Comparative Study on Phytochemical Composition and *In Vitro* Radical Scavenging Activity of Ethanolic Extracts of *Landolphia lanceolata* and Nutraceutical-C24/7

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Abstract. This study investigated the phytochemical composition and *in-vitro* radical scavenging activity of ethanolic extracts of Nutraceutical-C24/7 and *Landolphia lanceolata* fruit. Phytochemical screening of *Landolphia lanceolata* fruit extract indicated the presence of phenols, flavonoids, saponins, alkaloids, terpenoids, tannins, triterpenoids and steroids while nutraceutical-C24/7 showed presence of with exception of saponins, terpenoids and triterpenoids. Some of the identified phytochemicals and vitamins concentrations quantified were higher in *Landolphia lanceolata* fruit extract than nutraceutical-C24/7 with statistically significant difference ($p < 0.05$) with the former having flavonoids $0.062 \pm 0.00 \text{ mg/ml}$, phenol $0.097 \pm 0.00 \text{ mg/ml}$, vitamin C $206 \pm 42 \text{ mg/dL}$, vitamin B₁ $54.16 \pm 0.00 \text{ mg/dL}$ and vitamin B₂ $0.11 \pm 0.00 \text{ mg/dL}$ while, Nutraceutical-C24/7 extract had flavonoid $0.117 \pm 0.00 \text{ mg/ml}$, phenol 0.032 ± 0.00 , vitamin C $102.24 \pm 0.00 \text{ mg/dL}$, vitamin B₁ $23.36 \pm 0.00 \text{ mg/dL}$, and vitamin B₂ $0.067 \pm 0.00 \text{ mg/dL}$. The study also showed that at higher concentration, DPPH radical, ferric ion, and hydrogen peroxide the extracts radical scavenging activity increased. The study showed that the extracts scavenging activities at different concentrations had higher percentage inhibition on DPPH radical, reducing power of ferric ion capabilities and reducing composition of hydrogen peroxide indicated that ethanolic extract of *Landolphia lanceolata* fruit showed statistically significant different ($p < 0.05$) when compared with ethanolic extract of Nutraceutical-C24/7. Standard vitamin C percentage inhibition for DPPH radical compared well with that of *Landolphia lanceolata* fruit extract at higher concentration with no statistical significant different ($p > 0.05$). The indigenous functional food *Landolphia lanceolata* fruit can be concluded to be a good antioxidant that can scavenge, inhibit and quench free radicals. This suggests that *Landolphia lanceolata* may be considered a good source and alternative antioxidant for developing countries like Nigeria.

1. Introduction

Nutrition science offers increasing knowledge about macro, micronutrients and phytochemical compounds like carotenoids, flavonoids, anthocyanin, terpenes, phenols, saponins etc. on a molecular level [1]. A food can be defined as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutrition effect, in a way which is relevant to either the state of well-being and health or reduction of risk of disease [2]. Nutraceuticals are any non-toxic food extract supplement that has scientifically proven health benefits both the treatment and prevention of diseases [3]. Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of disease protection conferred from diets high in fruits, vegetables, beans, plant-based beverages such as tea and wine [4]. It is known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans from diseases [5]. Examples of these phytochemicals include terpenes, flavonoids, anthocyanin, phenolic compounds, saponins, alkaloids and many others.

Antioxidants are substances that prevent or delay some types of damages to the cell and different types are found in different fruits [6]. They are found in high fruits or vegetable diets. Antioxidants are inhibitors of free radicals which are highly unstable molecules that are naturally formed when one exercises or when the body converts food into energy [6]. Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis [7]. Indoles which are found in cabbages stimulate enzymes that make estrogen less effective and could reduce the risk of breast cancer [8]. Other phytochemicals which interfere with enzymes are protease inhibitors (soybean) and terpenes [7]. The phytochemical ‘allicin’ from garlic has very strong antibacterial properties [8]. Some phytochemicals bind physically to cell wall thereby preventing the adhesion of pathogens to human cell wall [9]. Proanthocyanidines are responsible for the anti-adhesion properties of cranberry [10]. Consumption of cranberries reduced the risk of urinary tract infections improved dental health [7]. Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells [8]. Capsaicin found in pepper protects DNA from carcinogens [11]. *Landolphia lanciolata* commonly known as “Ciwo” in Hausa, “Mba” in Igbo and “Eso” in Yoruba languages of Nigeria is a native African wild fruit which has a sunset yellowish color. The fruit is sweet, with high astringency, juicy. They have simple, glossy green leaves in opposite pairs, jasmine-like flowers with tubes, parts in fives and hard-shelled fleshy fruits with several seeds embedded in the pulp. *Landolphia lanciolata* fruits throughout the year, affordable and easily grown. Nutraceutical-C24/7 and *Landolphia lanceolata* are widely used by a wide range of people in Nigeria as well as neighbouring countries. But, the cost of nutraceutical-C24/7 makes it difficult for the low income earners to purchase it. Thus, the search for an indigenous functional, affordable and easily reached food with antioxidant property is best alternative. Nutraceuticals are very expensive supplement for the common man especially in developing countries including Nigeria. Whereas, most functional foods are indigenous, widespread, and affordable mostly reached at all seasons of the year.

