COMPARISON OF SOME*in-vitro* ANTIOXIDANT PROPERTIES OF *Cola acuminata* AND *Cola nitida*FRUIT TESTAS

ABSTRACT

Testas of *Cola* fruit are usually considered as agricultural waste by many farmers as their focus is always on the seed because of its economic importance. This study therefore aimed at evaluating and comparing the antioxidant potentials of *C. acuminata* and *C. nitida* fruit testas. The methanol extract of the fruit testas of *C. acuminata* and *C. nitida* were evaluated and compared for their antioxidant properties such as: their scavenging abilities on hydroxyl radicals, ABTS (2, 2– Azino-bis-3-ethylbenzothiazoline-6-sulphonic Acid), DPPH (2, 2-diphenyl-2-picrylhydrazyl) and nitric oxide.Also their ferric reducing ability and total antioxidant were investigated. In all the antioxidant parameters assayed, the fruit testas of *C. acuminata* and *C. nitida* compared favorably well with the standard (Butylated Hydroxytoluene) as they both showed positive correlation with the standard. The antioxidant ability of the methanol extract of *C. nitida* was significantly lower compared with that of *C. acuminata* in all the parameters tested. This can be attributed to the hydrogen atom donating potentials of the testaswhich can be as a result presence of vital secondary metabolites in therapeutic mopping up of free radicals in form of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

Keywords: Antioxidants, *Hydroxyl radicals, DPPH*, testas, therapeutic.

1.0 INTRODUCTION

Many health related problems such as some pathological conditions which present cardiovascular disease, cancer, neurological disorder, diabetes, other diseases and aging have been attributed to oxidative stress. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the major free radicals causing harm to the bio-system as they are directly or indirectly responsible for several of these degenerative ailments in human[1]. Diseases such as diabetes are responsible for the generation of reactive oxygen species which is as a result of the complications developed such as neuropathy, retinopathy, cardiovascular disease. This may result in cell damage through oxidative stress [1][2].

Blocking the process of oxidative stress requires the engagement of antioxidants which are well-known for their activity in neutralizing the effect of the presence of free radicals in the bio-system.This is achieved by the interruption of the chain reactions where free radicals changes other less-harmful molecules to free radicals like themselves[3].Oxidative enzymes like superoxide dismutase, catalase, some chemicals like ascorbic acid, carotenoids glutathione are known to be responsible for protection of the bio-system against cell damage from free radicals and when their functions are impaired, it results in disease and accelerated aging [4]. Highly reactive oxygen and nitrogen species damages human health by breaking the bonds in DNA molecules hence, altering the cell's genetic apparatus and subsequently resulting in proliferation of cancerous cells.

In recent times, many plant wastes from agricultural processes are now found to be of economic importance. For instance, the kolanut husk is proven to be good raw material for the formulation of animal feeds[5]. Many plant products are good natural source of many antioxidants at varying quantities, examples of which include: phenols, quinines, tocopherols, gallic acid, gallates e.t.c.[6][7].

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Cola which has its origin from West Africa, is from the family sterculiaceae and the fruit has a shape like capsule, fleshy with irregular-shaped pink, red or white seeds. The seeds may turn brown when dried[8].*Cola* is consumed culturally both by individuals or group settings and often for ceremonies such as weddings, funerals and entertainment of tribal chiefs in many parts of Western and sub-Saharan Africa precisely North and Eastern Nigeria, Ivory Coast, Sierra Leone [8][9]. In nutrition, the seed has been used as soft drink additives while in ethnomedicine, the seed is used in managing digestive impairment, food poisoning and even sometimes asthma[10][11].
For several decades the seeds of *Cola* have beenthe center of attraction in *Cola* fruit because of its wide usage and economic value. There is need to explore other part of the fruit which most times have been regarded as waste in the process of post-harvest handlings. Hence, this study's objective is to determine and comparethe antioxidant properties of the fresh *C. acuminata* and *C. nitida* fruit testas.

