J. Chem. Soc. Nigeria, Vol. 45, No.5, pp 835-843 [2020]

COMPARATIVE ASSESSMENT, PRELIMINARY SCREENING AND GC- FID PHYTOCHEMICAL ANALYSIS OF AQUEOUS AND PETROLEUM ETHER EXTRACTS OF *PHYLLANTHUS AMARUS* LEAVES.

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Received 02 July 2020; accepted 19 July 2020, published online 28 August 2020

Abstract:
The Gas Chromatography– Flame lonization Detector (GC-FID) method was employed in this study to evaluate the phytochemical constituents of both the aqueous and petroleum ether extracts of *phyllanthus amarus* leaves. Preliminary phytochemical screening showed the presence of Alkaloids, Flavonoids Glycosides, Phenols, Saponins, and Tannins. Further analysis revealed a total concentration of Phenol as 82.95mg/100g (4.46%) in the aqueous extract, Tannins 722.77mg/100g(38.82%),Alkaloids was 466.82mg/100(25.07%) while in the petroleum ether extract, Flavonoids was 472.59mg/100g (29.50%), Saponins 12.60mg/100g (0.79%) and Glycosides 18.63mg/100g (1.16%). These results are suggestive that *P.amarus* is endowed with phytochemicals that contains therapeutic potencies which support its use as traditional remedies for the treatment of variety of ailments.
Keywords*:* Gas Chromatography, Flame Ionization Detector, *Phyllanthusamarus,* Phytochemicals, Petroleum Ether Extracts.

Introduction

Medicinal plants are easily accessible and affordable resources available to over 80% of rural population in the developing world to treat and cure ailments [1]. Aside from their high consumption, natural products derived from plants origin play important role, such as source of pharmacological tools to enable the elucidation of biochemical pathways and drug etiology[2]. Researchers have yielded variety of compounds with potential properties useful for the development of synthetic drugs for disease treatment[3]. The therapeutic effects of these plants are due to the presence of secondary metabolites with bioactive components that are beneficial to human, apart from providing for the macro and micronutrients[4]. These secondary metabolites possess biological properties with antioxidant activities[5], antimicrobial effects [6,7] and the treatment of other chronic ailments [8,9]. These phytochemicals, function by detoxification and scavenging of free radicals that causes cancer, they also inhibit the activities of enzymes activated by carcinogens [10,11,12].

Phyllanthus Amarus is a herb that is widely found in most tropical and subtropical countries. The plant belongs to the Phyllanthaceae family with over 750 species. *P.amarus* had been used for the treatment of gastrointestinal disorders such as, diarrhoea, dysentery, stomach ache, ulcer, haemorrhoids, absence of menstruation or female fertility [13,14,15], liver treatment [16] and Ayurvedic formation [17,18]

Phytochemical screening is a test performed. to detect the presence of secondary metabolites in plants based on precipitation and formation of certain colours [19]. Gas Chromatographic – Flame lonization Detector(GC-FID) analysis of plant phytochemicals is a modern method employed to isolate and identify bioactive compounds. Therefore, this study is aimed to compare the constituents extracted by aqueous and petroleum ether solvents of *phyllanthusamarus* leaves.

Materials and Methods

Collection and Preparation of Plants Material

Fresh leaves of *phyllanthusamarus* were obtained from a bush farm in Kaiama town in Kolokuma/Opokuma Local Government Area of Bayelsa State, Nigeria. The leaves were thoroughly washed to remove extraneous matters and were sun dried, then pulverized with a blender.

Preparation of aqueous and petroleum ether extracts.

100gm of the powdered leaves were boiled in 500ml of distilled water for 15minutes. The decoction was taken and allowed to cool at room temperature. It was then filtered twice

using Whatman No1 filter paper. The filtrate was evaporated to dryness.

For the petroleum ether extract, the powdered leaves were soaked in 500 ml petroleum ether using Soxhlet extractor for 72 hours. The filtrate was concentrated to dryness in a rotary extractor under reduced pressure at a temperature of 65 ^{o}C.

Phytochemical screening
The qualitative phytochemical screening was performed using standard methods [20,21] on sample of both aqueous and petroleum ether extracts of *P. amarus*

Test for Tannins
3ml of 10% alcoholic $FeCl_3$ solution was added to a solution of the extract. A dark blue coloration was observed.

Test for Saponins
5ml of the extract was added with 5ml distilled water and vigorously shaken; to obtain a froth. 3ml of olive oil was then mixed with the froth, to obtain an emulsion mixture.

