

## Comparative Studies on the Antioxidant Potential of Three Medicinal Plants Commonly Used in the Treatment of Haemorrhoids

Salawu Sule Ola<sup>1\*</sup> Eseigbe Mercy Imade<sup>2</sup> Ige Joseph Kade<sup>1</sup> Adeniyani Oluwafisayo Opeoluwa<sup>1</sup> Ogunsina Olabode Isaiah<sup>1</sup>

1. Department of Biochemistry, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria.
2. Department of Basic Science, Edo State College of Agriculture, Agenebode Campus. Edo State, Nigeria.

\* E-mail of the corresponding author: [sosalawu@yahoo.com](mailto:sosalawu@yahoo.com)

### Abstract

The present study sought to evaluate the antioxidant properties of stem barks of *Anogeissus leiocarpus*, *Axonopus compressus* and *Senna fistula* that have been previously reported to be effective in the treatment of haemorrhoids. The amount of antioxidant agents such as phenols and flavonoids were determined in the methanolic extracts of their stem barks. In addition, the possible antioxidant mechanisms of the extracts were assessed by measuring their reducing property, iron (II) chelating ability, hydroxyl radical scavenging ability, ABTS radical scavenging ability and their ability to scavenge 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radicals. The results show that extracts of *Anogeissus leiocarpus* have a total phenolic content of (11.525±0.061 µg/mg), *Axonopus compressus* (12.080±0.041 µg/mg) and *Senna fistula* (12.813±0.054 µg/mg). The highest level of total flavonoids were obtained in the extracts of *Anogeissus leiocarpus* (1.321±0.034 µg/mg) followed by *Senna fistula* (1.127±0.034 µg/mg) and *Axonopus compressus* (0.681±0.034 µg/mg). The highest DPPH radical scavenging activity was found in *Anogeissus leiocarpus* while the least value was recorded for *Senna fistula*. In addition, extracts of *Anogeissus leiocarpus* was recorded to have the highest hydroxyl scavenging activity. Furthermore, ABTS radical scavenging activities of the three plants revealed that extracts of *Axonopus compressus* had the least scavenging activity. Taken together, we conclude that since extracts of stem barks of the three plants exhibited potent antioxidant potentials and haemorrhoids is intrinsically linked with oxidative stress, then *Anogeissus leiocarpus*, *Axonopus compressus* and *Senna fistula* possibly exerts their antihemorrhoidal action using a combination of mechanisms and their antioxidant potency possibly plays a major role in ameliorating secondary complications resulting from oxidative damage in haemorrhoids.

**Keywords** – Antioxidant, Haemorrhoids, Medicinal plants, Oxidative stress, Treatment

### 1. Introduction

Haemorrhoids or piles are a common ailment among adults. More than half of men and women aged 50 years and older will develop haemorrhoid symptoms during their lifetime (Madoff *et al.*, 2004). Haemorrhoids are rare in children but several reports state the occurrence of haemorrhoids in children, and in elderly people. Hospital based proctoscopy studies show prevalence rates of haemorrhoids with a symptomatic state in 86% of patient (Rutherford, 1999). Herbal treatments and nutritional therapy are safe and effective therapy for haemorrhoids and also varicose vein, although herbal treatments for haemorrhoids have been poorly researched. Several plant extracts have been shown to improve microcirculation, capillary flow, vascular tone, and strengthen connective tissue of the perivascular amorphous substrate.

One recent finding showed effects of plants taken orally for treatment of haemorrhoids, is due to the contribution of free radical scavenging properties to the pathogenesis of haemorrhoids and varicose veins (Madoff *et al.*, 2004). Plants have several properties which make them effective for the treatment of haemorrhoids, like antioxidant, anti-inflammatory, anti-oedema and hepatoprotective. Some plants, which were scientifically studied for their antihemorrhoidal properties include: *Ruscus aculeatus* (Butcher's Broom), *Collinsonia Canadensis* (Stone root), *Aesculus hippocastanum* (Horse Chestnut), *Hamamelis virginiana* (Witch Hazel), Onions, *Averrhoa bilimbi* (Cucumber tree), *Luffa acutangula* Lin (Luffa), White Oak, Barberry, Ampalaya (Bitter Melon), *Senna fistula* (Rangnekar *et al.*, 1974) and *Axonopus compressus*, *Anogeissus leiocarpus* (Gbedebo and Odukoya, 2011).

Medicinal plants have been a source of succor in the control of many diseases in developing countries and haemorrhoids is no exception (Olumide, 2008). In different parts of the world especially in Africa and Europe with high incidence of haemorrhoids, the people have learnt to manage the problem using plants which are God's gift of nature. Crude extracts from plants have been used in treating an array of diseases since ancient times although the bioactive components of such plants remain largely unknown (Sofowora, 1993). Most of the

plants used for medicinal purposes have been correlated to their possession of antioxidant activity (Middleton *et al.*, 2000).

