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PHYTOCHEMICALS AND ANTIOXIDANT PROPERTIES OF *LANDOLPHIA TOGOLANA* PLANT ROOT IN MAINTENANCE OF HEALTH AND WELL-BEING OF POST-PARTUM MOTHERS IN ONDO STATE, NIGERIA.

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Abstract

The study examined the phytochemical and antioxidant properties of *Landolphia togolana* root used in maintenance of health and well-being of post-partum mothers in Ondo State, Nigeria. Pure experimental design was used for the study. *Landolphia Togolana* root were harvested, washed, dried at 60°C for 24hrs and then milled into powder with a hammer mill. They were filled with a thick-gauged cellophane films, placed in air-tight bottles and stored in the freezer for the phytochemical and antioxidant analysis using standard procedure. Chemical analysis was carried out at the Department of Food Technology and Biotechnology system, Durban University of Technology, Steve Biko Campus, South Africa. Quantitative and Qualitative phytochemical analysis, anti-bodies activities determination, reducing potential assay, free radical scavenging and antioxidant properties were determined. Data obtained were analyzed using mean and standard deviation, t-test statistical tools. Results from the study revealed that the powdered root sample contained bioactive properties. The phytochemical analysis showed higher total flavonoids (392.49 ± 0.67 mg QE/g) followed by tannin (148.8 ± 0.59 mg TA/g) steroid (126.1833 ± 0.47 mg) triterpene content (95.68 ± 0.84), terpenoids, and coumarin (35.14 ± 0.17). However, it was observed that saponin (0.821 ± 0.03), alkaloids (0.41 ± 0.02) and glycosides (0.04 ± 0.01) showed low concentrations and the analysis further showed presence of seven secondary metabolites while fixed oil and alkaloids were not observed. The result of antioxidants activities of *Landolphia Togolana*, Diphenyl-2-picryl (DPPH) free radical scavenging activities of different concentrations of vitamin C and extract from the root of *Landolphia togolana*, the methanol extract of the root of *Landolphia togolana* demonstrated remarkable level of antioxidant properties and dose dependent increase in free radical scavenging activity. The methanol extract of the root of *Landolphia togolana* showed the highest inhibition percentage (100.1%) at 60 µg/ mL concentration compared to standard ascorbic acid with inhibition percentage of 98.53 % which make it stand as antioxidants which can fight against free radicals. Based on the findings the study concluded that *landolphia togolana* root contains bioactive properties which help to protect the body and fight against free radicals which makes it to be suitable for maintenance of post-partum mothers health and recommendations were made.

Keyword: Phytochemical, Antioxidant, Post-partum mothers, Nigeria.

Introduction

Plants have played very significant roles in the maintenance of health and well-being of humans throughout history. Plants are good sources of natural products commonly used in maintenance of health and well-being. The roots, leaves, seeds and bark of some plants are found to be good for foods and medicines because of their chemical and nutritional properties, such plants include: *landolphial owenriensis* and *landolphia togolana* among others. The therapeutic, culinary and nutritional effects of some plants commonly used in folklore remedies against many diseases may be attributed to the antioxidant properties of their phytoconstituents. The beneficial effects of plant antioxidants in promoting health are believed to be achieved through several possible mechanisms. Many plants are edible hence are used in food preparation and some are used as flavor enhancers, spices, sources of fibre, minerals and vitamins and sources of phytochemicals. The use of plants as a medium to maintaining health and well-being has increased substantially due to the increasing interests on natural substances. Among these plants is *Landolphia family* which are highly nutritious with antioxidant properties (Kossivi, Amegnona, & Messanvi 2015).

Landolphia togolana is a climber that belong to the family of Apocynaceace commonly called vine rubber. *Landolphia* has about 60 species on the African continent and it is known locally by various names in Nigeria, *Ibo – Eso/utu, Yoruba Mba / Alakitipa, Hausa – Ciwa*. It is locally called by people of Akoko south in Ondo State as *Jabajaba* and it is commonly found in the rain forest region of Nigeria and other parts of Africa. The plant flowers are seen mainly at the beginning of the rainy season, and also at the end of the dry season. Its fruits spans throughout the year. The rubber or gum of *Landolphia plants* is said to be of good quality in the Republic of Benin (Lewis & Lewis, 1977). In Akoko the plants are valuable in promoting food security and wellness due to their availability, affordability and their great nutritional value. The most common species in Africa includes, *Landolphia owerienses* and *Landolphia Toglana*. (Lewis & Lewis, 1977).