The study compared the *in-vitro* antioxidant property of an indigenous fruit - *Landolphia lanceolata* and nutraceutical-C24/7. This study aims at comparing the qualitative and quantitative phytochemical composition of *Landolphia lanceolata* and Nutraceutical-C24/7. Specifically, to identify the phytochemical composition of *Landolphia lanceolata* and Nutraceutical-C24/7. To determine the quantitative concentration of phytochemicals and some micronutrients present. Also, to determine the capability of the extracts to scavenge DPPH radical, hydrogen peroxide and reduce Ferric ion activity.

2. Materials and Methods

Functional food and nutraceutical

Landolphia lanceolata fruits were plucked from the wild in Riyom Plateau state, Nigeria located in the north central geopolitical zone of Nigeria. It lies between latitude 8°35'N and longitude 08°36'E. It was authenticated at the Department of Plant Science and Biotechnology, Nasarawa state University Keffi, Nigeria. The fruits were examined and approved for analysis. A Nutraceutical-C24/7 Caplet was procured from a Pharmacy in Keffi, Nigeria.

Chemicals and Reagents

DPPH, 2,4-dinitrophenyl hydrazine (DNPH), Folin Ciocalteu's phenol, quercetin and ethanol were product of Sigma Aldrich, St-Louis USA. Methanol was obtained from scharlab S.L. Gato Perez, 33p I mas D'En Cisa. Other chemicals and reagents used were of analytical grade and prepared in glass distilled water.

Extraction procedures

Landolphia lanceolata fruits were cut into small pieces, freeze dried and powdered. Then 1:15w/v was soaked in ethanol. Also 1:15 w/v of Nutraceutical-C24/7 powder was soaked in ethanol. Each was shaken at 200rpm for 24 hours at ambient room temperature. The mixtures were

filtered. The filtrate was evaporated using vacuum rotary evaporator and air dried at 40°C. Stock solutions of crude ethanolic extracts were prepared by diluting the extracts with 10% dimethyl sulphoxide solution to obtain a final concentration of 400mg/ml.

Phytochemical screening

The extracts of *Landolphia lanceolata* fruits and nutraceutical-C24/7 were screened for their secondary metabolite constituents according to the standard methods of [12-13].

Alkaloids were determined by adding 1% HCl to 3ml of the extracts. Mixture was heated for 20 minutes, cooled and filtered. A few drops of Wagner's reagent were added to 1ml of the filtrate. A reddish brown precipitate indicated the presence of alkaloids.

Saponins were determined by adding 5 drops of olive oil, 3 ml of the extract in a test tube and shaken vigorously. A stable emulsion indicated the presence of saponins.

Phenols were determined by adding 2 drops of 5% FeCl₃, 1 ml of the extract was added. A greenish precipitate indicated presence of phenols. Steroids were determined by adding 5 drops of concentrated H₂SO₄ to 1 ml of the extract. A red colouration indicated the presence of steroids.

Triterpens were determined by mixing 1 ml of the extract, 5 ml drops of acetic anhydride and a drop of concentrated H₂SO₄. The mixture was steamed for 1 hour and neutralized with NaOH, followed by chloroform. A blue-green colour indicated the presence of triterpenes.

Tannins were determined by adding 1ml of freshly prepared 10% KOH, 1ml of the extract was added. A dirty white precipitate indicated the presence of tannins. Flavonoids were determined by dissolving 1 ml of extract in diluted NaOH. A yellow solution that turns colourless on addition of concentrated HCl indicated the presence of flavonoids.

Quantitative determination of Total phenols, Total flavonoids and vitamins C, B₁ and B₂ in the extracts of *Landolphia lanceolata* fruit and nutraceutical C-24/7

Total Flavonoids

Total Flavonoids were assayed using the procedure described by Jagdash et al. [14]. 1.5 ml of the extract was added to methanol solution, the mixture was vigorously shaken on orbital shaker for 5 minutes at 200 rpm and the absorbance was read using UV-spectrophotometer at 367nm after 10 minutes of incubation. Quercetin was used as a standard for the calibration curve. The assay was carried out in triplicate.

