



Phytochemical Screening, Isolation and Characterization of Leaf Extract from *Paullinia pinnata* L. (Sapindaceae)

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Abstract

In Africa, the leaves of *Paullinia pinnata* L. (family Sapindaceae) are reported to be used in the treatment of gonorrhea, wounds and microbial infections. This study aimed at screening for phytochemicals, isolate and characterize bioactive compounds from the extracts of leaves from *Paullinia pinnata*. Sequential extraction using solvents of increasing polarity (*n*-hexane, chloroform, ethyl acetate, and methanol) was performed, with methanol yielding the highest amount of crude extract (6.08%). Phytochemical screening revealed the presence of flavonoids, phenols, tannins, saponins, terpenoids, cardiac glycosides, alkaloids, and anthraquinones across the various crude extracts. The chloroform crude extract (PLC) was selected based on the thin layer chromatography profile, for further purification using vacuum liquid chromatography (VLC) and column chromatography (CC). Pure compound (PLC-1-1) was obtained from repeated column chromatography of the chloroform extract. NMR (¹H and ¹³C) analysis and comparison with literature data for PLC-1-1, indicated a structure analogous to farnesol but with a methyl group substitution instead of a hydroxyl group, suggesting the compound to be methyl farnesene (2, 6, 10-trimethyl-trideca-2, 6, 10-triene). The findings support the traditional use of leaves from *P. pinnata* in the treatment of bacterial related ailments.

Keywords: *Paullinia pinnata*, Phytochemical screening, Sequential extraction, Isolation, Characterization.

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Introduction

Paullinia pinnata L. is a flowering plant belonging to the family Sapindaceae. This family is known to produce a wide range of secondary metabolites including saponins, diterpenoids, and cyanogenic glycosides (Buerki *et al.*, 2021). Among the Hausa people of northern Nigeria, the plant is commonly called 'Hannu' or 'Yaatsaa biyar'. It is a perennial climbing shrub that reaches heights between 2.5 and 8.0 metres. The plant has compound leaves, paired tendrils, and white flowers. Its fruits grow up to 2.5 centimetres long. *Paulinia pinnata* typically grows in

evergreen and mixed forests at altitudes up to 1200 metres (Buerki *et al.*, 2021).

Across sub-Saharan Africa, different parts of *P. pinnata* have long been used in traditional medicine. The leaves, for example, are used to treat gonorrhoea, wounds, and various microbial infections. The roots and stem bark are also used as fish poisons, arrow poisons, and remedies for dysentery and malaria (Nkansah, 2022; Adebayo and Krettli, 2023). Crude extracts of *P. pinnata* have shown significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. They have also demonstrated antiplasmodial activity against *Plasmodium berghei* (Ogbole

et al., 2021; Falodun *et al.*, 2022). In addition, the plant exhibits antioxidant and anti-inflammatory properties, which have been linked to the presence of polyphenols and flavonoids (Mbosso *et al.*, 2020). The analgesic and anti-inflammatory activities of both the ethanolic and aqueous extract of leaves from *P. pinnata* has been reported (Tseuguem *et al* 2019; Dingom *et al* 2017). The effects of methanol extracts from *P. pinnata* aerial part against *Salmonella typhimurium*-induced typhoid model in rats and the radical scavenging activities against stable DPPH free radical were reported with significant effects (Paul *et al*, 2014).

Despite these promising findings, specific compounds responsible for these activities have not been isolated from the plant. Previous studies have reported the presence of tannins, alkaloids, and saponins in the leaves of *P. pinnata*. The qualitative and quantitative phytochemical analysis of methanolic extract of *Paullinia pinnata* leaves reported the presences of carbohydrates, flavonoids, phenolics, tannins, glycosides, reducing sugar, steroids, saponins and terpenoids. (Onah *et al* 2025).

However, only a few attempts have been made to isolate pure compounds and determine their chemical structures (Nkansah, 2022; Buerki *et al.*, 2021). Six known terpenoids were reported from the aerial part of *P. pinnata* (Paul *et al*, 2014). More recent work using metabolomic approaches suggests that *Paullinia* species may contain novel triterpenoid saponins and acylated flavonoid glycosides that have not yet been fully characterised (Silva *et al.*, 2023). Therefore, the aim of this research work is to screen for phytochemicals, isolate compound(s), and characterise the pure isolated compound(s) obtained from the leaves extract of *Paullinia pinnata*.

