

Comparative Studies on the Phytochemical Composition, Phenolic Content and Antioxidant Activities of Methanol Leaf Extracts of *Spondias mombin* and *Polyalthia longifolia*

Ehimwenma S.Omoregie* and Ehigbai I. Oikeh

Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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Abstract

Spondias mombin and *Polyalthia longifolia* leaves are popular choices in traditional herbal medicine practice. *P. longifolia* leaf extracts have not enjoyed profound investigation as more attention is paid to its stem bark and roots. The present study, therefore, evaluates the phytochemical constituents, the phenolic content and the antioxidant activity of the methanol leaf extract of *P. longifolia* and draws comparisons with *Spondias mombin* leaf extracts. Results from the phytochemical screening test on *S. mombin* extract revealed the presence of alkaloids, reducing sugars, cardiac glycosides, flavonoids, tannins, saponins, steroids and terpenoids. *P. longifolia* extract contained alkaloids, flavonoids, tannins and reducing sugars. The *S. mombin* extract had significantly higher total phenol content, while the total flavonoid and proanthocyanidin content were significantly higher in the *P. longifolia* extract. The *S. mombin* extract had better antioxidant activities as evidenced by the lower IC₅₀ values for DPPH radical scavenging, a higher FRAP value and a higher inhibition of lipid peroxidation *in vitro*. The results suggest that the extracts of *S. mombin* and *P. longifolia* leaves possess moderate antioxidant activities, which may be of nutraceutical and pharmaceutical importance.

Keywords: *Polyalthia longifolia*, *Spondias mombin*, Antioxidant, Phenol, Flavonoid

1. Introduction

Spondias mombin Linn is a fructiferous tree that belongs to the family Anacardiaceae with about 14 species worldwide. In Nigeria, it is called with several local names, such as *okhikghan* (Bini), *akika* (Yoruba), *tsadermasar* (Hausa), *ijikara*. (Igbo) (Igwe *et al.*, 2011). A decoction of the plant leaves is used by the Senegalese and Edo people in Nigeria to treat dysentery and other intestinal disorders. The leaves have also been used as a remedy for malignant tumors in Nigeria. It is also documented to possess antimicrobial, anti-malaria, and anti-epileptic properties as well as wound healing properties (Asuquo *et al.*, 2013). *Spondias mombin* is also employed to manage several conditions arising within the female reproductive system. For instance, it is used to induce labor, reduce pain and bleeding during and after childbirth, to prevent miscarriage and treat uterine/vaginal infections (Asuquo *et al.*, 2013; Igwe *et al.*, 2013). Igwe *et al.* (2013) also reported that the extract has no detrimental toxicological effects on the organ/tissue function of the animals studied.

The genus *Polyalthia* includes about 120 species occurring mainly in Africa, South and South-Eastern Asia, Australia, and New Zealand. *Polyalthia longifolia* is a small medium-sized tree, native to India and Sri Lanka that has now been introduced in gardens of many tropical

countries across the world. It is an evergreen tree and can grow up to a height of 15-20 meters. The leaves are long, narrow, dark green and glossy (Jothy *et al.*, 2013). In the last few decades, a lot of phytochemical studies have been carried out on *P. longifolia*. However, most of these studies focused on the stem with very few reports on the leaves (Jothy *et al.*, 2013).

The search for new natural compounds with potent biologic activities has been increasing with an estimated eighty percent (80%) of the human population in developing countries relying, to some extent, on medicinal plant materials for their primary healthcare. Medicinal plant based drugs have the added advantage of being readily available, effective, and offering a broad spectrum of activity with a greater emphasis on preventive action. These medicinal plants are usually known to exert their diverse health benefits through the numerous phytochemicals they contain (Jeyachandran *et al.*, 2010; Jothy *et al.*, 2013). The present study, therefore, evaluates the phytochemical composition, the phenolic content and the antioxidant activities of methanol leaf extracts of *S. mombin* and *P. longifolia*. Hopefully, the extracts will be used in future in the pharmacological industry for drug discovery.

* Corresponding author. e-mail: ehimoregie@yahoo.co.uk.

2. Materials and Methods

2.1. Collection of Plant Leaves

Spondias mombin and *Polyalthia longifolia* leaves were collected during the period between January and March, 2013 from private farms at different locations in Benin City, Nigeria. The leaves were identified and authenticated by a Botanist in the Department of Plant Biology and Biotechnology, University of Benin. The leaves were then washed, dried and macerated.

