Caribbean to Carolina that was successfully sea dispersed to West Africa. (Mabbarhey, 1993)

Dioclea reflexa is a legume belonging to the family of Leguminosea and sub-family of Papilionoidea. It appears in three colours: black, dark brown and the light brown coloured covered with thick husk, but as soon as the husk is removed all appear white.
The seeds are spherical, they have ridge at the ‘hilum’, the scar on the seed coat where it was attached to the fruit; the seed is widely consumed as a soup thickener (Aiyesanmi and Oguntokun, 1996). Legumes are generally known to contain various natural constituents which affect their nutritional values. (Ologhobo and Fetuga, 1984). Some of these components inhibit the activities of specific enzymes are protease and amylase inhibitors and have been reported in some legumes. (Whitaker and Feeney, 1972).

Others are hemagglutinins, saponins, tannins and anti-vitamins (Liener, 1969), (Cuthbertson,1968) and (Oberleas, 1973), has also reported that the presence of phytic acid interferes with mineral elements absorption and utilization and it reacts with proteins to form complex products, which have inhibitory effects on peptic digestion (Barre, 1956).

Some researchers have done comprehensive work on the known tropical legumes grown in the developing countries and also reported legumes as a good source of protein (Martinez, 1970); (Ukhun and Ifeighigh, 1988) and (Aletor and Aladetimi,1998).

Unfortunately most developing countries are generally not producing enough food including legumes, despite the importance of the known conventional legumes such as (Soy bean, cowpea, and lima bean, pigeon pea). Legumes should be the common staple food in the diet of people with low incomes in the developing countries, but little awareness of their potentials have reduced the frequency of their consumption.

The nutritional contribution of legumes could be raised by increasing yield and by exploring the quality and quantity of the seed protein especially D. reflexa, through different processes (fermentation, roasting and boiling or parboiling).

However, the under-utilization of these edible plant protein sources may not be unconnected with long-established diets and eating habits, but there is little or no information about the effects of processing on the quality of nutrition, anti-nutritional as well as in-vitro protein digestibility of the seeds, which could be used to assess their protein value and potentials in the food industries.

Therefore, in pursuance of our studies on the utilization of tropical locally available but under-utilized legume seeds, and to exploit their nutritional values. This report presents the results of investigation on the nutritional, anti-nutritional, protein solubility pH dependent and in-vitro protein digestibility of Dicloea reflexa seeds.

**MATERIALS AND METHODS**

*Dicloea reflexa* seeds were obtained in dried form from the local market at Oye-Ekiti market in Ekiti State of Nigeria. The three different coloured varieties are; black (BLK), Dark brown (BRW) and light brown (LBWK), were identified and sorted out prior to treatment.

**Treatment of samples**

Each coloured varieties was divided into two equal parts; the seeds were split into two and boiled in water for 8-10minutes. This made the hard husk to be easily removed. The dehusked seeds were oven dried at 45°C and broken into pieces by pounding after which the dried seeds were dry milled into flour in a Kenwood blender and sieved with a screen mesh of aperture 425µm.

**Proximate Analysis**

The determination of proximate composition of *D. reflexa* seeds for moisture, total ash, crude fibre and ether extract, tannins, phytate were done according to (AOAC, 1990). Oxalate content was determined by the titrimetric method of (Moir, 1953), as modified by (Ranjan and krishna, 1980), in situation where extracts were coloured, they were decolourised with activated charcoal (Balogun and Fetuga,1980). Nitrogen was determined by the micro- kjeldahl methods as described by
(Pearson, 1976) and the percentage nitrogen was converted to crude protein by multiplying with 6.25. All determinations were performed in triplicates.

**Mineral Analysis**  
The minerals were analyzed by ashing the samples at 550°C to constant weight and dissolving the ash after which the mixture was filtered into 50ml volumetric flasks using distilled, deionised water with a few drops of concentrated hydrochloric acid., Sodium and potassium were determined using flame photometer (model 405, corning UK,) using standards calibration methods. Phosphorus was determined colourimetrically using Spectronic 20 Gallenkamp, UK, as described by (Pearson, 1976) with KH₂PO₄ as the Phosphorus standard. All other metals were determined by Atomic absorption spectrophotometer (Perkin-Elmer model 403, Norwalk CT, USA) and were done in duplicates.

