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# EVALUATION OF THE PHYTOCHEMICAL, ANTIOXIDANT AND NUTRITIONAL PROPERTIES OF *Phyllanthus muellerianus* LEAVES

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## *ABSTRACT*

***Plant-derived preparations and isolated phytochemicals or their model derivatives are now used to treat infectious diseases, especially in the light of the emergence of drug-resistant microorganisms and the need to produce more efficacious and cost-effective microbial agents. This study investigated the phytochemical, antioxidant and antinutrient properties of Phyllanthus muellerianus ethanol leaves extract. The extracts were screened for some antinutrient and phytochemical properties using various laboratory methods. Phytochemical examination of Phyllanthus muellerianus showed the presence of saponin, flavonoid, phlobatanin, tannin, steroids, cardiac glycosides and terpenoids. The antioxidant testing showed the increase in % inhibition of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), Nitric oxide and reducing power activity as concentration increases. Antinutrient analysis showed higher amount of phytate than oxalate and a higher amount of alkaloid -than tannin. These properties above may be the reason Phyllantus muellerianus plant has been reported to be useful in the treatment of several ailments.***

**Keywords:** *Phyllanthus muellerianus*, Antinutrient, Antioxidants, Phytochemicals.

## INTRODUCTION

Over the last decade, interest in drugs of plants origin and their uses in various disease management have increased in developed countries since plants used in traditional medicine are more likely to yield pharmacologically active compounds than developing new drugs synthetically (WHO, 1990).

Plant-derived preparations and isolated phytochemicals or their model derivatives are now used to treat infectious diseases, especially in the light of the emergence of drug-resistant microorganisms and the need to produce more efficacious and cost effective antimicrobial agents (Ncube *et al.*, 2008). Studies have shown that many plants have chemical components and biological activities that produce definite physiological actions in the body and, therefore, could be used to treat various ailments. Some important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Usunobun *et al*., 2015). Flavonoids (a group of polyphenolic compounds) and phenols with known properties, such as free radical scavenging activity (antioxidant), inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action and anticarcinogenic activity have been isolated from plants (Pourmorad *et al*., 2006).

Various herbal medicines derived from plant extracts are being used in the treatment of a wide variety of clinical diseases, though relatively little knowledge about their mechanisms of action is known (Ratheesh and Helen, 2007). There is need for research and developmental work in herbal medicine because apart from the social and economic benefits, it has become a persistent day healthcare in developing countries.

*Phyllanthus muellerianus* is a deciduous or evergreen shrub with numerous, it has straggling stems from the base with a climbing habit and sometimes becomes more tree-like in habit. It has the potential to grow up to 12 metres tall (PROTA,

2015). As a multipurpose plant, it is widely used medicinally and also provides food and various materials. It is found in Tropical African countries which include Senegal, Sudan, Uganda, Kenya, Angola, Zambia, Malawi, Mozambique and part of West Africa region (PROTA, 2015). In Nigeria, the plant is reported as a weed of rice field which is plainly caused by lack of timely cultivation (Burkil, 1994). The leaf extract of the plant has been reported to cause contraction of rat ileum and possesses antibacterial activity (Doughari and Sunday, 2008). In many parts of the world, *Phyllanthus muellerianus* has found usefulness as an herbal remedy (Siram *et al*., 2004). The fresh leaves can be crushed and applied to wounds and the decoction used as purgative bronchitis and relieving urethral discharges (Doughari and Sunday, 2008)

According to Assob *et al*. (2011), analysis done on *Phyllanthus muellerianus* plant revealed that it has antibacterial and anticandidal agents and could be used for the treatment of various urogenital and gastrointestinal ailments caused by multiresistant microbial agents. However, there is still limited available information with scientific backing to support the use of this plant medically. Hence, this study aimed to evaluate the phytochemical and antioxidant properties of *Phyllanthus muellerianus* ethanol leave extract with purpose of enriching the body of knowledge on it.

