# PHYTOCHEMICAL SCREENING AND INVITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF SELECTED NIGERIAN VEGETABLES

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## ABSTRACT

Antioxidants are substances known to protect the body from damage caused by reactive oxygen species induced oxidative stress. Studies have shown that the consumption of vegetables is capable of inhibiting the damaging effect of free radical in the body. Hence in this study, the invitro antioxidative properties of methanolic extract of *Adansonia digitata*(AD), *Celosia argentea var argentea (*CE), *Corchorus olitorius L.(*CO*), Gnetum africanum(GA), Gongronema latifolium(GL), Hibiscus sabdariffa(HS), Moringa oleifera(MO), Piper guineense(PI), Sesamum radiatum(SR), Solanum melongena(SG) and Pterocarpus mudbraedii (PM)* were investigated. Qualitative and quantitative phytochemical screenings of the vegetable extracts were determined using Standard method and the antioxidative activity was assessed using I,1-diphenyl-2-picrylyhydrazyl(DPPH) and reducing power method . The results obtained showed the presence of alkaloids, saponins, tannins, total phenols and flavonoids in all vegetable extracts. Methanolic extract of *Pteocarpus mildbraedi* showed a significantly high ($P < 0.05$) total phenol and tannin content of 499.78± 1.80mg/g and 466.23± 6.30mg/g respectively. *Corchorius olitorius* extracts had the most significant amount of flavonoids of 157.38mg/g when compared to other extracts and this was followed closely by *Pteocarpus mildbraedi extracts* with a value of 127.88±0.13mg/g. All vegetable extracts scavenge 1, 1, Diphenyl-2-picrylhydrazyl (DPHH) free radical scavenging activity in a dose dependent manner. *Pteocarpus mildbraedi* and *Sessasum radiatum was* observed to have the most significant DPPH scavenging activity and reductive potential compared to other extracts. These vegetables can be considered as good source of pharmacologically important phytoconstituents which possess strong antioxidant activities and can be harnessed in the prevention and treatment of degenerative diseases.

**Keywords:** Antioxidants, Free radicals; Phytochemicals; Reducing power.

## INTRODUCTION

Free radicals and reactive oxygen species are constantly formed in the human body during normal cellular metabolism for instance during energy production in the mitochondria electron transport chain, phagocytosis, arachidonic acid metabolism, ovulation, fertilization and in xenobiotic metabolism ( Halliwell & Gutteridge, 2007). They are also produced from external sources such as food, drugs, smoke and other pollutants from the environment (Miller, & Britigan, 1997). Organisms are endowed with endogenous (such as catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous (vitamin C, E, B-carotene) antioxidant defense systems which are capable of countering against the adverse reactions of free radicals (Sivakrishnan & Kottai Muthu (2013). The generation of free radicals in the body beyond its antioxidant capacity actually leads to oxidative stress and this seems to be the

apparent fundamental mechanism underlying a number of disorders (Hamzah, *et al*. 2013). As a result of this much attention is being focused on the use of antioxidants to inhibit and protect damage due to free radicals and reactive oxygen species. Synthetic antioxidant such as butylated hydroxyanisole (BHA), tert-butylated hydroxyquinone and butylated hydroxytoluene have been of utmost concern to many researchers because of their adverse side effects (Atiqur, *et al*.2008)). Therefore the current trend in drug discovery is to search for natural antioxidant with better safety than the orthodox drugs for the prevention and treatment of diseases arising from oxidative stress (Ashokkumar *et al*. 2008; Veerapur, *et al.* 2009).

Many vegetables are recognized as sources of natural antioxidants like phenolic acids, tannins, flavonoids and other metabolites [4]. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Sala *et al*. 2005; Rice-Evans, 1995). Thus the ingestion of many of these vegetables thus can play an important role in the chemoprevention of diseases that have their etiology and pathophysiology in reactive oxygen species (Atawodi, 2005; Dragland *et al.* 2003; Odukoya, *et al.* 2005). Therefore the focus of this study is to elucidate the phytochemical constituents and antioxidant activity of some uncommon vegetables in order to obtain readily available and safe antioxidants for subsequent prevention and treatment of diseases arising from oxidative stress.

