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IN-VITRO ASSESSMENT OF ANTIOXIDATIVE POTENTIALS OF ETHANOL SEED EXTRACT OF *COLA LEPIDOTA* K. SCHUM

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ABSTRACT

The aim of this study was to carry out an in-vitro assessment of antioxidative potential of ethanol seed extract of *Cola lepidota* K. Schum. In-vitro determination of antioxidant property of seed extract of *Cola lepidota* was done using ferric reducing antioxidant power (FRAP), 2,2-dyhenyl-l-picrylhydrazyl (DPPH) assay and nitric oxide radical (NO) scavenging activity. These assessments were carried out using standard antioxidant assays. Results obtained from the antioxidant assay show that the extract of *Cola lepidota* seed has an insignificant ferric reducing power ($p \geq 0.05$) (0.01, 0.05, 0.10, 0.20, 0.50µM) when compared to the ascorbic acid control (0.1, 0.25, 0.75, 1.50, 1.75µM). However, DPPH and NO radical scavenging ability of the seed extract (20.50, 36.20, 60.04, 92.20, 92.30 and 36.42, 58.00, 60.24, 62.10, 66.40 respectively) was statistically significant $p \leq 0.05$ when compared with the standard drug ascorbic acid 92.10, 94.00, 94.06, 94.05, 96.06 and 66.21, 71.06, 90.22, 94.88, 97.21 respectively, at 25,50,100,200 and 400 mg/ml. In conclusion, the result of this study lends credence to a probable role of the seeds of *Cola lepidota* as a potent antioxidant.

KEYWORDS: *Cola lepidota* seed, DPPH, $NO^{\cdot}$, FRAP, antioxidant potential.

INTRODUCTION

In most African homes, plants are used as sources of medication for the treatment or prevention of various diseases. Such plants with beneficial properties which are used for the management of various human diseases and which have been shown to contain

phytochemicals and other secondary bioactive compounds are called medicinal plants (Fasyi, 2006; Kumar *et al.*, 2009). Among these phytochemicals are flavonoids, alkaloids, phenols, saponins etc (Edeoga *et al.*, 2005 Ene-obong *et al.*, 2016). Different authors have noted that consumption of fruits and vegetables from these plants help to prevent and treat diseases like anaemia, cancer, hepatitis, ulcers etc (Umaru *et al.*, 2018; Okwu, 2005).

Cola lepidota is a member of a group of *Cola* species commonly known as monkey kola which produce edible tasty fruits. They belong to the same family Malvaceae and sub-family sterculioideae with *Cola* nitida (Kolanut) known for its maticastory and stimulation effects in the West African sub-region (Bosch *et al*, 2000; Pamplona-Roger, 2008). *Cola parchycampa* (with white pulp), *Cola lepidota* (with yellow pulp) and *Cola laterita* (with red pulp) are among the species commonly referred to as Monkey kola.

Cola lepidota which is commonly consumed fresh is edible, crunchy and tasty (Okudu *et al*, 2015) and grows up to 18m high with a twisted trunk and a calciferous lump. It is grown in western Cameroon, Gabon, lower Guinea and Southern part of Nigeria where it is commonly found between the months of June to November (Ogbu *et al*, 2007; Oghenerebo *et al*, 2013). *Cola lepidota* is locally known as achicha in south-east Nigeria and monkey kola in west Cameroon (Iwu, 1993) where native people relish the fruits as well as primates like monkeys, baboons (Essien *et al*, 2017).

Phytochemical studies show that monkey *Cola* contains bioactive compounds like flavonoids, saponins, glycosides, steroids, β-carotene (Adegboye *et al*, 2008; Eneobong *et al.*, 2016) as well as a considerable amounts of riboflavin, niacin, iron, zinc, selenium, calcium, carotene, vitamin C etc (Okudu *et al.*, 2015). Alternative medicine practitioners in Nigeria though not aware of the presence of the above bioactive compounds in *Cola lepidota* employ it in the treatment of febrifuge, pulmonary ailments and cancers (due to its antioxidant property) (Engel *et al.*, 2011; Oghenerebo *et al.*, 2013). According to Seitz *et al.*,(1992); Odion *et al.*, (2013); the monkey *Cola* species are also used in the African folk medicine as a remedy for headache, dysentery and to suppress sleep and enhance agility.