## MATERIALS AND METHODS

### Collection and preparation of material

Leaf samples were collected in Ijebu-Ikija in Ogun State Nigeria. Ijebu-Ikija is situated in the Eastern part of Ijebu in Ogun State, it boarders Ijebu-Ife, Isire and Ijebu-Imushin along Ijebu-Ode. It lies within latitude $6^{o}$ 47' N and$^{o}$ 2'longitudeE.Ithas4 an averagebovesea al level with an undulating topography and an annual rainfall of 1581 mm together with 81 – 91% relative humidity and a mean temperature of 27.1 $^{o}$C. The extraction process in this study was done using ethanol. This is because ethanol is easily evaporated and permits an easier estimation of extract concentration which is difficult to obtain with water as solvent.

### Determination of Proximate analysis of *Phyllanthus muellerianus* leave

**i. Moisture content**

AOAC (2005) method was adopted for the determination of moisture content of *Phyllanthus muellerianus*. The method was based on the removal of water from the sample and its measurement by loss of weight. A clean Petri dish was dried in the oven and weighed ($W_1$); 1.0 g of the sample was weighed into the crucible ($W_2$) and was dried at 105 °C, for twenty four hours. The Petri dish was then transferred from the oven to a desiccator, cooled and reweighed ($W_3$). The % moisture content was calculated from:

$$\% \text{ Moisture content} = 100 - [(W_3 - W_1) \times 100)] \quad \ldots\ldots\ldots \quad [1]$$

**ii. Determination of Ash Content**

Ash content was determined according to AOAC (2005) method. The porcelain crucible was dried in an oven at 100 °C for 10 mins, cooled in a desiccator and weighed ($W_1$). A portion (2 g) of the sample was placed into the previously weighed porcelain crucible and reweighed ($W_2$) and placed in a furnace for four hours at 600 °C to ensure proper ashing. The crucible containing the ash was removed cooled in the desiccator and weighed ($W_3$). The % ash content was calculated as:

$$\% \text{ Ash content} = (W_3 - W1) \div (W_2 - W_1) \times 100 \quad \ldots\ldots\ldots \quad [2]$$

**iii. Determination of Crude Protein**

Folin lowry method was used to determined crude protein *Phyllanthus muellerianus* leave. A portion (0.5 g) of the sample was homogenized using 10 mL of water and centrifuged. From the supernatant, I mL was taken and added to alkaline copper reagent. This was incubated for 10mins. After 10mins, 0.4ml of folin C was added and incubated for 5minutes. Absorbance was read at 750 nm (Lowry *et al.*, 1951).

**iv. Determination of Fat Content**

From the sample, 0.5 g was measured and homogenized using chloroform to methanol (1:2). To the homogenate, 10 mL of normal saline was added. The resulting mixture was separated with a separating funnel. The lower sample decanted was dried and weighed.

**v. Determination of Carbohydrate**

Anthrone method was used to determine Carbohydrate. A portion (1.0 g) of the sample was measured and homogenized with 20 ml of sulfuric acid. The resulting solution was heated for 10 minutes and filtered. The filtrate was made up to 250 mL and 1mL was taken from it which was also made up to 10 mL from the solution, 1 mL was measured and 4 mL of anthrone reagent was added. This was then boiled for 10 minutes. Absorbance was read at 620 nm.

**Preparation of the Extract for Phytochemical Screening**

Ethanol extract of *Phyllanthus muellerianus* was prepared by weighing 250 g of dried sample and dissolving in 2 L of ethanol in a glass jar. This was left standing for 3 days with occasional maceration. After 3 days, the filtrate was collected and dried over heat by evaporation to obtain a dried extract which was weighed to yield 5.9% and stored in a sample bottle before use.

**Qualitative Phytochemical screening of *Phyllanthus muellerianus* leave**

Phytochemical screening was carried out using methods described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973). 2 g of extract was dissolved in water and used for the following tests.