2.2. Preparation of Extracts

100g of the macerated *S. mombin* and *P. longifolia* leaves were soaked in 1000mL of absolute methanol for 72 hours with occasional stirring. The extracts were then filtered using a double layered muslin cloth and the filtrate concentrated to dryness by using a rotary evaporator at reduced pressure. The dried extracts were stored at 4°C until use.

2.3. Phytochemical Screening

Phytochemical screening was carried out on the plant samples using established protocols as described by Harbone (1998), Sofowora (1993) and Trease and Evans (1989).

A stock solution of each extract, with a concentration of 10 mg extract/mL distilled water, was prepared and used for the phytochemical screening.

2.3.1. Determination of Total Phenolic Content

The total phenolic content was determined using the Folin - Ciocalteu method as described by Cicco *et al.* (2009). Concentrations, ranging from 0.2 - 1 mg/mL of gallic acid or extracts, were prepared in methanol. Then, 4.5mL of distilled water was added to 0.5 mL of the extract and mixed with 0.5 mL of a ten-fold diluted Folin-Ciocalteu reagent. Five milliliters of 7% sodium carbonate was then added to the tubes and another 2mL of distilled water was added. The mixture was allowed to stand for 90 min at room temperature; absorbance was then read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the positive control. The total phenolic content was expressed as Gallic Acid Equivalent (GAE).

2.3.2. Determination of Total Flavonoid Content

The total flavonoid content was determined using the method of Miliauskas *et al.* (2004). Two milliliters of 2% AlCl₃ in ethanol was mixed with 2mL of varying concentrations of the extracts (0.1-1.0mg/mL), in methanol. The absorbance was measured at 420 nm after one hour incubation at room temperature. Similar concentrations of quercetin, the positive control were used. The total flavonoid content was calculated as mg quercetin equivalent /g of extract.

2.3.3. Determination of Proanthocyanidin Content

The determination of proanthocyanidin was carried out according to the method of Sun *et al.* (1998). To 0.5mL of 1.0mg/mL of each extract was added 1mL of 4 % methanol solution and 0.75mL of concentrated hydrochloric acid. The mixture was left undisturbed for

15 minutes and the absorbance was read at 500nm. Ascorbic acid was used as standard.

2.4. Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the leaf extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2mL of various concentrations (0.2 - 1.0 mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark; absorbance read at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5mL of 0.3 mM DPPH and 2mL methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1) / (A_0)] \times 100,$$

where A₀ was the absorbance of DPPH radical + methanol; A₁ was the absorbance of DPPH radical + sample extract or standard. The 50% inhibitory concentration value (IC₅₀) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

2.5. Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). To 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10mM 2,4,6-tripyridyls-triazine (TPTZ) in 40mM HCl, and 2.5 mL of 20 mM ferric chloride (FeCl₃ · 6H₂O) solution) was added to 1mL of the extracts at concentrations of 0.1 - 1.0 mg/mL. The reaction mixtures were incubated at 37°C for 30 min and the increase in absorbance at 593nm was measured. FeSO₄ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/g of the extract) for the extracts were then extrapolated from the standard curve.

2.6. Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS was estimated according to the method of Ohkawa *et al.* (1979). Egg yolk homogenate (0.5 mL of 10% v/v) and 0.1 mL of extract were added to a test tube and made up to 1mL with distilled water. 0.05mL of FeSO₄ (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 0.05 mL 20% TCA were added and the resulting mixture was vortexed; it was then heated at 95°C for 60 min. The generated color was measured at 532 nm.

Inhibition of lipid peroxidation (%) was calculated with the formula:

$$(C-E)/C \times 100\%;$$

where C is the absorbance value of the fully oxidized control and E is $(Abs_{532}+TBA - Abs_{532-TBA})$.

2.7. Statistical Analysis

All analyses were carried out in triplicate and the results were expressed as mean \pm SEM. The data were subjected to one-way analysis of variance (ANOVA) where applicable. P values of < 0.05 were regarded as significant.

3. Results

The results of phytochemical screening are shown in Table 1. More phytochemicals were detected in the *S. mombin* leaf extract than in the *P. longifolia* leaf extract. Alkaloids, flavonoids, tannins and reducing sugars were detected in both extracts while saponins, steroids, terpenoids and cardiac glycosides were detected in *S. mombin* extract only.