An antioxidant is defined as: "any substance that when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate (Halliwell and Gutteridge, 1995). They protect the cellular components of the body from oxidative stress and also constitute a defense system against free radicals and

reactive oxygen species that arise from the body's biochemical reactions (Boxion *et al.*, 2012). Free radical and ROS are produced either from exogenous sources like exposure to x-rays, cigarette smoking, some drugs, industrial solvents, pesticides etc or from normal enzymatic and non enzymatic cellular process (Pham-Huy *et al.*, 2008; Ebadi, 2001). Oxidative stress is implicated in diseases like lupus erythematosus, heart diseases, neurodegenerative diseases like Parkinson's and Alzheimers, arthritis, atherosclerosis, diabetes mellitus, cancer etc. (Pham-Huyy *et al.*, 2008; Kumpulainem and Salone, 1999).

A variety of free radical scavenging antioxidants are found in fruits, leaves and other parts of plants and these antioxidants help in converting the free ROS to less reactive species (Yadav *et al.*, 2016). According to reports by Dembinska-Kiec *et al* (2008); Sin *et al* (2013); Willis *et al* (2009), regular intake of plants fruits and vegetables with antioxidative potentials help in the fight against chronic diseases and in enhancing human longevity and well-being. Bioactive substances like vitamin C, E, carotenoids and phytochemicals like flavonoids have been shown to be abundant in various plants including *Cola lepidota* (Boskou *et al*, 2005; Oghenerebo and Falodun, 2013).

The antioxidative potentials of *Cola lepidota* leaf and stem bark extracts have been studied by various researchers (Essien *et al.*, 2015; Oghenerobo and Falodum, 2013; Engel *et al.*, 2011). Little research has however been done of the antioxidative potentials of the seed extract of *Cola lepidota* despite its high level of consumption in this part of Africa and this prompted this research on the assessment of the antioxidative potentials of *Cola lepidota* seed extract.

MATERIALS AND METHODS

Preparation of experimental materials

Fruits of *Cola lepidota* was purchased at Ekeukwu market, Owerri, Imo State Nigeria. The fruits were washed and the seed obtained by peeling off the bark and cutting open the pulp of the fruit. The seeds were sliced into pieces, sundried and then ground into fine particles with the help of electronic grinding machine.

Preparation of the extract was by mixing 100g of the powder with 100mls of ethanol and allowing it to stand for 48 hours after which the ethanol was evaporated using a rotary evaporator. The extract obtained after evaporation was then dissolved in normal saline after further concentration with electronic incubator at 40^{o}C.

In vitro Determination of Ferric Reducing Antioxidant Power (FRAP) of seed extract.

The ferric reducing antioxidant power of the seed extract was carried out using a protocol described by Benzie and Strain (1999) as follows:

1. Acetate buffer (30mM), pH 3.6 (3.1g sodium acetate: $3H_2O$ and 16ml glacial acetic acid in 1000ml buffer solution).
2. 2,4,6-triphridyl-S-triazine (TPTZ) (10mM) in 40mM HCl.
3. $Fecl_3$. $6H_2O$ (20mM) in distilled water.

A fresh working solution of FRAP was prepared by mixing 1,2 and 3 in the ratio of 10:1:1 respectively. FRAP reagent (3ml) and 100µL sample solution at concentrations 25,50, 100, 200 and 400µg/ml was mixed and allowed to stand for 4 minutes and the absorbance recorded at 593nm, at 37^{o}C. The ascorbic acid was tested in a parallel process. After the addition of the sample, the absorbance of each test tube was taken at 0 and 4 minutes.

