

Comparative Study of the Proximate and Phytochemical Analysis of *Polyalthia Longifolia* (Fresh Bark and Leaves)

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Abstract

Proximate and phytochemicals are chemical components that are derived from plants through metabolic activities, *Polyalthia longifolia* is a very important plant. The phytochemical analysis results obtained for the bark for alkaloid, saponins, flavonoid, total phenol, hydrogen cyanide and terpenoid are as follows; 8.13, 9.31, 9.41, 42.7, 0.01 and 4.47 and for the leaves. 4.84, 3.21, 7.14, 2.96, 1.73 and 4.11 respectively. The proximate results carried out on its bark using standard procedures shows it contains (%) carbohydrate 51.7, moisture content 12.8, ash content 10.52, crude fibre 15.31 and crude protein 7.22 and the proximate results for leaves 4.48, 3.21, 7.14, 2.96 and 4.11 respectively for carbohydrate, moisture content, ash content, crude fibre and crude protein. This result shows that the plant is a good source of saponin, alkaloid, phenols, and terpenoids which contribute to the nutritive and medicinal potency of the plant. The concentration of hydrogen cyanide in the plant leaves and bark is not significant and therefore will have no lethal effect and can easily be detoxified but comparatively the leaves contain more cyanide than the bark which could be related to the moisture content, the total phenol in the bark is also significantly higher than the leaves which show its effectiveness as an anti microbial agent. The high percentage of carbohydrate shows that the plant can be a good source of energy for both humans and animals. This plant has several benefits like anti-cancer, anti-oxidants and anti-haemorrhoids and can be used to reduce fibroid, it is applicable industrially as food preservatives, cosmetics and sweeteners.

Keywords: *Polyalthia Longifolia* Saponin, Hydrogen Cyanide, Total Phenol and Terpenoids.

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INTRODUCTION

Polyalthia longifolia has been of medicinal value in nature and a typical example is of the bark extract which is used in some parts of the west coast of Africa. In Cote d'ivoire particularly, it can be used for the treatment of haemorrhoids and fibroid, (Frank, and Vertkaik, 2006) the leaf oil has been demonstrated to exclusively compose of sesquiterpene derivatives while the leaf is used in Nigeria and elsewhere for treatment of skin diseases, fever, diabetes and hypertension. *Polyalthia longifolia* is popularly rich in minerals, vitamins, proteins, fibres and other substances which promote a healthy life. *Polyalthia longifolia* is originally known as Green Champa which is an evergreen plant, native to India, commonly planted due to its effectiveness in alleviating noise pollution (Fahey, and Jed, 2005). These plants are widely spread in Africa, Asia, Australia, tropical America and India. Reports showed that they contain phytochemicals like terpenes, carotenoids, tannins, alcohols, ketones, aldehyde, esters,

alkaloids, flavonoids and saponins among others at different concentrations which may be influenced by their geographical locations and content of soil on which the plants are grown. It is mainly used for landscaping purposes because of the exquisite beauty of its leaves arrangement and the unique height of the plant itself. The leaves and the roots are a major sources of vitamins E and B, dietary fiber, essential elements calcium and magnesium, monounsaturated fatty acids, and phytosterols with significant cholesterol-lowering effects (Hosseini, 2008). It is one of the most popular in nutritive food that can relieve different kinds of ailments. This review summarizes recent advances in the studies regarding this plant and its potential significances. The phytochemicals analyzed were found to be some of the parameters in this plant which has made it very useful to man and his environment. (Forster, and Hartonut, 2006) Some of the phytochemicals determined were saponin, flavonoid, cyanogenic glycoside and alkaloid using the leaves. This has also shown that some parts of the plant

are very edible and can be properly digested, also its usefulness for good health (Frank, 2006), they are majorly used for food, medicinal and industrial purposes (Lateef *et al.*, 2015). It is cultivated to use as a vegetable (leaves, green pods, flowers), for spice (Azeez *et al.*, 2015), (mainly roots) for cooking and cosmetic oil and as a medicinal plant (all plant organs). Medicinally, the parts are used for treatment of anaemia, anxiety, asthma, fever, semen deficiency (Frank, 2006). Nutritionally, have been used to combat malnutrition, especially among infants and nursing mothers. (Lindhorst 2007, Ted and Elevitch, 2010) It has high anti-oxidant properties making it a valuable source of vitamins A, C and E. it is one of the highest naturally occurring sources of anti-oxidants. (Hosseini, 2008). The review aims to renew the interest in this promising plant, thus stimulating researchers to go further with the study for discovering novel medicinal and nutritional benefits.

