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Evaluation of Phytochemical Composition, Antioxidant Activity, and Analgesic Effects of *Polyalthia Longifolia* Leaves (Masquaerade)

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Abstract
Polyalthia longifolia is a plant with a rich medicinal history, its name derived from the Greek words "poly," meaning many, and "althea," meaning cure. This study aimed to evaluate the phytochemical composition, antioxidant potential, and analgesic activity of the methanolic extract of Polyalthia longifolia leaves. Phytochemical analysis was conducted using standard qualitative methods, while the antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and analgesic activity was determined through the acetic acid-induced writhing test. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, cardiac glycosides, steroids, and triterpenes, whereas anthraquinones not detected. The extract exhibited significant antioxidant activity, with an IC_{50} value of 7.122 µg/mL, which was slightly less potent than that of Butylated Hydroxytoluene (BHT) with an IC_{50} value of 4.942 µg/mL. The methanol extract demonstrated high free radical scavenging activity (99.30% at 1000 µg/mL) compared to BHT (90.1% at 1000 µg/mL). The analgesic evaluation showed dose-dependent effects, with the 300 mg/kg dose achieving the highest pain inhibition (100%), followed by 150 mg/kg (98.1% inhibition) and 75 mg/kg (74.03% inhibition). The analgesic effect increased proportionally with dosage. These findings suggest that Polyalthia longifolia leaves contain bioactive compounds with notable antioxidant and significant analgesic activities, highlighting their potential for pharmaceutical and nutraceutical applications.

Keywords: Phytochemicals, Antioxidant, Analgesic, *Polyalthia longifolia*, Extract

INTRODUCTION
Polyalthia longifolia, often referred to as the Mast Tree, is a tropical tree belonging to the *Annonaceae* family, known for its flowering plants with notable ecological and economic roles (Flora of North America, 2008; Chatrou *et al*., 2012). The tree is native to India and Sri Lanka

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but has been widely cultivated in tropical regions as an ornamental plant due to its tall, slender, and conical structure. It is characterized by drooping branches, glossy dark green leaves, and delicate pale green flowers that bloom for a short duration. Traditionally, different parts of *P. longifolia*, particularly the bark and leaves, have been utilized in Ayurvedic medicine for treating fever, diabetes, indigestion, and inflammatory conditions (Chandaka *et al*., 2018).

The *Annonaceae* family, to which *P. longifolia* belongs, is one of the largest families in the Magnoliales order, comprising over 2,400 species distributed across tropical and subtropical regions (Subramanion *et al*., 2013). Many species in this family, including *Polyalthia*, are known for their diverse phytochemical composition, which contributes to their pharmacological significance. The genus *Polyalthia* itself consists of about 120 species, with *P. longifolia* being one of the most extensively studied due to its bioactive compounds and therapeutic potential (Mitra *et al*., 1999).

Several studies have reported the pharmacological properties of *P. longifolia*, highlighting its antibacterial, antioxidant, anti-inflammatory, analgesic, hepatoprotective, and anticancer activities (Thenmozhi and Sivaraj, 2010; Mundhe *et al*., 2011). The presence of bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, and saponins has been linked to these medicinal properties. Notably, the leaves of *P. longifolia* have demonstrated strong radical scavenging activity, supporting their potential as natural antioxidants in preventing oxidative stress-related disorders (Mundhe *et al*., 2011). Additionally, its analgesic effects have been observed in various animal models, indicating its possible application in pain management (Tanna *et al*., 2009). Despite these promising pharmacological findings, research on the therapeutic potential of *P. longifolia* remains limited, with many studies focusing on crude extracts rather than the isolation and characterization of specific bioactive compounds. Furthermore, while the antioxidant and anti-inflammatory activities of the leaves have been reported, their precise mechanisms of action and clinical relevance require further investigation. Most available studies have been conducted in vitro or on animal models, necessitating more comprehensive studies, including human clinical trials, to validate its medicinal efficacy.

This study aims to bridge the existing research gap by evaluating the phytochemical composition, antioxidant activity, and analgesic effects of *P. longifolia* leaves. By conducting a detailed phytochemical screening and bioactivity assessment, this study seeks to provide scientific validation for its traditional medicinal uses and explore its potential as a natural source of therapeutic agents.

MATERIALS AND METHODS

Collection and Identification of plant Materials

Polyalthia longifolia leaves were carefully collected from Rijiyar Zaki, located in Ungogo Local Government Area of Kano State, Nigeria. Identification and authentication of the plant were performed by botanical experts at the Herbarium, Plant Biology Department, Bayero University, Kano, and a voucher specimen number (BUKHAN 0006) was assigned.

