



Phytochemical and HPLC-UV-DAD chromatographic characterization of stem bark extracts of *Pentaclethra macrophylla* Benth used for management of diabetes mellitus in Nigeria

Samuel Ehiabhi Okhale^{1*}, Nnamdi Amuzie², Chinyere Imoisi³, Jemilat Aliyu Ibrahim¹

¹Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Idu Industrial Area, Idu, P.M.B. 21 Garki, Abuja, Nigeria.

²Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B. 5026, Awka, Anambra State.

³Department of Chemistry, University of Benin, Benin City, Nigeria.

* Corresponding author: Email: samuelokhale@gmail.com; Phone: +2348036086812

Abstract: *Pentaclethra macrophylla* is reputed to possess a number of medicinal properties. It is used widely as medication for a variety of ailments and even as treatment for injuries and sores. *Pentaclethra macrophylla* (*P. macrophylla*) stem bark has long history of use as herbal remedy for inflammation, fever, diabetes and debility. The phytochemical profile of different polar and non-polar solvent extractives of *P. macrophylla* stem bark namely hexane (PMHE), ethyl acetate (PMEE), acetone (PMAE), methanol (PMME) and hot water (PMWE) were investigated using colour reactions, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The phytochemical constituents included tannins, flavonoids, terpenoids, sterols, essential oils, alkaloids, phenolic acids and saponins. Thin layer chromatography analysis of the hexane extract (PMHE) revealed the presence of β -sitosterol. The HPLC analysis of the methanol extract revealed three principal components, two of which corresponded to gallic acid, and caffeic acid. These chemical constituents may be responsible for the antidiabetic, therapeutic effects of *Pentaclethra macrophylla* stem bark, and folkloric uses.

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Key words: *Pentaclethra macrophylla*, stem bark, antidiabetic, β -sitosterol, gallic acid, caffeic acid

1. Introduction

Human society has always been in close contact with the environment and humans have obtained food, medicine, fiber and shelter from plant parts such as roots, stems, flowers, fruit and leaves (Jamshidi *et al.*, 2018; Ibrahim and Fagbohun, 2014). Natural products from plants, animals and minerals are used for treating diseases. Plants play important roles in the ecosystem, and diverse plant species from different families are distributed all over the world (Jamshidi *et al.*, 2018).

Pentaclethra macrophylla Benth belongs to the family Fabaceae. It is also known as African oil bean tree. The local name in Igbo is "Ugba" or "Ukpaka" (Famurewa *et al.*, 2015). The tree has a characteristic low branching habit and an open crown. The compound leaves are usually about 20-45cm long and covered with rusty hairs. Its flowers are commonly yellow or pinkish white and sweet smelling. Its fruits are available at most periods of the year because the large woody pods are persistent. Its fruits split open explosively (Okoye, 2016).

P. macrophylla is reputed to possess a number of medicinal properties. It is used widely as medication

for a variety of ailments and even as treatment for injuries and sores. A decoction of the stem bark is used as medicine for the treatment of diarrhea. When the stem bark is made into a lotion it is used in the treatment of itching (Burkill, 1985). A decoction of the crushed leaves and bush pepper (*Piper nigrum*) is used for the treatment of fever. A one hundred milliliter wine glass of the decoction is administered as therapeutic dosage twice daily. Some undeveloped native African communities use a mixture of crushed seeds of *P. macrophylla* and red ant to make a contraceptive medicine used for abortion. The high protein content of fermented oil bean seeds makes it a good low-cost source of protein.

The oil bean has been reported to possess anticancer property; this may be due to its linoleic acid content, which is a precursor of gamma linolenic acid (Nwankwo, 2018). Oil bean is also a source of dietary phytoestrogens. It aids in the control of obesity (Abbiw, 1990). The stem bark is chewed for stomachache, appetizer, teeth cleaning and general weakness of the body (Idu *et al.*, 2009). The tree is 20-35 m high, bole to 1 m diameter, gnarled, twisted, irregularly buttressed, low branching, carrying a dense

evergreen crown; of river-banks and vicinity of water of the high forest and secondary jungle where it may

become dominant, and in coasts (Akindahunsi, 2004).