FRAP value = absorbance at 4min – absorbance at 0 minute.

In-vitro Determination of the antioxidant property of the extract using picrylhydrazyl (DPPH) photometric Assay

The protocol described by Mensor *et al* (2001) was used in determining the free radical scavenging activity of the *Cola lepidota* extract. The extract was analyzed by the DPPH assay using a spectrophotometer. 1mL of 0.5mM DPPH (in methanol) in a cuvette was mixed with crude extract at concentrations (25,50,100, 200 and 400) µg/ml. After 30 minutes of incubation in a dark room, the absorbance was taken at 517nm. The experiment was done in triplicate and the percentage antioxidant activities were calculated as follows.

% antioxidant activity (AA) = 100 – [{(sample abs-blank abs) x 100}/abs of control]
Where abs = absorbance

One millilitre of methanol plus 2.0mL of the test extract was used as the blank while 1.0 mL of the 0.5 nM DPPH solution plus 2.0 mL of methanol was used as the negative control. Ascorbic acid (Vitamin C) was used as reference standard (Iwalewa *et al.*, 2008).

In-vitro determination of nitric oxide radical ($NO^{\cdot}$) scavenging activity of seed extract of *Cola lepidota*

Nitric oxide which was generated in aqueous solution at physiological pH from sodium nitroprusside interacts with oxygen to produce nitrite ions which were measured by Griess

reaction (Marcocci *et al.*, 1994; Green *et al.*, 1982). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffer saline (PBS) and the extract from (25-400) µg/mL was incubated for 150 minutes at 25^{o}C. After incubation, 0.5 mL of the reaction mixture was removed and 0.5 ml of Griess reagent (1%w/v) sulfanilamide, 2% (V/V) H_3PO_4 and 0.1% (W/V) naphthylethylene diamine hydrochloride was added. The absorbance of the chromophore formed by the control and test samples was measured at 546 nm.

$$\text{Nitric oxide radical inhibition activity} = \frac{\text{ABS control} - \text{ABS test}}{\text{ABS Control}} \times \frac{100}{1}$$

Statistical Analysis

The data generated from this study were coded in excel sheets and then subjected to analysis using ANOVA test in the 1BM-SPSS software version 21. Mean ± SEM (standard error mean) were calculated and in all cases, the difference was considered statistically significant when p-value was ≤ 0.05.

RESULTS

The results of the in vitro antioxidative potentials of ethanolic seed extract of *Cola lepidota* K. Schum are presented in graphs.

