

Full Length Research Paper

Phytochemicals, antioxidant potentials and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Piper guineense* (Schumach & Thonn) seed

Fajobi O. Adeniyi*, Fasakin O. Wilson and Oyedapo O. Oluboade

Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Received 30 November, 2016; Accepted 8 February, 2017

The study involved preparation, phytochemical screening and evaluation of antioxidant potentials of the extract of *Piper guineense*. This was with a view to investigating the possibility of using the plant in the treatment and management of oxidant and inflammatory related disorders. Spices are herbs that contain complex mixtures of phytochemicals which give characteristic odours, flavours and protect living systems from deleterious effect of reactive oxygen species (ROS). Phytochemical screening of the plant revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, triterpenoids and saponins. The extract exhibited DPPH-free radical scavenging activity which was concentration dependent. The results revealed that the extract is rich in antioxidant components which make the seed useful in the prevention and management of various forms of diseases.

Key words: Phytochemicals, antioxidant potentials, *Piper guineense*, methanolic extract.

INTRODUCTION

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS), free radicals and antioxidant defences (Eboh, 2014). This imbalance leads to damage of important molecules such as proteins, lipids, nucleic acids and is also involved in the pathogenesis of various forms of diseases including cardiovascular diseases; cancer etc. Moreover, antioxidants are different group of chemicals that inhibit the oxidation of other molecules, neutralising free radicals and protect specific organs against deleterious effect of dangerous xenobiotics (Durackova, 2010; Sachdeva et al., 2014). The harmful

effects of ROS are eliminated by antioxidants which are of two types, enzymatic and non-enzymatic (Eboh, 2014). Despite the presence of the endogenous antioxidant defense systems, ROS still accumulate during the life cycle. They are implicated in a number of diseases, aging and age dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders and other chronic conditions (Eboh, 2014; Sachdeva et al., 2014). Accumulation of ROS may also be triggered from compromised homeostatic pathways which normally result in the damage of cellular components such as proteins, lipids and deoxyribonucleic acids (Sachdeva et

*Corresponding author. E-mail: fajobi2009@gmail.com.

al., 2014).

Medicinal plant extracts (including herbal/spices) contain different phytochemicals with varieties of biological activities of valuable therapeutic indices. The protective effects of plant are attributed to the activities and potentials of phytochemicals (Poongothai et al., 2011; Etim et al., 2013), which are the non-nutrient compounds. They are classified into three main groups namely alkaloids, isoprenoids (terpenoids and steroids) and phenols (phenolic acid, flavonoids, tannins and coumarin). The plant derived phytoconstituents are capable of terminating free radical reactions and prevent the body or biological systems from oxidative damage (Saikat et al., 2010) and protect against chronic diseases (neurodegenerative and cardiovascular) (Prior et al., 2005).

Piper guineense commonly known as West African black pepper is a climbing perennial plant belonging to the family Piperaceae. It is found in the tropical regions of Central and Western Africa. It is very similar to cubeb pepper in terms of flavour, but is much less bitter and has a fresher, more herbaceous flavor. It is used as spices whose fruits contain pungent piperine an essential oil, which constitutes 5 to 8% of the weight of black pepper. It is used in the beverage and pharmaceutical industries as flavouring and preservative agent (Oyemitan et al., 2014; Opara, 2014; De LaTorres et al., 2015). The pungency of the pepper is due to the presence of resins particularly chavicine and a yellow alkaloid. The dried fruits of *P. guineense* is usually ground and used as spices in food (Hassan et al., 2010). It has also been reported to have medicinal and health benefits including treatment and prevention of morning sickness, allergy, keep the body warm and prevent cold (Ekanem et al., 2010; Tankan and Ito, 2013; Etim et al., 2013; Opara, 2014). This paper sought to evaluate the phytochemical constituents, the antioxidant potentials and DPPH-radical scavenging activity of defatted methanolic extract of *P. guineense* seeds with a view to studying the possibility of utilizing the plant in the treatment and management of disorders.