2.0 METHODS:

2.1 Sample Preparation: The pods of*C. acuminata* and *C. nitida* fruits were opened after which the seeds were removed, and then washed with distilled water to remove unwanted particles. The seeds were soaked in water for few days thenthe testas were removed and air-dried at room temperature under the shade to avoid loss of bioactive components through irradiation. The dried testas were pulverized to fine powder using high speed blender and finally extracted using methanol as solvent. 10g of the pulverized testa was mixed with 100ml of methanol at room temperature for 24hours after which the supernatant was removed. The residue was re-extracted under the same conditions until the solvent became colorless. The supernatant was then filtered through whatman No. 1 filter paper.The filtrate was made to pass through a rotary evaporator to remove the methanol solvent at 40 ^{0}C. The methanolic extract was dried and stored in the refrigerator for further analysis.

Comment [A1]: rewrite

2.2 Determination of2, 2-diphenyl-2-picrylhydrazylScavenging Ability: The determination of DPPH (2, 2-diphenyl-2-picrylhydrazyl) radical scavenging activities was evaluated based on the method described by Singh *et al.*[12]. Methanol extracts of the*Cola* testas (1ml) of the different concentrations (i.e., 25, 50, 100, 200, 400, 800, and 1000μg/mL^{-1}) made by reconstituting in respective solvents were added to DPPH solution (5mL, 0.1mM) in methanol and mixed. After 20 minutes of reaction at 25°C, the absorbance was measured at 517nmagainst a blank (methanol) in a UV-Vis spectrophotometer. Methanolic DPPH solution (5ml) without antioxidant was used as control. The DPPH scavenging activity of the extract was expressed as IC_{50} (inhibitory concentration), that is, the concentration of the extract at which DPPH radicals were scavenged by 50%. Butyl hydroxy toluene (BHT) was used as standard antioxidant.

Comment [A2]: variations in the concentration of the DPPH test not match the results (Figure 3)

The percentage quenching of DPPH was calculated as follows:

$$\text{DPPH Quenching Capacity (\%)} = \frac{(Abs_{control}) - (Abs_{sample})}{(Abs_{control})} \times 100$$

Where: Abs_{sample} is the absorbance of the sample (extract and standard antioxidant) and $Abs_{control}$ is DPPH solution without the added extract.

2.3 Determination ofScavenging Capacity onNitric Oxide Radical

Nitric oxide (NO) generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH was estimated by the use of the Griess reaction [13]. The reaction mixture (3ml) containing SNP (10mM, 2ml), phosphate buffer saline (0.5ml, pH7.4), and the extracts (0.5ml) at different concentrations (25, 50, 100, 200, 400, 800, and 1000μgml^{-1}) were incubated at 25°C for 150min. After incubation, 0.5mL of the incubated solution containing nitrite was pipetted and mixed with 1mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1ml of N-(1-naphthyl) ethylenediamine dihydrochloride was added, mixed, and allowed to stand at 25°C for 30 min. The absorbance of pink colored chromophore formed during diazotization was measured at 540nm. The NO scavenging activity of the extract was expressed as

Comment [A3]: variations in the concentration of the DPPH test not match the results (Figure 4)

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IC_{50} (inhibitory concentration), that is, the concentration of the extract at which NO radicals were quenched by 50%. Butyl hydroxy toluene (BHT) was used for comparison.

$$\text{Nitric Oxide Scavenging Capacity (\%)} = \frac{(Abs_{control}) - (Abs_{sample})}{Abs_{control}} \times 100$$

Where: Abs sample is absorbance of the sample and Abs control is absorbance of control.