MATERIAL AND METHODS

Materials

Bark and Leaves of *Polyalthia longifolia* plant were freshly obtained from Akwaka village, thoroughly washed and immediately sent to End-point Laboratories and Equipment Agip, Rivers-State. Nigeria.

All determinations were then carried out in triplicates using standard methods of analysis.

Methods

The phytochemical and proximate analysis of the bark and leaves were determined using the method of AOAC with the absorbance measured using spectrophotometer.

Sample Preparation and Extraction

The fresh *Longifolia Polyalthia* leaves and bark upon arriving the laboratory were first washed with water to remove any trace of dirt. The leaves and bark were then chopped into small pieces using electric blender. 100g of the processed sample was weighed and soaked in 500ml of 50% methanol (1:1 vol/vol absolute methanol: distilled water) in a clean flat bottomed flask for 48hours. The flask with the mixture were accompanied with intermittent stirring and agitation during the 48hours period. The mixture was then subjected to filtration using Whatman No. 1 filter paper. The resulting filtrate was evaporated on water bath at 60°C and allowed to concentrate. The resulting concentrates were regarded as the crude methanol and water extracts. The extract was used for the determination of the phytochemicals and proximate properties of the leaves and bark

MOISTURE CONTENT

Procedure

1. Place a clean dry petridish in an oven at 105°C for 15 min.
2. Cool petridish in desiccator, weigh and record weight as (W_1)

3. Weigh 5g of the sample and record weight of petridish plus sample as (W_2)
4. Dry petridish plus sample in oven at 105°C for 18 - 24hrs or overnight.
5. Cool in desiccator, weigh back, and record weight
6. Repeat the heating process and weighing until a constant weight (W_3) is obtained

ASH CONTENT

Procedure

1. Dry empty crucible in an oven at 105°C for at least 2hours.
2. Cool crucible in desiccator, weigh and record weight as (W_1).
3. Accurately weigh 5g of sample in the crucible and record weight of crucible plus sample as (W_2)
4. Place crucible plus samples in preheated muffle furnace and ash at 550°C for 4 hours
5. Transfer crucible into a desiccator and allow to cool to room temperature (approximately 45 minutes).
6. Weigh the dish and record weight as (W_3).

CRUDE PROTEIN

Method summary

The protein content of the sample was determined using the Macro-Kjeldahl method. 1.0 g of the dry powdered sample was digested with 20 ml of concentrated H_2SO_4 to which was added Kjeldahl catalyst tablet in a fume cupboard. The digest was made up with distilled water to 80 ml on a volumetric flask. The digested solution was distilled and titrated with 0.1M HCl. The crude protein was therefore equaled to the N multiplied by a conversion factor, 6.25.

Procedure

- Weigh accurately 1.0g of well-mixed ground sample and place in Kjeldahl digestion tubes. In each batch use a flask without sample as blank test
- Add two Kjeldahl tablets and 20ml of sulphuric acid. If fuming is a problem, add a few drops of anti-forming agent.
- Place the tubes in a digestion unit and connect to the fume removal manifold.
- Digest the sample for at least for 1 hour at $420 \pm 20^\circ C$.
- Allow the content of the tubes to cool.
- Add distilled water into the cool Kjeldahl digestion tubes to a total volume of 80ml.

Distillation and Titration

- Place a conical flask containing 25-30ml of the concentrated boric acid under the outlet of the condenser of the distillation unit in such a way that the delivery tube is below the surface of the boric acid solution.

- Gently add 50ml of sodium hydroxide solution and distill the ammonium.
- Titrate the content of the conical flask with hydrochloric acid standard solution after adding a few droplets of indicator solution using a titration unit and read the amount of titrant used. The endpoint is reached at the first trace of pink colour in the contents.
- Record the amount of acid used to the nearest 0.05ml for the blank test (Vb) and for each sample (Vs).