## MATERIALS AND METHODS

### Collection of Plant Material

The following vegetables Adansonia digitata(AD), Celosia argentea var argentea (CE), Corchorus olitorius L.(CO), Gnetum africanum(GA), Gongronema latifolium(GL), Hibiscus sabdariffa(HS), Moringa oleifera(MO), Piper guineense(PI), Sesamum radiatum(SR), Solanum melongena(SG) and Pterocarpus midbraedii (PM) were purchased from Kure market, minna, Niger State, Nigeria. They were identified at the Biological Science Department of Federal University of Technology, Minna, Nigeria.

### Plant Preparation and Extraction

The vegetables were picked to remove debris, cut into small pieces and air dried at room temperature with adequate ventilation and pulverized using a blender. The pulverized samples were extracted with methanol by reflux. Exactly 50g of the powdered samples were weighed into 400ml of methanol in a reflux flask and refluxed for 2 hours. The extracts were filtered hot using a muslin cloth and subsequently evaporated using a rotary evaporator. The semi- dry extracts were weighed, placed in sterile sample bottles and stored in a refrigerator until required for use.

### Qualitative Phytochemical Screening

The extracts were screened for phytochemical properties using standard methods (Sofowora, 1993).

**Quantitative Determination of the Chemical Constituents in Samples**
**Total flavonoids determination**

Aluminum chloride colorimetric method was used for flavonoid determination (Chang et al. 2002). Each plant extracts (0.5 ml) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M sodium acetate and 2.8 ml of distilled water. This was left at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a a double beam Perkin Elmer (Waltham, MA, USA) UV-visible spectrophotometer.The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg ml-1 in methanol.

**Determination of total phenol content**

The total phenol content of the extracts was determined using the method reported by Singleton *et al.* ( 1999). Appropriate dilutions of the extracts (0.5ml) was oxidized with 2.5mL of 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated using gallic acid as standard.

**Determination of Alkaloids**

Exactly 0.5 g of the sample was dissolved in 5ml of 96% ethanol -20% $H_2$ $SO_4$ (1:1). Then 1 ml of the filtrate was added to 5 ml of 60% tetraoxosulphate (VI) and allowed to stand for 5 min. Then, 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was then taken at absorbance of 565 nm (Oloyed, 2005). Vincristine was used as the standard for the preparation of standard curve.

**Saponin Determination**

Exactly 0.5 g of the extract was added to 20 ml of 1NHCl and boiled for 4 h. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. Five milliliter of acetone ethanol was added to the residue and 0.4mls of each taken into 3 different test tubes. Ferrous sulphate reagent (6ml) was then added each followed by 2 ml of conc $H_2SO_4$. This was thoroughly mixed after 10 min and the absorbance taken at 490 nm (Oloyed, 2005). The absorbance of saponin standard solution was read after color development at same wavelength of 490nm.

**Tannin Determination**.

Sample (0.2g) was measured into a 50ml beaker. Then 20ml of 50%methanol was added and covered with para film and placed in a water bath at 77-80$^o$ for one hour. It was shaken thoroughly to ensure a uniform mixing .The extract was quantitatively filtered using a double layered whatman NO 41 filter paper into a 100ml volumetric flask. 20mls of water, 2.5 ml folin-Denis reagent and 10ml of 17% $Na_2CO_3$ was then added and mixed properly. The mixture was then made up to mark with water, mixed well and allowed to stand for 20min.A bluish-green

colour was developed at the end of range 0-10ppm.The absorbance of tannin acid standard solution as well as sample shall be read after color development on a spectrophotometer at wave length of 760nm ( AOAC,1984 ).

**In vitro Antioxidant Determinations**
**Free radical scavenging activity by DPPH method**

The free-radical-scavenging ability of the extracts against DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al.* (1999). Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals; the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free-radical-scavenging ability was subsequently calculated. Methanol was used to blank the spectrophotometer while the control contained 1ml of methanol and 1ml of DPPH. DPPH scavenging activity was calculated as

$$\% \text{ scavenging activity} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

**Determination of reducing property**

The reducing property of the extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution as described Oyaizu (1987). In total, 2.5 ml aliquot was mixed with 2.5 ml 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50$^{o}$C for 20 min and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min when necessary. Five milliliters of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm and the ferric reducing antioxidant property was subsequently calculated.