**Statistical analysis of the samples**  
Data was analyzed using Statistical Package for Social Science (SPSS) version 14 (SPSS Inc. Chicago IL, USA). Statistical differences between means were compared using, Anova and Duncan Multiple Range Test (DMRT), to determine differences in means of the proximate composition, mineral and anti-nutrient composition between and within mean values of cultivars and were statistically significant at p< 0.05.

**Determination of in-vitro Protein Digestibility (IVPD)**  
The IVPD was determined using the procedure of Hsu et al.,(1977). The multi-enzyme {mg/ cm³} used contained 1.6mg porcine pancreatic trypsin (EC 3.4.21.4), 3.1mg bovine chymotrypsin (EC3.4.21.1) and 1.3mg bovine intestinal peptidase (EC.3.4.23..1), and were purchased from Sigma-Aldrich, Dublin, Ireland.  
50 cm³ of the aqueous suspension of the respective D. reflexa seed flour, (6.25mg/sample /cm³)in distilled water was adjusted to pH 8 with 0.1M HCl and or 0.1M NaOH while stirring in water bath maintained at 37°C. A 0.2ml aliquot of multi-enzyme was added to the sample suspension with constant stirring at 37 ± 2°C. The pH of the suspension was recorded after 15minutes. The in vitro digestibility was thereafter calculated using the regression equation of (Hsu et al., 1977): Y=210.46-18.10X, where Y is the in vitro digestibility in (%) and X is the pH of the sample after 15minutes digestion with the multi-enzyme solution.

**RESULTS AND DISCUSSION**  
Table1 shows the data on the proximate composition of the dehusked Dioecleus reflexa seeds cultivars. The crude protein (CP) ranged between 26.11 ± 0.18 - 33.75 ± 0.35%. This is higher than those of commonly consumed legumes such as chick pea (Cicer arietium), green pea (Pisum sativum) common bean (Phaseolus vulgaris); Pigeon pea ( Cajanus cajan) and Lentil (Lens culinaris) which had a range of concentrations from 18.5 to 21.9%,(Costa et al.,2006)and (Kumar et al., 1991), except for soybean that contains average of 38 - 40% crude protein (Augustin and Klein, 1989).

Crude protein content of D. reflexa bean species compared well with that of cowpea (Vigna unguiculata) at 29.3 % (Mwasaru et al.,1999) and Mung bean (Phaseolus aureus) at 26.5 %, (Sathe and Salunkhe, 1984), but higher than the value of crude protein content 44.43% as reported (Aijesammi and Oguntokun, 1996). The crude protein content in D. reflexa bean species grown in different locations of the tropics and sub tropics vary, the variation may be attributed to interaction between genetic makeups and the environment. The ether extract (EE) of lipid was in the range of 5.23± 0.18–6.96 ± 0.22 %. However light brown species had the highest value than the other cultivars,
the fats content value reported by Aiyesannmi and Oguntokun (1996) was higher at 14.59 ± 0.36 
%, but D. reflexa spp. contain higher crude fat 
content than Mucuna bean (3.65%) (Mugendi 
et al., 2010). With the exception of chickpea 
having 6.7 % (Costa et al., 2006), soybean at 
21% and peanut at 49% where higher (Augustin 
and Klein, 1989).
Fat is important in diets because it promotes the 
absorption of fat soluble vitamins (Bogert 
et al., 1994), it is a high energy nutrient and 
does not add to the bulk of the diet. The crude 
fibre content 0.98±0.04 – 1.29±0.08 % was 
lower than the range for Mucuna bean reported by 
The ash contents ranging between 7.87±0.28 – 
8.6±0.19% and this compared well with chick 
pea (9.8%) and (10.4%) reported for pea (Costal 
et al., 2006). Ash is important in legumes as it 
contains nutritionally important mineral 
elements. D. reflexa was observed to contain 
good amount of minerals both macro and micro 
elements. However, the ash content of other 
Mucuna varieties reported by (Mugendi et al., 
2010) was 3.53% and Adenopus breviflorus 
benth seed was within the range of 2.06 – 3.49%.
(Oshodi, 1992). Dehulled D. reflexa seed species 
contained higher Gross energy (G.E) values 
ranging between 1486.94 ± 1.97– 1497.97±1.54 
ocal/kg, but the light brown D. reflexa seed had 
the highest value 1497.97 ± 1.54 kcal/kg.
The Gross energy values showed that most of 
the samples were concentrated sources of energy, the energy from cereals ranged between 
1.3 – 1.6 MJ/kg, (Paul and Southgate, 1978). 
Indicating that, most of the samples have energy concentration, favourably comparable with 
or less to cereals.
The respective mean values for potassium, 
sodium, calcium, phosphorus and magnesium in 
the dehulled samples (Table 2.) were in the 
range of 406.50± 6.39 - 466.61 ± 5.49 mg/100g. 
174.56 ± 3.46 – 223.51±4.80 mg/100g, 197.30 
± 1.99 – 182.31 ± 1.37 mg/100g, 110.64 ± 2.1 
– 156.14 ± 1.06 mg/100g and 264.10 ± 5.65 – 
335.74 ± 3.1 mg/100g respectively.
Copper was the least abundant micro element 
with trace values in all the dehulled D. reflexa 
seed samples having the lowest value in black 
seed (0.47±0.02 mg/100g).
Table 3, showed the values of tannins, phytic 
acids and oxalate contents in the dehulled dried 
D. reflexa seeds and were significantly different 
at p <0.05. The concentration of Phytic acid, 
tannins and oxalate were respectively in the 
range: 327.43 ±0.40 - 360.27 ± 1.30 mg/100g, 
93.77±0.14 – 115.34 ± 0.11mg/100g, 40.35 ± 
0.35 – 80.35 ± 0.38 mg/100g. Black species of 
D. reflexa has the lowest values of phytic acid, 
tannins and oxalate; 327.43 ±0.40 mg/100g, 
99.77 ± 0.14 mg/100g, and 40.35 ± 0.35 mg/ 
100g, respectively, when compared with the 
other two species, Dark brown and light brown 
coloured species.