Materials and Methods

Materials

The materials used include: Vacuum Liquid Chromatography (VLC) apparatus, pressure pump, glass column, silica gel (mesh sizes; 200–400 and 70–230), NMR machine, JEOL spectrometer (600 MHz) at King Fahd University of Petroleum and Minerals, Saudi

Arabia was used, aluminum foil, capillary tube, beaker, spatula, conical flask, funnel, filter paper, precoated Thin Layer Chromatography (TLC) plate, measuring cylinder, wash bottle, *n*-hexane, chloroform, ethylacetate, methanol, hydrochloric acid, ammonia, acetic acid, magnesium ribbon, ferric chloride, sulphuric acid, sodium hydroxide, distilled water, acetic anhydride and ascorbic acid.

Plant Collection, Authentication and Preparation

The leaves of *P. pinnata* were collected at Loko Development Area, Nasarawa Local Government Area of Nasarawa state on 16th of July 2023 by Baba Sabo (a traditional herbalist) and Ibrahim Ali Gambo. The plant sample was authenticated by taxonomist; Dr. Kolawole S. O. of Department of Biological sciences, Federal University of Kashere (FUK), Gombe State. Sample was deposited at the FUK herbarium against a voucher number of FUKH0101. The leaves of *P. pinnata* were washed with distilled water and air dried under shade for a period of two weeks. The dried plant material was ground into powder with a wooden mortar and pestle (Peiris *et al.*, 2023).

Sequential Extraction of Plant Sample

The air-dried powdered leaves of *P. pinnata* (500 g) were sequentially extracted by maceration at room temperature using *n*-hexane, chloroform, ethyl acetate, and methanol for 7 days each. After each extraction, the mixture was filtered using filter paper (Whatman No.1) and the solvent was allowed to evaporate to dryness. The resulting *n*-hexane, chloroform, ethyl acetate, and methanol extracts were weighed and labeled PLH, PLC, PLE, and PLM for *P. pinnata* leaves of *n*-hexane, chloroform, ethyl acetate, and methanol extract respectively (Alara, *et al.*, 2021).

Phytochemical Analysis of the Crude Extracts

Test for Alkaloids: To the filtrate (2 ml) of the extract was added 1% HCl + water. Then drops of Wagner's reagent was added to the mixture. A reddish brown colour indicate the presence of alkaloid (Zakari and Kubmarawa, 2016).

Test for Anthraquinone: 1 ml of dilute (10%) ammonia was added to 2 ml of the extract. A pink-red color in the ammoniacal (lower) layer indicate the presence of Anthraquinone (Pandey and Tripathi, 2023).

Test for Cardiac glycosides: To the filtrate (2 ml), 1ml of glacial acetic acid, 1ml ferric chloride and 1ml concentrated sulphuric acid was added. A Green-blue coloration of solution indicates the presence of Cardiac glycosides (Pandey and Tripathi, 2023).

Test for Flavonoid: To 2-3ml of extract, a piece of magnesium ribbon and 1ml of concentrated hydrochloric acid was added. Pink red or red coloration of the solution indicate the presence of flavonoid (Zakari and Kubmarawa, 2016).

Test for Phenol: Spot the extract on a filter paper. Add a drop of phosphomolybdic acid reagent and expose to ammonia vapors. Blue coloration of the spot indicates the presence of a phenol (Pandey and Tripathi, 2023).

Test for Saponin: Add 0.5 ml with 5 ml of distilled water and shake well. Persistence of frothing indicate the presence of saponin (Pandey and Tripathi, 2023).

Test for Steroid: To 1 ml extract, add 1ml of chloroform, 2-3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid. Dark green coloration indicates the presence of a steroid (Pandey and Tripathi, 2023).

Test for Tannin: 10% alcoholic ferric chloride will be added to 2-3ml of methanolic extract (1:1). Dark blue or greenish grey coloration of the solution indicate the presence of tannin (Pandey and Tripathi, 2023).

Test for Terpenoid: To 1 ml extract, add 1ml of chloroform, 2-3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid. Pink or red coloration indicate the presence of Terpenoid (Pandey and Tripathi, 2023).