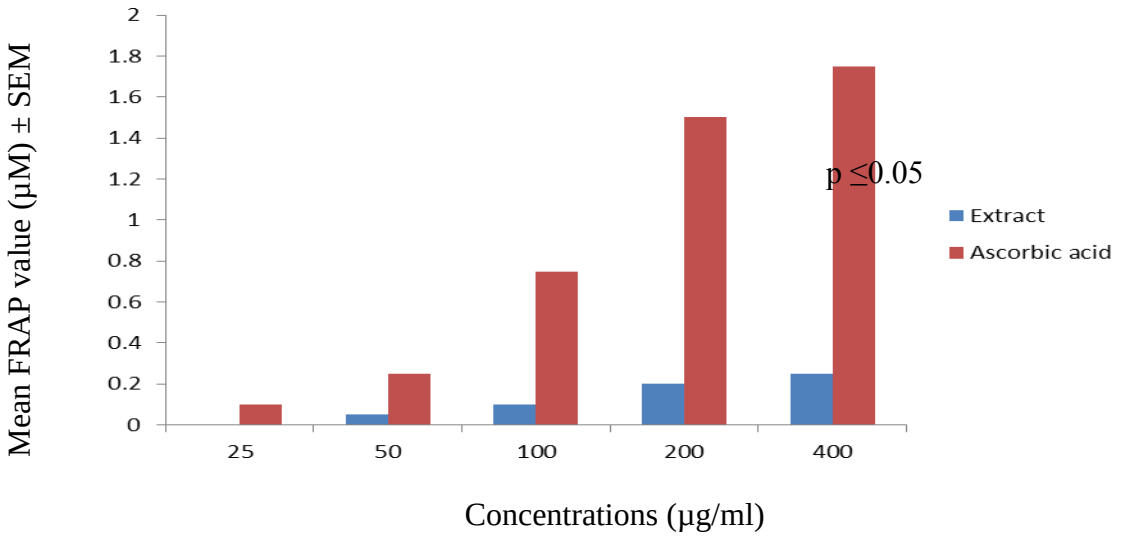

Figure 1: Ferric reducing antioxidant power (FRAP) of Seed extract.

Figure 1 shows the graphical representation of the mean FRAP concentrations of ethanolic seed extract of *Cola lepidota K. Schum* and ascorbic acid which serves as the standard. The vertical axis represents mean FRAP value in µm of both extract and ascorbate standard while

the horizontal axis represents the concentrations in µg/mL of the ethanolic seed extract and the ascorbic acid standard. The legend shows seed extract in blue colour and ascorbic acid in red. From the graph, it can be deduced that the extract of *Cola lepidota* does not possess a strong reducing power as it was not effective in reducing Fe^{3+} in ferric chloride to Fe^{2+} (0.01, 0.05, 0.10, 0.20 and 0.25) µM when compared with the standard (ascorbic acid) (0.10, 0.25, 0.75, 1.50 and 1.75) µM at concentrations of 25,50,100, 200 and 400 µg/ml.