Landolphia togolana (jabajaba) is one of the species found in the African region and is locally believed to have some nutrients which could be responsible for its wide use for soup preparation by mothers in Ondo State, Nigeria to elevate and maintain the well-being of post-partum mothers immediately after delivery and women's health in general. A post-partum period begins immediately after the birth of a child. It is the period when the mother's physiological and emotional changes occur, including hormone levels and uterus size which is expected to return to normal state within forty days. World Health Organization (2014) reported that the term "post-partum" period is commonly used to refer to the first six weeks following childbirth. The World Health Organization (2014) describes the post-natal period as the most critical and yet the most neglected period in the lives of the mothers and the babies and most maternal and new-born deaths occur during the post-partum period (Kamile, Altuntuğ, Yeşim, Anikand, Emel & Ege 2015). In this period, significant physiological, social, and emotional changes occur in the woman, hence various traditional foods are used to protect the health of the mother and that of the baby.

Landolphia togolana root species are mostly used in soup preparation to help the mother deal with problems associated with child birth and complications after birth such as hemorrhage, pain and inflammations, aneamia, exhaustion, stress, infections, failure of breast milk production, psychological and emotional disorders often associated with child birth. (Kamile,

Yeşim & Emel 2018). In Ondo state it is believed that women are vulnerable to infections and diseases in the post-partum period and the use of *Landolphia togolana* root extract as soup preparation could help alleviate the problems associated with such diseases, this may due to their phytochemicals and antioxidants properties.

Phytochemicals such as flavonoids, carotenoid, lycopene, terpenes and phytoestrogens are chemicals associated with foods and also referred to as "bioactive properties" present in a plant. The phytochemicals present in the *landolphia togolana* root plants family have anti-microbial properties, cholesterol lowering, immunological modulators and anti-carcinogenic effect (Olanipekun, Kayode & Akinyemi, (2013). These may provide many added nutritional benefits and can help reduce risk of developing chronic health conditions such as heart disease, stroke, cataract, Parkinson's disease and even cancer (Olanipekun, Kayode, & Akinyemi, (2013). Phytochemicals are not necessarily nutrients and are not consumed by the human body to support life, but they do have valuable properties that can help avoid or treat certain common diseases. Many of these benefits mean that phytochemicals can play a role in disease prevention and care. The plant family has been reported to contain large quantity of iron and zinc, trace elements which play important role in many enzymes for their functions and also act as anti-aging agent when consumed (Owoyele, Olayele & Elegba, (2002). *Landolphia togolana* species (root) is traditionally used for soup preparation in Ondo State among experienced women especially for post-partum mothers because of its health and nutritional value. It can also be used as supplement and as a functional food. *Landolphia togolana* has continued to be an important plant in South west Nigeria where it is widely consumed mainly as "Jabajaba" soup. However, little is known about these plant's phytochemicals and antioxidant properties.

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves. Though oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases (Hamid et al. 2010). Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging (Amit and Priyadarsini 2011). Besides, there are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β -carotene, and vitamin C, and hence these must be supplemented in the normal diet (Teresa et al. 2011). Antioxidants can also act as prooxidants when these are not present at the right place at the right concentration at the right time (Tourinho et al. 2008).

Antioxidants can be classified into two major types based on their source, i.e., natural and synthetic. Natural antioxidants are gotten directly and entirely from natural sources which are used in food and pharmaceutical industries. While the synthetic antioxidants are substances

created from chemical processes. Natural antioxidants are widely distributed in food and medicinal plants. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer (Boeing, 2012). The effective extraction and proper assessment of antioxidants from food and medicinal plants are crucial to explore the potential antioxidant sources and promote the application in functional foods, pharmaceuticals and food additives. The present provides comprehensive information on the green extraction technologies of natural antioxidants, assessment of antioxidant activity at chemical and cellular based levels and their main sources from food and medicinal plants (Boeing, 2012). The exogenous antioxidants are mainly derived from food and medicinal plants, such as fruits, vegetables, cereals, mushrooms, beverages, flowers, spices and traditional medicinal herbs (Amiot, 2016). Besides, the industries processing agricultural by-products are also potentially important sources of natural antioxidants (Koosha, 2016). These natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C).