Vacuum Liquid Chromatography of the Crude Extract

The various extracts; PLM, PLC, PLH, and PLE crude extracts were subjected to TLC, with the chloroform extract showing the most prominent results. Consequently, the chloroform crude extract was selected for further purification using VLC. Silica gel of mesh size 200-400 was packed in the VLC funnel using *n*-hexane. The chloroform crude

extract was then mixed with silica gel until a homogeneous mixture was obtained, after which it was loaded onto packed VLC flask. A pressure pump was connected to the flask, and solvents of increasing polarity were used to elute and 17 fractions were obtained (Zhang *et al.*, 2023).

Column Chromatography of Fraction PLC-1

Fraction 3 to 16 obtained from VLC were pooled together due to similar R_f value and labeled as PLC-1 which was subjected to column chromatography. The PLC-1 fraction was re-columned to obtain 120 fractions. Fractions 1 to 5 showed similar R_f value with single spot on the TLC plate. The pooled fraction was labelled PLC-1-1.

Characterization of PLC-1-1

The ^1H and ^{13}C -NMR spectral data of PLC-1-1 were recorded in deuterated chloroform (CDCl_3) using a JEOL spectrometer (600 MHz) at King Fahd University of Petroleum and Minerals, Saudi Arabia. Chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethyl silane (TMS), and coupling constants were reported in Hertz (Hz) (Jibril *et al.*, 2019).

Results and Discussion

Sequential Extraction: Extraction of *Paullinia pinnata* leaves using solvents of varying polarity produced crude extracts with different yields and similar dark green, gel-like appearances. Methanol (a highly polar solvent) gave the highest yield (30.4 g, 6.08%), followed by chloroform (14.5 g, 2.9%), *n*-hexane (7.9 g, 1.58%) and ethyl acetate (5.2 g, 1.04%). This agrees with Ogbale *et al.* (2021) and Falodun *et al.* (2022), who also reported methanol as the most effective solvent for *P. pinnata* due to its ability to dissolve both polar and moderately polar compounds. However, our ethyl acetate yield (1.04%) was lower than the 2.8% reported by Mbosso *et al.* (2020) for samples from Cameroon, possibly due to geographic variation, soil composition, or plant age (Adebayo & Krettli, 2023). The dark green, gel like appearance of all extracts matches Nkansah (2022), indicating co extraction of

chlorophyll, which necessitates further purification.

Phytochemical Screening: Phytochemical screening of *P. pinnata* extracts using solvents of varying polarity revealed diverse distributions of bioactive compounds. Flavonoids and phenols were present in all extracts, consistent with Buerki *et al.* (2021). Tannins appeared only in *n*-hexane and chloroform, contradicting Nkansah (2022) who found them in methanol extract. Saponins, terpenoids, and cardiac glycosides were absent in *n*-hexane but present in the chloroform, ethyl acetate and methanol extracts. This result concor with previous report (Silva *et al.*, 2023). Unlike Ogbole *et al.* (2021) report of the presence of cardiac glycosides in chloroform extract, it was not found in the present study. This could be possibly due to geographical or genetic variations. Alkaloids were found only in ethyl acetate and methanol extracts. This agrees with Falodun *et al.* (2022) but contrasts with Mbosso *et al.* (2020), who detected alkaloids in the chloroform extract. Anthraquinones were reported in methanol extract (Nkansah *et al.*, 2022; Buerki *et al.*, 2021. However, Silva *et al.* (2023) detected trace anthraquinones in *Paullinia* using metabolomics, supporting the result obtained in this research. From this findings, chloroform and methanol extracts showed the richest profiles, confirming that multiple solvents are needed for extraction of *P. pinnata* (Adebayo and Krettli, 2023).

Vacuum Liquid Chromatography of PLC

The results obtain from the vacuum liquid chromatography is shown in Table 3. Using vacuum liquid chromatography, fractions 3 to 16 from the chloroform extract were pooled as PLC 1 based on similar TLC spots, a standard practice also used by Ogbole *et al.* (2021) and Falodun *et al.* (2022). Column chromatography of PLC 1 yielded four subfractions: PLC 1-1, PLC 1-2, PLC 1-3, and PLC 1-4. The R_f values (0.375 in 100% *n*-hexane; 0.825 in *n*-hexane:chloroform 4:1) indicate that PLC 1-1 is non polar, consistent with Silva *et al.* (2023), who isolated non polar terpenes from *Paullinia* species using similar solvent systems.

