



(RESEARCH ARTICLE)



Bioactive chemical constituents, acute toxicity and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of *Polyalthia longifolia* root

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GSC Biological and Pharmaceutical Sciences, 2021, 14(01), 018–026

Publication history: Received on 15 December 2020; revised on 30 December 2020; accepted on 01 January 2021

Article DOI: <https://doi.org/10.30574/gscbps.2021.14.1.0407>

Abstract

Polyalthia longifolia (masquerade tree) is a plant which is believed to possess varied pharmacological and therapeutic values among different populations. The present report investigated the phytochemical composition, proximate, acute toxicity and antioxidant potential of *P. longifolia* root. All analyses were carried out using established methods; the antioxidant activity of the crude methanol extract and its fractions (n-hexane and ethyl acetate) were examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay while the total phenolic and flavonoid contents were assessed using the Folin-Ciocalteu and the aluminum chloride calibration methods respectively. The phytochemical analysis revealed the presence of alkaloids, carbohydrate, reducing sugars, tannins, saponins, flavonoids, phenolic compounds and protein in aqueous extract. The proximate analysis showed moisture content, total ash, alcohol extractive value, water extractive value, acid insoluble ash and water soluble ash at 8.80, 9.35, 3.28, 3.29, 2.27 and 7.29% respectively. The ethyl acetate fraction showed the highest antioxidant property compared to the n-hexane fraction and crude methanol extract in all assays conducted. Also, the methanol fraction was found to have the highest flavonoids and phenolic content among the extract and fractions. Oral administration of crude methanol extract of *P. longifolia* to Swiss mice was relatively non-toxic at a maximum dose of 5000 mg/kg. The root extract and fractions of *P. longifolia* indicated moderately high level of some phytochemicals with outstanding radical scavenging activity, and therefore substantiate its use as a conventional and comparatively non-toxic plant antioxidant.

Keywords: *Polyalthia longifolia*; Total phenolics; Total flavonoids; Antioxidant activity

1. Introduction

Plant derived medicines have been used across the globe (by different cultures and races) for many millennia, in the management of many diseases. These medicinal plants have active compounds, some of which have been isolated and their mechanisms of action assessed [1-5].

Free radicals such as reactive oxygen and reactive nitrogen species (ROS and RNS) are usually produced during cellular metabolism and by exogenous means [6]. Apart from their significance in maintaining the redox status, cellular signaling and immune adjustment [7], they are well known for their harmful effects on cellular biomolecules such as DNA destruction, tissue wound and protein mortification [8-10]. Antioxidants help in destroying these free radicals as well as the oxidative reactions they initiate, thereby inhibiting cell destruction which may occur because of these oxidative reactions [6]. Plants represent a wealthy reservoir of natural antioxidant that can be used to prevent these oxidative mutilations [11,12]. Antioxidants can be sub-categorized into synthetic and natural, depending on their sources. It is now widely accepted that those from natural sources could be relatively safe and can help improve nutritional values of diets [13].

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Polyalthia longifolia is well-known locally in Nigeria as the 'Masquerade tree'. It is a lofty evergreen tree native to India [14]. Its popular names include greenchampa, Indian Mast Tree, false ashoka and Indian Fir Tree [15]. It was introduced to gardens in several tropical countries around the world including Nigeria. The fruits are borne in clusters of 10 -20 which are originally green, but turn purple or black when ripe [14]. This plant is used as an antipyretic agent in indigenous system in the field of traditional medicine [16]. Pharmacological studies on the bark and leaves of this plant showed antimicrobial activities, [17-20] cytotoxic functions [21, 22] and hypotensive effects [23]. Major uses reported about *P. longifolia* has been of medicinal nature and a typical example is the bark extract being used in certain part of the west coast of Africa, in particular; Cote D'Ivoire, to treat haemorrhoids and febrile pains [14]. There is a claim of the existence of sesquiterpenes in the essential oils of *P. longifolia* leaf [24]. The leaf oil has been verified to exclusively contain sesquiterpene derivatives. Meanwhile, the leaf is used in Nigeria and some other countries for treatment of skin diseases, fever, diabetes and hypertension [19, 20, 25, 26]. The antimicrobial activity of clerodane diterpenoids from *P. longifolia* seeds had been recorded [27]. However, there seems to be inadequate scientific reports on the ethno-medicinal uses of *P. longifolia* root [28]. It therefore became very important to undertake this present study which was aimed at evaluating the bioactive chemical composition, acute toxicity and antioxidant potential of the extract and fractions of *P. longifolia* root.