Preparation of Plant extracts

Freshly collected leaves of *Polyalthia longifolia* were thoroughly washed, air-dried, and grounded into coarse powder using a grinding machine. The powdered material was stored in airtight containers to maintain its quality for further analysis. About 2 kg of the powdered

sample was soaked in 5 L of 80% methanol in a suitable container. The mixture was allowed to stand at room temperature (28 ± 2°C) for 72 hours with regular agitation to improve the extraction process. After this period, the mixture was filtered through muslin cloth to remove solid particles, followed by filtration using Whatman No. 1 filter paper. The resulting filtrate was evaporated in a water bath at 50°C until all the methanol was removed, leaving behind a concentrated extract. The final extract weighed 60.9g and was stored in properly labeled containers under appropriate storage conditions (Namadina *et al*., 2020).

Qualitative Phytochemical screening of Methanol extract of *Polyalthia Longifolia* leaves

The *Polyalthia longifolia* leaf extract was subjected to phytochemical analysis to determine its chemical components using the following procedures:

Tests for carbohydrates

Molisch's Test: To detect carbohydrates, 1 mL of the filtrate was mixed with 1 mL of Molish's reagent in a test tube. Then, 1 mL of concentrated sulphuric acid was carefully added down the side of the test tube to form a lower layer. The formation of a reddish color at the interface indicates the presence of carbohydrates (Evans, 2009).

Tests for Saponins

Frothing Test: Approximately 10 mL of distilled water was added to a portion of the extract and shaken vigorously for 30 seconds. The tube was left to stand vertically for 30 minutes. A honeycomb-like froth persisting for 10-15 minutes confirms the presence of saponins (Evans, 2009).

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2 mL of 50% methanol. Metallic magnesium chips were added, followed by a few drops of concentrated hydrochloric acid. The appearance of a red color confirms the presence of flavonoids (Evans, 2009).

Test for Alkaloids

Wagner's Test: A few drops of Wagner's reagent were added to a portion of the extract. The formation of a whitish precipitate indicates the presence of alkaloids (Evans, 2009).

Test for Steroids and Triterpenes

Liebermann-Burchard's Test: Equal volumes of acetic acid anhydride were added to a portion of the extract and mixed gently. Then, 1 mL of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A color change observed immediately or later indicates the presence of steroids and triterpenes. A red, pink, or purple color indicates triterpenes, while a blue or blue-green color confirms steroids (Evans, 2009).

Test for Cardiac Glycosides

Kella-Killiani's Test: A portion of the extract was dissolved in 1 mL of glacial acetic acid containing traces of ferric chloride solution. The mixture was transferred into a dry test tube, and 1 mL of concentrated sulphuric acid was carefully added down the side of the test tube to form a lower layer. A purple-brown ring at the interface confirms the presence of deoxy sugars, while a pale green color in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric Chloride Test: Three to five drops of ferric chloride solution were added to a portion of the extract. A greenish-black precipitate indicates condensed tannins, while hydrolysable tannins yield blue or brownish-blue precipitates (Evans, 2009).

Test for Anthraquinones

Borntrager's Test: Approximately 5 mL of chloroform was added to a portion of the extract in a dry test tube and shaken for at least 5 minutes. The mixture was filtered, and the filtrate was shaken with an equal volume of 10% ammonium solution. A bright pink color in the aqueous upper layer confirms the presence of free anthraquinones (Evans, 2009).

Antioxidant activity Procedure

The antioxidant activity of *Polyalthia longifolia* leaf extracts was determined based on their radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The method used was a modified version from Sani and Dailami (2015). A 200 µL aliquot of 100 µM DPPH in methanol was mixed with 100 µL of sample fractions prepared at various concentrations in methanol (1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.8 µg/mL). The reaction took place in the dark for 30 minutes at room temperature. Absorbance readings for the blank, test, and control samples were taken at 517 nm. The experiment was conducted in triplicate, and the scavenging activity was calculated as a percentage of inhibition using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \text{ X } 100$$

The concentration causing 50% inhibition (IC_{50}) was calculated through probit analysis using SPSS 16.0 software. The obtained IC_{50} values were compared to ascorbic acid, a standard antioxidant.