2.4 Determination of Ferric Reducing Ability

Ferric reducing capacity was determined according to Oyaizu [14]. From the solution (25, 50, 100, 200, 400, 800, and 1000 µgml^{-1}) of the *Cola* testas extract , 0.5ml portion was mixed with 2.5ml of sodium phosphate buffer (0.2 M,pH6.6) and 2.5mL of potassium ferricyanide [$K_3Fe_3(CN)_6$] (1%) in test tubes. The resulting solution was mixed and incubated at 50°C for 20 min. Then, 2.5ml of trichloroacetic acid (TCA) (10%, w/v) was added to all tubes and the solutions were centrifuged at 3000 × *g* for 10 min. The aqueous solution of $FeCl_3$ (1%, 1 ml) was diluted by adding deionized water (5 ml). To this solution, the upper layer of the centrifuged solution (5 ml) was mixed and incubated at 35°C for 10 min. Absorbance of the developed color was measured at 700nm. BHT was used as standard.Reducing capacity of the *Cola* testas extracts towards Fe^{3+} was interpreted from relation of absorbance and reducing capacity: when absorbance increases, reducing capacity also increases.

Comment [A4]: These data are not matching in the resultsl

Comment [A5]: Nothing in the results and discussion

2.5 Determination of Hydroxyl Radical Scavenging Ability

The hydroxyl radical scavenging assay was performed by the method described by Klein *et al.* [15]. The testas methanolic extracts and BHT were dissolved separately in DMSO and water. 1 ml ofeachextracts with various concentrations was mixed with 1 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.2% EDTA in a ratio of 1:1). After mixing, 0.5ml of 0.02% EDTA solution and 1ml of DMSO were added.

The reaction was initiated by adding BHT (0.2%) and incubated at 90^0 C in a water bath for 20 min. Then, 2ml of 15% TCA was added to the termination of the reaction. 2ml of Nash reagent (ammonium acetate (75g), 3mlof glacial acetic acid, and 2 ml of acetylacetone were mixed, and volume was made up to 1L with distilled water) was added and kept at room temperature for 20 min. The reaction mixture without the testa extracts served as control, and BHT was used as standard. All the experiments were done in triplicates. A UV-VIS spectrophotometer measured the absorbance of the reaction mixture at 412 nm against a blank solution. The percentage inhibition was calculated from the standard, and the IC_{50} value was determined.

Comment [A6]: Add data in result and discussioan

2.6 Determination of 2, 2– Azino-Bis-3-Ethylbenzothiazoline-6 Sulphonic Acid (ABTS) Scavenging Ability

The ABTS radical scavenging ability of *Cola acuminata* and *Cola nitida*fruittestas extracts were determined by method used by Zhou *et al.*[16]. A solution of ABTS radical cation was prepared by reacting 7mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) at room temperature in the dark for 16 hours. The mixture was then diluted with deionized water to obtain an absorbance of 0.700 ± 0.005 at wavelength of 734nm. The sample solution (100µl) at a range of concentrations was mixed with the $ABTS^+$ solution (100µl) in 96-well plates. After incubation at 30 ^oC for 30 min in the dark, the absorbance at wavelength of 734 nm was immediately recorded. BHT was used as control. The activity of $ABTS^+$ radical scavenging was calculated.

2.7 Determination of Total Antioxidant Capacity

The total antioxidant capacities of the testas were determined using the thiocyanate method [17]. Briefly, 10mg of the methanolic extract of the *Cola acuminata* and *Cola nitida* fruit testas were dissolved in 10ml ethanol. Then, 100 or 250µg/ml of each testa extract or a standard sample in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5ml of linoleic acid emulsion in

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potassium phosphate buffer (0.04 M, pH 7.0). Alternatively, a 5ml control solution was prepared consisting of 2.5ml linoleic acid emulsion and 2.5ml potassium phosphate buffer (0.04 M, pH 7.0). Each solution was incubated at 37 °C in a glass flask in the dark. After reaction with $FeCl_2$ and thiocyanate, the peroxide values were determined at 3 minutes intervals by reading the absorbance at 500nm in a spectrophotometer. During the linoleic acid oxidation, peroxides form and oxidize Fe^{2+} to Fe^{3+}. The Fe^{3+} ions form a complex with SCN^- which has a maximum absorbance at 500nm. Therefore high absorbance indicates high linoleic acid oxidation. The solutions without extract were used as blanks. All antioxidant activity data were reported as the average of triplicate analyses. The inhibition of lipid peroxidation in % was calculated by following equation:

% Inhibition = 100 – [(A_1/A_0) X 100]

Where; A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the testa extract.