MATERIALS AND METHODS

Reagents and chemicals

All the reagents and chemicals used in this study are of analytical grade and were procured from British Drug House (BDH) Chemicals Limited, Poole, England. 2, 2'- α -bipyridyl, 2, 2' -diphenyl-1-picrylhydrazyl (DPPH), L-Ascorbic acid and Folin - Ciocalteu's phenol reagent were obtained from Sigma-Aldrich Laboratories, Switzerland. All the solutions and buffers were prepared with glass distilled water.

Plant material: Collection and identification

Seeds of *P. guineense* were purchased from a local market in Ile-Ife, Osun State, Nigeria. The identification and authentication were carried out at IFE Herbarium, Department of Botany where specimen copy with specimen number (17480) was deposited. The

seeds were sun-dried, carefully cleaned and ground to fine powder using manual grinder.

Preparation of methanolic extract of *P. guineense*

The methanolic extract of *P. guineense* (MEPG) was prepared by suspending 100 g defatted powdered seed in 500 ml of methanol-water mixture (3:1 v/v) for 72 h at room temperature with intermittent shaking. The suspension was filtered using Whatman no. 1 filter paper. The filtrate was concentrated *in vacuo* to dryness under reduced pressure at 40°C on Buchi (Switzerland) Vacuum Pump V-700 rotary evaporator. The dark brown residue was kept at 4°C for further analysis.

Phytochemical screening

Phytochemical screening of the extract was carried out according to earlier standard methods (Sofowora, 2006; Tiwari et al., 2011; Prohp and Onoagbe, 2012) with the following specific detecting chemicals and reagents. Alkaloids were screened with 0.5 M Picric acid, Mayer's reagent, Wagner's reagent and Dragendorff's reagent; flavonoids [1% (w/v) Lead acetate solution and 0.5 N ethanolic KOH]; Tannins [0.1% (w/v) Ferric chloride in glacial acetic acid]; saponins (Frothing and haemolytic tests); cardiac glycosides (chloroform/conc. sulphuric acid); anthraquinone (benzene/10% ammonia solution); triterpenoids (chloroform/concentrated sulphuric acid); steroids (concentrated sulphuric acid); and xanthoproteins (concentrated nitric acid/ammonia solution).

Determination of total phenolics and flavonoids levels in MEPG

The concentration of phenolics in the extract was determined spectrophotometrically according to the method of Singleton et al. (1999). The reaction mixture consisted of 0.5 ml (1 mg/ml stock) of the extract, 0.5 ml of distilled water, 1.5 ml of 7.5% (w/v) sodium carbonate (Na_2CO_3) and 1.5 ml of 10% (v/v) Folin-Ciocalteu's phenol reagent and followed by incubation at room temperature for 1 h 30 min. The absorbance was read at 725 nm against the reagent blank on vis - spectrophotometer. The concentration of total phenolics was extrapolated from the standard calibration curve and expressed in milligram tannic acid equivalent per gram of extract (mg TAE/g extract).

The concentration of flavonoids in the extract was determined spectrophotometrically according to the method of Sun et al. (1999) with slight modification. The analysis was based on the formation of yellow-coloured flavonoid-aluminum complex. The reaction mixture consisted of 0.5 ml (1 mg/ml) of the extract, 1.5 ml of distilled water, 0.3 ml of 5% (w/v) sodium nitrite (NaNO_2) freshly prepared, 0.3 ml of 10% (w/v) aluminum chloride (AlCl_3) and 2 ml of 4% (w/v) sodium hydroxide (NaOH). The reaction mixture was incubated at room temperature for 10 min and the absorbance taken at 500 nm against the reagent blank. The concentration of total flavonoid was extrapolated from the standard calibration curve and was expressed in milligram rutin equivalent per gram of extract (mg RE/g extract).

Determination of total vitamins C and E levels in MEPG

The vitamin C concentration in the extract was quantified using Folin-Ciocalteu's phenol reagent reaction method according to procedure that was based on the methods of Omaye et al. (1979); and Japota and Dani, (1982). 0.5 ml of the extract was mixed with 1.5 ml of 10% (v/v) acetic acid and 0.5 ml of Folin-Ciocalteu reagent (1:10 dilution). The mixture was incubated at room temperature

Table 1. Phytochemicals of methanolic extract of *Piper guineense* (MEPG) seed.