Test for Flavonoids
1% dilute Ammonia solution (NH_4OH) was added to a portion of the extract sample, followed by a few drops of concentrated H_2SO_4 down the side of the test tube. A yellow coloration of the mixture was observed.

Test for Glycosides
2ml of Acetic acid added with $FeCl_3$solution was mixed with a few drops of the extract. 1ml concentrated H_2SO_4 was carefully added to the mixture. No visible change in colour was observed.

Test for Alkaloids
2gm of KI and 1.27 gm of Iodine was mixed with 100ml distilled water. 2ml of Wagner's reagent was then added to some quantity of the extract and stirred. A reddish-brown precipitate was observed.

Test for Phenols
A portion of the extract sample was mixed with 5% $FeCl_3$. A greenish coloration was observed.

GasChromatographic–Flame lionization Detector(GC-FID) Phytochemical Analysis

Phytochemical Extraction
2gm of powdered *P. amarus*was mixed with 15ml of ethanol and 10ml of 50%w/v KOH. The mixture was boiled in a water bath for 1 hour at 60^{o}C. The mixture was then transferred into another tube using a separation funnel, and thoroughly rinsed with 20ml ethanol, 10ml of hot water and 3ml of hexane. The extract in the test tube was washed thrice with 10ml of 10% v/v ethanol solution. The extract solutionwas then dried with anhydrous Sodium Sulphate. The resulting residue was dissolved in 100µL of pyridine out of which 20µL of the solution was transferred into a vial in the gasechromatographic machine for analysis [(22)].

Quantitative Analysis
The GC-FID quantitative analysis was performed using BUCK M910 Gas Chromatograph (GC) (BUCK scientific, USA).The apparatus is equipped with a flame Ionization detector (FID), a RESTEK 15metre MXT-I column (15m X 250µm X 0.15µm). The temperature of the injector was maintained at 280^{o}C with a 2µL sample size Spitless Injector at a linear velocity of 30cmper sec. The carrier gas was Helium (5.0pa) with a flow rate of 40mL/min. the oven was initially operated at 200^{o}C and gradually increased at a rate of 3^{o}C per min to be maintained at 320^{o}C.

Results
Results of the qualitative phytochemical components of the aqueous and petroleum ether extracts of *Phyllanthus Amarus* leaves indicated the presence of Alkaloids, Tannins, Saponins, Flavonoids, Glycosides and Phenols as shown in Table 1, 2 and 3.

Table 1: Results of the preliminary phytochemical screening of aqueous and petroleum ether extracts of *Pyllanthus amarus*.

Phytochemical	Aqueous Extract	Petroleum Ether Extract
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Glycosides	+	+
Phenols	+	+

+= present.

Table 2: Percentage compositions of Phytochemicals in Petroleum ether extracts and Aqueous extract

PAAq mg/100g		**%**	**PAPEE mg/100g**	**%**
Phenols	82.95	4.46	69.91	4.36
Tannins	722.77	38.82	602.28	37.59
Alkaloids	466.82	25.07	426.16	26.60
Glycoside	27.35	1.47	18.63	1.16
Saponins	50.69	2.72	12.60	0.78
Flavonoid	511.21	27.46	472.59.	29.50
Total	**1861.79**	**100**	**1602.05**	**100**

Table 3: **Concentration in mg/100g of selected components in Aqueous and Pet. Eth. Extracts**

Flavonoids	Components	Catechin	Astragin	Kaemferol	Rutin
	Aq Ex	2.61	103.32	1.27	173.71
	Pet Ex	1.24	100.89	4.68	157.31
Alkaloid	Components	Phyllatene	Phyllantindine	Norsecurinine	
	Aq Ex	229.61	133.57	22.80	
	Pet Ex	257.37	142.12	22.99	
Tannins	Components	Tannic acid	Amarinic acid	Phylathusin – D	Corilagin
	Aq Ex	107.77	98.69	170.70	84.76
	Pet Ex	124.72	69.12	149.59	74.68

The Gas Chromatograhpy- Flame Ionization Detector (GC-FID) results are as presented in Figures la (Alkaloid Aq Ex), 1b (Alkaloid Pet eth ext), 2a (Tannins Aq Ex), 2b (Tannins Pet eth ext), 3a (Saponins Aq Ex), 3b (Saponins Pet eth ex), 4a (Flavonoid Aq Ex), 4b (Flavonoid Pet eth ex), 5a (Glycoside Aq Ex), 5b (Glycoside Pet eth ex), 6a (Phenol Aq Ex), 6b (Phenol Pet eth ex) respectively.