*Anogeissus leiocarpus* commonly known as Ayin in Yoruba is a tall evergreen tree. It germinates mostly in wetlands, along river banks forming gallery forests. The bark of the tree is used as a human and livestock antihelmintic for treating worms, and for treatment of a couple of protozoa diseases in animals. The bark is used as decoction and also as a chewing stick in Nigeria for the treatment of haemorrhoids (Neuwinger, 2000).

*Axonopus compressus* is commonly called blanket grass, or broad leaf carpet grass (Sajise and Lales, 1974). The plant contains tannins and is considered an astringent that is able to draw tissues together (both internally and externally). As such, they can help to stop bleeding (including bleeding from mucous membranes) and control excess menstrual flow. It has also been used to relieve colitis, haemorrhoids, diarrhoea, dysentery, vomiting and bedwetting in children and incontinence in the aged. Most parts of *Axonopus compressus* such as the root, stem bark and seed oil have been used in folk medicine in Africa (Fahey, 2005). *Senna fistula* is widely grown as ornamental plant in tropical and subtropical areas. It contains elevated quantities of anthraquinones and consequently is mainly useful against gastrointestinal conditions and to stop bleeding particularly in haemorrhoids. Edeoga (2005) reported that the majority of the medicinal uses of *Anogeissus leiocarpus* are likely to be based on its tannin, saponin and lipid content with no event of toxicity at the pharmacological dose tested while Esuruoso (1971) reported no event of toxicity with *Axonopus compressus*.

However, despite the widely reported pharmacological relevance of *Anogeissus leiocarpus*, *Axonopus compressus* and *Senna fistula*, there is dearth of information correlating the observed antihemorrhoidal potentials of these three plants with their antioxidant properties. Hence, there is dire need to unravel the mechanisms by which these plants exert their pharmacological action. This study therefore aims to determine and compare the possible antioxidant mechanisms associated with the methanolic extracts of the stem bark of *Anogeissus leiocarpus*, *Axonopus compressus* and *Senna fistula*.

## 2. Materials and Methods

### 2.1 Chemicals

2-deoxyribose sugar, DPPH (1, 1-diphenyl-2-picrylhydrazyl) and 1, 10 phenanthroline were obtained from Fluka Chemie and Merck (Germany). All other chemicals from standard chemical suppliers and were of analytical grade.

### 2.2 Sample Collection and Identification

The stems of *Anogeissus leiocarpus*, *Axonopus compressus* and *Senna fistula* were obtained from Ilara-mokin, in Akure, Ondo State, Nigeria and were identified by Professor Olawoye of biochemistry department of Federal University of Technology, Akure, Ondo State, Nigeria.

### 2.3 Sample Preparation

The stems of the three plants were shade dried for about three weeks at room temperature after which they were pulverized with the aid of a blender, until a fine powdered sample was obtained.

### 2.4 Sample Extraction

100g of each powdered sample was extracted with mixture of methanol and water in ratio 4:1. The extracts were filtered and the solvent evaporated to give concentrated extract.

### 2.5 Antioxidant Properties

#### 2.5.1 Total Phenolic Content

The total phenolic content of the methanolic extracts was determined by Folin-Ciocalteu colorimetric method as described by McDonald *et al.*, (2001). Tannic acid standard solution was prepared by dissolving 0.05g of the dry tannic acid in 50ml distilled water in a standard flask. Concentration of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml tannic solution were prepared by serial dilution of the standard solution. 0.5ml methanolic phenol acid extract/tannic acid standard were added to 10ml distilled water. 2.5ml Folin-Ciocalteu was then added. The solution was then mixed and left for two minutes. 7.5ml of NaCO<sub>3</sub> solution (20g/100ml) was added and mixed vigorously and made up to volume with deionised water. The flask is allowed to stand for 2 hours; the absorbance is measured at 760nm using a spectrophotometer. The results were expressed as µg tannic acid equivalents per mg of the sample.

#### 2.5.2 Total Flavonoid Content

The total flavonoid is determined using slightly modified method reported by Chong *et al.*, (2002). 0.5ml of appropriately diluted plant extract was separately mixed with 50 µg of 10% aluminum chloride (AlCl<sub>3</sub>). 50 µl of 1M potassium acetate and 1.4ml distilled water and left at room temperature for 30 minutes to incubate. The absorbance of the reaction mixture was measured at 415nm using a spectrophotometer. The calibration curve was prepared by preparing quercetin solution 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml and the results were expressed as µg quercetin equivalents per mg of the sample.