**Statistical Analysis**

All values were expressed as Mean±SEM. Statistical analysis will be performed by one-way Analysis of Variance (ANOVA) and individual comparisons of the group mean values will be done using Duncan test.

**RESULTS**
**Qualitative Phytochemical Screening**

Result of the qualitative phytochemical screening of the methanolic extract of selected Nigeria vegetables is given in Tables 1. Alkaloids, flavonoids, phenols, saponins and tannin were observed to be present in all vegetable extracts. Cardiac glycosides was observed to be present in all extracts except in *Adansonia digitata* and *Hisbiscus sadariffa* while terpenoids and steroids were found in all vegetable extracts except in *Gnetum africanum* and *Pterocarpus mildbraedi*. Phlobatannin was found present only in Pterocarpus mildbraedi while anthraquinone was found in the methanolic extract of *Pterocarpus mildbraed, Sesasum radiatum, Piper guineense* and *Gongronema latifolium* only.

**Table 1: Phytochemical constituents of Methanolic extract of selected Nigerian vegetables**

| Vegetables | Alkaloids | Anthraquinones | Cardiac glycosides | Flavonoids | Phenols | Phlobatannins | Saponins | Tannin | Terpenoids | Steroids |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Adasonia digitata*** | ++ | - | - | ++ | ++ | - | ++ | +++ | + | + |
| **Corchorus olitorius *L.(Tiliaceae*** | ++ | - | + | ++ | +++ | - | ++ | ++ | ++ | + |
| ***Celosia argentea var argentea*** ***(Amaranthaceae)*** | + | - | ++ | ++ | ++ | - | +++ | +++ | + | + |
| ***Gnetum Africanum*** | +++ | - | +++ | ++ | +++ | - | ++ | ++ | - | - |
| ***Gongronema latifolium*** | +++ | + | ++ | ++ | ++ | - | +++ | + | + | +++ |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Hibiscus sabdariffa*** | +++ | - | - | ++ | +++ | - | ++ | ++ | ++- | +++ |
| ***Moringa oleifera*** | ++ | - | +++ | ++ | +++ | - | ++ | ++ | + | ++ |
| ***Piper guineense*** | ++ | + | ++ | ++ | +++ | - | +++ | ++ | + | ++ |
| ***Sesamum radiatum*** | +++ | ++ | ++ | ++ | +++ | - | + | +++ | + | ++ |
| ***Solanum melongena*** | +++ | - | ++ | ++ | ++- | - | ++ | ++ | + | + |
| ***Pteocarpus mildbraedi*** | +++ | + | ++ | ++ | +++ | + | +++ | +++ | - | - |

KEY -Absent, +Faintly present, ++ Moderately present, +++ Highly present

## Quantitative Phytochemical Analysis

The quantitative phytochemical content determination of the methanolic extract of selected Nigeria vegetables result as given in Table 2 indicates that the alkaloid content in the extracts of *Piper guineense (*224.94±0.48 µg/g*), Hibiscus sabdariffa (*220.85±0.7 µg/g*), Pteocarpus mildbraedi (*209.50±0.20*)* and *Gnetum africanum* (200.58±1.2 µg/g) were significantly higher ($P < 0.05$) than other vegetable extracts .On the other hand, the saponin content in *Pteocarpus mildbraedi* extract *(*106.20±4.61mg/g) was significantly higher ($P < 0.05$) than that of other vegetables. Also methanolic extract of *Pteocarpus mildbraedi* showed a significantly high ($P < 0.05$) total phenol and tannin content of 499.78± 1.80mg/g and 466.23± 6.30mg/g respectively. *Corchorius olitorius* extracts had the most significant amount of flavonoids of 157.38mg/g when compared to other extracts and this was followed closely by *Pteocarpus mildbraedi extracts* with a value of 127.88±0.13mg/g.