Statement of the Problem: The post-partum period is a time for a mother to recover from trauma of childbearing and take on new responsibilities. In many cultures, it is also seen as a period when a new mother is prone to illness and specific traditional practices are observed such as diet modification to ensure quick recovery and avoid sickness latter on. The World Health Organization in Akerele, (1991) explained that 80% of people in developing countries, (Nigeria inclusive) still rely on indigenous plants to meet their nutritional and health needs. Despite this, negligence and under-utilization of indigenous plants still remain a major problem. Among these plants is *Landolphia togolana* which is widely consume in south west Nigeria as soup during post-partum period. It has been observed that post-partum dietary habit varies greatly among different cultures and ethnic groups especially those influenced by the concept of traditional system, they follow special dietary practices during post-partum period, this is due to the high physiological stress of pregnancy and blood loss during delivery. Post-partum period is a period when major changes occur during and after childbirth which possess significant health risks for both the mother and the baby. This soup known as "Jabajaba soup", is a special traditional soup prepared with *landolphia togolana* root specie for southern Nigeria mothers during post-partum period.

People may have been eating this plant *landolphia togolana* root specie traditionally without knowing the nutritional and medicinal values. Despite its wide consumption it is neglected, unpopular, underutilized and gradually going into extinction. In order to solve the above problems, the study therefore, determined the phytochemical and antioxidant properties of *landolphia togolana* root specie used for maintaining health and well-being of postpartum mothers in Ondo State, Nigeria. Adequate knowledge of *Landolphia* chemical composition is needed for successful utilization. Given its remarkable nutritional potential, nutritional and anti-nutritional factor evaluation is required, as this will serve as the foundation for its constant use as a formulation of a new diet source in the fabrication of new food products in the food industry. *Landolphia togolana* root contains anti-nutritional factors that have health advantages, such as phytic acid, which has antioxidant, anti-carcinogenic, and hypoglycemic properties. This research will contribute to a better understanding of these advantages. The outcome of this study will help

to create awareness on the phytochemicals, antioxidant and medicinal values as well as the usage of the *landolphia togolana* root specie among women for maintenance of well-being.

Purpose of the Study

The main purpose of this study was to determine the phytochemical and antioxidant properties of (*Landolphia Togolana*) root used in maintaining health and well-being of post-partum mothers in Ondo State, Nigeria. Specifically, the study,

1. Determined the phytochemical properties of *Landolphia Togolana* root extract.
2. Determined the antioxidant properties of *Landolphia Togolana* root extract

Materials and Methods; Sources of Raw Materials: The plant was obtained from Adelabu Salami's farm in Owo, Owo Local Government Area of Ondo State, Nigeria in June, 2017. The plant was carefully up-rooted, with the leaf and stem and was identified by a Professor of Botany in the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Nigeria. *Landolphia togolana* root species were extracted at the Department of plant pharmacology, laboratory, University of Benin. The root extract was used specifically for this study because of its chemical, nutritional and medicinal properties.

Laboratory Experimental design was performed in the food and biotechnology laboratory of the Department of Food Technology, Steve Bako Campus, Durban University of Technology, Durban South Africa. Chemical analysis such as antioxidants determination and phytochemical analysis were carried out according to standard procedures described by the Association of Official Analytical Chemist (2005) and Odebiyi and Sofowora, (1978).

Extraction with methanol by the described by the Association of Official Analytical Chemist (2005). The root powder of 500g was weighed and soaked with 1500ml of methanol concentrate for 24hrs under dark cupboard. The sample was filtering and evaporated at 60°C for 30mins, the yield gotten from extract was 2.65ml and this was bottled and store in a refrigerator and used for the antioxidant (DPPH) and phytochemicals analysis.

Qualitative Phytochemical Analysis

Phytochemical analysis of *Landolphia togolana* root extract was evaluated according to the method described by Odebiyi and Sofowora (1978) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

Determination of Total Phenol Content

Total phenolic content was determined according to Folin and Ciocalteu's method (1927). Concentrations ranging from 0.01-0.15 mg/ml of gallic acid were prepared from a stock solution (1mg/ml). Concentrations of the extracts (1mg/ml) were also prepared in methanol. Four and a half milliliter of distilled water was added to 0.5 ml of the extract/fractions and mixed with 0.5 ml of a ten-fold diluted FolinCiocalteu reagent. Five milliliters of 7% sodium carbonate were then added to the tubes and another 2 ml of distilled water was added. The mixture was allowed to stand for 90 min at room temperature then absorbance read at 760 nm. All determinations

were performed in triplicates with gallic acid utilized as the standard. The total phenolic content was expressed as gallic acid equivalent (GAE).

Antioxidant Determination. Diphenyl-2-picryl-hydrazyl radical scavenging assay

Diphenyl-2-picryl-hydrazyl (DPPH) scavenging effect of crude methanol extract/fractions of *T. conophorum* seed was determined by the method described by Jain *et al.* 2008. A stock solution of 0.1 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract/fractions in methanol containing 0.01–0.2 mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was read spectrophotometrically at 517 nm. Ascorbic acid was used as standard. Percentage inhibition of the samples were calculated using the equation below:

$$\text{Percentage Inhibition (\%)} = [(A_0 - A_1) / (A_0)] \times 100,$$

Where A_0 = absorbance of DPPH radical + methanol; A_1 = absorbance of DPPH radical + sample extract / standard.