### 2.5.3 DPPH Photometric Assay

The DPPH was determined by the method of Mensor *et al.*, (2009) with slight modification. Sample stock (0.004g/10ml) was diluted to final concentration of 200, 100, 50, 25, 12.5 µg/ml in methanol. 1ml portion of 0.3mM DPPH methanol solution was added to 1ml of sample solution of different concentration and allowed to react at 100m temperature. After 30minutes, the absorbance values were measured at 517nm using a spectrophotometer. The standard used was tannic acid and the results were expressed in percentage antioxidant activity

### 2.5.4 ABTS Radical Scavenging Assay

ABTS radical scavenging activity was determined using slightly modified method reported by Perlegrini *et al.*, (1999). ABTS radical cation was produced by reacting ABTS solution (8mM) with 3mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 hours. Trolox standard solution (1000 µg/ml) was prepared and serially diluted to obtain concentration of 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml. The ABTS radical cation solution (working solution) 2.9ml was added to each concentration of the Trolox stock solution (0.1ml) prepared and allowed to react for 30 minutes. The absorbance of both the standard and samples were measured at 734nm using a spectrophotometer and results were expressed in percentage antioxidant activity. A Trolox standard curve was obtained by plotting the absorbance against concentration.

### 2.5.5 Hydroxyl Radical Scavenging Assay

The ability of the extract to prevent Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> induced decomposition of deoxyribose is determined using the method of Halliwell and Gutteridge (1981). Each extract solution was serially diluted to concentration of 200 µg/ml, 100, 50, 25 and 12,5 µg/ml in 100% methanol. Mannitol used as standard was also prepared and diluted to the serial concentration as described for the plant extract solution 1.2ml of 0.1M phosphate buffer (PH7.4) was mixed with 7.5 µl of 20mM hydrogen peroxide, 30 µl of FeCl<sub>3</sub>, 45 µl of 1, 10-phenanthroline and 0.5ml extracts of different concentration and then left for 30minutes after which absorbance was read at 532nm using a spectrophotometer and results were expressed in percentage antioxidant activity.

### 2.5.6 Reducing Power Assay (FRAP)

The Fe<sup>3+</sup> reducing power of the extract was determined by the method of Oyizu (1989) with a slight modification. 0.5ml of different concentrations of the extract was mixed with 1.25ml phosphate buffer (0.2M, P<sup>H</sup> 6.6) and 1.25ml potassium ferricyanide (0.1%), followed by incubations at 50<sup>C</sup> in a water bath for 20mins. After incubation, 1.25ml of TCA (10%) was added to terminate the reaction. The upper portion of the solution (1.25ml) was mixed with 1ml distilled water, and 0.25ml FeCl<sub>3</sub> solution (0.01%) was added. The reaction mixture was left for 10mins at room temperature and the absorbance was measured at 700nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA) against an appropriate blank solution. A higher absorbance of the reaction mixture indicated greater reducing power. The standard used was ascorbic acid.

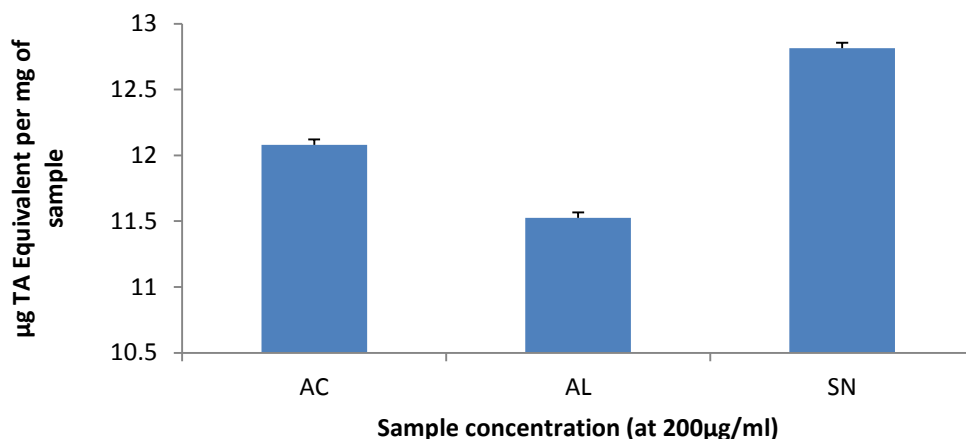
## 2.6 Statistical Analysis

All experiments were performed in triplicates. Analysis at every time point from each experiment was carried out in triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyzed using Microsoft Excel XP.