2. Material and methods

2.1. Collection and preparation of plant materials

The root bark of *P. longifolia* was collected in University of Benin, Benin City, Edo State, Nigeria. The plant was identified and authenticated by a plant taxonomist in the Department of Pharmacology, University of Benin. The root was severed into smaller pieces, washed with water and air dried under shade for 21 days (at room temperature) after which, the sample was ground to powder. The powdered sample was weighed and kept for further analysis.

2.2. Extraction and fractionation of plant sample

The powdered plant material (200 g) was macerated in 1 L of methanol for 7 days. The extract was concentrated using rotary evaporator at reduced pressure and allowed to dry. The dried extract was weighed and a weight of 30.20 g was obtained.

Twenty grams (20 g) of the crude sample was weighed into a clean beaker and 5 ml of methanol was added to dissolve sample, after which it was turned into a separating funnel. Water and methanol in ratio (1:4) was added to the separating funnel and shaken vigorously. Two hundred (200 ml) of n-hexane was added to the column and shaken, and the column was allowed to settle down. Thus, formation of two layers; the less dense, which is the n-hexane fraction was collected.

Ethyl acetate was added to the column and the remaining extract dissolved completely and was collected. Thus, the two fractions; n-hexane (10.0294 g) and ethyl acetate (6.0450 g) were concentrated using the rotary evaporator. These were stored in air tight bottles for further investigation.

2.3. Proximate analysis

The following quantitative parameters were carried out using established methods [33,34]: Moisture content/water loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value analyses were carried out in this study.

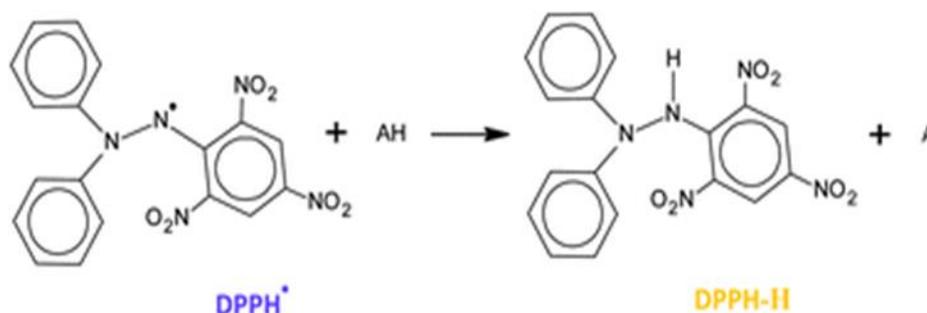
2.4. Estimation of antioxidant activity

The scavenging effect of the crude methanol extract of *P. longifolia* on DPPH radical was determined using the method described by Jain et al [29].

A solution of 0.2 mM DPPH in methanol was prepared. 1 ml of this solution was mixed with 3 ml of extract in methanol containing 0.001-0.200 mg/ml of the extract. It was then thoroughly vortexed and left in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm with a spectrophotometer. Ascorbic acid was used as standard. The ability to scavenge DPPH was calculated using the following equation:

$$\text{DPPH radical Scavenging Activity}\% = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 =absorbance of DPPH radical plus methanol; A_1 =absorbance of DPPH radical plus sample



Scheme 1: 1,1-diphenyl-2-picrylhydrazyl (DPPH)

2.5. Determination of total phenolic content

The total phenolic content of the extract and fractions were determined according to the method previously described by Kim et al [30]. The extract (0.5 ml) with a concentration of 1 mg/ml was added to 4.5 ml of distilled water and 0.5 ml of Folin-ciocalteu's reagent (previously diluted with water 1:10, v/v). After mixing, the tubes were then allowed to stand at room temperature for 5 minutes and thereafter 5 ml of 7% sodium carbonate and 2 ml of deionized water were added. The mixtures were afterwards incubated for 90 minutes at room temperature. The absorbance of each was measured at 750 nm using a spectrophotometer. The standard curve was prepared using gallic acid at concentrations of 12.5, 25, 50, 75, 100, and 150 μgml^{-1} . The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract) [31].

2.6. Determination of total flavonoid content

This test was determined according to the method described by Ighodaro and Ogbeide [11]. The extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride followed by 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured using a spectrophotometer at 415 nm. The standard Quercetin was prepared in six different concentrations (12.5, 25, 50, 75, 100 and 150 $\mu\text{g/ml}$). The result was expressed as milligram Quercetin per gram of extract (mg QE/g extract).