**Table 2: Quantitative Phytochemicals of methanolic extracts of Selected Nigeria Vegetables**

| Phytochemicals | *Adasonia digitata* | Corchorus olitorius *L* | *Celosia argentea var argentea* | *Gnetum africanum* | *Gongronema latifolium* | *Hibiscus sabdariffa* | *Moringa oleifera* | *Piper guineense* | *Sesamum radiatum* | *Solanum melongena* | *Pteocarpus mildbraedi* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Alkaloids (µg/g)** | 81.56±0.56$^{g}$ | 68.65±2.05$^{f}$ | 45.26±00$^{e}$ | 200.58±1.26$^{m}$ | 146.36±6.04$^{k}$ | 220.85±0.75$^{o}$ | 84.63±2.19$^{g}$ | 224.94±0.48$^{o}$ | 116.81± 2.25$^{h}$ | 175.61±4.89$^{l}$ | 209.50±0.2$^{n}$ |
| **Saponins (mg /g)** | 16.59±1.85$^{c}$ | 22.17±0.24$^{e}$ | 14.05±1.70$^{b}$ | 99.60±4.80$^{i}$ | 18.69±1.01$^{d}$ | 54.80±1.15$^{g}$ | 9.45± 1.69$^{a}$ | 106.45±1.21$^{j}$ | 33.07±0.6$^{f}$ | 95.18±1.58$^{h}$ | 106.20±4.6$^{j}$ |
| **Tannins (mg /g)** | 311.98±0.01$^{f}$ | 287.07±0.16$^{ef}$ | 103.02±0.60$^{a}$ | 143.67±0.50$^{ab}$ | 163.48±0.00$^{abc}$ | 402.19±0.0$^{g}$ | 191.00±0.0$^{b}$ $^{cd}$ | 233.50±0.50$^{de}$ | 213.12±0.02$^{cd}$ | 175.36±0.80$^{bcd}$ | 466.23±6.2$^{h}$ |
| **Flavonoids (mg /g)** | 25.38 ±2.88$^{a}$ | 157.38±0. 38$^{f}$ | 47.88±9.13$^{bc}$ | 91.75±3.50$^{d}$ | 51.8750± 3.63$^{c}$ | 87.00± 6.25$^{d}$ | 34.16±3.79$^{b}$ | 85.41±2.28$^{d}$ | 48.50±0.25$^{bc}$ | 85.33±5.92$^{d}$ | 127.88±0.1$^{e}$ |
| **Phenols(mg /g)** | 170.90±0.68 $^{a}$ | 330.07±0.32$^{cd}$ $^{e}$ | 212.16±4.30$^{ab}$ | 272.47±3.11$^{bc}$ | 186.60±5.37$^{a}$ | 388.46±70$^{e}$ | 366.66±4.9$^{d}$ | 319.17±2.96$^{cd}$ | 273.32±8.02$^{bc}$ | 178.74±6.85$^{a}$ | 499.78±1.8$^{f}$ |

Results are presented as mean ± SEM. Letters represent the level of significance

## Antioxidative Activities of Selected Nigeria Vegetables

### Diphenyl picryl Hydrazyl radical Scavenging Activities

Scavenging activity result is shown in Figure 1 and is expressed as percentage of inhibition of DPPH free radical. DPPH Scavenging acitivities of the selected vegetables shows that *extract of Pteocarpus mildbraedi and Sesasum radiatum* has a stronger scavenging activity than all other extracts at all concentrations (Figure 1). It was also shown that scavenging activity occurs in a dose-dependent manner.in all *the selected indigenous vegetable and* the standard (gallic acid).