Analgesic Studies

Acetic Acid-Induced Writhing in Mice: The evaluation of analgesic activity followed the acetic acid-induced writhing method described by Koster *et al*. (1959). Writhing behavior, as defined by Mishra *et al*. (2011), includes stretching, tension to one side, hind-leg extension, abdominal contraction that causes the abdomen to touch the floor, and trunk twisting. Thirty Swiss albino mice of both sexes were divided into five groups. Groups 1 and 5 served as the negative control (distilled water at 10 mL/kg) and the positive control (Piroxicam at 20 mg/kg), respectively. Groups 3, 4, and 5 were administered the extract orally at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg. One hour after administration, each mouse received 0.6% acetic acid (10 mL/kg) intraperitoneally to induce pain. Five minutes after the acetic acid injection, the mice were observed for 15 minutes, and the number of writhes per mouse was recorded. The percentage inhibition of writhing was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Average number of writhes (negative control)} - \text{Average of writhes(test)}}{\text{Average number of writhes (negative control)}} \text{ X } 100$$

RESULTS AND DISCUSSION

Results

Qualitative Phytochemical screening of methanolic extract of *Polyalthia Longifolia* leaves

Alkaloid, flavonoids, saponins, tannins, steroid, triterpenes, phenols, carbohydrate and cardiac glycosides were detected in methanolic extracts of *Polyalthia Longifolia* leaves while anthraquinones were absent (Table 1).

Table 1. Qualitative Phytochemical screening of methanolic extract of *Polyalthia Longifolia* leaves

Metabolites	Inferences
	***Polyalthia Longifolia* leaves**
Alkaloid	+
Flavonoid	+
Saponins	+
Cardiac glycoside	+
Tannins	+
Steroid	+
Triterpenes	+
Anthraquinones	-
Carbohydrate	+

The antioxidant activities different levels of Methanol extracts of *Polyalthia Longifolia leaves*

Free radical scavenging ability of the methanol extract of *Polyalthia Longifolia* leaves were evaluated using DPPH radical. Botilated hydroxyl toluene (BHT) was used as positive control. It was determined that methanol extract of *Polyalthia Longifolia* leaves possessed higher radical scavenging ability of 99.30 % at the highest concentration of 1000 µg/mL and was compared with standard where it showed 90.1 % activity (Table 2).

Table 2. Antioxidant activities of different levels of Methanol extracts of *Solanum aethiopicum* fruit and *Polyalthia Longifolia* leaves

Analyes		Concentration (µg/mL) / % Inhibition							
		1000	500	250	125	62.5	31.25	15.6	7.8
Polyalthia leaves	*Longifolia*	99.3	98.7	99.8	74.9	56.2	48.7	34.1	32.3
BHT		90.1	88.8	88.8	87.6	81.5	70.1	57.6	48.3

The antioxidant activities of Methanol extracts of *Polyalthia Longifolia* leaves

The inhibition of DPPH radical by the extracts were concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extracts showed that the extract had higher antioxidant activities than standard. The higher the IC_{50} the lower the antioxidant activities (Table 3).

Table 3: Antioxidant activities of Methanolic extracts of *Polyalthia Longifolia* leaves

Sample	IC_{50} (µg/mL)
Polyalthia Longifolia leaves	7.122
BHT	4.942

BHT= Butylated Hydroxytoluene

The effect of methanol extract of *Polyalthia Longifolia leaves* on acetic acid induced writhing in mice

The extract significantly ($p<0.05$) decreased the number of writhes caused by acetic acid in a dose-dependent manner compared with distil water as shown in Table 4. The effects observed at 300 mg/kg and 150 mg/kg were higher than that of the control. Piroxicam, the positive control at 20 mg/kg, shows 78.8% inhibition, indicating strong pain relief. *Polyalthia longifolia* at 300 mg/kg achieves 100% inhibition, meaning it completely stops the writhing,

showing very strong pain relief. At 150 mg/kg, the extract shows 98.1% inhibition, nearly as effective as the higher dose and more effective than piroxicam. At 75 mg/kg, the extract has 74.03% inhibition, which is slightly less effective than piroxicam but still significant. This indicates that the pain-relieving effect of the extract increases with the dose (Table 4).