Total Phenols

Total phenols were determined using Folin-Ciocalteu's method described by Olajire and Azeez [15]. About 0.5ml of the extract was added to 10ml deionized distilled water and 2.5ml of 0.2N Folin-Ciocalteu's phenol reagent. The mixture was allowed to stay for 5 minutes and then 2ml of 2% sodium carbonate was added. The absorbance was read using UV-spectrophotometer at 780nm. The analysis was run in triplicate.

Ascorbic acid (vitamin C)

Vitamin C was determined by the method described by Omaye et al. [16]. About 0.5ml of the extract was mixed with 1.5 ml of 6% TCA and centrifuged for 10 minutes at 3000 rpm, after which 0.5 ml of the supernatant was mixed with 0.5 ml of DNPH reagents and allowed to stand at room temperature for an additional 3 hours then added 2.5 ml of 80% sulphuric acid and left undisturbed for 30 minutes. The absorbance was read using UV-spectrophotometer at 530nm. A set of standards containing 10-50 µg of ascorbic acid were taken and processed similarly along with a blank. The assay was carried out in triplicate.

Thiamine (vitamin B₁)

Vitamin B₁ was assayed as described by Gezer [17]. Briefly 5g of the samples were homogenized with ethanolic sodium hydroxide (50ml). It was filtered into a 100ml flask and 10ml of the filtrate was pipetted. The colour developed by addition of 10ml of potassium dichromate and

the absorbance was read using UV-spectrophotometer at 360nm. A blank sample was prepared and treated as the sample. The assay was carried out in triplicate.

Riboflavin (vitamin B₂)

Vitamin B₂ was also assayed as described by Gezer [17]. About 5g of the sample was extracted with 100ml of 50% ethanol solution and shaken for one hour and filtered into a 100ml flask. Exactly, 10 ml of the extract was pipetted into 50ml of volumetric flask followed by addition of 10ml of 5% potassium permanganate and 10ml 30% H₂O₂ and allowed to stand over a hot water bath for 30 minutes. Thereafter, 2ml of 40% sodium sulphate was added. This was made up to 50ml mark. The absorbance was read using a UV-spectrophotometer at 510nm. The assay was carried out in triplicate.

Determination of *in-vitro* free Radical scavenging activity of *Landolphia lanceolata* fruit and Nutraceutical-C24/7

DPPH radical scavenging capacity

Free radical scavenging activities of the extracts were measured according to Oyaizu [18]. 2 ml of various concentrations (0.2-1.0 mg/ml) of *Landolphia lanceolata* fruit and nutraceutical-C24/7 extracts were added separately to 2 ml of 0.1 mmol/L methanolic solution of DPPH. Incubated for 30 minutes in the dark at room temperature, the absorbances were read against a control using UV-spectrophotometer at 517 nm. The scavenging rates (1%) on the DPPH radical were calculated using the equation:

$$1\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 ,$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The procedures were carried out in triplicates.

Ferric ion reducing ability

The ability of the extracts to reduce Fe³⁺ to Fe²⁺ was evaluated by adopting the procedure described by Oyaizu [18]. Briefly, various concentrations of the extracts (0.2 - 1.0 mg/ml) were suspended in 1 ml of distilled water, mixed with 250 µl of 1% potassium ferricyanide [K₃Fe(CN)₆]. The mixture was incubated at 50°C for 20 minutes, and 250 µl of trichloroacetic acid was added. Following centrifugation at 604 × g for 10 minutes, 250 µl of the supernatant was mixed with an equal amount of distilled water and 0.5 ml of 0.1% FeCl₃. The absorbance of the resulting solution was read at 700 nm using UV-spectrophotometer. The procedure was carried out in triplicate.

Hydrogen peroxide scavenging activity

The ability of the extracts to break down hydrogen peroxide to water and oxygen was determined according to the method described by Ruch et al. [19]. About 4mM of hydrogen peroxide was prepared in phosphate buffered saline of pH 7.4. Exactly, 4mls of various concentrations (0.2-1.0mg/ml) of each extract was added to 0.6ml of hydrogen peroxide. The absorbance was read after 10 minutes at 230nm using a UV-spectrophotometer against a blank solution containing sample without hydrogen peroxide. The inhibition rate (1%) on the hydrogen peroxide was calculated using the expression below:

$$1\% = [(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}})] \times 100 ,$$

where, A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The procedure was carried out in triplicate.