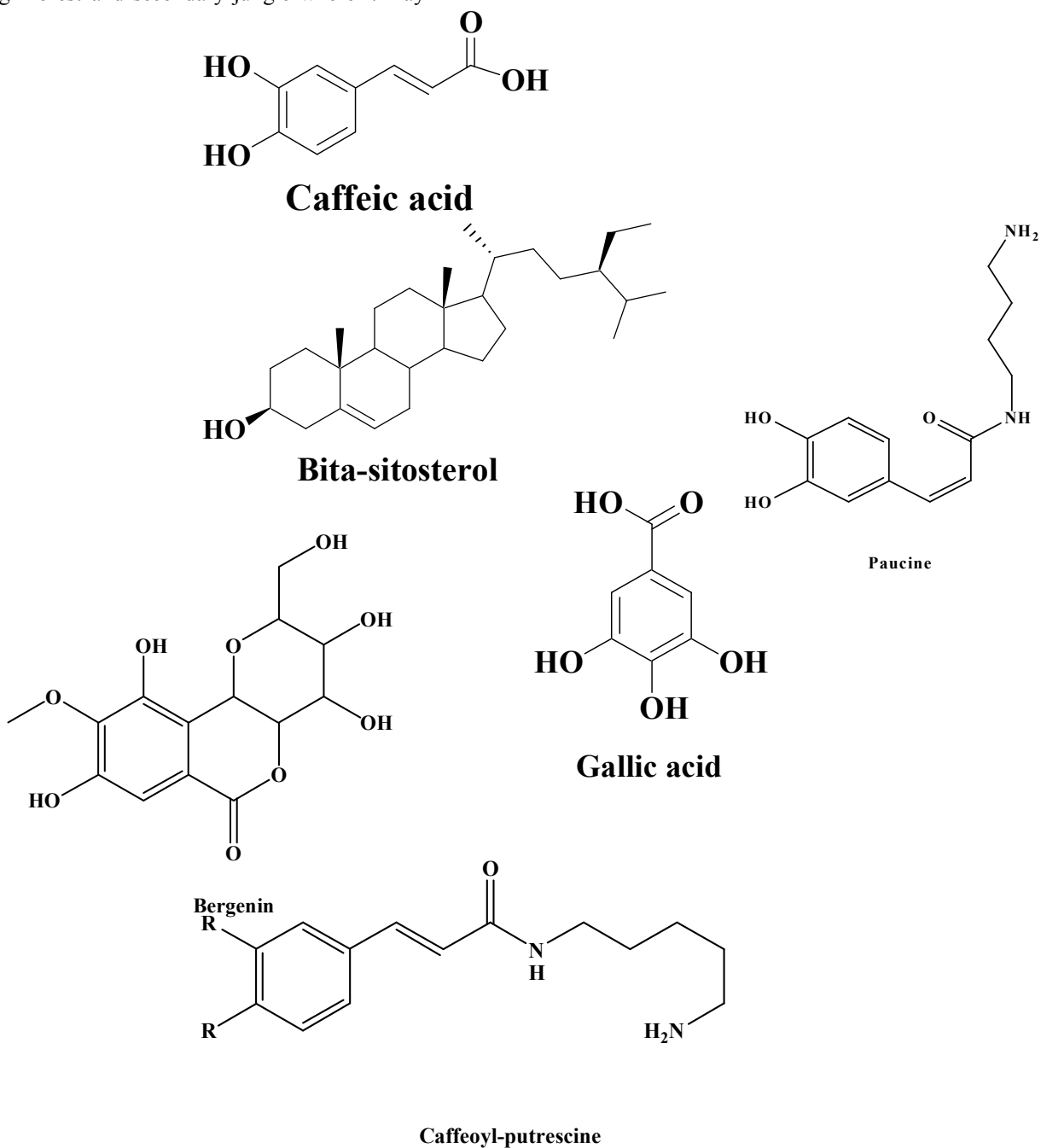


Fig 1: Structure of some chemical constituents of *Pentaclethra macrophylla*

Pentaclethra macrophylla has been reported to have numerous medicinal uses. Various extracts of the stem bark is widely used in ethnomedicine (Ameyaw, 2009). For appropriate use in herbal medicine practice, these extracts need to be standardized. There is however no reported simple chemical or chromatographic standardization on this drug plant. One aspect of chemical standardization