2.7. Determination of acute toxicity

Acute toxicity test was carried out on Swiss mice using the procedure previously described by Lorke [32]. This method consists of two phases.

2.7.1. Phase 1

Nine mice of 3 animals per group were used.

Each group of animals was administered different doses of 10, 100 and 1000 mg/kg of crude extract of *P. longifolia* root bark.

The animals were placed under observation for 24 hours to monitor their behaviour as well as mortality rate.

2.7.2. Phase 2

Three animals were used which were distributed into three groups. The animals were administered higher doses of 1600, 2900 and 5000 mg/kg of crude extract of *P. longifolia* root

They were placed under observation for 24 hours for any sign of toxicity.

The LD_{50} is calculated by the formula.

$$\text{LD}_{50} = (\text{LD}_0 \times \text{LD}_{100})^{1/2}$$

Where

LD_0 = Highest dose that gave no mortality

LD_{100} = Lowest dose that produced mortality

LD₅₀= Lethal dose is the dose that kills 50% of the total animal population.

2.8. Statistical analysis

The statistical significance between antioxidant activity values of the extracts was evaluated by analysis of variance (ANOVA) followed by Dunnett's test. P values less than 0.05 were considered to be statistically significant [8]. The experiments were carried out in triplicates unless otherwise specified. The test was carried out for statistical analysis and results are presented as mean \pm standard error of the mean (Mean \pm SEM).

3. Results

3.1. Preliminary Phytochemical Investigation

Table 1 Results for phytochemical screening

Phytochemicals	Result
Alkaloids	+
Carbohydrate	+
Reducing sugar	+
Saponins	+
Tannins	+
Flavonoids	+
Phenolics	+
Steroidal saponin	-
Protein	+

3.2. Proximate analysis of the powdered *P. longifolia* Root.

The values of the proximate analysis of the powdered root of *P. longifolia* are shown in Table 2.

Table 2 Proximate analysis of *P. longifolia* root.

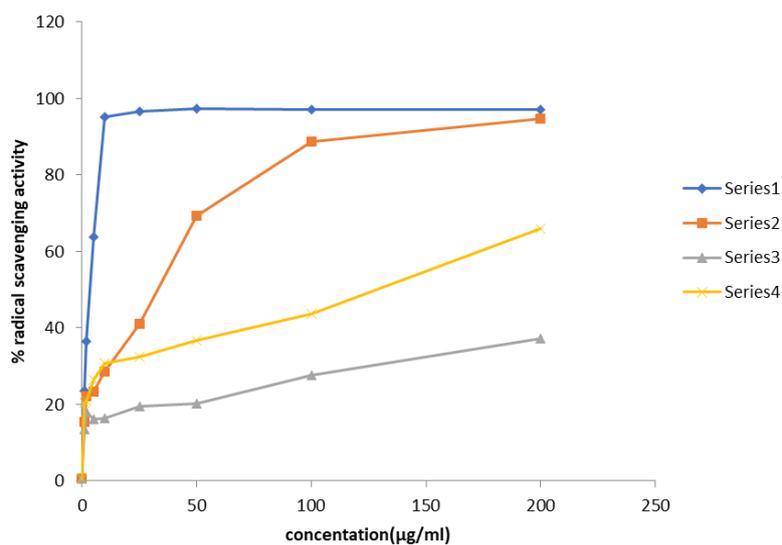
Parameters (%)	Mean \pm SEM
Moisture content	8.80 \pm 0.05
Total Ash	9.35 \pm 0.01
Acid insoluble Ash	2.27 \pm 1.01
Water soluble Ash	7.29 \pm 0.44
Alcohol extractive value	3.28 \pm 0.00
Water extractive value	3.29 \pm 0.01

3.3. Acute Toxicity

Table 3 Acute Toxicity

Mean Weight (g)	Dose(mg/kg)	Mortality rate
24.45	10	No mortality
21.83	100	No mortality
22.99	1000	No mortality
19.38	1600	No mortality
19.51	2900	No mortality
21.67	5000	No mortality

3.4. DPPH Scavenging Activity (Antioxidant Property)



Keys: Series 1=Ascorbic acid; Series 2=Ethyl acetate fraction; Series 3= n-Hexane fraction; Series 4=Crude extract.