Statistical Analysis

Statistical analysis was determined using the Student T-test and the results were tabled and presented as the mean $\pm$ standard deviation and p which is the level of statistical significance for differences was set up at ($p < 0.05$).

3. Results and Discussion

Phytochemicals are the secondary metabolites, bioactive non-nutrient plant compounds in fruits, vegetables, grains and other plant foods. The presence of secondary metabolites such as flavonoids, saponins, phenols and many others in plants or extracts influence their biological effects [16]. They have been linked to reductions in the risk of major chronic diseases [14]. The study investigated the phytochemical screening and composition of ethanolic extracts of *Landolphia lanceolata* fruits and Nutraceutical-C24/7, the study also looked at some of the micronutrients. The extracts were also investigated for their free radical scavenging activities on the ability to reduce DPPH radical, ferric ion and hydrogen peroxide. The presence of phenols, flavonoids, saponins, alkaloids, terpenoids, tannins, triterpenoids, and steroid in *Landolphia lanceolata* fruit extract would confer a host of pharmacological abilities on these plant extracts. The nutraceutical-C24/7 contains some of the phytochemicals but not all (saponins, terpenoids and triterpenoids) compared to the *Landolphia lanceolata* fruit extract. Mireku et al. [20] reported presence of many phytochemicals in roots of *Landolphia heudelotti* methanolic extract whereas Shuma et al. [21] studied ethanolic, methanolic, acetone and chloroform extracts of *Planteogo lanceolata* and found various phytochemicals in the leaves. Antoni et al. [22] studied flowering parts of *Coreopsis lanceolata* and found a variety of phytochemicals in the methanolic and chloroform extracts.

Flavonoids have been reported to possess free radical scavenging and anticancer activities [18] [19]. Phenols have also been reported to have tumour inhibiting activity and to inactivate carcinogens and mutagens [23-25]. Alkaloids are anaesthetics, stimulants and anticancer agents [26]. Saponins have been reported to be cytotoxic and cholesterol lowering agents [27-28] Saponins possess antimicrobial, antiviral, antifungal, hypocholesterolaemic, immunostimulant, membrane permeabilising, insecticidal and molluscicidal [29-31]. Tannins possess antioxidant, antimicrobial and antiviral, protein precipitating and iron-binding properties [30]. Studies have shown that triterpenoids have been reported to have antimicrobial activity [32]. Vitamin B₁ and vitamin B₂ act as coenzymes in dehydrogenase enzymes and redox reactions. Vitamin C is an oxygen scavenger, a good antioxidant and it can activate reduced α -tocopherol to get activated as an antioxidant α -tocopherol [33]. Vitamin C also reduces vitamin deficiencies diseases [34-33]. The healing properties, antimicrobial, radical scavenging, antioxidant and cytotoxic properties might have been due to the phytochemical components which have been also found in *Landolphia lanceolata* fruit extract and some in nutraceutical-C24/7.

The ability of extracts to reduce DPPH radical has been used to assess the in-vitro free radical scavenging abilities of extracts [35-37]. Antioxidants scavenge DPPH radical by donating a phenolic hydrogen atom or an electron to it [29] [38]. Reduction of hydrogen peroxide has been widely used to assess the *in-vitro* antioxidant scavenging capabilities of extracts [39] [29]. Studies have shown that flavonoids scavenge hydrogen peroxide by donating electron which results in formation of water [29] [37] [39]. The chelating property of extracts to reduce Fe³⁺ to Fe²⁺ in the ferric ion assay has been used to assess the antioxidant capacity of extracts [40-41].

4. Conclusion

The result of the study have shown that *Landolphia lanceolata* fruit extract could be said to have better healing and micronutrient properties than nutraceutical-C24/7. The study showed that *Landolphia lanceolata* fruit extract had higher percentage inhibition on DPPH scavenger and hydrogen peroxide as well as higher percentage reducing power on ferric ion reducing activity than nutraceutical-C24/7 extracts. This may be due to its higher flavonoids, phenolic, vitamin C

concentrations and presence of more phytochemical compounds than nutraceutical-C24/7 extract. The ability of *Landolphia lanceolata* fruit extract to reduce these oxidized molecules is an indication of an in vitro antioxidant capacity which can be related to possibly *in vivo* antioxidant capacity and ability to prevent oxidative damage to cellular macromolecules such as lipids, protein and DNA. The *Landolphia lanceolata* fruit extract should therefore be investigated for *in-vivo* antioxidant activity.