Phytochemical constituents	Results
Alkaloids	Mayer
	Wagner
	Picric
	Dragendoff
Flavonoids	
Tannins	
Triterpenoids	
Cardiac glycosides	
Saponins	

Table 2. Antioxidant potentials of the methanolic extract of *P. guineense*.

Property	Level
Total phenolics (mgTAE/g)	141.50 ± 1.60
Flavonoids (mgRE/g)	86.09 ± 3.10
Vitamin C (mg/g)	11.85 ± 0.10
Vitamin E (µgTE/g)	18.95 ± 0.05
DPPH scavenging activity extract of <i>P. guineense</i> (IC ₅₀ µg/ml)	74.00
DPPH scavenging activity of ascorbic acid (IC ₅₀ µg/ml)	31.17

Results were expressed as the mean ± SD, n=5 readings. TAE, Tannic acid equivalent; RE, rutin equivalent; TE, Trolox equivalent.

for 10 min. The absorbance was read at 760 nm against the reagent blank. The concentration of vitamin C was obtained from the standard calibration curve and expressed in milligram per gram of extract (mg/g extract).

The total vitamin E in the extract was determined according to a procedure that was based on the earlier methods of Baker and Frank (1968) and Santhosh et al. (2013). The analysis was based on the reduction of ferric to ferrous ion by vitamin E and the formation of red coloured complex with 2, 2'- bipyridyl. Vitamin E (0.5 ml) extracted in heptane was mixed with 0.5 ml of 2', 2', α-bipyridyl reagent (0.12% w/v) and 0.5 ml of ferric chloride (0.12% w/v). 0.5 ml of Trolox (25 µg/ml) in ethanol was treated similarly. The absorbance was read at 492 nm against the reaction blank. The vitamin E concentration was expressed in milligram Trolox equivalent per gram (mg TE/g) using the expression:

$$\text{Concentration of Vitamin E} = \frac{\text{Abs of extract} \times \text{Conc. of Std}}{\text{Abs of Std}}$$

where Abs = absorbance, Conc. of Std = concentration of Trolox.

Assay of DPPH radical scavenging activity of MEPG

The free radical scavenging activity of the extract was measured according to the modified method of Blois (1985). To 1 ml each of the different concentrations of extracts (62.5, 125, 250, 500 and 1000 µg/ml) or standard (vitamin C) (7.8125, 15.625, 31.25, 62.5 and 125 µg/ml) in a test tube, 1 ml of 0.3 mM DPPH in methanol was added. The mixture was mixed and incubated in the dark chamber for 30 min. The absorbance was measured at 517 nm against a blank that contained methanol in place of the extract. The percentage DPPH radical scavenging activity was calculated using the expression:

$$\text{Percentage scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

The antioxidant activity of the extract was expressed as IC₅₀. The IC₅₀ value was defined as the concentration (µg/ml) of extract that scavenged 50% the DPPH radical.

Statistical analysis

Data were expressed as mean ± SD of n = 5 readings, mean and standard deviation were processed using Ms Excel.

RESULTS

Phytochemical constituents and antioxidant potentials

The methanolic seed extract of *P. guineense* (MEPG) gave positive reactions for the presence of phenolics, flavonoids, alkaloids, cardiac glycosides, tannins, triterpenoids and saponins. Further analysis showed that it contained total phenolics (141.50 ± 1.60 mgTAE/g of extract), flavonoids (86.09 ± 3.10 mgRE/g of extract), vitamin C (11.85 ± 0.10 mg/g of extract) and vitamin E (18.95 ± 0.05 µgTE/g of extract) (Tables 1 and 2). The results revealed that the plant is very rich in phenolic compounds as well as in non-enzymatic antioxidants.

DPPH radical scavenging activity of the extract

The extract exhibited DPPH radical scavenging activity with IC_{50} of 74.0 $\mu\text{g/ml}$, and ascorbic acid 31.17 $\mu\text{g/ml}$ as positive control. The radical scavenging activity of the extract is low (42%) when compared with that of ascorbic acid. This might be due to the interference with or presence of other phytochemicals.