Figure 1 Antioxidant Activity of extract and fractions of the root of *P. longifolia*

Table 4 Evaluation of IC_{50} of Extract and Fraction of *P. longifolia* Root and Ascorbic Acid.

Samples	IC_{50} values (µg/ml)
Ascorbic acid	3.54 ± 0.03
Crude	5.75 ± 0.11
Ethyl acetate	4.73 ± 0.81
n-hexane	13.56 ± 1.54

3.5. Total flavonoid

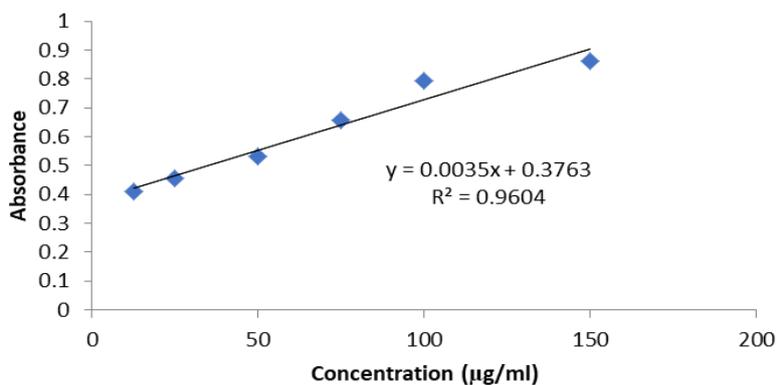
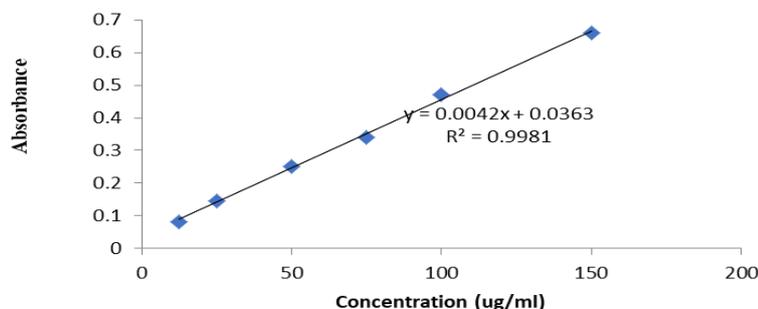


Figure 2 Quercetin calibration curve (mg QE/g extract)

Table 5 Flavonoid content of the extract and fractions

Fractions	Flavonoid Content (% mg QE/g extract) value±SEM
Crude	242.4±0.090
Ethylacetate	188.5±0.033
n-hexane	206.9±0.023

3.6. Total phenolic

**Figure 4** Gallic acid calibration curve.**Table 6** Phenolic content of the extract and fractions.

Extract/fraction	Phenolic Content (% mg QE/g extract value ±SEM)
Crude	29.75±0.00
n-hexane	39.66±0.01
Ethylacetate	73.93±0.00

4. Discussion

The crude powdered root of *P. longifolia* contained alkaloids, carbohydrates, reducing sugars, saponins, tannins, phenolics, flavonoids, and proteins. Phytochemicals are compounds that act as free radical scavengers to help eradicate the highly charged oxygen molecules that are by-products of metabolized oxygen and are believed to provide several health benefits [4]

The moisture content (8.80 ± 0.05) in *P. longifolia* root showed that it is slightly higher than African Pharmacopoeia tolerable limits of 6-8. This value could have effect on the crude powdered sample by increasing its susceptibility to microbial and hydrolytic degradation during storage. The ash content is generally recognized as a measure of quality for the assessment of the functional properties of food. Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, carbohydrates, proteins, vitamins, organic acid, etc.). Ash content is usually taken as the measure of mineral content of the original food. In *P. longifolia* root, the ash content was 9.35%. This value is slightly below the result obtained by Uraku and Ogbanshi [35]. The ash content obtained in this report (Table 2) revealed that the roots of *P. longifolia* are rich in minerals and as a result could be an important source of natural minerals. The extractive values obtained showed that water would be a better solvent for the extraction of phytoconstituents (Table 2). The extractive values also signified that the plant sample is majorly made up of highly polar compounds. In much the same way, it gives an insight on the method to be employed during isolation and purification of phyto-constituents from *P. longifolia* root.