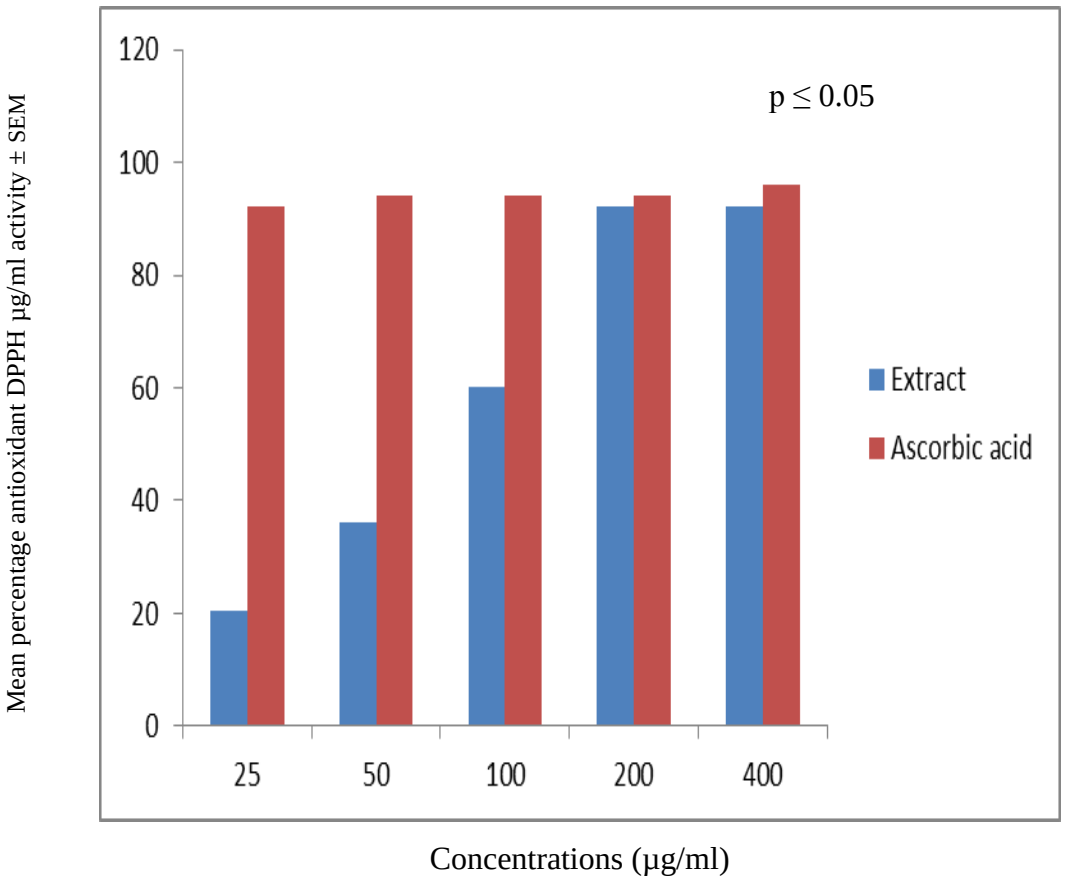

Figure 2: 2,2-Diphenyl-1-picryhydrazyl (DPPH) radical scavenging photometric assay.

Figure 2 shows the graphical representation of the mean DPPH radical scavenging activity obtained by photometric assay. The horizontal axis represents the concentration of seed extract of *Cola lepidota* and a standard drug ascorbic acid measured in µg/ml while the vertical axis represent the mean percentage antioxidant activity of both the standard ascorbic acid and seed extract.

The legend shows seed extract in blue colour and ascorbic acid in red colour. From the graph it can be deduced that the extract possess good DPPH radical scavenging activity (2.50, 36.20, 60.04, 92.20, 92.30) when compared with the standard ascorbic acid (92.10, 94.00,

94.04, 94.05 and 96.06) µg/ml at 25, 50, 100, 100 and 400 µg/ml. It can also be deduced from the graph that the DPPH radical scavenging activity of the extract is dose dependent.

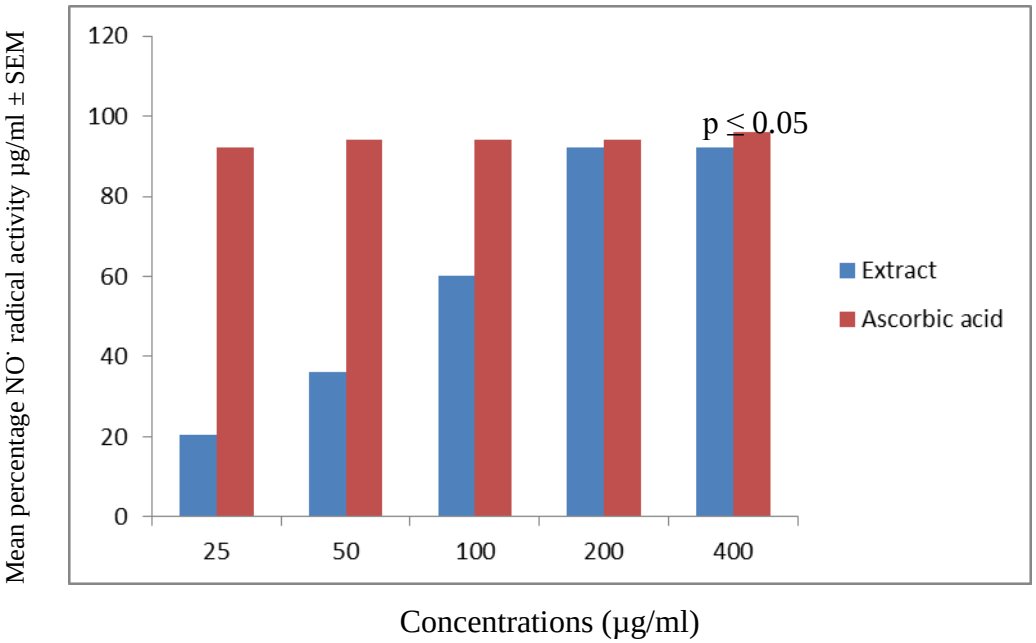

Figure 3: Nitric oxide radical ($NO^{\cdot}$) scavenging activity of the seed extract.