The presence of anti-nutrient in all the D. reflexa 
dehulled seeds agrees with an earlier reports of 
wide spread occurrence in tropical legumes 
(Aletor et al.,1994) Kidney bean; (D'Mello,1995), 
grass pea; (Aiyesannmi and Oguntokun,1996), 
Diocelea reflexa seed; (Ologboho and Fetuga, 
1994) and (Alonso et al., 2000 ) on Jack beans 
and Faba beans respectively. Despite its 
potentials, D. reflexa remains a minor food crop, 
and poorly adopted in agricultural systems. 

Nevertheless, the level of phytic acid in D. 
reflexa seeds secured is much lower than that 
reported for Moth bean cultivars (852 - 899 mg/ 
100g) (Santish and Chauhan, 1986), and other 
beans such as kidney beans. (Lolas and 
Markakus; 1975), Soybean, (DeBoland et al., 
1975) and (Ologboho and Fetuga,1984).

Several studies have criticized and also 
implicated dietary phytic acid and oxalic acid 
in black Mucuna for reducing vital minerals 
due to its chelating effect, especially for diets 
already had low concentration of mineral 
contents (Siddhuraju and Becker, 2000). Also 
as well as impairment of the efficient utilization 
of the minerals, especially lowering of bio-
availability of divalent minerals such as zinc, 
calcium, and magnesium, and inhibiting several 
Proteolytic enzymes (amyloses).
Tannins are characteristically present in the 
legumes grains having pigmented seed coat and 
decortication result in removal of 83- 97% of
tannins, (Santish and Chauhan, 1986); (Alonso et al., 2000) and (Mugendi et al., 2010).

Although, the values of tannins in D. reflexa seeds are significantly different (p<0.05), within the range of 93.99 ±0.14 mg/100g – 115 ± 0.15 mg/100g, they had comparative lower value in the black seed species of D. reflexa. These showed better nutritive values than those pulse seeds having high significant level of tannins compared with those reported in legumes, such as; Chicken pea (Singh, 1988); Mucuna pruriens (Siddhuraju, 2000); Faba and kidney beans (Alonso et al.,2000) and also Cowpea (Pretc and Punia, 2005).

Tannins are known to affect the digestibility of proteins, carbohydrates, fats, and bio-availability of minerals due to the formation of insoluble enzyme resistant complexes, (Santish and Chauhan, 1986; Mugendi et al., 2010).