DISCUSSION

The study evaluated the phytochemicals, antioxidant potentials and DPPH-radical scavenging activity of methanolic seed extract of *P. guineense*. The extract weighed 12 g which was 12% of the starting material which was considerably high when compared with extraction with other solvents. The observations agreed with that of Udoh et al. (2012). It implied that methanol could be regarded as a suitable solvent for the extraction of phytochemicals for biological studies. Phytochemical screening of the extract revealed that it contained alkaloids, cardiac glycosides, flavonoids, tannins, triterpenoids and saponins. Further analyses of the extract revealed that total phenolics and flavonoids contents of *P. guineense* extract as measured spectrophotometrically were 141.50 ± 1.60 mg/g expressed as an equivalent of tannic acid and 86.09 ± 3.10 mg/g expressed as an equivalent of rutin.

Phenolic compounds exhibit strong anti-oxidant activity, protect cells against the oxidative damage, are well-known scavengers, metal chelators, reducing agents, hydrogen donors and single oxygen quenchers (Ghasemzadeh and Ghasemzadeh, 2011). Studies have revealed that phenolics are ubiquitous groups of plant metabolites with varying biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, anti-atherosclerosis and cardiovascular protection (Aiyegroro and Okon, 2010; Akinpelu et al., 2012).

Naturally occurring antioxidants are mainly synthesized in plants in form of phenolics such as flavonoids, phenolic acids, tocopherols, tannins etc. (Ali et al., 2008). Flavonoids constitute one of the most important and widely distributed plant phenolics and are hydroxylated substances known to be synthesized by plants in response to microbial infections found to elicit antimicrobial activity against wide array of microorganisms *in vitro* (Yadav and Agarwala, 2011). The biological activity of flavonoids could probably be due to their ability to complex with extracellular soluble proteins and bacterial cells (Yadav and Agarwala, 2011; Duraikannu et al., 2014). Tannins on the other hand bind to proline-rich proteins and interfere with protein synthesis, contributing to the acceleration of healing in ulcer patients and wound cuts (Yadav and Agarwala, 2011; Ekpo et al., 2013).

Moreover, saponins possess the ability of precipitating

and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, and cholesterol binding properties and in the management and treatment of hypercholeostreolemia which implies that saponins might be acting by interacting with intestinal absorption of cholesterol (Malinow et al., 1977; Malinow, 1984; Yadav and Agarwala, 2011). Saponins have also been reported to possess hypoglycemic and anti-inflammatory activities (Poongothai et al., 2011). The presence of these compounds explains the ability of the seed extract to exhibit antioxidant properties. The results agreed with the essential observations of Ekpo et al. (2013) and Oyemitan et al. (2014) that ethanolic extract seed and essential oil of *P. guineense* contained alkaloids, flavonoids, tannins, saponins, terpenes, phenols and cardiac glycosides and exhibited potent and appreciable biological activities.

Vitamins are chemically unrelated organic entities that cannot be synthesized in right quantities by humans, as such must be supplied in the diets. They are classified into two major classes, (water and lipid soluble), based on their solubility and perform specific cellular functions (Champe et al., 2008). The water soluble vitamins include non-B complex which is L-ascorbic acid (vitamin C) and B-complex (energy releasing, hemopoietic and others) while the fat soluble vitamins include A, D, E and K (Bellows and Moore, 2012).

Ascorbic acid (vitamin C) is one of the most powerful antioxidants that scavenges harmful free radicals, reactive oxygen species (ROS) and regenerates tocopherol to its functional state (Eboh, 2014). It has a 6-carbon lactone ring structure with 2, 3-enediol moiety with its antioxidant mechanisms based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen. Gayathri et al. (2010) reported that vitamin C can prevent the oxidation of lipids by trapping water soluble peroxy radicals before their diffusion into lipid membranes. Denre (2014) reported that high intake of ascorbic acid has been linked to lower risk of cardiovascular diseases and several types of cancers by stimulating the immune system.