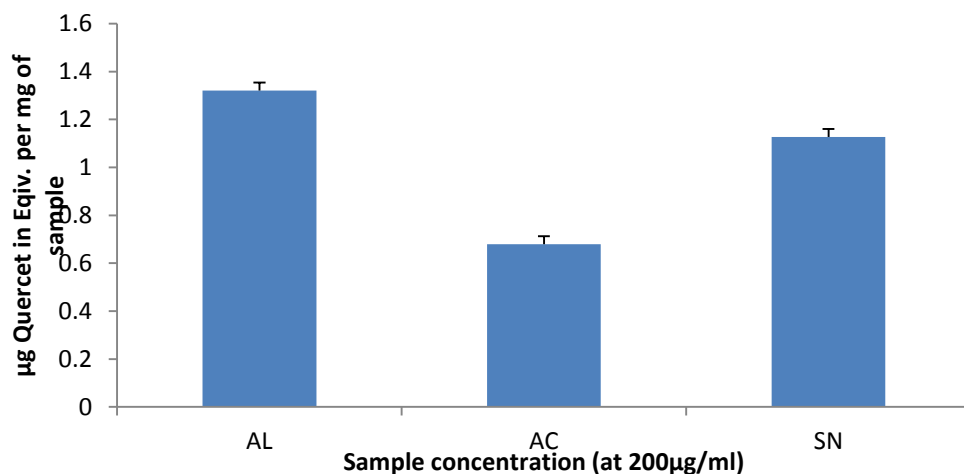
## 3. Results

### 3.1 Antioxidant Constituents

The total phenol content of each plant extract in tannic acid equivalent at concentration of 200 µg/ml is shown in Figure 1. *Senna fistula* had the highest total phenolic content; (12.813 ± 0.054 µg/mg) followed by *Axonopus compressus* (12.080 ± 0.041 µg/mg) and then *Anogeiessus leiocarpus* (11.525 ± 0.061 µg/mg). The result of the total flavonoid content (µg quercetin Equivalent /mg sample) of the study plants at concentration of 200 µg/ml is as shown in Figure 2. *Anogeiessus leiocarpus* has the highest flavonoid content (1.321 ± 0.034 µg/mg) and the least content is recorded for *Axonopus compressus* (0.681 ± 0.034 µg/mg).



**Figure 1 :** Total phenol content of the study plants in tannic acid equivalent. Reported values are the mean  $\pm$  S.D (n=3). AC= *Axonopus compressus*, AL= *Anogeiessus leiocarpus*, SN= *Senna fistula*



**Figure 2:** Total flavonoid content of the study plants in Quercetin Equivalent. Reported values are the mean  $\pm$  S.D (n=3). AC= *Axonopus compressus*, AL= *Anogeiessus leiocarpus*, SN= *Senna fistula*

### 3.2 Antioxidant Mechanisms

In order to better ascertain the antioxidant potentials of *Anogeiessus leiocarpus*, *Axonopus compressus* and *Senna fistula*, several antioxidant mechanisms such as reducing property, metal chelating ability, hydroxyl radical scavenging ability, ABTS radical scavenging ability and DPPH radical scavenging ability. In general terms, it is noteworthy that the all three extracts of stem barks of the plants exhibited potent antioxidant action in a concentration dependent manner.

#### 3.2.1 DPPH radical scavenging activities

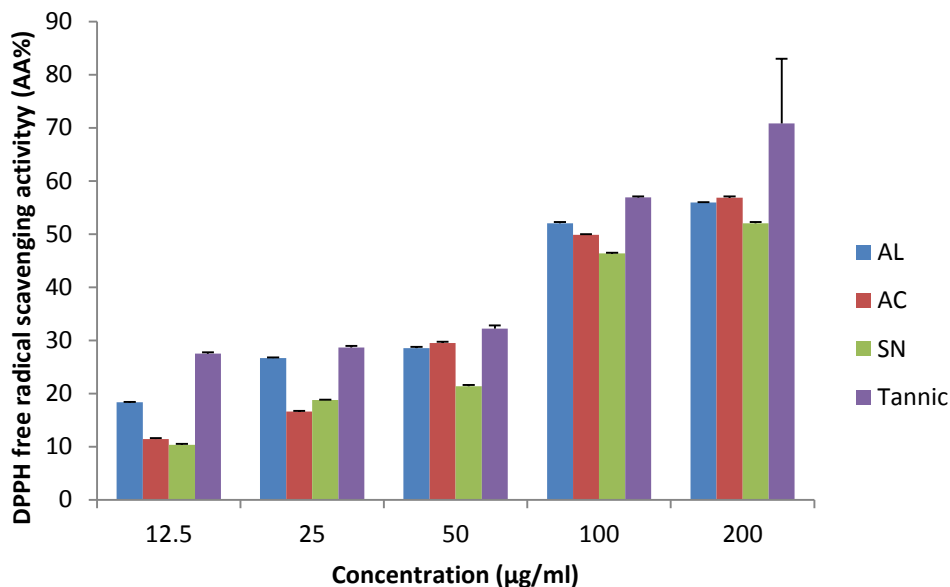
Figure 3 shows the DPPH free radical scavenging abilities of the extracts of the stem bark of *Anogeiessus leiocarpus*, *Axonopus compressus* and *Senna fistula*. One important routine *in-vitro* antioxidant parameter used for testing the potency of agents is their ability to scavenge 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radicals. The reaction involves protonation of the unstable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radicals turning it to stable diamagnetic molecule which is visually noticeable as a discoloration from purple to golden yellow. The effect of antioxidants on 1, 1-diphenyl-2-picryl hydrazyl (DPPH) is thought to be due their hydrogen donating ability (Baumann *et al.*, 1979). The result of the DDPH scavenging activity of *Axonopus compressus*, *Senna fistula*, and *Anogeiessus leiocarpus* at different concentration (12.5, 25, 50, 100, 200 µg/ml) is as presented in figure 3. *Senna fistula* is observed to have the lowest antioxidant activity at all concentrations (10.3%, 21.3%, 46.3%, and 52%) except at 25 µg/ml (18.8%) where it is higher than *Axonopus compressus* (11.4%).