Column Chromatography for Sample PLC-1

The result obtained from the column chromatography is shown in Table 4. Based on the TLC results, fractions 5 to 9, 10 to 13, 19 to 26 and 35 to 41 were pooled together and labeled PLC-1-1, PLC-1-2, PLC-1-3 and PLC-1-4 respectively. Fractions PLC-1-1 and PLC-1-2 had an R_f value of 0.375 in *n*-hexane (100 %) and 0.825 in an *n*-hexane: chloroform (4:1) solvent system. These fractions were combined and assigned the code PLC-1-1. Fractions PLC-1-3 had an R_f value of 0.1621 in *n*-hexane (100 %) and 0.7 in an *n*-hexane: chloroform (4:1) solvent system.

Characterization of Fraction PLC-1-1

Fraction PLC-1-1 was isolated as a colorless liquid with R_f value of 0.375 in *n*-hexane (100 %) solvent system. The ^1H NMR and ^{13}C NMR spectra of fraction PLC-1-1 were obtained on 600 MHz JOEL spectrometer in CDCl_3 as solvent. The ^1H NMR spectrum from literature (Wellburn, *et al.*, 1974) also revealed notable variations. In farnesol, a proton signal at 4.02 ppm corresponds to the hydroxyl-bearing carbon. In contrast, fraction PLC-1-1 exhibited a signal at 0.87 ppm, consistent with the presence of a terminal methyl group replacing the hydroxyl functionality. Additional chemical shift values indicated that fraction PLC-1-1 retains a conjugated diene system similar to farnesol but with structural modifications.

The ^{13}C NMR spectrum of fraction PLC-1-1 showed key differences compared to farnesol. Notably, in literature-reported data (Pandey and Tripathi, 2023), a carbon signal at 58.5 ppm corresponds to a hydroxyl-bearing carbon. However, in PLC-1-1, this signal was absent, and instead, a signal at 14.27 ppm was observed, suggesting the presence of a methyl ($-\text{CH}_3$) group replacing the hydroxyl group at carbon 1.

Based on comparisons of the ^1H NMR and ^{13}C NMR data obtain with literature, compound PLC-1-1 is proposed as methyl farnesene (2,6,10-trimethyl-trideca-2,6,10-triene).

Table 1: Crude Extract obtained from *n*-Hexane, Chloroform, Ethylacetate and Methanol

Crude extract	Colour	Texture	Weight (g)	Percentage yield (%)
PLH	Dark green	Gelly	7.9	1.58
PLC	Dark green	Gelly	14.5	2.9
PLE	Dark green	Gelly	5.2	1.04
PLM	Dark green	Gelly	30.4	6.08

Keys: PLC = Chloroform Leave extract of *P. pinnata*; PLE = Ethyl acetate Leave extract of *P. pinnata*; PLH = *n*-Hexane Leave extract of *P. pinnata*; PLM = Methanol Leave extract of *P. pinnata*

Table 2: Phytochemical Screening of the Crude Extracts

Phytochemicals	PLH	PLC	PLE	PLM
Flavonoids	+	+	+	+
Tannins	+	+	-	-
Saponins	-	+	+	+
Steroids	-	+	-	+
Phenols	+	+	+	+
Alkaloid	-	-	+	+
Terpenoids	-	+	+	+
Anthraquinone	-	-	-	+
Cardiac glycosides	-	+	+	+

Keys: PLC = Chloroform Leave extract of *P. pinnata*; PLE = Ethyl acetate Leave extract of *P. pinnata*; PLH = *n*-Hexane Leave extract of *P. pinnata*; PLM = Methanol Leave extract of *P. pinnata*

Table 3: Vacuum Liquid Chromatography Fractions obtained from PLC

Solvent system (%)	Fraction	Volume (mL)
<i>n</i> -hexane (100)	1 and 2	200
<i>n</i> -hexane: chloroform (80:20)	3 and 4	300
<i>n</i> -hexane: chloroform (70:30)	5	200
<i>n</i> -hexane: chloroform (60:40)	6	100
<i>n</i> -hexane: chloroform (50:50)	7	100
<i>n</i> -hexane: chloroform (20:80)	8	100
chloroform (100)	9	200
Chloroform: ethylacetate (90:10)	10	100
Chloroform: ethylacetate (70:30)	11	100
Chloroform: ethylacetate (50:50)	12	100
Ethylacetate (100)	13 to 15	400
Ethylacetate: methanol (50:50)	16	100
Methanol (100)	17	250