Figure 3 is a graphical representation of the mean nitric oxide ($NO^{\cdot}$) radical scavenging activity of ascorbic acid and ethanolic seed extract of *cola lepidota*. The horizontal axis represents the concentrations of the seed extract and the standard drug (ascorbic acid) in µg/ml while the vertical axis represents the mean percentage nitric oxide ($NO^{\cdot}$) scavenging activity of both extract and ascorbic acid. The legend shows seed extract in blue colour and ascorbic acid standard in red colour. The graph shows that the seed extract of *Cola lepidota* possesses nitric oxide (NO) radical scavenging activity (36.42, 58.00, 60.24,63.10, 66.40) when compared with ascorbic acid (66.21, 71.06, 90.22, 94.88 and 97.21) at concentrations of 25,50,100,200 and 400 µg/ml.

DISCUSSION

Antioxidants are dietary supplements taken in addition to food to prevent chronic diseases (Ohlsson and Bengston, 2002). Antioxidants are at the centre of protective roles exerted by plant foods like vegetables, fruits and seeds against chronic diseases (Liyana *et al.*, 2006; Dembinska *et al.*, 2008). They prevent the cell damaging effects of free radicals and other toxic products and reduce the risks of diseases like cancer, obesity, coronary heart disease as well as enhancing longevity (Kalcher *et al.*, 2009; Halvorsen *et al.*, 2006). There are four

possible mechanisms through which antioxidants exert their action which include hydrogen donation by antioxidants, electron donation, formation of complex with lipids and addition of lipid to the antioxidants (John, 1989).

This study showed a strong DPPH free radical scavenging activity at concentrations 200 and 400mg/ml when compared with that of ascorbic acid. This is consistent with the findings of Essien *et al.,* (2015); Oghenerebo and falodun (2013) where high DPPH free radical scavenging ability of *Cola lepidota* methanolic seed extract was demonstrated. *Cola lepidota* seed is rich in polyhphenols which have the ability to donate hydrogen atoms or electrons and to capture free radicals (Essien *et al.,* 2015). The ability of the polyphenols to act as electron donors and terminate free radical chain reactions also explains the antioxdative property of the extract. The percentage inhibition of the free radical was dose dependent and the higher the concentration, the higher the percentage inhibition. Vitamin C which is a potent and safe antioxidant in medicine (Jain *et al.,* 2008) has a higher antioxidative ability than the extract probably because it is a refined drug. The finding of this study agrees with the reports of Ashok *et al*., (2002), Omotosho *et al.,* (2013), Nwankpa *et al.,* (2015) on the antioxidative properties of Gingo biloba, *Chrysophylum* fruit extract and *Sida acuta* leaf extracts, respectively. This may possibly be linked to the role of scavenging free radical species of the extracts and reducing them to non-toxic products.

Figure 1 showed minimal ferric reducing antioxidant activity of the extract at 50, 100, 200 and 400 mg/ml when compared to the standard ascorbic acid control. This result agree with the finding of Essien *et al* (2015) which showed that *Cola lepidota* seed extract possesses strong ferric reducing antioxidant activity. Method of sample preparation and extraction may be responsible for the difference in the results. The nitric oxide radical scavenging activity of the seed extract was also dose dependent with increasing concentration resulting in increased percentage inhibition. This result supports the DPPH free radical scavenging ability of *Cola lepidota* seed extract.

CONCLUSION

In conclusion, this research has shown that *Cola lepidota* seed has antioxidant property as is affirmed by its $NO^{\cdot}$ and scavenging ability. It therefore validates the role of *Cola lepidota* seed as a natural source of antioxidant which should be used in the management of diseases in which free radicals and oxidative stress are implicated.

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