**Flavonoids**

To 1 mL of extract, 2 mL of diluted ammonia and small quantity of concentrated sulfuric acid was added. A yellow coloration was observed but disappeared on standing which indicates a positive result.

**Tannins**

To 1 mL of extract, few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black coloration.

**Phenols**

To 1 mL of extract, 2 mL of iron chloride was added and observed for a deep blue or green coloration.

**Alkaloid**

From the extract, 1 mL of was taken and 4mL of 1% HCl was added. This was boiled in a water bath for 5mins and filtered. 2 mL of the filtrate was treated with 2-3 drops of dragendorff reagent. Turbidity shows the presence of alkaloid.

**Saponin**

From the extract, 1 mL was boiled in 10 mL of distilled water in a water bath and filtered. 5 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and then observed for the formation of emulsion.

**Phlobatanin**

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 3 mL of 1% aqueous HCl indicates the presence of phlobatanin.

**Steroids**

Acetic anhydride (2 mL) was added to 1 mL of ethanol extract of sample with 2 mL sulfuric acid. The color change from violet to blue or green in some samples indicates the presence of steroids.

**Anthraquinones**

To 1 mL of extract, 10 ml of 10% HCl was added and boiled for few minutes and filtered. Equal volume of chloroform and few drops of 1% $NH_3$ were added to the filtrate and heated. The formation of rose-pink colour indicates positivity.

**Terpenoid**

To 1 mL of extract, 2 mL of chloroform and few drops of $H_2SO_4$ were added to form a layer. A brownish coloration indicates a positive result.

**Cardiac Glycoside**

Glacial acetic acid of 1 mL containing one drop of ferric chloride solution was used to treat 1 mL of each ectract. This was underplayed with 1 mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

**Quantitative Phytochemical screening of *Phyllanthus muellerianus* leave**

**Tannin**

From the sample, 1 mL was taken and added to 10 mL distilled water. This was incubated for 1 hour and filtered then made up to 25 mL. From the filtrate, 1 mL was added to distilled water of 5 mL with 5 drops of tannin reagent. Absorbance was read after 10 minutes at 720 nm using tannin as standard.

**Saponin**

From the sample, 1 mL was taken and 25 mL of isobutyl alcohol with shaking. This was filtered after 5 mins and 10 mL of 40% magnesium carbonate sodium was added. The resulting mixture was then filtered again to obtain a colorless solution. From the filtrate, 1 mL was taken, 2 mL of 5% $FeCl_3$ was added which was then made up to 50 mL with distilled water and allowed to stand for 30 mins for the development of a blood red colour. Absorbance was then read at 380 nm.

**Flavonoid**

A portion (1.5 mL) of sample was taken and added to 1.5 mL of 2% Aluminium chloride. Incubation was then done for 5mins and absorbance was then read at 510 nm using quercetin as the standard.

**Determinations of Anti-nutrients composition of Phyllanthus muellerianus leave**

**Determination of Phytate**

The phytate was determined using the method reported by Hassan *et al*., (2011). Powdered sample (4 g) were soaked in 100 $cm^3$ of 2% HCl (v/v) for 3 h and filtered. To 25 $cm^3$ of the filtrate in a conical flask, 5 $cm^3$ of 0.3% $NH_4SCN_{(aq)}$ and 53.5 $cm^3$ of distilled water were mixed together and titrated against standard $FeCl_{3(aq)}$ solution containing 0.00195 g Fe/$cm^3$ until yellow colour persisted for 5 minutes. Phytin-Phosphorus (1 $cm^3$ Fe = 1.19 mg Phytin-Phosphorus) was determined and the phytate content calculated by multiplying the value of the Phytin-Phosphorus by 3.55.