#### 3.2.2 Ferric Reducing Property

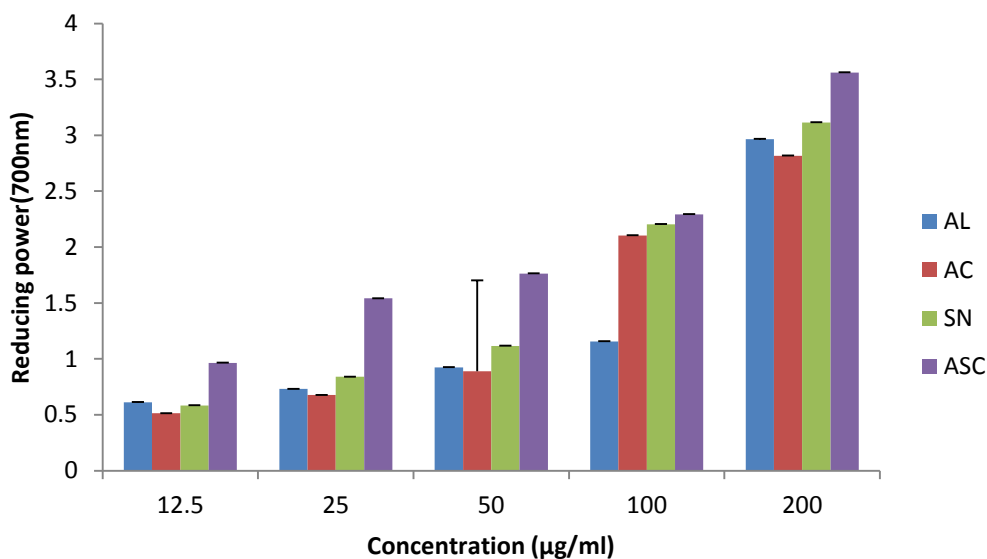
Ferric reducing antioxidant properties of *Senna fistula*, *Axonopus compressus* and *Anogeiessus leiocarpus* as shown in Figure 4 revealed that the plant extracts possess good antioxidant activity since there is little or no difference in the values obtained. The values obtained at concentration of (12.5, 25, 50, 100, 200 µg/ml) for *Senna fistula* is (0.583, 0.840, 1.116, 2.204, 3.114), for *Axonopus compressus* is (0.513, 0.676, 0.890, 2.104, 2.966) and for *Anogeiessus leiocarpus* is (0.612, 0.730, 0.924, 1.154).

### 3.2.3 Hydroxyl radical scavenging activities

Hydroxyl radical scavenging activity of the plant extracts is shown in Figure 5. *Axonopus compressus* has the highest hydroxyl scavenging activity (19.4%) at 12.5 µg/ml. *Anogeiessus leiocarpus* has the highest hydroxyl scavenging activity (73.7%) at 100 µg/ml and ( 74.9%) at 200 µg/ml. *Senna fistula* is also observed to have the highest hydroxyl scavenging activity (32.9%) at 25 µg/ml and ( 46.2%) at 50 µg/ml.