involves chromatographic profiling of constituent compounds. Currently there are different chromatographic techniques employed in the standardization of herbal drugs which include high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), ultra performance liquid chromatography (UPLC), liquid chromatography-mass spectroscopy (LC-MS), liquid

chromatography–nuclear magnetic resonance (LC-NMR), gas chromatography (GC) and gas chromatography–mass spectroscopy (GC-MS), super critical fluid chromatography (SFC) and capillary electrophoresis (CE) (Neeraj *et al.*, 2011).

The present study aims to establish simple reference profiles for the extractives of *Pentaclethra macrophylla* stem bark using thin layer chromatography and high performance liquid chromatography-UV-DAD, towards its chemical and chromatographic standardization.

2. Materials and Methods

Collection of plant sample

The plant material was collected at the University of Ibadan campus and identified at the Department of Botany herbarium.

Preparation of plant extract

The stem bark of *Pentaclethra macrophylla* was cleaned, chopped, air dried at room temperature (28-30°C) and milled into coarse powder using mortar and pestle. Extraction was carried out by cold maceration of 30 g each of the coarse powder with 500 ml of hexane, ethyl acetate, acetone methanol at ambient temperature (28-30°C) for 24 h and hot distilled water for 24 h. The resultant mixtures were filtered using Whatman filter paper (No.1) and the filtrates were concentrated to dryness at 40°C using rotary evaporator. Yield of the hexane extract (PMHE), ethyl acetate extract (PMEE), acetone extract (PMAE), methanol extract (PMME) and hot water extract (PMWE) were determined.

Phytochemical analysis

The phytochemical analyses of the powdered stem bark extractives were conducted to explore the secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids using standard procedures of Evans (2002); Pascaul *et al.* (2002) and thin layer chromatographic (TLC) analysis. The TLC plates were developed in a suitable solvent system and sprayed with various visualizing agents including Dragendoff's reagent, Bontrager's reagent, Kedde's reagent, Ferric chloride solution, vanillin/HCl reagent and 1% ethanolic Vanillin followed by 10% ethanolic sulphuric to detect the presence of alkaloids, anthraquinones, cardiac glycosides, tannins, triterpenes and flavonoids, respectively. The analyses were carried out in triplicate.

Thin layer chromatography of extracts

Several mobile phase mixtures were investigated in order to establish the most suitable solvent system for the thin layer chromatographic separation of the extracts individually.

In order to get a suitable solvent system that will give good separation for all three extracts on the same plate, the average polarity was determined using the average polarity index of all three individual mobile phase

systems. The mobile phase system thus applied consisted of ethyl acetate-chloroform-methanol (9:5:1). This mobile phase system separated components of all three extracts with optimum clarity. Average polarity needed to separate all three extracts was determined as follows: Polarity of ethyl acetate-methanol system = $\{(9 \times 4.4) + (1 \times 5.1)\} / 2 = 22.35$. Polarity of ethyl acetate-methanol-acetone system = $\{(9 \times 4.4) + (1 \times 5.1) + (2 \times 5.1)\} / 3 = 18.30$. Polarity of ethyl acetate-methanol-acetone-chloroform system = $\{(9 \times 4.4) + (1 \times 5.1) + (2 \times 5.1) + (12 \times 4.1)\} = 26.03$. Average polarity of all three systems = $(22.35 + 18.30 + 26.03) / 3 = 22.225$. In order to obtain a mobile phase system of an average polarity near 22.225, ethyl acetate, chloroform and methanol were mixed in the ratio 9:5:1 and the polarity of the mobile phase system was calculated thus $\{(9 \times 4.4) + (5 \times 4.1) + (1 \times 5.1)\} / 3 = 21.733$. The developed and dried TLC plates were viewed in day light, under UV and in iodine vapour tank. A general mobile phase system that separated PMAE, PMEE and PMME when spotted on the same plate was also developed. Mobile phase solvent mixtures investigated were as follows: PMAE, PMEE and PMME: chloroform-methanol (3:2); Chloroform-methanol (7:2); Hexane-chloroform-methanol (3:2:1); Methanol-ethyl acetate-acetic acid (3:2:1); Ethyl acetate-Chloroform-methanol (9:5:1). PMME: Ethyl acetate-methanol (9:1). PMAE: Ethyl acetate-acetone-methanol (9:2:1). PMEE: Chloroform-ethyl acetate-acetone-methanol (12:9:2:1). PMHE: Hexane and ethyl acetate (4:1).