**Determination of Oxalate**

For oxalate estimation, the Munro and Basir method as reported by Monago and Akhidue (2002) was used to obtain the total oxalate. Ground sample of 1 g was taken and extracted three times by warming and stirring with magnetic stirrer for 1 h in 20 $cm^3$ of 0.3 moldm-3 HCl each time. The combined extract was made up to 100 cm3 in a volumetric flask with distilled water. From this, 5 cm3 was taken and made alkaline with 1.0 $cm^3$ 5 $moldm^{-3}$ ammonium hydroxide. Drops of glacial ethanoic acid were added after 3 drops of phenolphthalein indicator were added until a colourless solution was obtained. $CaCl_2$ of 1.0 $cm^3$ was then added; the mixture was allowed to stand for 3 h and finally centrifuged at 3000 rpm for 15 mins. The supernatant was discarded and the precipitate was washed with hot water. The precipitate was then dissolved in 2.0 $cm^3$ warm 1.5 $moldm^{-3}$ $H_2SO_4$ in a water bath. This was then titrated with a freshly prepared 0.01 $moldm^{-3}$ $KMnO_4$ initially at ordinary temperature until the first pink colour appeared throughout the solution. This was allowed to stand until it becomes colourless and warmed afterwards for further titration until a pink colour which persisted for at least 30 seconds was obtained. The obtained values were used to calculate the oxalate content of the samples.

**Determination of Alkaloid**

Aliquots (0.4, 0.6, 0.8, 1.0 and 1.2 mL) of atropine standard solution was measured. Each was transferred to different separating funnels and 5 mL of pH 7 phosphate buffer with 5 mL of BCG was added. The mixture was shaken with 1 mL, 2 mL, 3 mL and 4 mL of chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank solution which was also prepared

according to the method above but without atropine.

**RESULTS**

The result from figure 1 shows the proximate analysis of dry sample of *Phyllanthus muellerianus* leaves. According to the result, a very high amount of crude fibre (55 %) was present in the leaves. Carbohydrates show the next high value (15 %) with a low amount of protein (6 %).