On the other hand, vitamin E is a fat-soluble vitamin with its main function to protect against lipid peroxidation (Pryor, 2000). Vitamin E is the major powerful membrane bound antioxidant employed by the cell in humans (Hensley et al., 2004). Antioxidant mechanisms of tocopherols include the transfer of a hydrogen atom at 6-hydroxyl group on the chroman ring, and scavenging of singlet oxygen and other reactive species (Eboh, 2014). In the present study, the concentrations of vitamin C and vitamin E were observed to be 11.85 ± 0.10 mg/g of extract and 18.95 ± 0.05 $\mu\text{g/g}$ trolox equivalent of extract respectively which is quite high when compared with other sources.

Anti-oxidants (exogenous and endogenous) are vital substances which possess the ability to protect the body

from damage caused by free radical induced oxidative stress (Ghasemzadeh and Ghasemzadeh, 2011). Naturally occurring antioxidants have attracted considerable interest among nutritionists, food manufacturers and consumers due to their presumed safety and potential therapeutic values (Kumar and Pandey, 2013). Fruits and vegetables contain many different antioxidant components which are related to three major groups- vitamins, phenolics and carotenoids (Baladi et al., 2014). The consumption of fruits and vegetables has been associated with low incidences and mortality rates of cancer and heart diseases (Thaipong et al., 2006; Doss and Pugalenth, 2012). Eating fruits and vegetables reduces blood pressure, boosts the immune system, detoxifies contaminants, pollutants and reduces inflammation.

The antioxidant potential assays in the study showed that the plant possess antioxidant activities. Anyasor et al. (2011) reported that plant phenolics are the major group of compounds acting as primary free radical scavengers or antioxidants. 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) assay is a well-known method for the evaluation of free radical-scavenging activity (Rasooli, 2007). It is a stable nitrogen centred free radical, the colour changes from violet to yellow to colourless on reduction by either the process of hydrogen or electron-donation. Molecules or compounds which are capable to perform the above reactions are considered as antioxidants and therefore radical scavengers (Brand-Williams et al., 1995; Hinneberg et al., 2006; Egemole et al., 2007). DPPH stable free radical method is an easy, rapid and sensitive method employed to analyze the antioxidant potential of a specific compound or plant extracts (Koleva et al., 2002). A potent scavenger of free radicals may serve as a possible preventive intervention for the diseases (Gyamfi et al., 1999).

The present study showed that the free radical scavenging power of the extract of *P. guineense* increased in a concentration dependent manner, though significantly low compared to standard ascorbic acid (21.17 µg/ml). The result of the study is in close agreement with the observation of Ayoola et al. (2008) who observed a dose-dependent scavenging activities of some medicinal plants used for malaria therapy in south-western Nigeria.