The mobile phase solvent system that gave the best separation for PMME consisted of ethyl acetate and methanol (9:1). The mobile phase solvent system that gave the best separation for PMAE consisted of ethyl acetate-acetone-methanol (9:2:1). The mobile phase solvent system that gave the best separation for PMEE was chloroform-ethyl acetate-acetone-methanol (12:9:2:1). Hexane and ethyl acetate (4:1) gave the best separation for PMHE. The developed and dried plates were viewed in day light, under UV and in iodine vapour tank or sprayed with appropriate visualizing reagent.

High performance liquid chromatography-UV-DAD analysis.

The methanol extractive (PMME) was subjected to HPLC-UV-DAD analysis. The chromatographic system includes Shimadzu HPLC system consisting of Ultra-Fast LC-20AB prominence equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector (UV-DAD); column oven CTO-20AC, system controller CBM-20A Lite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, VP-ODS 5µm and dimensions (150 x 4.6 mm). The chromatographic conditions included mobile phase:

solvent A: 0.2% v/v formic acid (80%); solvent B: acetonitrile (20%); mode: isocratic; flow rate 0.6 ml/min; injection volume 10 µl of 40 mg/ml solution of PMME in methanol; detection UV 254 nm. Flavonoids and phenolic acid standards such as rutin, quercetin, caffeic acid, gallic acid and ferulic acid were employed for the identification of the phytoconstituents of the methanol extract by comparing the retention time under similar experimental conditions (Krishna and Manohar, 2014). The HPLC operating column oven temperature was 40°C and the total run time was 10 minutes.

3. Results and Discussion

The extraction was carried out by cold maceration of 30 g each of the coarse powder with 500

ml of hexane (PMHE), ethyl acetate, acetone, methanol for 24 h at ambient temperature (28-30°C) and hot water by decoction. The resultant mixtures were filtered using Whatman filter paper and the filtrates were concentrated to dryness *in vacuo* at 40°C using rotary evaporator. The extraction yielded dried hexane extract (PMHE), ethyl acetate extract (PMEE), acetone extract (PMAE), methanol extract (PMME) and hot water extract (PMWE) as shown in Table 1, to be 1.3%, 2.0% w/w, 2.6%, 2.9% and 3.8% w/w of respectively. Hot water extraction by decoction gave the highest extractive value of 3.8% w/w.

Table 1: Yields of *Pentaclethra macrophylla* stem bark extracts.

Extract	Yield (g)	Yield % (w/w)
PMHE	0.4	1.3
PMEE	0.6	2.0
PMAE	0.78	2.6
PMME	0.87	2.9
PMWE	1.14	3.8

Key: PMHE = Hexane extract; PMEE = Ethyl acetate extract; PMAE = Acetone extract; PMME = methanol extract; PMWE = Hot water extract by decoction

Table 2: Phytochemical analysis of *Pentaclethra macrophylla* stem bark extracts by colour reaction methods.