Reducing Potential Assay: The reduction of potential activity of extract was determined with the method described by Lai *et al* (2001). One milliliter of different concentrations of extracts (0.1–1.0 mg/ml) in water was mixed with 2.5 ml (0.2 M phosphate buffer, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (10%) was added and the mixture was centrifuged at 1000 g for 10 min. The supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl_3 . Absorbance was read in a spectrophotometer at 700 nm. Higher absorbance values indicated higher reducing power. Ascorbic acid was used as a standard.

Results and Discussions

Table 1: Result of Determination of qualitative phytochemical content of *landolphia togolana*

Phytochemical	Methanol Extract
Saponin	+
Tannins	+
Phenolics	+
Phlobatannin	-
Steroids	+
Flavonoids	+
Cumarins	+
Amino acid	-
Terpenoids	+
Glycosides	+
Triterpenes	+
Alkaloids	+
Fixed Oil	+

Key: + = Present - = Absent.

Qualitative phytochemical investigation of methanol extract *Lanolphia togolana* root bark demonstrates the presence of seven secondary metabolites (saponins, phenols, steroids,

cumarins, terpenoids, glycosides, and triterpenes). However, no phlobatannin, amino acid was observed in the crude extract and fractions.

Table 2: Result of Determination of quantitative Phytochemical Analysis of methanol extract of *Landolphia togolana* root.

Phytochemical	Concentration
Saponins	0.821±0.03 g/100 ml
Steroid	126.00±0.39g/100ml
Tannins	1.488±0.59 mg TA/g extract
Flavonoids	392.49±0.67 mg QE/g extract
Coumarins	35.14±0.17 mg GA/g extract
Terpenoids	59.36±0.19 g/100 ml
Glycosides	0.036±0.01 g/100ml
Triterpene	95.68±0.84 g/100 ml
Alkaloids	0.413±0.02 g/100ml

Values are expressed as Mean ±SEM, n=3

Results from the quantitative phytochemical investigation showed higher total flavonoids (392.49±0.67 mg QE/g), followed by tannins/steroids (148.8±0.59 mg TA/g), triterpene content (95.68±0.84), terpenoids, and coumarin. However, it was observed that saponin, alkaloids and glycosides showed low concentration.

Table 3: Result of DPPH Radical Scavenging Activity of Different Concentrations of Ascorbic acid and Extract From the root of *Lanolphia togolana*

Concentration(µg/mL)	% Inhibition of methanol Extract of <i>Lanolphia togolana</i>	% Inhibition of Ascorbic acid (Standard)
20	84.8±0.03 ^a	63.59±0.01 ^a
40	97.8±0.04 ^b	96.97±0.07 ^b
60	100.1±0.05 ^b	98.53±0.01 ^b
80	98.7±0.02 ^b	98.82±0.08 ^b
100	101.2±0.25 ^b	99.41±0.01 ^b

Values are expressed as Mean ±SEM, n=3. Values with different lowercase letters are significantly different between means p<0.05

DPPH radical scavenging activities of different concentrations of vitamin C and extract from the root of *Lanolphia togolana* showed that the methanol extract of the root of *L. togolana* demonstrated remarkable and dose dependent increase in radical scavenging activity. The methanol extract of the root of *Lanolphia togolana* showed the highest inhibition percentage (100.1%) at 60 µg/ mL concentration compared to standard ascorbic acid with inhibition percentage of 98.53 %.

The result of Phytochemical analysis revealed that the methanol extract of *L. togolana* root is rich in saponins 0.821±0.03mg/g, phenols, steroids 126.18±0.47mg/g, alkaloid 0.413,