2.8 Statistical Analysis

All the experimental results were expressed as mean ± SEM of three replicates. Data were evaluated using one way variance analysis. *P* values< 0.05 were regarded as significantly different.

3.0 RESULT AND DISCUSSION

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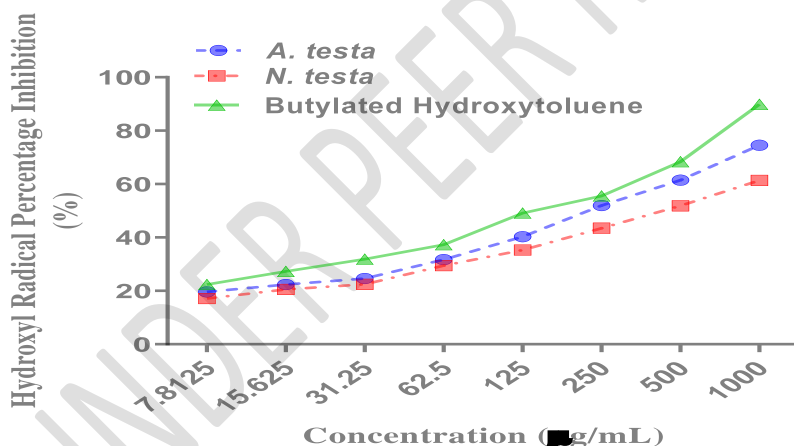

Figure 1: *In-vitro* Hydroxyl Radical Scavenging Ability of testas of *Cola acuminata* and *Cola nitida* Fruits

Values are expressed as mean of three replicates ± Standard Error of Means

Key: A. testa = Cola acuminata seed testa

N. testa = Cola nitida seed tetsa

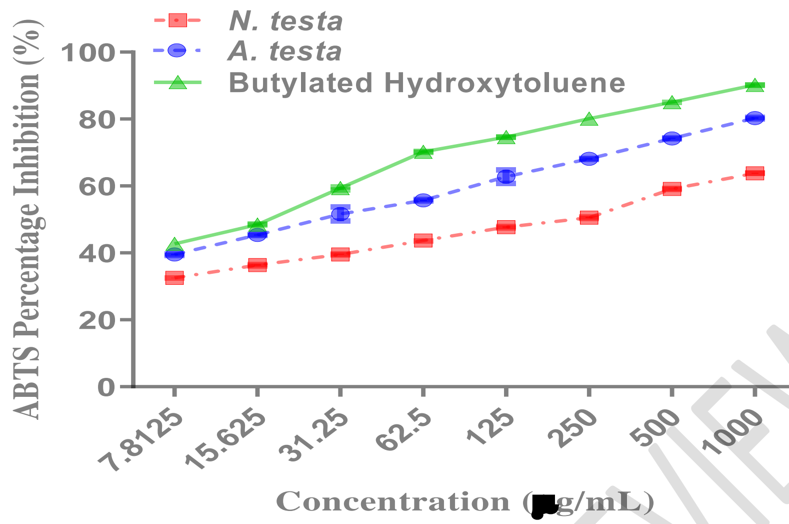

Figure 2: ABTS (2,2^{1}-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) Scavenging Ability of testas of *Cola acuminata* and *Cola nitida* Fruits