The oral administration of crude root extract of *P. longifolia* in the dose 10, 100, 1600, 2900 and 5000 mg/kg body weight did not cause any sign of acute toxicity. No death of the Swiss mice was recorded even after 72 hours of close

monitoring from the lowest to the highest dose. The physical appearance such as raised tails, salivation, paw licking were not observed which pointed out that the crude extract do not have adverse effect on the animals. Hence, *P. longifolia* root has not caused acute toxicity effects. This result is in accordance with what was stated by Syahmi et al [36]. Based on Hodge and Sterner scale [37], a test drug administered orally is deemed to be extremely toxic if the LD₅₀ is 1 mg kg⁻¹ and below, highly toxic at 1-50 mgkg⁻¹, moderately toxic at 50-500 mgkg⁻¹, only slightly toxic at 500-5000 mgkg⁻¹, practically non-toxic at 5000-15000 mgkg⁻¹ and relatively harmless at ≥15,000 mg kg⁻¹ [4, 38]. Therefore, based on the findings of this study, *P. longifolia* extract is comparatively non-toxic as the highest dosage of 5000 mgkg⁻¹ did not lead to any death in the test animals. It is also reported [39] that any substance with LD₅₀ ≥ 1000 mgkg⁻¹ is considered to be of low toxicity or relatively safe.

Figure 1 shows the antioxidant activity of the extract and fractions of the root of *P. longifolia*. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with maximum absorption at 517 nm (purple colour) in the UV spectrum that can readily undergo reduction in the presence of an antioxidant. The degree of discoloration indicates the scavenging potentials of the antioxidant [40, 41]. Due to the ease and convenience of this reaction, it now has widespread use in the free radical-scavenging activity assessment [42]. The plant showed radical scavenging capacity, which is in agreement with Ogbeide et al and Ogbeide et al [43, 44]. This activity may be related to the phenolic compounds present. Figure 1 shows the radical scavenging activity of the crude and fractionated extracts of *P. longifolia* root. The result of DPPH assay was expressed in IC₅₀ values. IC₅₀ values are negatively related to the antioxidant activity, as it expresses the concentration that will inhibit 50% of the initial DPPH radical. Lower IC₅₀ value represents higher antioxidant activity of the tested sample [43, 44]. The result showed more activity of antioxidant in the ethyl acetate fraction than the other fraction. DPPH free radical scavenging activity of *P. longifolia* root extract and fractions showed significant, dose-dependent increase in scavenging ability from 1 µg/ml to 200 µg/ml for both the standard (ascorbic acid), crude and fractionated extracts (Figure 1). The ethyl acetate fraction showed great antioxidant power quite comparable to that of the standard. The crude extract did not give 50% inhibition until well above 100 mg/ml while that of the n-hexane extract recorded 37.20% inhibition at the highest concentration. This could be due to variations in the phytochemicals present in each extract as a result of differences in polarity of solvent [11]. On the whole, the percentage inhibition of the crude extract and fractions ranged from 37.20 to 94.90% at the highest concentration (200 µg/ml), whereas, the reference standard had a percentage inhibition of 97.10%.

The total flavonoids and phenolic content present in the extract and fraction of *P. longifolia* root were estimated and results were expressed in terms of milligrams quercetin and gallic acid per gram of extract respectively (Table 5 and 6). The result obtained revealed that the total flavonoid and phenolic content were significantly higher in the crude extract and ethyl acetate fraction of *P. longifolia* root respectively, (Table 5 and 6). The high values of polyphenolic content of the ethyl acetate fraction may be due to the moderately polar nature of the phyto constituents in *P. longifolia* root. Phenolic compounds are generally polar and solvent appears to play significant role in their extraction so that polar solvents tends to contain more of those components compared to the less polar or non-polar solvent. Hence, the antioxidant capacity reported in this research could be due to the flavonoid and phenolic content of *P. longifolia* root. The acidic nature of the hydroxyl (OH) groups on the flavonoid and phenolic compounds may have significantly influenced the antioxidant power of *P. longifolia* root.

5. Conclusion

The study has shown that *P. longifolia* root has antioxidant property and relatively non toxic at dosages up to 5000 mg/kg. Thus, the root extract and fractions of *P. longifolia* indicated moderately high level of some phytochemicals with outstanding radical scavenging activity, and therefore substantiate its use as a conventional and comparatively non-toxic plant antioxidant. Consequently, the root of *P. longifolia* could also be an important source of natural antioxidants.

Compliance with ethical standards

Acknowledgements

The Authors are grateful to Dr. O. Erharuyi and Professor A. Falodun of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy for guidance and use of their laboratory equipment.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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