The analysis carried out showed high concentration of Tannins 722.77mg/100g (38.82%), Flavonoids 511.211.21 mg/100 (27.46%) Alkaloids 466 466.82 (25.07%), Phenols 82.95 mg/100g (2.72%) and Glycosides 27.35mg/100g (1.47%) in the aqueous extract. The petroleum ether extract revealed the highest concentration of Tannins 602.28(Flavonoids 472.59mg/100g (29.50%, Alkaloids 426.16mg/100g (26.60%) Phenols 69.91mg/100g (4.36%), Glycoside 18.63mg/100g (1.16%) and Saponins 12.60gm/100g (0.79%).

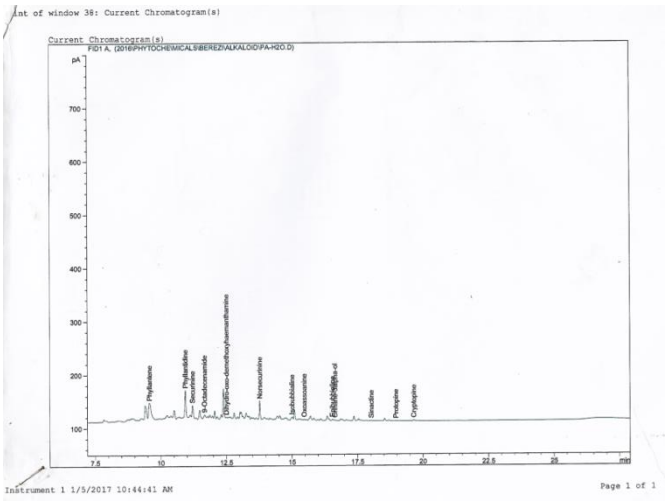


Fig 1A : Spectra of Aqueous extract of alkaloids

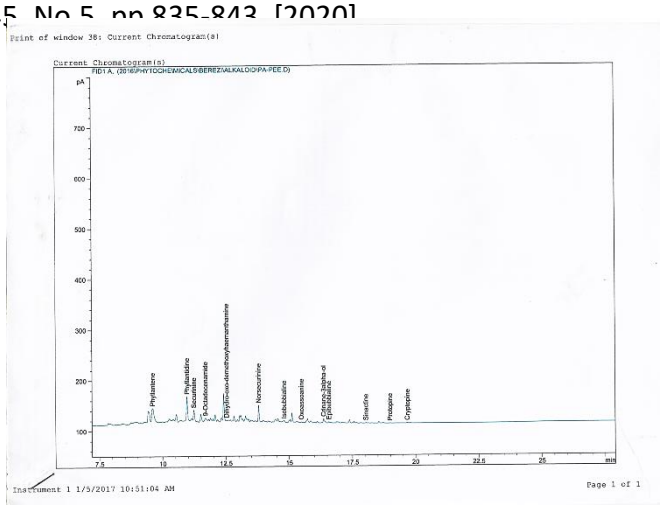


Fig 1B: Spectra of Pet. Ether extract of alkaloids.