Figure 1. Showed the results of the in-vitro protein digestibility of all the three D. reflexa dehulled seeds; Dark brown, black, and light brown coloured seeds. The values which differed significantly (p<0.05) ranged between 72.38 ± 0.13 – 83.95 ± 0.67 %, with the highest value observed in the black coloured Diolea reflexa (83.95±0.67%). It may be due to the differences in the sample origin or the environment of the cultivars.

### Table 1: Proximate composition (%) and Gross Energy (kcal/kg) of Diolea reflexa seeds analyzed (dry weight basis) with respect to the groups.

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Dry matter</th>
<th>Crude protein</th>
<th>Ether extract</th>
<th>Crude Fibre</th>
<th>Ash</th>
<th>NFE</th>
<th>GE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Brown</td>
<td>12.95±0.28</td>
<td>87.05±1.46</td>
<td>27.33±0.25</td>
<td>6.13±0.02</td>
<td>1.29±0.08</td>
<td>7.79±0.28</td>
<td>44.29±1.14</td>
</tr>
<tr>
<td>Black SPP</td>
<td>7.75±0.37</td>
<td>92.55±2.13</td>
<td>33.75±0.35</td>
<td>5.23±0.18</td>
<td>0.92±0.04</td>
<td>8.65±0.19</td>
<td>43.70±0.69</td>
</tr>
<tr>
<td>Light Brown</td>
<td>10.60±0.41</td>
<td>89.4±±2.81</td>
<td>26.11±0.18</td>
<td>6.96±0.23</td>
<td>1.25±0.02</td>
<td>7.87±0.18</td>
<td>47.21±1.31</td>
</tr>
</tbody>
</table>

### Table 2: Mineral composition of Diolea reflexa seeds analyzed (dry weight basis) with respect to the groups.

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>Mg mg/100g</th>
<th>P</th>
<th>Ca</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td>446.61±5.49</td>
<td>335.74±3.1</td>
<td>156.14±4.47</td>
<td>197.30±1.99</td>
<td>1.07±0.04</td>
</tr>
<tr>
<td>Black</td>
<td>406.30±6.36</td>
<td>283.31±3.81</td>
<td>110.64±2.7</td>
<td>182.31±4.37</td>
<td>0.63±0.03</td>
</tr>
<tr>
<td>Light Brown</td>
<td>424.96±5.59</td>
<td>264.10±5.65</td>
<td>143.45±2.19</td>
<td>161.65±1.91</td>
<td>0.85±0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>Zn</th>
<th>Fe ppm</th>
<th>Cu</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td>174.30±3.46</td>
<td>15.15±3.46</td>
<td>7.91±0.07</td>
<td>0.97±0.13</td>
<td>1.07±0.04</td>
</tr>
<tr>
<td>Black</td>
<td>223.51±4.80</td>
<td>13.20±0.29</td>
<td>8.31±0.13</td>
<td>0.47±0.02</td>
<td>0.63±0.03</td>
</tr>
<tr>
<td>Light Brown</td>
<td>206.55±6.13</td>
<td>12.10±0.14</td>
<td>4.84±0.08</td>
<td>0.73±0.03</td>
<td>0.85±0.03</td>
</tr>
</tbody>
</table>

Each value represents means of duplicate ± Standard deviation. Mean having the same superscripts are not significantly different (p>0.05) from each other.

### Table 3: Tannins, Oxalate and Phytic acid (mg/100g) of Diolea reflexa seeds analyzed (dry weight basis) with respect to the groups.

<table>
<thead>
<tr>
<th></th>
<th>Tannins</th>
<th>Oxalate</th>
<th>Phytic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Brown spp.</td>
<td>106.10±0.07</td>
<td>50.33±0.38</td>
<td>360.27±1.13</td>
</tr>
<tr>
<td>Black spp.</td>
<td>93.77±0.14</td>
<td>40.35±0.35</td>
<td>327.43±0.40</td>
</tr>
<tr>
<td>Light Brown spp.</td>
<td>115.34±0.14</td>
<td>46.32±0.46</td>
<td>352.93±0.11</td>
</tr>
</tbody>
</table>

Each value represents means of duplicate ± Standard deviation. Mean having the same superscripts are not significantly different (p>0.05) from each other.
This result indicated that the black seed of *Dioclea reflexa* is an excellent protein supplement for balancing the deficiencies in other grain crops. In *D. reflexa* seeds samples, all cultivars showed a highly significant variability (P<0.05), while highest digestibility value was observed in the black seed of *D. reflexa* 83.90 ± 0.67%. The variability among cultivars in other crop grains different from legumes were also shown by several authors, (Duodu *et al.*, 2002); (Nunes *et al.*, 2004); (Mokrane *et al.*, 2010) on *Sorghum bicolor (L)* Moench.