Table 4. Effect of Methanolic extract of *Polyalthia Longifolia* leaves on Acetic Acid Induced writhing in mice

Treatment	Dose (mg/kg)	Mean No. of Writhes ± SEM	Inhibition (%)
Distilled water	10ml/kg	17.33±0.45	-
Piroxicam	20	3.67±0.34	78.8
Extract	300	0.00±0.0	100
Extract	150	0.33±0.01	98.1
Extract	75	4.50± 0.11	74.03

DISCUSSION

The qualitative phytochemical screening of the methanolic extract of *Polyalthia longifolia* leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, triterpenes, phenols, carbohydrates, and cardiac glycosides, while anthraquinones were absent. This aligns with earlier studies by Okwu and Josiah (2006) and Alhassan *et al*. (2014), which reported the presence of similar metabolites in plant species. The absence of anthraquinones, as previously observed by Evans (2002), could be attributed to differences in geographical location, maturity, or time of collection. The bioactive metabolites identified have been associated with various therapeutic benefits. Alkaloids are known for their analgesic, antispasmodic, and bactericidal properties (Okwu and Okwu, 2004). Flavonoids exhibit strong antioxidant, anti-inflammatory, and antibacterial effects (Alan and Miller, 1996). Saponins, tannins, and steroids have also been associated with antibacterial, wound-healing, and aphrodisiac properties (Njoku and Akumufula, 2007; Cushine and Lamb, 2005). These findings emphasize the potential of *Polyalthia longifolia* leaves as a rich source of bioactive compounds for pharmaceutical applications.

The methanolic extract of *Polyalthia longifolia* leaves demonstrated remarkable antioxidant activity, with a free radical scavenging ability of 99.3% at a concentration of 1000 µg/mL, surpassing the activity of the standard antioxidant, butylated hydroxytoluene (BHT), at 90.1%. The activity was concentration-dependent, as inhibition increased with higher extract concentrations (Table 2). However, at lower concentrations (e.g., 31.25 µg/mL), the extract (48.7%) was less effective than BHT (70.1%).

The IC_{50} value of the extract (7.122 µg/mL) was higher than that of BHT (4.942 µg/mL), suggesting the extract's effectiveness at lower concentrations is less than BHT. Nonetheless, its superior activity at higher concentrations highlights its potential as a natural antioxidant. These findings are consistent with previous studies that have demonstrated the strong antioxidant potential of plant extracts rich in flavonoids, phenols, and tannins, and other secondary metabolites in plants (Okwu, 2004; Jonathan and Tom, 2008).

Antioxidants play a critical role in neutralizing free radicals, reducing oxidative stress, and preventing cellular damage. The high radical scavenging activity observed in *Polyalthia longifolia* indicates its potential application in food preservation, nutraceuticals, and the treatment of diseases caused by oxidative stress, including cardiovascular and neurodegenerative disorders.

The methanol extract of *Polyalthia longifolia* leaves significantly reduced acetic acid-induced writhing in mice in a dose-independent manner, with 300 mg/kg and 150 mg/kg doses showing 100% and 98.1% inhibition, respectively. These values surpass the inhibition observed with the standard drug, piroxicam (78.8%), at 20 mg/kg (Table 4). At 75 mg/kg, the extract still achieved 74.03% inhibition, comparable to piroxicam.

The acetic acid-induced writhing test is commonly used to evaluate peripheral analgesics, as it involves the activation of nociceptive neurons and the release of pain mediators like prostaglandins (Abubakar *et al*., 2016; Gupta *et al*., 2015). The significant inhibition observed suggests that the analgesic effect of the extract may result from the suppression of cyclooxygenase and lipoxygenase pathways, thereby reducing the synthesis of prostaglandins and other pain mediators (Paschapur *et al*., 2009).

The presence of bioactive compounds, such as flavonoids and alkaloids, further supports the analgesic potential of the extract. Flavonoids are known to inhibit the enzymes involved in prostaglandin synthesis, while alkaloids act as mood enhancers and analgesics (Salawu *et al*., 2008; Gurib *et al*., 2013). Similar results have been observed in other plants with high flavonoid and alkaloid content (Safari *et al*., 2016).

The dose-independent behavior at higher doses could be due to the saturation of active sites or the presence of metabolites that exhibit maximum effect at specific concentrations. This aligns with findings from Nthiga *et al*. (2016), where similar patterns were observed in plant extracts tested for antinociceptive activity.

CONCLUSION

The study establishes that *Polyalthia longifolia* leaves are a rich source of bioactive phytochemicals with significant antioxidant and analgesic properties. The methanolic extract exhibited superior antioxidant activity at higher concentrations, making it a potential natural alternative to synthetic antioxidants like BHT. Additionally, its strong peripheral analgesic effect, surpassing that of piroxicam, highlights its therapeutic potential for pain management. These findings suggest that *Polyalthia longifolia* could be further explored for its application in pharmaceuticals, nutraceuticals, and related fields.

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