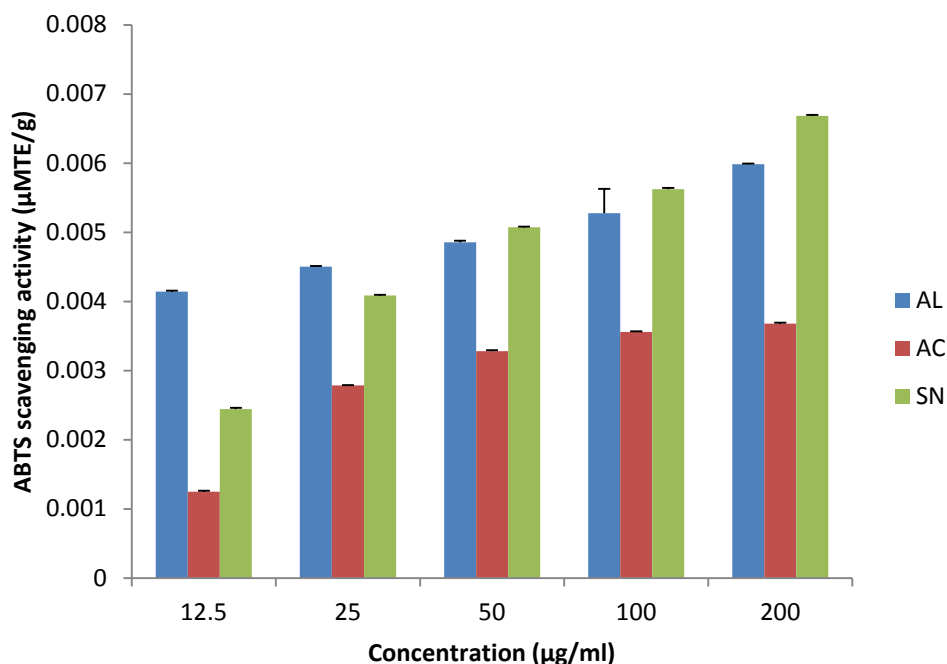
**Figure 3: DDPH scavenging activity of *Axonopus compressus*, *Senna fistula*, *Anogeiessus* and Tannic acid. Reported values are the mean ± S.D (n=3). AC= *Axonopus compressus*, AL= *Anogeiessus leiocarpus*, SN= *Senna fistula***



**Figure 4: Ferric Reducing Antioxidant Properties of the three plant extracts and Ascorbic acid. Reported values are the mean ± S.D (n=3). AC= *Axonopus compressus*, AL= *Anogeiessus leiocarpus*, SN= *Senna fistula*, ASC= Ascorbic acid**

### 3.2.4 ABTS radical scavenging activities

Figure 6 shows the ABTS radical scavenging activities of the three plant extracts. It was observed that *Senna fistula* had the highest ABTS radical scavenging activities at 200  $\mu\text{g/ml}$ , 100  $\mu\text{g/ml}$  and 50  $\mu\text{g/ml}$  (0.007  $\mu\text{MTE/g}$ , 0.006  $\mu\text{MTE/g}$ , 0.005  $\mu\text{MTE/g}$ ) respectively, followed by *Anogeiessus leiocarpus* with the highest ABTS scavenging activity at 12.5  $\mu\text{g/ml}$  and 25  $\mu\text{g/ml}$  (0.004  $\mu\text{MTE/g}$ , 0.005  $\mu\text{MTE/g}$ ), while the least activity was recorded for *Axonopus compressus* at all the selected concentrations (0.001  $\mu\text{MTE/g}$ , 0.003  $\mu\text{MTE/g}$ , 0.003  $\mu\text{MTE/g}$ , 0.004  $\mu\text{MTE/g}$ , 0.004  $\mu\text{MTE/g}$ ).



**Fig 6:** ABTS radical scavenging activity of *Anogeiessus leiocarpus*, *Senna fistula* and *Axonopus compressus*. Reported values are the mean  $\pm$  S.D (n=3). AC = *Axonopus compressus*, AL = *Anogeiessus leiocarpus*, SN = *Senna fistula*

## 4. Discussion

It is of particular interest to investigate the antioxidant properties of medicinal plants, especially those traditionally used in folk medicine. Medicinal chemical compounds that exhibit antioxidant properties are widely present in a large number of aromatic and other medicinal plants (Mathew and Abraham, 2006). Antioxidants protect the human system by neutralizing the free radicals interactively and synergistically. Plants are rich sources of free radicals scavenging molecules and other metabolites which are rich in antioxidant activity (Halliwell and Gutteridge, 1989).

Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Plants polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stresses associated with diseases (Shahidi and Wana, 1992). The total phenolic content ( $\mu\text{g/mg}$ ) was determined using the Folin-Ciocalteu method in terms of tannic acid equivalent and is as shown in figure 1. The total phenolic content of the studied plant extracts are not significantly different with *Anogeiessus leiocarpus* having a total phenolic content of  $11.525 \pm 0.061$ , *Axonopus compressus* ( $12.080 \pm 0.041$ ) and *Senna fistula* ( $12.813 \pm 0.054$ ). This result shows the importance of plant cell as an alternative system for the production of biologically active metabolites, indicating *Axonopus compressus*, *Senna fistula* and *Anogeiessus leicarpus* could represents an interesting supply of potential antioxidative and chemoprotective components (Bhakta *et al.*, 1998).