Conclusion

The results of this study suggest that the defatted methanolic leaf extract of *P. guineense* has potential as an antioxidant agent. However, further investigation on the isolation and characterization of the antioxidant components is required.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Aiyegboro OA, Okoh AI (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of aqueous extract of *Helichrysum longifolium* DC. BMC Complement. Altern. Med. 10(1):21.
- Akinpelu BA, Dare CA, Adebesein FI, Iwalewa EO, Oyedapo OO (2012). Effect of stem - bark of *Erythrophloeum suaveolens* (Guill. & Perri.) saponin on fresh water snail (*Lanistes lybicus*) tissues. Afr. J. Environ. Sci. Technol. 6(11):446-451.
- Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U (2008). Indian medicinal herbs as source of antioxidants. Food Res. Int. 41:1-15.
- Anyasor GN, Aina DA, Olushola M, Aniyikaye AF (2011). Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena Odorata*. Ann. Biol. Res. 2(2):441-451.
- Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Trop. J. Pharm. Res. 7(3):1019-1024.
- Baker H, Frank O (1968). Tocopherol. In. Clinical vitaminology, methods and interpretation. New York Interscience Publishers, John Wiley and Sons Inc., Pp.172-173.
- Baladi RM, Moghaddaszadeh-Ahrabi S, Afrouziyeh M (2014). Extraction of phenolic composition and quantification of the total phenolic of pomegranate pomace. Eur. J. Zool. Res. 3(1):133-135.
- Bellows L, Moore M (2012). Water-Soluble Vitamins: B-Complex and Vitamin C. Food and Nutrition Series. Fact Sheet No. 9: pp. 312.
- Blois MS (1985). Antioxidant determination by use of stable free radicals. Nature 29:1199-1200.
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT Food Sci. Technol. 28:25-30.
- Champe PC, Harvey RA, Ferrier DR (2008). Vitamins. In. Lippincotts Illustrated Reviews: Biochemistry, Fourth Edition, Pp. 373-394.
- De LaTorres JE, Gassara F, Kouassi AP, Brar SK, Belkacemi K (2015). Spice Use in Food: Properties and Benefits. Crit. Rev. Food Sci. Nutr.
- Denre M (2014). The determination of vitamin C, total phenol and antioxidant activity of some commonly cooking spices crops used in West Bengal. Int. J. Plant Physiol. Biochem. 6(6):66-70.
- Doss A, Pugalenth M (2012). Evaluation of Antioxidant Activity and Phytochemical Screening of *Malus domestica* Borkh (Apple) and *Phaseolus vulgaris* L. (Green beans). J. Pharm. Sci. Innov. 3:1-4.
- Durackova Z (2010). Some current insights into oxidative stress. Physiol. Res. 59:459-469.
- Duraikannu D, Chandrasekaran SD, Selvakumar JN, Vaithilingam M (2014). A preliminary study of *in vitro* Antioxidant and Antibacterial Activity of *Streptomyces gancidius* VITSD1 isolated from Marine soil. Curr. Bioact. Compd. 10:292-297.
- Eboh AS (2014). Biochemistry of Free Radicals and Antioxidants. Scholars Acad. J. Biosci. 2(2):110-118.
- Egemole AN, Odekanyin OO, Akinpelu BA, Akinwunmi KF, Oyedapo OO (2007). An examination of the extracts of *Icacina tricantha* Oliv. Niger. J. Biochem. Mol. Biol. 22:39-42.
- Ekanem AP, Udoh FV, Oku EE (2010). Effects of ethanol extract of *Piper guineense* seeds (Schum. & Thonn.) on the conception of mice (*Mus musculus*). Afr. J. Pharm. Pharm. 4(6):362-367.
- Ekpo IA, Osuagwu AN, Agbor RB, Okpako EC, Ekanem BE (2013). Phytochemical composition of *Aframomum melegueta* and *Piper guineense* seeds. J. Curr. Res. Sci. 1(1):24-27.
- Etim OE, Egbuna CP, Odo CF, Udoh NM, Awah FM (2013). *In Vitro* Antioxidant and Nitric oxide Scavenging Activities of *Piper*