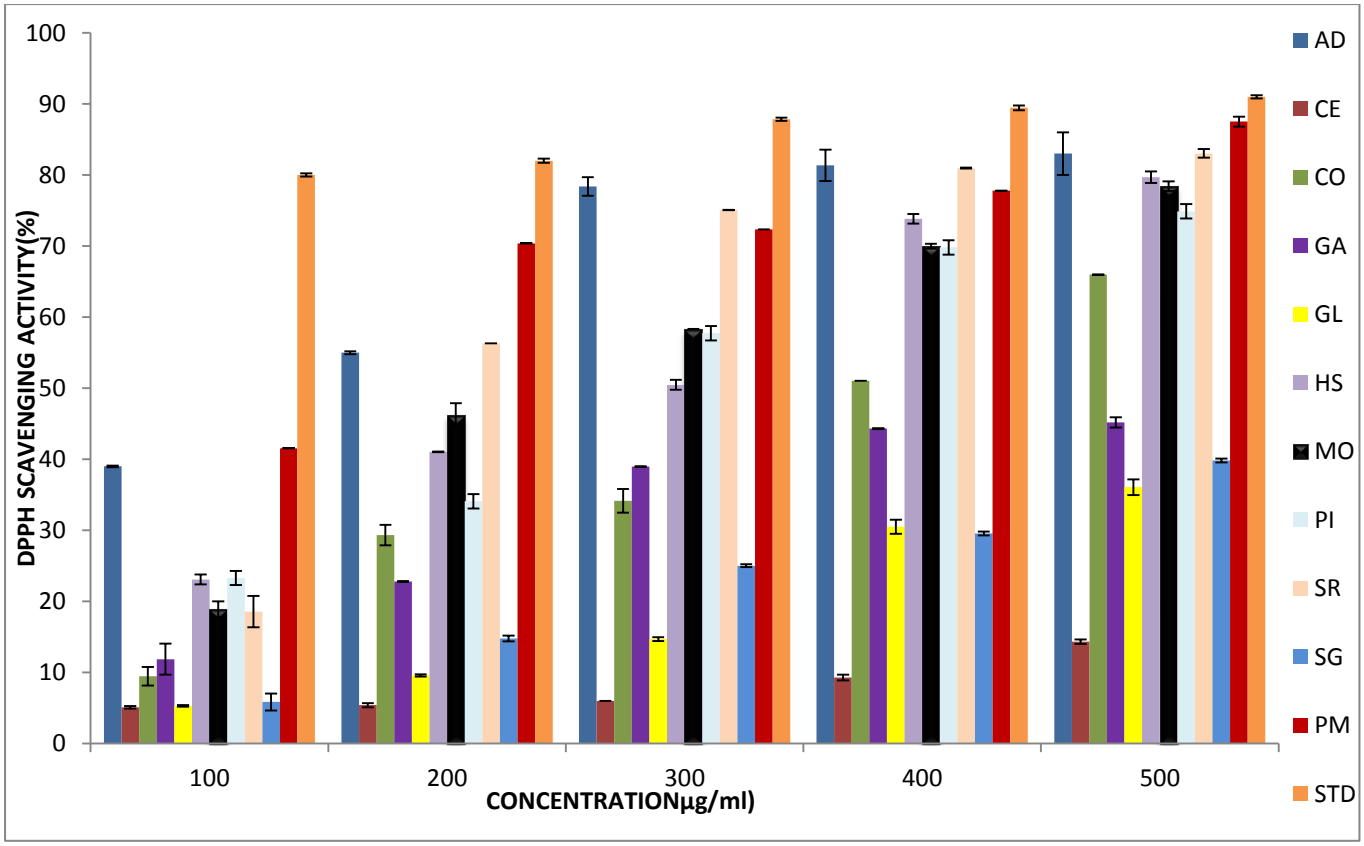


**Figure 1: DPPH Scavenging Activity of methanolic extract of selected Nigerian vegetables**

### Reductive Power

The reductive power of the vegetable extracts increases as the concentration increases however *Pteocarpus mildbraedi* extract exhbited better ($P < 0.05$) reductive potential at all concentrations when compared to other vegetables extracts (Figure 2).