It has been recognized that flavonoid shows antioxidant activity and their effect on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al.*, 2003). The content of the total flavonoid were measured spectrophotometrically by using aluminium chloride colorimetric assay in terms of quercetin equivalent. The highest level of total flavonoids were obtained in the extracts of *Anogeiessus leiocarpus* ( $1.321 \pm 0.034$ ) followed by *senna fistula* ( $1.127 \pm 0.034$ ) and *Axonopus compressus* ( $0.681 \pm 0.034$ ). The results confirmed the presence of flavonoid in the three plant



extracts. Anti-haemorrhoidal properties of flavonoid from *Senna fistula*, *Anogeiessus leiocarpus*, *Axonopus compressus* has been reported from previous work (Miski *et al.*, 2003) and that flavonoid act as modulators of immune system in several biological systems. This stems from the fact that they are powerful antioxidants protecting the biosystem against damaging effects of free radicals (Miski *et al.*, 2003).

When DPPH is exposed to antioxidant compounds the purple colour of DPPH changes to yellow. The more yellowish colour of DPPH observed, then the greater the antioxidant activity of the compound tested (Shirwaiker *et al.*, 2004). The results at concentration of 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml revealed *Senna fistula* with DPPH scavenging activity of (10.2%, 18.8%, 21.4%, 46.3%, 52%), *Anogeiessus leiocarpus* with (18.4%, 26.7%, 28.6%, 52%, 56%) and *Axonopus compressus* with (11.4%, 17%, 29.5%, 49.9%, 57%). The three methanolic extracts of the studied plants have some level of DPPH radical scavenging activity. Similarly ethanolic extracts of the three medicinal plants have been previously reported to have DPPH radical scavenging action (Neuwinger, 2010).

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediate of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yildirim *et al.*, 2000). The reducing power of all the studied plant extracts increased with increasing concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. From figure 4 the reducing power ( absorbance unit) at concentration of 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml for *Anogeiessus leiocarpus* is 0.612, 0.730, 0.924, 1.61, 2.966, *Axonopus compressus* is 0.513, 0.676, 0.890, 2.104, 2.817, and *Senna fistula* is 0.583, 0.840, 1.116, 2.204, 3.114. The results are in agreement with that of Adekunle *et al.*, (2011) and Eprahim (2007) who studied the methanolic extract of *Anogeiessus leiocarpus*, *Axonopus compressus*, *Senna fistula* stem bark against haemorrhoids and reported that the reducing power of all the plant extracts increased with increased concentration.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. They were produced in this by incubating ferric EDTA with ascorbic acid and H<sub>2</sub>O<sub>2</sub> at pH 7.4 and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA) – like product. This compound forms a pink chromogen upon heating with TBA at low pH. When the plant extracts were added to the reaction mixture, it removed hydroxyl radicals from the sugar and prevented the reactions. Figure 5 showed the hydroxyl radical scavenging activity of the plant extract at concentration of 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml. The radical scavenging activity of *Axonopus compressus* at the selected concentration is 19.4%, 27.2%, 33.7%, 62.3%, 62.9%, *Senna fistula* is 17.33%, 46.2%, 64.9%, 71.4% and 13%, 23.2%, 39.8%, 73.7%, 78.9% is recorded for *Anogeiessus leiocarpus*. The result shows that the three plants extracts play a major role in the inhibition of ribose fragmentation.

ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical, it is a decolorization reaction and thus the radical cation assay is performed prior to addition of antioxidant test system, rather than the generation of the radical to occur continuously in the presence of antioxidants. Figure 6 showed the ABTS radical scavenging activities of the studied plants at different concentrations (12.5, 25, 50, 100, 200 µg/ml). The results revealed an increasing radical scavenging activity with increasing concentrations. At 200 µg/ml the highest radical scavenging activity was recorded for *senna fistula* (0.007 µMTE/g), while the least activity was recorded for *Axonopus compressus* (0.004 µMTE/g). The results demonstrated that the extracts are potent antioxidants by virtue of their ability to scavenge the radicals generated by ABTS. The results obtained are in agreement with an earlier report that revealed the potential activity of the studied plant extracts to scavenge ABTS cation radical (Salah and Evans 1995).

## 5. Conclusion

The exact cellular and molecular mechanisms which underlie the etiology of haemorrhoids are not still fully understood. However, the onset of haemorrhoids can be complex and may involve several processes and free radicals-induced oxidative stress is thought to play a central role in the development of haemorrhoidal complications. Since *Anogeiessus leiocarpus*, *Axonopus compressus* and *Senna fistula* have recently been reported to be effective in the treatment of haemorrhoids. This study therefore sought to investigate a component of the antihaemorrhoidal mechanisms of the plants by evaluating their antioxidant properties. The antioxidant assays shows that *Anogeiessus leiocarpus*, *Axonopus compressus* and *senna fistula* are plants that have high antioxidant activity, and this activity is due to the presence of phytochemicals in the plant. Therefore, these plants can be harnessed in preventive health care. This study has to some extent validated the medicinal potentials of the stem bark of *senna fistula*, *Anogeiessus leiocarpus* and *Axonopus compressus* which has been shown to contain some level of polyphenols. We therefore partially conclude that the stem bark of *Anogeiessus*

*leiocarpus*, *Axonopus compressus* and *Senna fistula* utilizes various antioxidative mechanisms which among others include free radical scavenging and reduction of transition metals involved in the initiation of free radical induced macromolecular damage among others.