cumarins 35.14 ± 0.17 mg/g, terpenoids 59.36 ± 0.1 mg/g, glycosides 0.04 ± 0.01 mg/g, and triterpenes 95.68 ± 0.84 mg/g. These phytochemicals are known to exhibit medicinal and physiological activities confirming its use in ethnomedicine. The presence of these phytochemical constituents in the methanol extract of *Landolphia togolana* root has made it to have medicinal properties. Sofowora (1993) reported that these secondary metabolites possess antimicrobial, anti-inflammatory, anti-hypertensive and analgesic activities. Medicinal plants and the use of herbs are now sources of many important drugs and food supplements of the modern world and have been documented by some researchers especially Holetz, Pessini, Sanches, Cortez, Nakamura, & Filho (2002) who asserted that *landolphia* plants root contain bioactive constituents such as steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, glycosides, saponins, phenols etc. Addae-Mensah, (1992) and Ojinnaka & Nwokonkwo (2012) buttressed these with the fact that these bioactive substances are found well distributed in medicinal plants and that they are the active principles used as antioxidants, antifungals, antibiotics, anti-inflammatory, hemolytic, antipyretic, analgesics and diuretics. In herbal and traditional medicine, the part of the plant that contains the active ingredient; leaves, stem, root, seeds and so on is taken in form of an extract, infusion or decoction before they are medicinally utilized. This is in agreement with the findings of this study that the root of *landolphia togolana* contained bioactive properties and showed higher total flavonoids (392.49 ± 0.67 mg QE/g). Flavonoids are important antioxidants, and promote several health effects. Aside from antioxidant activity, these molecules provide the following beneficial effects: Anti-virus, Anti-cancer, Anti-inflammatory, Anti-allergic. Aiyegoro, O.A., & Okoh, A.I. (2010).

Landolphia plant species especially the *owariensis* and *togolana* have been utilized locally for various medicinal advantages. The leaves and roots are used in herbal medical practice. Different parts of this plant are used for the treatment of many ailments. The decoction of leaves is used as purgative and to cure malaria. The root is soaked in local gin for a week and the extract given two glasses a day to cure gonorrhea (Gill, 1992). Owoyele, Olaleye and Elegbe (2002) also reported that aqueous, methanol and chloroform extracts of *L. owariensis* leaves have anti-inflammatory and analgesic activities. The latex is used as an enema for intestinal worms in parts of Ivory Coast. The latex is also used as a natural preservative (Anthony, 1995).

Phytochemicals may display their health protective effects in diverse ways; they can act as antioxidants and protect cells against free radical damage for example polyphenols. The presences of saponins 0.821, flavonoid 392.49mg, tannin 148.8 and alkaloid 0.413 in the methanol extract of *L. togolana* root supports its use as an antidiabetic agent, hypocholesterolemic and anti-tumour activities as this is in agreement with the study of Olumesi and Onoagbe, (2017) which reported that alkaloid 2.25%, tannin 0.06, flavonoids 10.47% are present in *uvaria chamae* root. However, the anti-oxidative abilities of the plant extract could be attributed to the presence of phytochemicals. In the present study the methanol extract of *Landolphia togolana* root exhibited high antioxidant capacity. The DPPH radical assay is employed as a substrate to evaluate the antioxidant activity of methanol extract of *L. togolana* root. The methanol extract exhibited high percentage of inhibition in a concentration dependent manner, and compares favorably with ascorbic acid. The higher the percentage of inhibition of a plant extract/sample, the lower the IC_{50} value. IC_{50} is the amount of antioxidant required to scavenge DPPH radical concentration by 50% (Chada et al., 2011). DPPH radical scavenging activities of different concentrations of vitamin C and extract from the root of *Lanolphia togolana*

were evaluated. The methanol extract of the root of *L. togolana* demonstrated remarkable and dose dependent increase in radical scavenging activity. The methanol extract of the root of *L. togolana* showed the highest inhibition percentage (100.1%) at 60 µg/ mL concentration compared to standard ascorbic acid with inhibition percentage of 98.53 %. This shows that the plant contains a high percentage of antioxidants that can fight against free radical and protect the body

Conclusion

Based on the findings of the study, it was concluded that, the study revealed that qualitative and quantitative phytochemical and antioxidant properties present are significantly higher in quantity compare to the standard value. This makes the root to stand out in prevention of various diseases, fighting against free radical and in relation to maintaining health and well-being of post-partum mothers. The study also demonstrated that *landolphia togolana* root is also good for functional food, food supplement and additive.

Recommendation: Based on the findings of the study, the study made the following recommendations:

1. Home Economists and Manufacturers in the food industry should consider formulating a unique food supplement and soup thickener product from *Landolphia togolana* root to increase its availability and utilization.
2. Researchers in the field of Home Economics should further and widely disseminate the findings of studies carried out on the antioxidant and phytochemical properties of *Landolphia togolana* to promote awareness of the plant regardless of people's socio-economic status.
3. Agriculturist and Food scientists should invent new and more presentable ways to produce *Landolphia togolana* root specie in a large scale for women of high and middle socio-economic status.
4. The utilization and consumption of *landolphia togolana* root specie should not be limited to post-partum women alone but to the household in general.
5. Pharmacist should consider producing drugs for steroid hormone fertility, cancer and vitamin E supplement from *landolphia togolana* root specie.

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