Figure 1 depicts the total phenol, flavonoid and proanthocyanidin content of extracts of *S. mombin* and *P. longifolia* leaf. *S. mombin* extract had a higher total phenol content (213.50 ± 1.25 mg gallic acid equivalent / g extract) than the *P. longifolia* extract (110.00 ± 10.00 mg gallic acid equivalent / g extract). However, the *P. longifolia* extract had higher total flavonoid and proanthocyanidin contents (98.58 ± 1.75 mg quercetin equivalent/ g extract and 47.25 ± 0.15 mg ascorbic acid equivalent / g extract, respectively) than the *S. mombin* counterpart (78.75 ± 0.42 mg quercetin equivalent/ g extract for total flavonoid and 20.00 ± 0.00 mg ascorbic acid equivalent / g extract for proanthocyanidin content).

The results of the DPPH radical scavenging activities of *S. mombin* and *P. longifolia* leaf extracts are shown in Figure 2 with the IC_{50} values in Table 2. The results show that at lower concentrations, (2 – 10 μ g/mL) the *P. longifolia* extract was a better inhibitor of the DPPH radical than the *S. mombin* extract. Whereas, at higher concentrations (25 – 100 μ g/mL), the *S. mombin* extract had a better DPPH radical scavenging activity than the *P. longifolia* extract. The IC_{50} values (Table 2) were 144.89 μ g/mL for the *S. mombin* extract and 139.72 μ g/mL for the *P. longifolia* extract in contrast to that of ascorbic acid (118.55 μ g/mL).

The Ferric Reducing Antioxidant Potential (FRAP) results are presented in Figure 3. The results revealed that the *S. mombin* extract had a significantly higher ($p < 0.05$) FRAP value (68.13 ± 2.13 mg Fe(II)/0.05g extract) than the *P. Longifolia* extract (23.63 ± 1.88 mg Fe(II)/0.05g extract).

Figure 4 shows the thiobarbaturic acid reactive substances (TBARS) inhibitory activities of the *P. longifolia* and *S. mombin* extracts. The percentage inhibition of lipid peroxidation was significantly higher ($p < 0.05$) in the *S. mombin* extract ($11.7 \pm 0.00\%$) than in the *P. longifolia* extract ($1.40 \pm 0.01\%$).

Table 1. Phytochemical composition of methanol extracts of *P. longifolia* and *S. mombin* leaves.

Phytochemicals	<i>Polyalthia longifolia</i>	<i>Spondias mombin</i>
Cardiac glycosides	–	++
Terpenoids	–	+
Alkaloids	++	+++
Reducing sugars	++	+++
Flavonoids	+	+
Tannins	++	+
Saponins	–	+
Steroids	–	++

KEY:+++ = Very highly detected; ++ = Highly detected; + = Less detected; – = Not detected

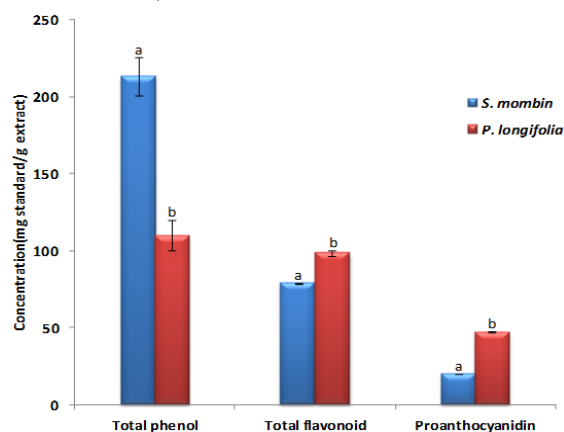


Figure 1. Total phenol, flavonoid and proanthocyanidin content of extracts of *S. mombin* and *P. longifolia* leaves. Total phenol is expressed as mg Gallic Acid Equivalent / g extract, Total flavonoid is expressed as mg Quercetin Equivalent / g extract and Proanthocyanidin content is expressed as mg Ascorbic acid Equivalent / g extract. Values are expressed as mean \pm SEM, $n = 3$ /group. Different lowercase letters represent significant difference between means at $P < 0.05$.