Metabolites	Name of Sample/ Inference					
	Herb	PMHE	PMEE	PMME	PMAE	PMWE
Carbohydrate	+++	-	-	-	-	+++
Alkaloids	++	-	-	++	-	++
Phenols	++	++	++	++	++	++
Flavonoids	+++	-	+++	+++	+++	++
Anthraquinones	-	-	-	-	-	-
Saponins	+++	-	-	+++	-	+++
Terpenes	+++	+++	+++	+++	+++	+++
Sterols	+++	+++	+++	+++	+++	+++
Phlobatannins	++	-	-	++	-	++
Cardiac glycosides	-	-	-	-	-	-
Tannins	+++	-	-	+++	-	+++

Key: +++: Significantly present; ++: Moderately present; +: Present; -: Absent

Herb = Powdered stem bark of *Pentaclethra macrophylla*

PMHE = Hexane extract; PMEE = Ethyl acetate extract; PMME = Methanol extract; PMAE = Acetone extract; PMWE = Hot water decoction.

The phytochemical analysis performed using various colour reaction methods showed the presence of tannins, phlobatannins, saponins, flavonoids, terpenes and sterols, alkaloids (Table 2). Presence of saponins in the stem bark of *Pentaclethra macrophylla* had been reported (Akaniro-Ejim *et al.*, 2016).

Several mobile phase mixtures were investigated in other to determine the most suitable solvent system for the thin layer chromatographic separation of the extracts individually. The mobile phase solvent systems that gave the best separation for PMME, PMAE

and PMEE consisted of ethyl acetate and methanol (9:1), ethyl acetate-acetone-methanol (9:2:1) and chloroform-ethyl acetate-acetone-methanol (12:9:2:1) respectively. Four spots were detected each for PMAE (Table 3), PMEE (Table 4) and PMEE (Table 5).

In all no component was visible in day light. The plate developed with mobile phase system

comprising ethyl acetate-chloroform-methanol (9:5:1) for PMME, PMEE and PMAE showed five spots when viewed in iodine vapour tank (Table 6). However, no spot was detected in daylight and under UV 254 nm. The observation that no spot was detected under UV 254 nm may be due to fluorescence quenching by chloride ions (Joseph, 2006).

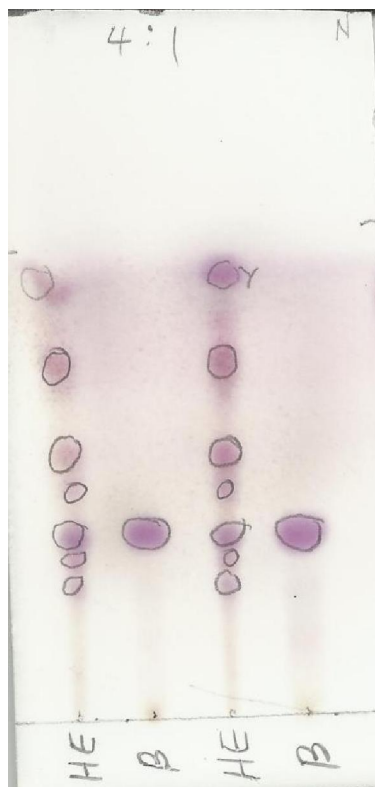


Fig 2: Thin layer chromatogram of hexane extractive (PMHE). Mobile phase: hexane and ethyl acetate (4:1). Spray reagent: 10% vanillin in sulphuric acid. β -Sitosterol reference was used as control gave R_f value of 0.38 (Table 2).

Table 2: Thin layer chromatography of hexane extractive (PMHE). Mobile phase comprised hexane and ethyl acetate (4:1).

R _f value	Colour under UV 254 nm	Colour with spray reagent*
0.2	Purple	Pink
0.35	Nil	Pink
0.38	Nil	Pink†
0.46	Lilac	Pink
0.54	Nil	Pink
0.72	Nil	Pink
0.91	Nil	Pink

*Spray reagent was 10% vanillin in concentrated sulphuric acid. †The constituent with R_f value of 0.38 corresponded to β -sitosterol.