- guineense* seeds. Glob. J. Res. Med. Plants Indig. Med. 2(7):485-494.
- Gayathri K, Jayachandran KS, Vasanthi HR, Rajamanickam GV (2010). Cardioprotective effect of lemon grass as evidenced by biochemical and histopathological changes in experimentally induced cardiotoxicity. Hum. Exp. Toxicol. 30(8):1073-1082.
- Ghasemzadeh A, Ghasemzadeh N (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J. Med. Plants Res. 5(31):6697-6703.
- Gyamfi MA, Yonamine M, Aniya Y (1999). Free- radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally- induced liver injuries. Gen. Pharm. 32:661-667.
- Hassan DI, Ogah DM, Yusuf ND, Musa MM, Saidu GM (2010). The effect of acute and chronic (short and long term) oral administrations of black pepper (*Piper guineense*) aqueous extract on the body weight and haematological values of albino-Wistar rat. J. Med. Plants Res. 4(12):1122-1125.
- Hensley K, Benakass EJ, Bolli R (2004). New perspectives on vitamin E: α -tocopherol and carboxyethylhydroxychroman metabolites in biology and medicine. Free Rad. Biol. Med. 36:1-15.
- Hinneberg L, Drmen HJD, Hiltunen R (2006). Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. 97:122-129.
- Japota SK, Dani HM (1982). A new Colorimetric Technique for the estimation of vitamin C using Folin-Ciocalteu's phenol reagent. Anal. Biochem. 127:178-182.
- Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13:8-17.
- Kumar S, Pandey AK (2013). Chemistry and Biological Activities of Flavonoids: An Overview. Sci. World J. 2013, Article ID 162750, 16 pages.
- Malinow MR (1984). Saponins and Cholesterol Metabolism. *Atherosclerosis* 50, 117 – 119.
- Malinow MR, Mehaughlin P, Kohler GO, Livingstone AC (1977). Prevention of elevated cholesterolemia in Monkeys. Steroids 29:105-110.
- Omeye ST, Turnbull JD, Sauberlich HE (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCormick D.B. and Wright D.L. (eds). Methods of Enzymology New York: Academic Press. Pp. 3-11.
- Opara MN (2014). Some Edible Spices of Southeastern Nigeria with Antiparasitic Properties: A Review. Am. J. Ethnomed. 1(6):384-392.
- Oyemitan IA, Kolawole F, Oyedeji AO (2014). Acute toxicity, antinociceptive and anti-inflammatory activity of the essential oil of fresh fruits of *Piper guineense*, Schum & Thonn (Piperaceae) in rodents. J. Med. Plants Res. 8(40):1191-1197.
- Poongothai A, Sreena KP, Sreejith K, Uthiralingam M, Annappoorani S (2011). Preliminary phytochemicals screening of *Ficus racemosa* linn. bark. Int. J. Pharma Bio Sci. 2(2):431-434.
- Prior RL, Wu X, Schaich K (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53:4290-4302.
- Prohp TP, Onoagbe IO (2012). Determination of phytochemical composition of the stem-bark of *triplochiton seleroxylon* K. Schum (Sterculiaceae). Int. J. Appl. Biol. Pharm. Technol. 3(2):68-74.
- Pryor WA (2000). Vitamin E and heart disease: basic science to clinical intervention trials. Free Rad. Biol. Med. 28:141-64.
- Rasooli I (2007). Food preservation: A Biopreservation Approach. Food 1:111-136.
- Sachdeva M, Karan M, Singh T, Dhingra S (2014). Oxidants and Antioxidants in Complementary and Alternative Medicine: A Review. Spatula DD 2(1):1-16.
- Saikat S, Raja C, Sridhar C, Reddy YSR, Biplab DE (2010). Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. Int. J. Pharm. Sci. Rev. Res. 3(1): 91-100.
- Santhosh KS, Samyudurai P, Ramakrishnan R, Nagarajan N (2013). Polyphenols, Vitamin-E Estimation and *in vitro* Antioxidant Activity of *Adiantum capillu-veneris*. Int. J. Innov. Pharm. Res. 4(1):258-262.
- Singleton VL, Orthofer R, Lamuela RRM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu's reagent. Methods Enzymol. 299:152-179.
- Sofowora A (2006). Phytochemical screening. In. Medicinal Plants and Traditional Medicine in Africa. Second Edition, Spectrum Books Limited, Ibadan, Nigeria. pp. 150-153.
- Sun PX, Yie LK, Zhang ZL, Hu M, Lu L (1999). Colometric determination of the total content of the flavonoids in epimedium capsules. J. Shenyang Pharm. Univ. 16:68-70.
- Tankan JM, Ito M (2013). Inhalation of the Essential oil of *Piper guineense* from Cameroon shows Sedative and Anxiolytic-like Effects in Mice. Biol. Pharm. Bull. 36(10):1608-1614.
- Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal. 19:669-675.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011). Phytochemical screening and extraction: A review. Int. Pharm. Sci. 1(1):98-106.
- Udoh FV, Ekanem AP, Eyo VO (2012). Pharmacodynamic Effect of Methanolic Extract of *Piper guineense* Leaf on Uterine Physiology. Pharmacologia 3(7):200- 203.
- Yadav RNS, Agarwala M (2011). Phytochemical analysis of some medicinal plants. J. Phytol. 3(12):10-14.