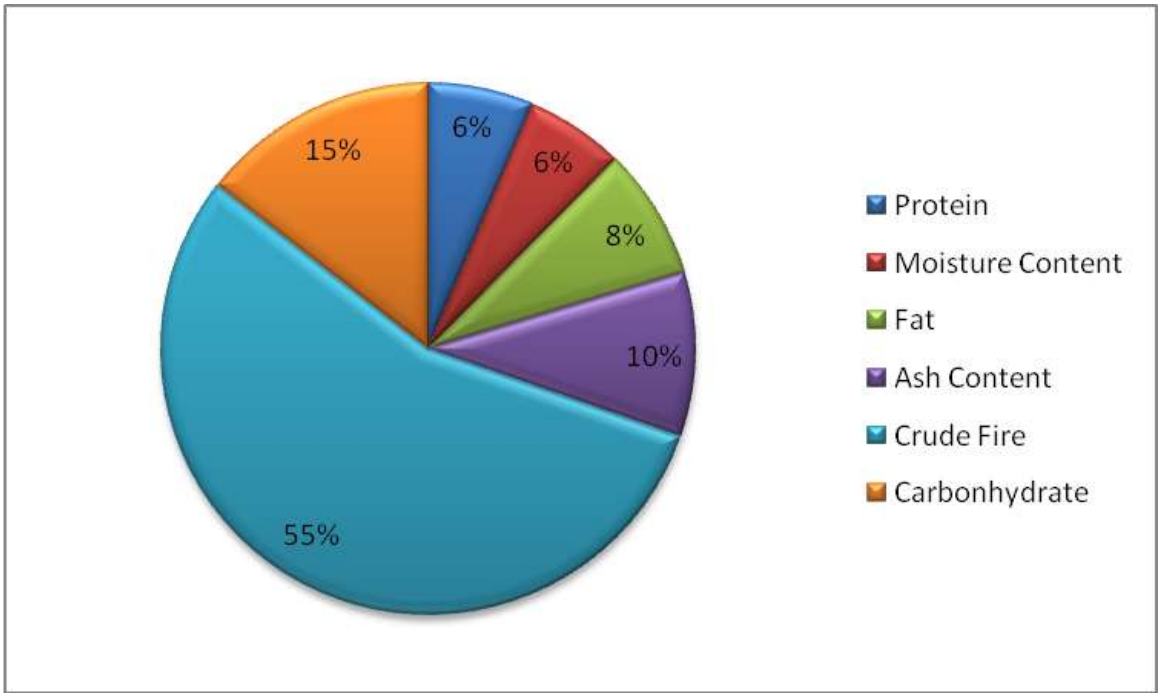


**Figure 1: Proximate analysis of dry sample of *Phyllanthus muellerianus* leaves**

Table 1 shows the qualitative phytochemical analysis of *P. muellerianus* ethanol extract revealing the presence (+) of saponin. flavonoid, phlobatanin, tannin, steroid, cardiac glycoside and terpenoid while phenol, alkaloid and anthraqinone were absent (-).

**Table 1: Summary of qualitative phytochemical screening of *Phyllanthus muellerianus***

| Test | Inference |
|---|---|
| Flavonoids | + |
| Tannin | + |
| Phenols | - |
| Alkaloid | - |
| Saponin | + |
| Phlobatanin | + |
| Steroids | + |
| Anthraquinones | - |
| Terpenoids | + |
| Cardiac Glycosides | + |

The quantitative phytochemical analysis in ethanol extract of *P. muellerianus* in table 2 shows the presence of Saponins (8.18 ± 0.04 mg/100 g), Flavonoids (3.59 ± 0.19 mg/100 g), Tannins (31.52 ± 2.81 mg/100g).

**Table 2: Quatitative phytochemical screening of *Phyllanthus muellerianus***

| Phytochemical | Mean±Sdv mg/100g |
|---|---|
| Tannin | 31.52 ± 2.81 |
| Saponin | 8.18 ± 0.04 |
| Flavonoid | 3.59 ± 0.19 |

Table 3 shows numeric value of the antinutrients present in *P. muellerianus.* From the result, it shows that phytate, oxalate, tannin and alkaloid have 2.5mL, 0.3mL, 0.052nm and 0.264 nm, respectively.

Values of Total Antioxidant Capacity in *P. muellerianus* (Table 4) which reveals a very high value of total antioxidant capacity.

**Table 3: Summary of the numeric value of the antinutrients present in *Phyllanthus muellerianus.***

| Anti-nutrient | Value |
|---|---|
| Phytate | 2.5 mL |
| Oxalate | 0.3 mL |
| Tannin | 0.052 nm |
| Alkaloid | 0.264 nm |

**Table 4: Summary values of Total antioxidant Capacity in *Phyllanthus muellerianus***

| Name of Species | Plant Total flavonoids mg/100g | Total phenol mg/100g | TotalAntioxidant Capacity mg/100g |
|---|---|---|---|
| *Phyllanthus muellerianus* | 3.59 ± 0.19 | 10.89 ± 0.17 | 46.69 ± 1.21 |

## DISCUSSION

The results of the proximate food analysis show that *P. muellerianus* is enriched with carbohydrate, protein, fibre, ash content, fat and moisture content which make the leave of the plant to have potential as food supplement in developing countries. The high level of crude fibre obtained from this result means it has anti-tumorigenic and hypocholestrolaemic agent which makes the plant good for people with cholesterol related problems (Peter and Tolulope, 2015). The low moisture content account for its long shelf life when harvested and dried. Proximate analysis Flavonoids presence in *P. muellerianus* leaves gives an antioxidant property which acts as a free radical scavenger. Research has shown that antioxidants aid in the oxidation of low density lipoproteins (LDL), inhibition of lipid peroxidation, to promote vascular relaxation and helps to prevent artherosclerosis (Mohammad *et al*., 2015). Flavonoids have been reported to be synthesized by plants in response to microbial infection and have been shown to have antibacterial activities (Kujumgeiv *et al.,* 1999; Gorniak *et al*., 2019). It is also known to provide colour, texture and taste present in varieties of fruits and vegetables such as citrus fruits and juices, berries, apples, tea, red cabbage and various botanicals including *P. muellerianus* plant which is known as a good source of nutrients and play preventive roles against diseases in the body (Pietta, 2000).