Table 3: Thin layer chromatography of methanol extractive (PMME). Mobile phase comprised ethyl acetate and methanol (9:1).

R _f value	Colour under UV 254 nm	Colour in iodine vapour tank
0.6	Purple	Yellow
0.7	Nil	Yellow
0.77	Lilac	Yellow
0.94	Nil	Yellow

Table 4: Thin layer chromatography of acetone extractive (PMAE). Mobile phase comprised ethyl acetate, methanol and acetone (9:1:2).

R _f value	Colour under UV 254 nm	Colour in iodine vapour tank
0.36	Purple	Yellow
0.55	Nil	Yellow
0.74	Lilac	Yellow
0.94	Nil	Yellow

Table 5: Thin layer chromatography of ethyl acetate extractive (PMEE). Mobile phase comprised chloroform-ethyl acetate- acetone-methanol (12:9:2:1)

R _f value	Colour under UV 254 nm	Colour in iodine vapour tank
0.12	Purple	Yellow
0.25	Nil	Yellow
0.66	Nil	Yellow
0.94	Purple	Yellow

Table 6: Thin layer chromatography of PMME, PMEE and PMAE on the same TLC plate. Mobile phase consisted of ethyl acetate, chloroform and methanol (9:5:1)

Extract	R _f value	Colour under UV 254 nm	Colour in Iodine tank
PMME	0.11	Nil	Yellow
	0.33	Nil	Yellow
	0.54	Nil	Yellow
	0.61	Nil	Yellow
	0.74	Nil	Yellow
PMEE	0.11	Nil	Yellow
	0.33	Nil	Yellow
	0.54	Nil	Yellow
	0.61	Nil	Yellow
PMAE	0.74	Nil	Yellow
	0.11	Nil	Yellow
	0.33	Nil	Yellow
	0.54	Nil	Yellow
	0.61	Nil	Yellow
	0.74	Nil	Yellow

Key: PMEE = Ethyl acetate extract; PMME = Methanol extract; PMAE = Acetone extract

The HPLC mobile phase for PMME consisted of solvent A: 0.2% v/v formic acid in water (80%) and solvent B: acetonitrile (20%) isocratic. Three principal peaks designated CP347, CP387 and CP414 were eluted at 3.47, 3.87 and 4.14 minutes respectively (Fig 3). CP347 corresponded to gallic acid and CP414 corresponded to caffeic acid.