CRUDE FAT

Procedure

The fat content of the sample was determined by (AOAC, 1980). 5.0 g of the sample was introduced into an ether-extracting thimble and placed on a soxhlet reflux flask connected to a round bottomed flask of known weight. This was placed on a heating mantle filled with about 250 ml of petroleum ether. The fat content in the sample was extracted by a reflux system. After a series of refluxes, a clear solution was obtained in the flask, and then the sample was removed from the flask. Further heating separated the ether from the extraction oil. The round-bottomed flask containing the oil was finally dried in an oven at 70°C and determination by gravimetric method was done and expressed as a percentage of the sample weighed

CARBOHYDRATE

Procedure

This was determined by the percentage difference in sum of other proximate parameters (Ash, Moisture, Protein and Fat):

$$\text{Carbohydrate (\%)} = 100 - (\% \text{Ash content} + \% \text{Moisture} + \% \text{Protein} + \% \text{Fat})$$

Flavonoids

Procedure

- The total flavonoids content of the *L. Polyalthia* leaves extract was determined by (AOAC,2006).
- 1.0ml of the sample extract was mixed with 4ml of distil water and 0.30ml of 10% sodium nitrate (NaNO₃) was added.
- After 5 minutes, 0.30ml of 10% Aluminium chloride (AlCl₃) solution was added followed by 2.0ml of 1% NaOH solution.
- The mixture was thoroughly mixed and absorbance was then read at 510nm using Axiom UV spectrophotometer.

To quantify the amount of flavonoids in the sample, standard curve of quercetin was prepared and the results were expressed as quercetin equivalents (mg quercetin/100g of sample).

SAPONINS

Procedure

- The saponins content of the mango leaves extract was determined by using spectrophotometric method.
- 0.2ml of the sample extract was mixed with 0.2ml of vanillin reagent (8% vanillin in ethanol) and 2.5ml of 72% aqueous sulphuric acid in a test tube.
- The resultant mixture was thoroughly mixed and heated for 10minutes in a water bath at 60oC.
- The test tube containing the mixture was cooled in an ice bath and allowed to attain room temperature.

The absorbance was then read at 544nm using Axiom UV-Vis spectrophotometer, and total saponins content of the sample was expressed as standard saponin equivalent in mg/100g of sample weight.

Total Phenols

Procedure

- The total phenolic content of the mango leaves extract was determined by Folin Cocalteu method.
- 0.2ml of the sample extract was mixed with 0.2ml of Folin-Cocalteu reagent and 0.8ml of 2% sodium carbonate was added after standing for 5minutes.
- The mixture was thoroughly mixed and allowed to stand for 30minutes at room temperature.
- Absorbance was then read at 760nm using Axiom UV spectrophotometer.

To quantify the amount of total phenols in the sample, standard curve of gallic acid was used as a standard and the total phenolics were expressed as gallic acid equivalents (mg GAE /100g of sample).

Alkaloids

Procedure

The alkaloid content of the *L. Polyalthia* leaves extract was determined by using spectrophotometric method. 1.0ml of the sample extract was mixed with 1.0ml solution of Iron (III) chloride (0.025M of FeCl₃ in 0.5M HCl) and 1ml of 0.05M of 1, 10-phenanthroline in ethanol. The resultant mixture formed was incubated for 30minutes in a water bath maintained at 70oC. The absorbance of red coloured complex formed was measured at 510nm against reagent blank. Alkaloid content of sample was separated as quinine equivalent in mg/100g of sample weight.

Hydrogen Cyanide

Procedure

10g of sieved sample (sieve No. 20) in 800ml Kjeldahl flask was added 200ml water and allowed to stand for 3hrs. Steam distillation was employed and

155ml was distilled into sodium hydroxide solution (0.5g in 20ml H₂O) and diluted to 250ml. 10ml of the distillate was titrated against 0.02N silver nitrate using micro-burette. End-point was determined at permanent mixture turbidity.

Terpenoids

Procedure

The alkaloid content of the *L. Polyathia* leaves extract was determined by using spectrophotometric method. 1.0ml of the sample extract was mixed with

1.0ml solution of Iron (III) chloride (0.025M of FeCl₃ in 0.5M HCl) and 1ml of 0.05M of 1, 10-phenanthroline in ethanol. The resultant mixture formed was incubated for 30minutes in a water bath maintained at 70oC. The absorbance of red coloured complex formed was measured at 510nm against reagent blank. Alkaloid content of sample was separated as quinine equivalent in mg/100g of sample weight.