## References

- Adekunle, P.B., Alabi, D.A. and Yakubu, F.A. (2011). Nigeria Flora and its Pharmaceutical Potential. *Journal of Ethnopharmacology* **19**:116
- Bhakta, S., Mavi, A., Sukru, L. and Sies, H. (1998). An approach to free radical in medicine and biology. *Journal of Biotechnology* **492**:153-168.
- Chong, K., Apel, O. and Hirt, H. (2002). Oxidative stress and antioxidant system in plants. *Nutrition* **82**:12271238.
- Edeoga, H.O. (2005). Phytochemical Constituents of Some Nigeria Medicinal plants. *African Journal of Biotechnology* **6**:578 – 685.
- Halliwell, B. and Gutteridge, J.M. (1981). Antioxidant in Nutrition, Health and Disease. Oxford University Press, Oxidative stress; Pp.90-102
- Halliwell, B. and Gutteridge, J.M. (1989). Chemoprevention of Cancer. *CA Cancer Journal of Clinic* **54**:150-180.
- Kessler, P., Abdullahi, M., Mohammed, G. and Abubaka, N.U. (2003). Medicinal and Economic Plants of Nupe Land. Jube-Evans Publishers. 1<sup>st</sup> Edition, Pp.56-66.
- Madoff, K., Gbile, Z.O. and Pillay, V. (2004). Conservation and cultivation of local medicinal plants for natural medicine production. Paper Presented at the Seminar / Workshop and Exhibition of Natural Medicine Products.
- Mathew and Abraham (2006). Induced Oxidative Damaged in Sickle red blood Cells and the Role of Flavonoids. *Biomedical Pharmacology* **57**:124-129
- Mezzetti, A., Lappenna, D., and Pierdomenico, S.D. Atherosclerosis 1995; 112: 91.
- Middleton, A.B., Gazi, M.R. and Kanda, R. (2000).Some Properties of Radical Scavenging substances from some Medicinal Plants. *Parkistan Journal of Biological Science* **7**:1365-1370.
- Miski, M., Uhubelon, A. and Johonson (2003). Antibacterial activity studies of flavonoid from *Salvia palestina*. *Journal of Nutritional Production* **46(6)**: 874-875.
- Morimitsu, I., Kris, P.M. and Hills, A.F. (2000). Economy Botany. A textbook of useful plants and plant Products. 3<sup>rd</sup> edition.
- Neuwinger, (2000). Studies on the characterization and glyceride composition of *Anogeissus leiocarpus*. *Life Science* **34**:144-148.
- Neuwinger, (2010). Anti-inflammmatory activities of constituents isolated from *Phyllanthus polyphyllus*. *Journal of Ethnopharmacology* **103**:181-186.
- Olu, (2004). Antimicrobial properties of *Senna fistula*. *Journal of Agriculture and Food Chemistry* **5**:686-87.
- Olumide, (2008). Bioactive Compounds in Medicinal Foods: Their Role in the Prevention Cardiovascular Disease. *Biomedical Pharmacology* **20**:237-248.
- Oyizu, (1989). Antioxidant activity. *Free Radical Research* **19**:110-114.
- Rangnekar, K., Palis, R.K. and Norcio (1974). The biology of *C. odorata L*. *Weed Science* **1**:17-24
- Sadique, (1989). Chemoprevention of Cancer in Traditional Medicine.
- Sajise, P.E. and Lales (1974). Flowering behavior pattern of growth and nitrate metabolism of *Axonopus compressus*. *Weed Science* **1**:18 – 22.
- Salah, W. and Evans, C. (1995). Polyphenolic Flavonoids as Scavenger of Aqueous Phase Radicals as Chain Breaking Antioxidant. *Archeological Biochemistry Broph.* **2**:339 – 346.
- Shahidi and Wanna (1992). Composition of polyphenols in fresh tea leaves. *Journal of Agriculture and Food Chemistry* **99**:1387-1396.
- Sharp, D.S. (1981). Biochemical and Therapeutic Effects of Azheimer’s Disease, Parkinso’s disease and Amyotrophic Lateral Selerosis. *Current Drug Target* **2**:95 – 107.
- Shirwaiker, Suja, K.P. and Arumughen, C. (2004). Free radical scavenging behavior of antioxidant compounds of sesame in DPPH system. *Journal of Agriculture and Food Chemistry* **52**:912-915
- Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria 289.
- Williams, (1999). Antioxidant and Free Radicals Scavengers of Natural Origin. *Current Organic Chemistry* **2**:650-785.
- Yildirim, F.D., Miller, W. and Rice, E. (2000). Spectrophotometric Determination of Antioxidant Activity. *Redox Report* **6**:161- 165.