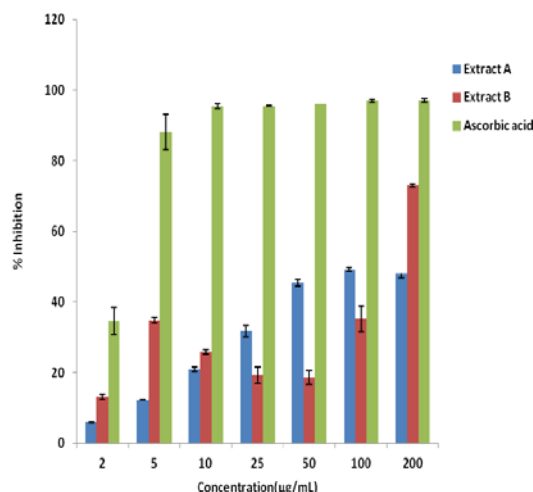


Figure 2. DPPH's radical scavenging activity of methanol extracts of *S. mombin* and *P. longifolia* leaves. Extract A= *S. mombin*; Extract B= *P. longifolia*, Values are expressed as mean \pm SEM, $n = 3$ /group. Different lowercase letters represent significant difference between means at $P < 0.05$.

Table 2. IC₅₀ values of extracts of *S. mombin* and *P. longifolia* leaves.

Plant	IC ₅₀ (µg/mL)
Ascorbic acid	118.55 ^a
<i>Spondias mombin</i>	144.89 ^b
<i>Polyalthia longifolia</i>	139.72 ^b

Data represent mean ± SEM of triplicate analysis. Different lowercase letters within column indicate significant difference at $P < 0.05$.

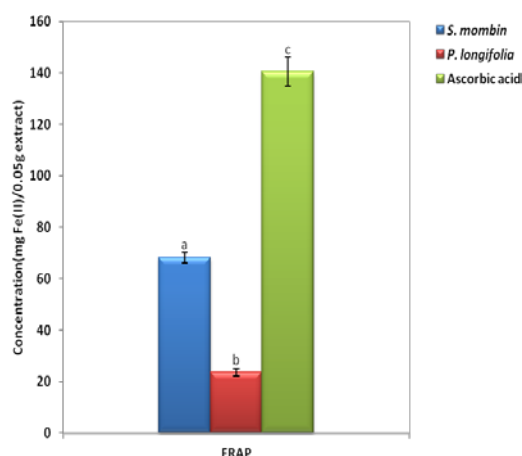


Figure 3. Ferric acid reducing antioxidant potential (FRAP) assessment of methanol extracts of *P. longifolia* and *S. mombin* leaves. Values are expressed as mean ± SEM, $n = 3$ /group. Different lowercase letters represent significant difference between means at $P < 0.05$.

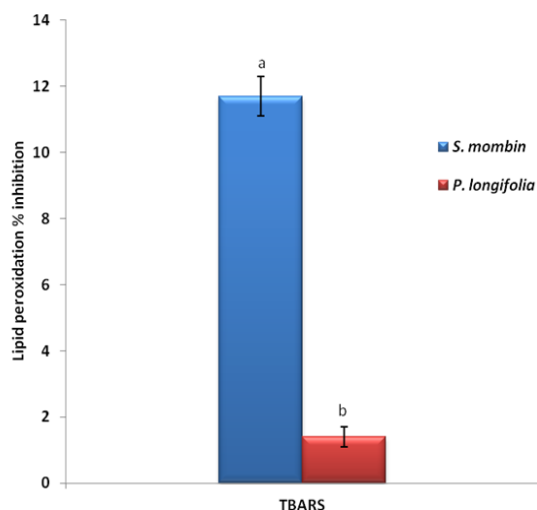


Figure 4. Thiobarbituric Acid Reactive Substances (TBARS) of extracts of *S. mombin* and *P. longifolia*. Values are expressed as mean ± SEM, $n = 3$ /group. Different lowercase letters represent significant difference between means at $P < 0.05$.

4. Discussion

The WHO describes a medicinal plant as any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or that are precursors for chemo-pharmaceutical semi synthesis. These non-nutrient plant chemical compounds or bioactive components often referred to as phytochemicals

or phyto-constituents are responsible for protecting the plant against microbial infections or infestations by pests (Doughari, 2012). Screening plant materials for their phytochemicals is usually the first step in research protocol aimed at the isolation and the purification of natural compounds with bioactivities. It allows the researcher to see at a glance the phytochemicals present in a plant material and, hence, gives an idea of the potential use of the plant material (Oikeh *et al.*, 2013).