Cardiac glycosides are also noticed to be present in this plant. They are often used to treat severe heart failure and atrial fibrillation that can occur with congenital heart defects (Newman *et al.,* 2008). It helps to control rapid irregular heartbeats, reduce the back up of blood and fluid in the body and increase blood flow through the kidney which helps get rid of salt.

Saponins which is present in this plant has several benefits which include; helping to inhibit the growth of cancer cells, serving as cholesterol lowering agent (Shi *et al*., 2004). They also serve as an immune boost, as a natural antibiotic, energy boost and as a sweetener. Their anticarcinogenic properties include antioxidant effect, direct and selective cytotoxicity of cancer cells, immune-modulation, acid and neutral sterol metabolism and regulation of cell proliferation.

According to the results gotten from the

antinutrients, it was deduced that there was a higher level of phytate (2.5 mL) than oxalate (0.3 mL). As an antinutrient, phytate has its advantages and disadvantages. Phytate has a strong binding affinity to important minerals such as calcium, zinc and iron. When binding takes place, there is the formation of insoluble precipitate (usually zinc or iron) which is less absorbable in the intestines. This can contribute to zinc and iron deficiencies in people that rely on these foods for their mineral intake (Hurell, 2003). Phytate for its alleged anti-cancer properties, based on a research by Shamsuddin (2002) has a strong binding affinity for trace elements and helps in its regulation. High value of total antioxidant capacity in *P. muellerianus* makes the herb a potent herb (Anbudhasan *et al*., 2014)

## CONCLUSION AND RECOMMENDATIONS

*Phyllanthus muellerianus* plant showed the presence of saponins, flavonoids, phlobatanin, tannins, steroids, cardiac glycosides and terpenoids. The study also revealed a significant high amount of antioxidants which might help in the inhibition of free radicals in the body. Results of *Phyllanthus muellerianus* ethanol leaves extract indicated that its plant-derived active could be considered for clinical evaluation for the production of improved phytomedicine in the treatment of multi-resistant microbial infections.

## REFERENCES

Anbudhasan, P., Surendraraj, A., Karkuzhali, S. and Sathishkumaran, S. (2014). Natural Antioxidants and Its Benefits. *International Journal of Food and Nutritional Sciences.* 3(6): 225-232.

AOAC, (2005). Official methods of analysis (18ed). Washington D C, USA. Association of official Analytical Chemists. 88 (3): 673-678. https://doi.org/10.1093/jaoac/88.3.673

Assob, C. N., Kamga, L. F., Nsagha, D. S., Njunda, A. L. Nde, P. F., Asongalem, E. A., Njouendou, A.J., Sandjon, B. and Penlap, V. B. (2011). Antimicrobial and toxicological activities of five medicinal plant species from Cameroun Traditional medicine. BMC Complementary and Alternative medicine. Pp 11-70.

Burkil, H. M. (1994). *The useful Plants of West Tropical Africa.* 2nd Edition. Royal Botanic Gardens. Kew, Richmond, United Kingdom. Pp 636.

Doughari, J. H. and Sunday, D. (2008). Antibacterial activities of *Phyllanthus muellerianus. Pharmaceutical Biology*. 46 (6): 400-405.

Gorniak, I., Bartoszewski, R. and Kroliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochem Rev.* 18 (1): 241-272.

Harborne, J. B. (1973). *Phytochemical methods: a guide to modern techniques of plant analysis*. Chapman and Hall Ltd, London. Pp 279. https://doi.org/10.1002/food.19730170231.