RESULT AND DISCUSSION

Table 3.10 Phytochemical Analysis Results for *P. longifolia* bark and leaves samples (mg/100g)

S/N	Parameters	Fresh Bark Mg/100g	Fresh Leaves mg/100mg
1	Alkaloid(mg/100g)	8.13	2.55
2	Saponin (mg/100g)	9.31	5.23
3	Flavonoid (mg/100g)	9.41	6.13
4	Total phenol (mg/100g)	42.7	6.92
5	Hydrogen Cyanide (mg/100g)	0.01	1.24
6	Terpenoids (mg/100g)	4.47	3.45
7	Tanins (mg/100g)	2.96	3.53

Table 3.20 Results for *P. longifolia* bark and leaves samples (%) Proximate Analysis

S/N	Parameters	Fresh Bark (%)	Fresh Leaves (%)
1	Carbohydrate (%)	51.7	4.84
2	Moisture content (%)	12.8	3.21
3	Ash content (%)	10.52	7.14
4	Crude fibre (%)	15.31	2.96
5	Crude Protein (%)	7.22	4.11
6	Fat content	2.46	3.71

DISCUSSION

The chemicals and the nutritional contents produced by plants which represents the phytochemicals and proximates analyzed were found to be some of the parameters in *P. Longifolia* leaves and bark (fresh) which has made it very useful to both biotic and abiotic factors (man and his environment) according to (Forster and Hartonut, 2006), Some of the phytochemicals and proximates determined were saponin, flavonoid, Hydrogen Cyanide, Total phenol, Terpenoids, carbohydrates, fats, crude fibre, crude protein and Alkaloid using the bark and leaves (Fugile and Olson, 2010) . This has also shown that some parts of the plants are very edible and can be properly digested, also, its usefulness for good health according to (Frank, 2006). Results of some phytochemicals and proximates analysis of *P. Longifolia* leaves and bark are presented in the tables 3.10 and 3.20 above. From our analysis, it shows that phytochemicals; flavonoid, terpenoids, total phenol and alkaloid have high concentrations with total phenol having a higher concentration in *P. Longifolia* bark than its leaves as analyzed with concentration of 42.7mg. The high concentration of total phenol in the *P. Longifolia* bark is responsible of its anti-microbial activity and anti-oxidant properties, it is also interesting to note that the total phenol concentration makes it more applicable

industrially and medically as food supplements. This confirms that the plant leaves and bark are good sources of saponin and flavonoid which contain high amount of lipids. Saponin helps in protecting the plant against microbes and fungi and may also enhance nutrient absorption and aid in animal digestion. The presence of saponins have many health benefits which includes; reduction of blood cholesterol level, cancer and improvement of the immune system (Bohm, and Kocipal-Abyazam, 1999). The results revealed that the proximates and phytochemical parameters analyzed in the sample of *P. Longifolia* leaves and bark are of good health benefits and therefore, *P. Longifolia* is a good source of food (Castello *et al.*, 2002). This result shows that the plant is a good source of saponin, alkaloid, phenols, and terpenoids which contribute to the nutritive and medicinal potency of the plant. The concentration of hydrogen cyanide in the plant leaves and bark is not significant and therefore will have no lethal effect and can easily be detoxified but comparatively the leaves contain more cyanide than the bark which could be related to the moisture content, the total penol in the bark is also significantly higher than the leaves which show its effectiveness as an anti microbial agent. The high percentage of carbohydrate shows that the plant can be a good source of energy for both humans and animals. This

plant has several benefits like anti-cancer, anti-oxidants and anti-haemorrhoids and can be used to reduce fibroid, it is applicable industrially as food preservatives, cosmetics and sweeteners,

CONCLUSION

The results of this comparative study have shown that phytochemical and proximate components of the aqueous leaf and bark extracts of *P. Longifolia* have antioxidant property thereby making them a prospect in drug production. The comparative analysis determined gives the plant its improved quality as a medicinal and nutritive plant. The level of cyanide determined is also an indication that the plant can be used as a good source of food nutrients and the toxicity level is very low, more importantly is the high percentage of carbohydrate which indicates a good source of energy and vegetable. Further work is hereby recommended on the plants potential as anticancer and anti microbial agents and to isolate and evaluate the active chemical constituents.

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