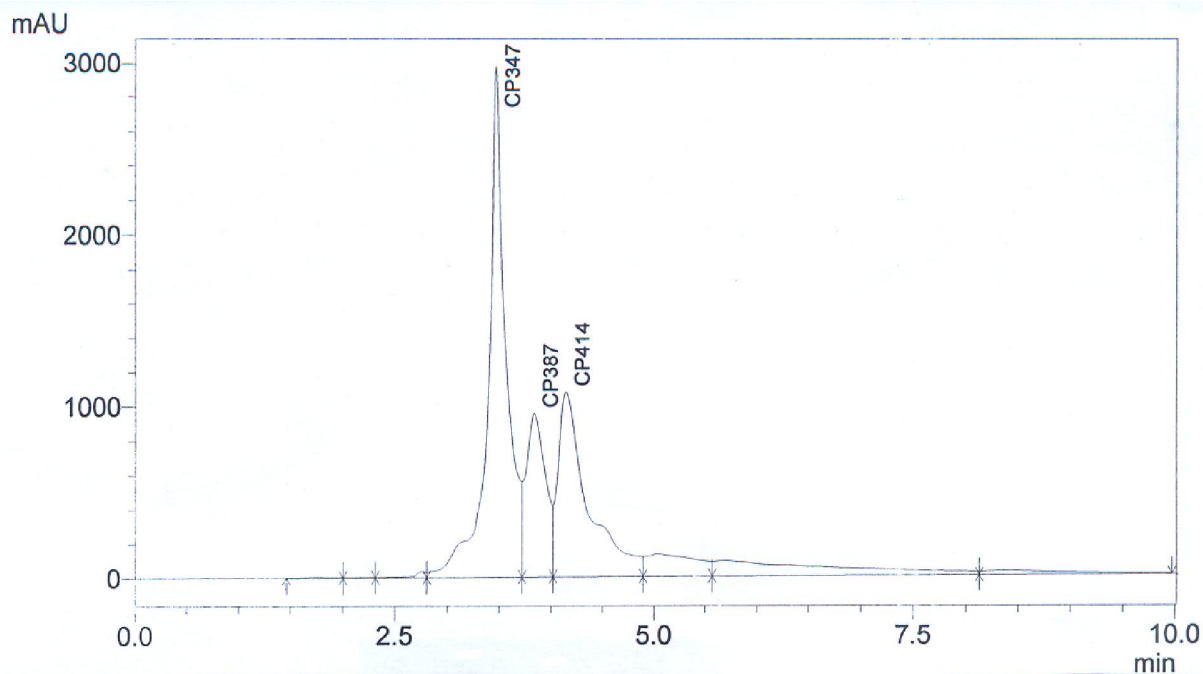


Fig 3: High Performance Liquid Chromatogram of methanol extract (PMME) of *Pentaclethra macrophylla* stem bark. CP347 and CP414 corresponded to gallic acid, and caffeic acid respectively.

The results of qualitative phytochemical analysis to identify the secondary metabolites present in the herb and extracts, revealed the presence of carbohydrates, tannins, flavonoids, saponins, phlobatannins, saponins, flavonoids, terpenes and sterols, alkaloids (Table 2). The TLC and HPLC chromatograms of *Pentaclethra macrophylla* stem bark extractives revealed the presence of β -sitosterol, gallic acid, and caffeic acid. *Pentaclethra macrophylla* stem bark is a renewable source of β -sitosterol, gallic acid and caffeic acid. These bioactive phytochemical compounds may be responsible for therapeutic effects of *Pentaclethra macrophylla* stem bark used as herbal remedy for inflammation, diabetes and debility (Sofowora, 2008; Evans, 2002). Ethanol extract of the stem bark had been reported to possess antimicrobial activity (Idonije *et al.*, 2011). The methanol extract and aqueous fraction of the stem bark exhibited antinociceptive activity (Okunrobo *et al.*, 2009). Antihyperglycemic and hypoglycemic effects of aqueous and hydroethanolic stem bark extracts of *Pentaclethra macrophylla* had been reported (Gilles *et al.*, 2008).

The leaves contain saponins, tannins, alkaloids, phenols, glycosides, cyanogenic glycosides. The stem bark contains tannins, alkaloids, glycosides,

cyanogenic glycosides, phenols, and saponins. Four compounds bergenin (Fig 1), vakerin, methyl gallate and ardisic acid had been isolated from the plant (Folefoc *et al.*, 2005; Nnennaya *et al.*, 2017). The fatty acid composition of the seed oil had been reported (Jones *et al.*, 1987). The alkaloids paucine and caffeoyl-putrescine (Fig. 1.) were isolated from the seed. The fermented seed have been reported to contain a wide range of aroma compounds (Nwokeleme and Ugwuanyi, 2015; Eziuche *et al.*, 2020).

Conclusion

Phytochemical evaluation of *Pentaclethra macrophylla* stem bark revealed it contained tannins, phlobatannins, flavonoids, terpenes, sterols, alkaloids and saponins. *Pentaclethra macrophylla* stem bark is a renewable source of bioavailable β -sitosterol, gallic acid and caffeic acid. A solvent system of average polarity index of 22.225 is recommended for the thin layer chromatography of *Pentaclethra macrophylla* stem bark extracts.

Conflict of Interest

The authors declare no conflict of interest.

Corresponding Author:

Dr. Samuel Ehiabhi Okhale
Department of Medicinal Plant Research and
Traditional Medicine, National Institute for
Pharmaceutical Research and Development,
Idu Industrial Area, P.M.B. 21 Garki, Abuja, Nigeria.
Tel: +2348036086812
Email: samuelokhale@gmail.com

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