Values are expressed as mean of three replicates ± Standard Error of Means

Key: A. testa = Cola acuminata seed testa
N. testa = Cola nitida seed tetsa

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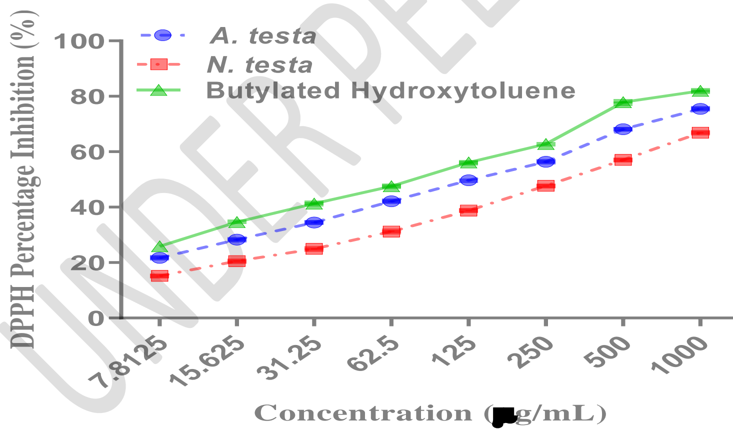

Figure 3: DPPH (2, 2-diphenyl-2-picrylhydrazyl) Scavenging Ability of testas of *Cola acuminata* and *Cola nitida* Fruits

Values are expressed as mean of three replicates ± Standard Error of Means

Key: A. testa = Cola acuminata seed testa
N. testa = Cola nitida seed tetsa

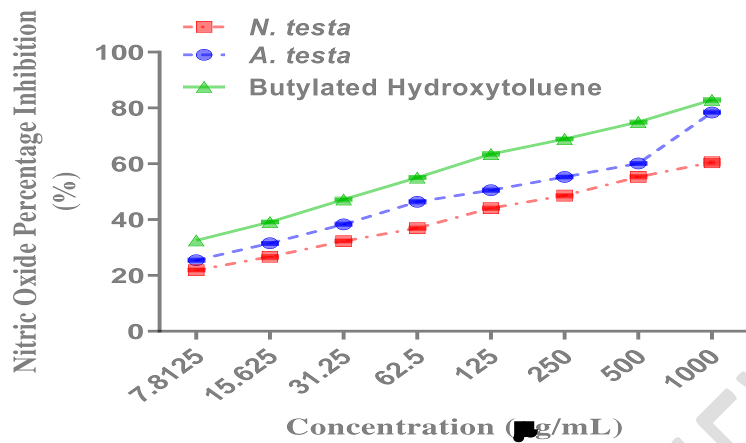

Figure 4: Nitric Oxide Scavenging Ability of *Cola acuminata* and *Cola nitida* Fruits

Values are expressed as mean of three replicates ± S.E.M

Key: A. testa = *Cola acuminata* seed testa

N. testa = *Cola nitida* seed tetsa

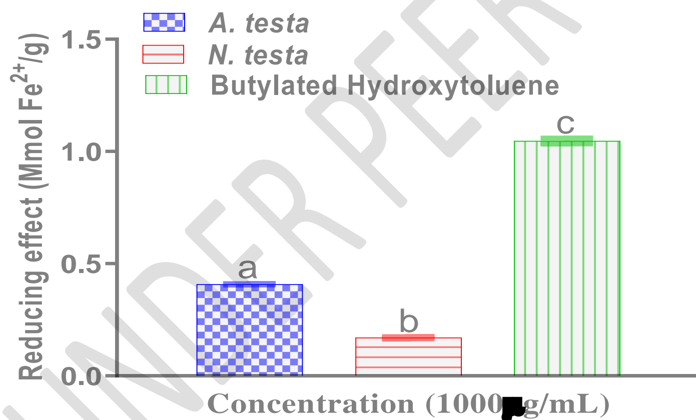

Figure 5: Ferric Reducing Ability of testas of *Cola acuminata* and *Cola nitida* Fruits

Values are expressed as mean of three replicates ± S.E.M and bars with different alphabets are significantly different (P<0.05) from each other.