Table 4: Column Chromatography Collections from PLC-1

Solvent system (%)	Volume (mL)	Fraction
<i>n</i> -hexane (100)	500	1 to 2c
<i>n</i> -hexane: chloroform (99:1)	700	3 to 42
<i>n</i> -hexane: chloroform (97:3)	100	43 to 48
<i>n</i> -hexane: chloroform (95:5)	100	49 to 53
<i>n</i> -hexane: chloroform (90:10)	100	54 to 59
<i>n</i> -hexane: chloroform (80:20)	350	60 to 74

<i>n</i> -hexane: chloroform (70:30)	150	75 to 82
<i>n</i> -hexane: chloroform (60:40)	700	82 to 120

Table 5: ¹H NMR Data for Fraction PLC-1-1 and of Farnesol from Literature

Position of Proton	Experimental data of PLC-1-1 (ppm)	Literature Data of Farnesol (ppm)
1	1.25 (CH ₂ CH ₃)	4.20 (CH ₂ OH)
2	5.12 (CH=CH ₂)	5.39 (CH=CH ₂ OH)
3	1.60 (=C-CH ₃)	1.71 (=C-CH ₃)
4	2.02 (=CH-CH ₂)	2.0 (=CH-CH ₂)
5	4.85 (=CH)	5.20 (=CH)
6	0.87 (CH ₃ CH ₂)	-

Table 6: ¹³C NMR Data for Fraction PLC1-1 and of Farnesol from literature

Position of Carbon	Experimental data of PLC-1-1 (ppm)	Literature Data of Farnesol (ppm)
1	39.87 (CH ₂)	58.5 (HO-CH ₂)
2	124.44 (=CH)	124.1 (=CH)
3	135.26 (=C)	138.73 (=C)
4	29.84 (=C-CH ₂)	31.77 (=C-CH ₂)
5	25.84 (CH ₂ CH ₂)	25.60 (CH ₂ -CH ₂)
6	124.54 (=CH)	124.79 (=CH)
7	135.26 (=C)	135.59 (=C)
8	28.41 (=C-CH ₂)	32.11 (=C-CH ₂)
9	26.89 (CH ₂ -CH ₂)	26.45 (CH ₂ -CH ₂)
10	124.41 (=CH)	124 (=CH)
11	131.42 (=C)	131.14 (=C)
12	17.82 (C=CH ₃)	23.31 (C=CH ₃)
13	16.14 (C=CH ₃)	17.57 (C=CH ₃)
14	26.79 (C=CH ₃)	26.20 (C=CH ₃)
15	16.18 (C=CH ₃)	23.2 (C=CH ₃)
16	14.27 (CH ₂ -CH ₃)	-

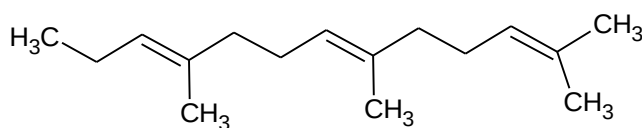


Figure 1: Proposed Structure for Compound PLC-1-1



Plate 1: Collections from VLC of PLC

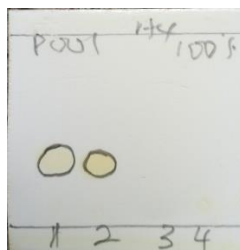


Plate 2: TLC Plate of fraction PLC-1-1 and PLC-1-3 in *n*-hexane (100 %)

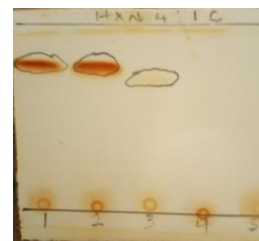


Plate 3: TLC Plate of fraction PLC-1-1 and PLC-1-3 in *n*-hexane: chloroform (4:1)

Conclusion

The sequential extraction of *P. pinnata* leaves yielded varying percentages, with methanol extract showing the highest yield (6.08%), followed by chloroform (2.9%), n-hexane (1.58%), and ethyl acetate (1.04%). Phytochemical screening confirmed the presence of key bioactive compounds, including flavonoids, tannins, alkaloids, and terpenoids, supporting the plant's traditional medicinal use. Chromatographic purification of the chloroform extract (PLC) led to the isolation of multiple fractions, with NMR (¹H and ¹³C) analysis of fraction PLC-1-1 revealing a farnesol-like structure modified by a methyl substitution instead of a hydroxyl group, suggesting a novel derivative.

Conflict of Interest

Authors declare no conflict of interest

Ethical Statement

This work required no ethical statement

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