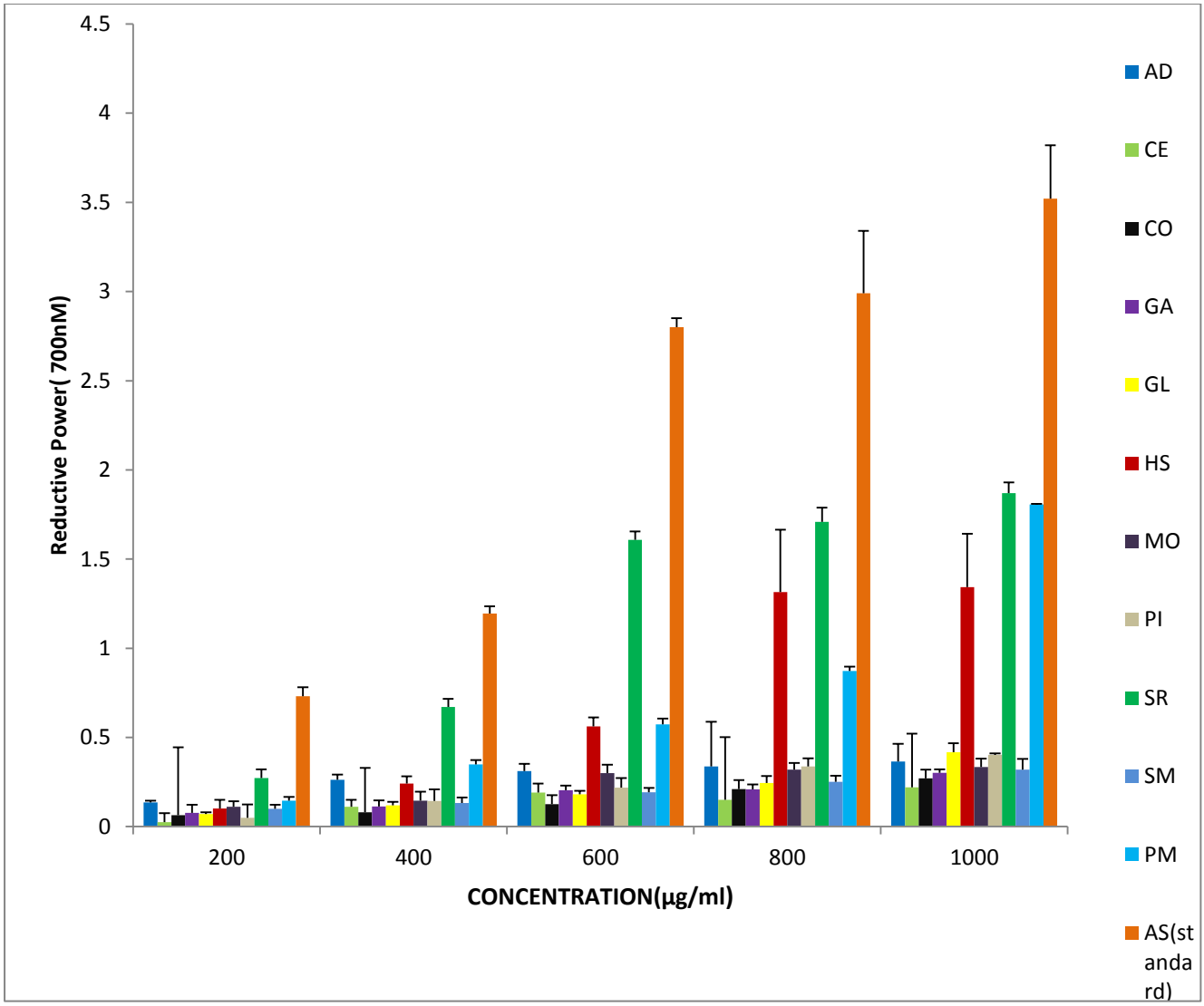


**Figure2: Reductive power of the Methanolic extract of selected Nigerian Vegetables**

## DISCUSSION OF RESULTS

The result obtained from the qualitative phytochemical screening of methanolic extracts of selected indigenous vegetable showed the presence of tannin, saponin, cardiac glycoside, alkaloids, steroid, anthraquinone, terpene, phenol, and flavonoid in varying concentration (Table 1).