The result of the phytochemical screening of *S. mombin* leaf extract revealed the presence of several phytochemicals, viz saponins, flavonoids, terpenoids, cardiac glycosides, reducing sugars, alkaloids, tannins and steroids. These findings are consistent with those of Njoku and Akumefula (2007) who also detected the presence of some of these phytochemicals. Phytochemical screening of *P. longifolia* leaf extract revealed the presence of flavonoids, tannins, alkaloids and reducing sugars. Cardiac glycosides, steroids, terpenoids and saponins were not detected. These results corroborate, in part, with the findings of Saha *et al.* (2008). The differences between both research findings may be due to the different extracting solvents used.

Reactive Oxygen Species (ROS) are continuously produced *in vivo*. At low concentrations, they exert beneficial effects on cellular response and immune function, but at high levels, these radicals become toxic and disrupt the antioxidant defense system of the body, which may lead to oxidative stress (Jothy *et al.*, 2012). Antioxidants scavenge free radicals in biological systems and hence, help in disease prevention. The antioxidant activity of plants is believed to be due to their phenolic compounds. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching reactive oxygen species (Jothy *et al.*, 2012). Phenolics are chemical components that occur ubiquitously in plants and play important roles in plant defense against pathogens and herbivore predators, hence their use in the control of human pathogenic infections. Flavonoid phenolics are a major group of plant phenolics with proanthocyanidins, being a sub group of flavonoids (Doughari, 2012). The proanthocyanidins (condensed tannins) are believed to possess potent antioxidant activities and exert several protect effects on humans (Gu *et al.*, 2003).

The *S. mombin* extract contained a higher total phenolic content but a lower total flavonoid and proanthocyanidin content than the *P. longifolia* extract. The lower flavonoid content of the *S. mombin* extract may suggest that its total phenolic content is majorly of non-flavonoidal origin. Proanthocyanidins are condensed tannins formed as a result of coupling reactions between flavanyl units (Prakash and Gupta, 2009). The higher quantification of proanthocyanidins in the *P. longifolia* extract than in the *S. mombin* extract is consistent with the result of the phytochemical screening wherein tannins gave more intense coloration in the *P. longifolia* extract when compared with the *S. mombin* counterpart.

Antioxidants can deactivate radicals by three major mechanisms, namely via Hydrogen Atom Transfer (HAT), Electron Transfer (ET), and combination of both

HAT and ET (Jothy *et al.*, 2012). HAT measures the ability of an antioxidant to quench free radicals by hydrogen donation, while ET detects the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls. Ferric Reducing Antioxidant Power (FRAP) is an ET assay while DPPH assay combines both HAT and ET mechanisms (Jothy *et al.*, 2012). As a rapid and simple measure of antioxidant activity, the DPPH radical scavenging capacity is based on the reduction of the stable radical DPPH to yellow colored diphenylpicrylhydrazine in the presence of a hydrogen donor (Jothy *et al.*, 2012). The IC₅₀ values obtained from the extracts were relatively low and compared favorably with that of the ascorbic acid, the standard antioxidant. These plant extracts may thus have potent radical scavenging properties. The ferric reducing antioxidant potential test is based on the ability of antioxidants present in the test extracts to reduce Fe³⁺ to Fe²⁺. The *S. mombin* extract was observed to have a significantly higher ($P < 0.05$) FRAP value than the *P. longifolia* extract. Both extracts however have significantly lower ($P < 0.05$) FRAP values than the standard antioxidant.

Lipid peroxidation has been implicated in many diseases. The results on the inhibition of lipid peroxidation revealed that the *S. mombin* extract had a significantly greater inhibitory effect on lipid peroxidation than the *P. longifolia* extract. Moreso, polyphenols are strong natural antioxidants that possess free radical scavenging activity (Prakash and Gupta, 2009). This may explain why the *S. mombin* extract, which was observed to contain a higher total phenolic content, is a better inhibitor of lipid peroxidation and a better ferric reducing antioxidant potential than the *P. longifolia* extract.

5. Conclusions

This study reveals that *S. mombin* contains higher amounts of the tested phyto-constituents than the *P. longifolia* extract. The *S. mombin* extract also contains a higher total phenolic content than the *P. longifolia* extract. These may be responsible for the better antioxidant properties observed, especially in the *S. mombin* extract which may be of importance in the drug discovery process.

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