Hassan, L. G., Umar, K. J., Dangoggo, S. N. and Maigandi, A. S. (2011). Anti-nutrient Composition and Bioavailability Prediction as Exemplified by Calcium, Iron, and Zinc in *Melocia corchorifolia leaves. Pakistan Journal of Nutrition.* 10 (1): 23-28.

Hurrel, R. F. (2003). Influence of vegetable protein sources on trace elements and mineral bioavailabity. *The Journal of nutrition.* 133 (1): 2973-2977.

Kujumgiev, A., Tsvetkoval, S., Bankova, V., Christo, R. and Popov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis geographic origin. *Journal of Ethnopharmacology.* 64(3): 35-40. https://doi.org/10.1016/s03788741(98)00131-7.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randal, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 193 (1): 265-275.

Mohammed, A. R., Noorlidah, A., and Norhaniza, A. (2015). Antioxidative Effect and Inhibition of Human Low Density Lipoprotein Oxidation In Vitro of Polyphenolic Compounds in *Flammilina velutipes* (Golden Needle Mushroom). *Journal of Oxidative Medicine and Cellular*

*Longevity*. Article ID 403023, 10p. https://doi.org/10.1155/2015/403023

Monago, C. C. and Akhidue, V. (2002). Estimation of Tanin, Saponin, Oxalate, Cynagenic and Cardiac Glycosides in *Garcinia Kola. Journal of Applied Sciences and Environmental Management.* 16 (1): 22-25.

Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (2008). Assessment Techniques of Antimicrobial Preperties of Natural Compunds of Plant Origin: Current methods and future trends. *African Journal of Biotechnology*. 7 (1): 1997-1806.

Newman, R., Yang, P., Pawlus, A. and Block, K. (2008). Cardiac glycosides as novel cancer therapeutic agents. *Journal of Molecular Interventions.* 8 (1): 36.

Peter, T. O. and Tolulope, A. O. (2015). Proximate Analysis and Chemical Composition of *Cortinarius Species*. *European Journal of Research in Biological and Life Sciences.* 3(3): 1-9.

Pietta, P. G. (2000). Flavonoids as Antioxidants. *Journal of Natural Product.* 63: 1035-1042. Plant Resources of Tropical Africa, (2015). Protabase. http;//www.prota.org.

Pourmorad, F., Hosseinimehr, S. J. and Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology.* 5 (11): 1142-1145.

Ratheesh, M. R. and Helen A. (2007). Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats. *African Journal of Biotechnology*. 6 (10).

Shamsuddin, A. M. (2002). Anti-cancer function of Phytic Acid. *International Journal of Food Science and Technology.* 37 (7):769-782.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G. and Jiang, Y. (2004). Saponins from edible legumes: Chemistry, Processing and Health benefits. *Journal of Medicinal Food,* 7 (1): 67-78.

Siram, S., Patel, M. A., Patel, K. V. and Punjani, N. H. (2004). Compendium on Medicinal Plants, S A Gujarat. Agricultural University, Ahmedabad, India: Pp 1-54.

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. 2nd Ed. Sunshine house, Ibadan, Nigeria: Spectrum Books Ltd, Ibadan, Nigeria: Pp 289.

Trease, G. E. and Evans, M. O. (1989). *A textbook of Pharmacognosy*. 13th Ed. Builler Trindall and Canssel London: Pp 176-180.

Usunobun, U., Okolie, N. P., Anyawu, O. G., Adegbegi, A. J. and Egharevba, M. E. (2015). Phytochemical Screening and Proximate composition of *Annona muricata* leaves. *European Journal of Botany, Plant Science and Phytology.* 2 (1): 18-28.

World health Organization. Regional Publication, (1990). Medicinal plants in Vietnam. Institution of Material Medical, Hanoi. WHO regional office for the western pacific, Manila, Philippines. Series 3. http//www.iris.wpro.who.int/handle/10665.1/6736.