Although the specific roles of these phytochemicals were not investigated in this study, it has been reported that most active ingredients in plants and vegetables are frequently saponins, tannins, alkaloids, flavonoids and phenols and these may be responsible for many of the pharmacological actions of such plant and vegetable.

Specifically Phenolic compounds have been reported to serve as antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti-cancer, ant-iinflammatory and diabetes (Hamzah *et al.* 2013b; Nagavani *et al.*, 2010).

Flavonoids are one of the most diverse and widespread group of natural compounds and it has been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergenic, antiviral, antiinflammatory, and vasodilating actions (Parajuli *et al*. 2012, Pereira *et al*., 2009). The antioxidant properties of flavonoid depend on their structure particularly hydroxyl position in the molecule and their ability as electron donor to free radical (Odukoya, *et al.* 2005). Thus the methanolic extract of the studied vegetables may be a good alternative for the treatment of diseases associated with excessive free radical generation.

Methanolic extract of *Pteocarpus mildbraedi* showed a significantly high ($P < 0.05$) total phenol and tannin content of 499.78± 1.80mg/g and 466.23± 6.30mg/g respectively. *Corchorius olitorius* extracts had the most significant amount of flavonoids of 157.38mg/g when compared to other extracts and this was followed closely by *Pteocarpus mildbraedi extracts* with a value of 127.88±0.13. The results of these phytochemical especially that of flavonoid and phenolic content was shown to be higher than that found in some previously studied plants (Omoregie & Osagie 2012; Amir *et al*. 2012]. Phenols and flavonoids which are important plant constituents with radical scavenging ability have been shown to contribute directly or indirectly to the antioxidative action of such plants (Keleş *et al.*, 2012). The high content of total phenols and flavonoids contents in the *Pteocarpus mildbraedi* and *Sesasum radiatum* extracts might account for the better results found for their antioxidant activity.

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh *et al.*, 2008). DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. DPPH scavenging activities of the methanolic extract of the selected Nigeria vegetables (Fig. 1) showed that all the extract investigated scavenge DPPH radical in a dose dependent manner. That is the scavenging ability of all the sample investigated increases as their concentration increases (100-500/µg/ml). *Pteocarpus mildbraedi* (87.51 % at500/µg/ml) and *Sesasum radiatum* (83.04% at 500/µg/ml) had a significantly higher percentage of DPPH scavenging activity when compared with other extracts amongst the vegetable. The DPPH scavenging activity of these extracts is higher than that of most commonly consumed vegetables and fruits investigated (Kim *et al.* 2003). The result of this study also correlates to that obtained in a recent study on DPPH scavenging ability of some vegetables (Azeez *et al.* 2012). This significant scavenging ability in the above extracts could be attributed to the high amount of phenolic and flavonoids in these extracts. Thus these vegetables can be use as cheap source of antioxidants for the prevention of free radical associated diseases.

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action of Phenolics (Nabavi *et al.* 2009). In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of $Fe_3^+$ to $Fe_2^+$ by donating an electron. Amount of $Fe_2^+$ complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. It was found that the reducing powers of all the studied extracts increased with increase in their concentrations. Amongst these vegetables, the reducing power was found to be highest in *Sesasum radiatum* (Figure 2) which was significantly followed by *Pteocarpus mildbraedi* and *Hisbiscus sabdariffa.* In this study a positive relationship between reducing power and the amount of phenolic

compounds was observed for the vegetable *Pterocarpus Mildbraedi*. Thus for this extract, the total phenol content might be the major contributor to their antixodant properties via the reductive mechanism. However for other vegetables, the highest amount of flavonoid and phenol does not necessarily corresponds to the reducing potential of such extracts. Therefore for those extracts other non-phenolic compounds may be responsible for their antioxidant activities.

This study showed that the selected vegetables generally could be potential source of natural antioxidants that could be used as therapeutic agent in preventing or treatment of degenerative diseases associated with oxidative stress. Specifically *Pterocarpus Mildbraedi* and *Sesasum radiatum* exhibited better free radical scavenging ability and reductive power. Isolation and characterization of bioactive compounds responsible for these activities is ongoing in our Laboratory.

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