With this information it is possible to use the protein characteristics of several *D. reflexa* samples as a yardstick to selecting suitable cultivars for specific application for food processing or as source of bioactive protein regarding their digestibility and their protein contents.

Fig 3. Depicts the pH solubility profile of tropical *D. reflexa* seed of different colours, pH exerted pronounce effect on solubility profile of black, dark brown and light brown coloured *D. reflexa* seed flour, indicated that protein solubility reduces as the pH increases from pH 1 to 4 respectively, with minimum solubility which correspond to their iso-electric point (pI), after which subsequent increase in pH increased protein solubility progressively. The highest protein solubility was also obtained at pH 7 and 12. Several investigation had been done and reported on Jack bean (Lawal and Adebowale, 2006); soy bean (Achouri *et al.*, 1998); wheat gluten (Barber and Wartheson, 1982) and (Paulson and Tung, 1987) and Peanut (Neto *et al.*, 2001). Also the pH profile of dark brown *D. reflexa* has indicated that the seeds flour has a minimum solubility at pH of about 6.2 and its protein is soluble in both the acidic and basic pH region.

Protein solubility is an index of functionality showing potential application in food products since the results showed that *D. reflexa* seed species suggests its usefulness in alkaline foods but generally *D. reflexa* seeds may find good use in both acidic and alkaline foods.

![Image](image-url)

**Figure 1:** Variability In-vitro digestibility of three *Dioclea reflexa* seeds cultivars analyzed with respect to the groups, mean with different letters are significantly different (p<0.05).

BRW=Brown coloured seed, BLK=Black coloured seed, LBWK=Light brown seed

Prevalent change on the constituent amino acids of proteins at various pH determine protein solubility as follows. (Figure 2) ‘I’ is a Zwitterions or dipolar ion which predominates at the region of Isoelectric point (pI) in protein. At this pH, minimum solubility takes place because of minimum repulsion among the constituent amino acids. The balance in positive and negative charges minimized the electrostatic repulsion and this reduced solubility of protein at pH of 3, 4 and 6.2, when pH of the solution reduced further, cation ‘III’ predominates, while in alkaline medium anion II takes preponderance. In both cases, electrostatic
repulsion improved and this enhanced solubility as it is observed in pH of 1 and 12. (Lawal and Adebowale, 2006).

Fig 3, indicates that protein is least soluble in the black seed flour in the acidic pH region and maximum at basic pH region, Dark brown seed flour showed highest maximum solubility in acidic pH region but the least in protein solubility in the basic pH region. This pattern is similar to those of some of other legumes reported earlier on Jack bean (Lawal and Adebowale, 2006) and Cowpea (Prinyawiwatkul et al., 1997).

![Figure 2: Schematic diagram for prevalent change on proteins at various pH.](image)

![Figure 3: Protein solubility as a function pH of three Dioclea reflexa seeds cultivars. BRW=Brown coloured seed, BLK=Black coloured seed, LBWK=Light brown seed](image)

The proximate composition and mineral contents of *Dioclea reflexa* bean seeds compared favourably with that of conventional edible legumes. Black coloured seed of *D. reflexa* are notably rich in protein with highest in-vitro digestibility value and depicts highest solubility in basic pH region. Also the Dark brown and light brown coloured *D. reflexa* have relatively similar values to the black coloured *D. reflexa*. The study had reveals the high potentials of *D. reflexa* seeds, which are under-utilized. If substantially consumed, it could help to alleviate the nutrient deficiency prevalent and could be a

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substitute for the high price plant protein food in Nigeria West Africa diets. The study further showed that it is possible to use the protein characteristics of several \emph{D. reflexa} samples as a yardstick for selecting most suitable cultivars for specific food processing or as a source of bioactive proteins.

\section*{ACKNOWLEDGEMENT}
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