Key: A. testa = *Cola acuminata* seed testa

N. testa = *Cola nitida* seed tetsa

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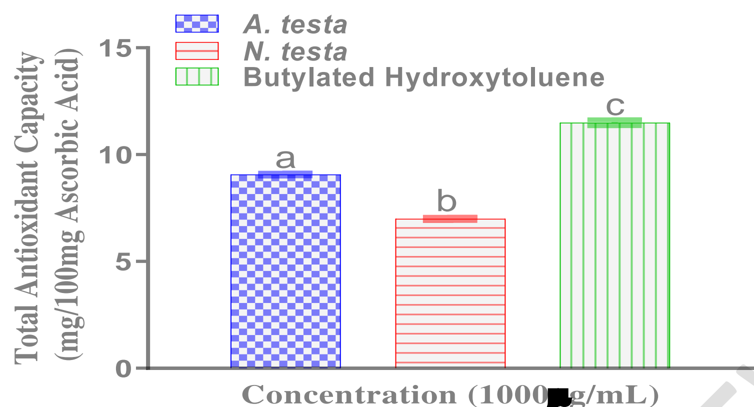

Figure 6: Total Antioxidant Capacity of testas of *Cola acuminata* and *Cola nitida* Fruits

Values are expressed as mean of three replicates ± S.E.M and bars with different alphabets are significantly different (P<0.05) from each other.

Key: A. testa = *Cola acuminata* seed testa
N. testa = *Cola nitida* seed tetsa

The antioxidantparameters;*in-vitro* inhibitory capacity of the testas of *C. acuminata* and *C. nitida* fruitson Hydroxyl Radical, ABTS (2,2[1]-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2, 2-diphenyl-2-picrylhydrazyl) and Nitric Oxideinvestigated in this study were found to have a correlating progression with that of the Butylated Hydroxytoluene standardlikewise their ferric reducing and total antioxidant capacities. Although the testasinhibitory effects on these parameters are not significantly as high as that of the standard Butylated Hydroxytoluenebut testa of *C. acuminata* showed significant increase in all the parameters assayed for when compared with that of *C. nitida*. Hydroxyl radical is important reactive oxygen specie which can react with some other biomolecules such as polyunsaturated fatty acid units in phospholipids of the cell membrane subsequently leading to cell damage and responsible for lipid peroxidation, protein oxidation and disruption of the DNA [18][19]. The hydroxyl radical scavenging power of these testas significantly increases with increase in their concentrations.

ABTS is commonly known for its use by many food and agricultural research based industries to estimate the antioxidant potentials of foods because the radical cation reacts to most antioxidants such as phenolic, thiol and vitamin C also for determining the antioxidant capacity of substances such as phenolics, carotenoids e.t.c[20][21][22].DPPH is one of the most important molecules for the in vitro antioxidant assays. It is known to have hydrogen acceptor capability. The hydrogen atom donating capability of the extracts of the testas was estimated by the decolorization of methanol solution of DPPH. Nitric oxide on the other hand is important for the physiologic role it plays in the generation of reactive nitrogen species which is known for its cell injury or death causing ability by induction of nitrosative stress [23]. Nitric oxide reacts with superoxide anion radical (O_2^{*-}) to produce peroxynitrite anion ($ONOO^-$) a Reactive Nitrogen Species.The inhibitory effect of the testas of both *C. acuminata* and *C. nitida* on the hydroxyl radical, DPPH, ABTS and Nitric oxide compared

Comment [A7]: Include quantitative data, Each method must use a standard antioxidant

Figure are not referenced in the discussion

Comment [A8]: It should be discussed the chemical composition that has been found from each of these species

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favourably well with the results of some of the positive antioxidant properties as reported by Olorunfemi *et al*.[24] and Sudha *et al*.[25].

Comment [A9]: Compare the results quantitatively

4.0 CONCLUSION:

From the present study, the antioxidant properties of the extracts of *C. acuminata* and *C. nitida* fruit testas showed a positive correlation with the standard they were compared with which is the butylated hydroxyltoluene. This may be attributed to strong hydrogen donating ability and scavenging ability of the testas on free radicals which are important precursors for the generation of reactive oxygen species and reactive nitrogen species. Hence, these testas can serve as potential precursor for discovery of novel drugs in the pharmaceutical industry.

Comment [A10]: not clear, more clarified whether the meaning here is that the activities are equivalent, higher or weaker than the standard.

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