Conflict of interest

The Authors declare no conflict of interest.

Table of results

Table 1. Phytochemical composition of the ethanolic extract

Phytochemical	Nutraceutical-C24/7	<i>Landolphia lanceolata</i>
Phenol	+	+
Flavonoids	+	+
Saponins	-	+
Alkaloids	+	+
Terpenoids	-	+
Tannins	+	+
Triterpenoids	-	+
Steroid	+	+
Volatile oil	-	-

Keyword: Positive (+) = present, Negative (-) = absent

Table 2. Phytochemicals and vitamins Quantitative compositions of the ethanolic extracts

Phytochemical/vitamin	Nutraceutical extract	<i>Landolphia lanceolata</i> extracts
Total phenols (mg/ml)	0.011±0.002	0.063±0.000 ^a
Total Flavonoids (mg/ml)	0.032±0.000	0.097±0.001 ^b
Vitamin C (mg/dL)	102.341±0.000	206.417±0.000 ^c
Vitamin B ₁ (mg/dL)	23.362±0.001	54.156±0.000 ^d
Vitamin B ₂ (mg/dL)	0.067±0.000	0.113±0.000 ^e

^a: statistically significant when compared to total phenols of nutraceutical-C24/7 extract; ^b: statistically significant when compared to total flavonoids of nutraceutical-C24/7 extract; ^c: statistically significant when compared to vitamin C concentration of nutraceutical-C24/7 extract; ^d: statistically significant when compared to vitamin B₁ concentration of nutraceutical-C24/7 extract; ^e: statistically significant when compared to vitamin B₂ concentration of nutraceutical-C24/7 extract.

Table 3. DPPH radical scavenging activity of the ethanolic extracts

Concentration (mg/ml)	% inhibition vitamin C (standard)	% inhibition nutraceuticalC-24/7	% inhibition <i>Landolphia lanceolata</i>
0.2	74.01±0.01	10.19±0.01	31.88±0.01 ^{af}
0.4	74.80±0.02	15.96±0.01	43.40±0.01 ^{bg}
0.6	70.39±0.01	37.69±0.01	66.28±0.02 ^c
0.8	79.96±0.01	41.90±0.01	76.39±0.01 ^d
1.0	81.22±0.02	56.90±0.01	79.38±0.01 ^e

^a: statistically significant when compared to DPPH % inhibition nutraceutical-C24/7 at 0.2mg/ml; ^b: statistically significant when compared to DPPH % inhibition nutraceutical-C24/7 at 0.4mg/ml; ^c: statistically significant when compared to DPPH % inhibition nutraceutical-C24/7 at 0.6mg/ml; ^d: statistically significant when compared to DPPH % inhibition nutraceutical-C24/7 at 0.8mg/ml; ^e: statistically significant when compared to DPPH % inhibition nutraceutical-C24/7 at 1.0mg/ml; ^f: statistically significant when compared to standard DPPH % inhibition vitamin C at 0.2mg/ml; ^g: statistically significant when compared to standard vitamin C at 0.4mg/ml.

Table 4. Ferric ion reducing activity of ethanolic extract

Concentration mg/ml	Ferric ion reducing power of Nutraceutical-C24/7	Ferric ion reducing power of <i>Landolphia lanceolata</i>
0.2	56.96±0.03	91.39±0.01 ^a
0.4	59.75±0.01	91.75±0.02 ^b
0.6	61.75±0.02	94.79±0.02 ^c
0.8	65.04±0.02	96.83±0.01 ^d
1.0	68.03±0.01	99.88±0.02 ^e

^a: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.2mg/ml; ^b: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.4mg/ml; ^c: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.6mg/ml; ^d: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.8mg/ml; ^e: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 1.0mg/ml.

Table 5. Hydrogen peroxide decomposition activity of the ethanolic extracts

Concentration (mg/ml)	% inhibition nutraceuticalC-24/7	% inhibition <i>Landolphia lanceolata</i>
0.2	40.00±0.01	60.40±0.00 ^a
0.4	43.31±0.02	66.81±0.02 ^b
0.6	48.22±0.01	71.11±0.00 ^c
0.8	56.70±0.00	74.35±0.02 ^d
1.0	60.05±0.00	78.61±0.01 ^e

^a: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.2mg/ml;

^b: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.4mg/ml;

^c: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.6mg/ml;

^d: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 0.8mg/ml;

^e: statistically significant when compared to % Fe chelating activity of nutraceutical-C24/7 at 1.0mg/ml.

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