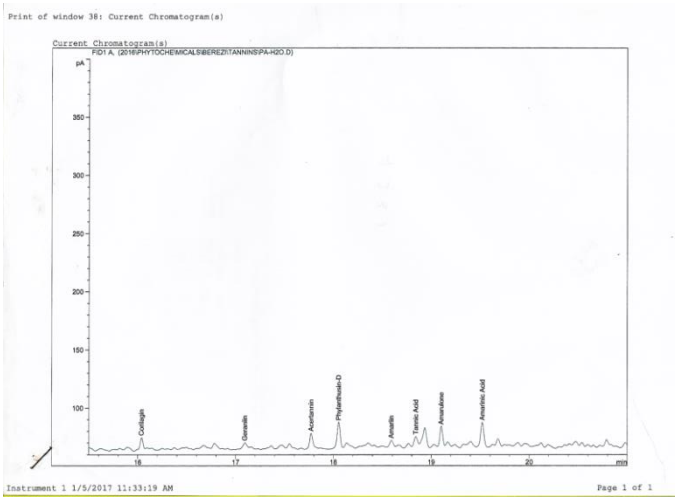


Fig 2A: Spectra of Aqueous extract of Tannins

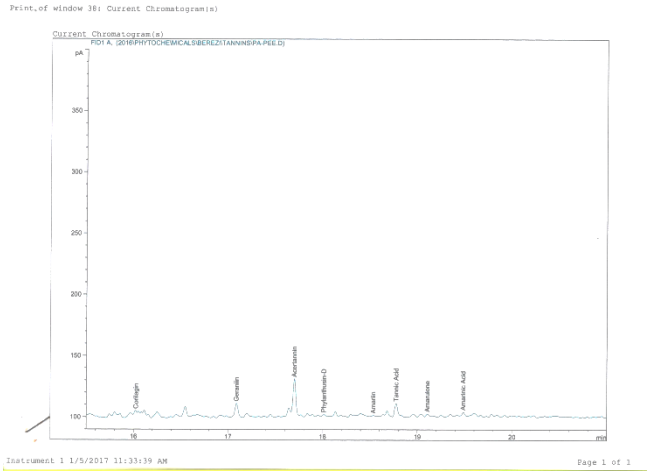


Fig 2B: Spectra of Pet. Ether extract of Tannins

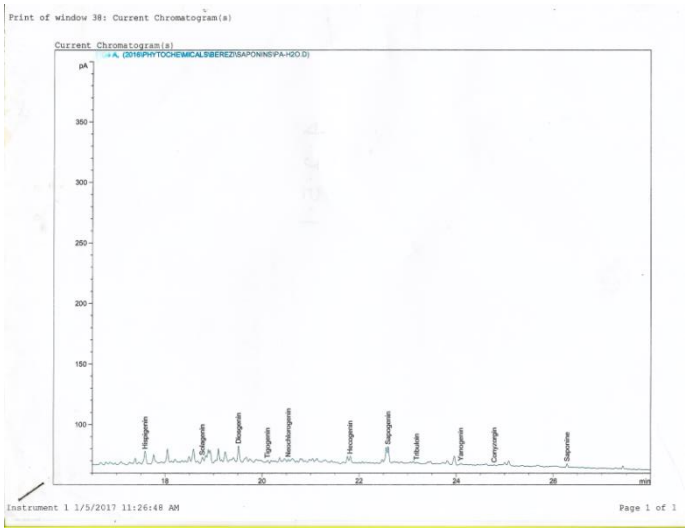


Fig 3A: Spectra of Aqueous extract of Saponins

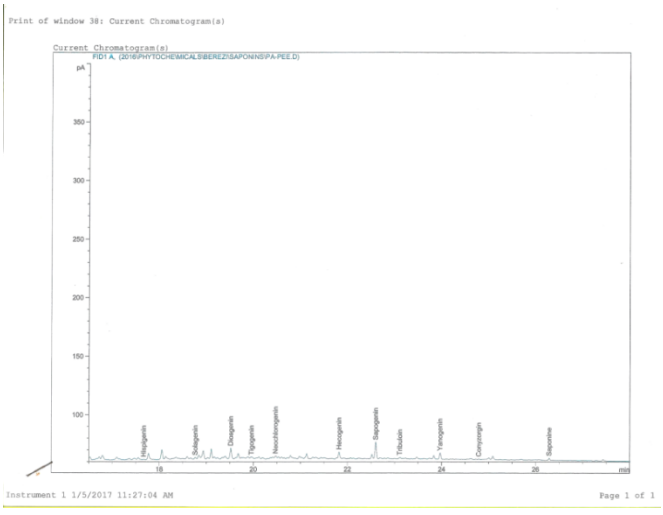


Fig 3B: Spectra of Pet. Ether extract of Saponins

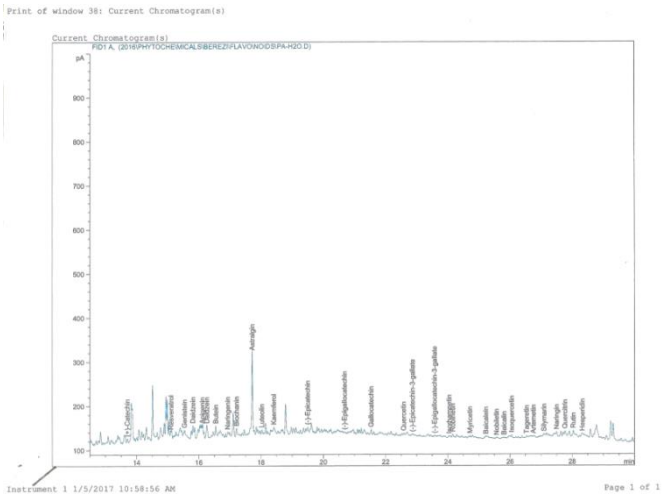


Fig 4A: Spectra of Aqueous extract of Flavonoids

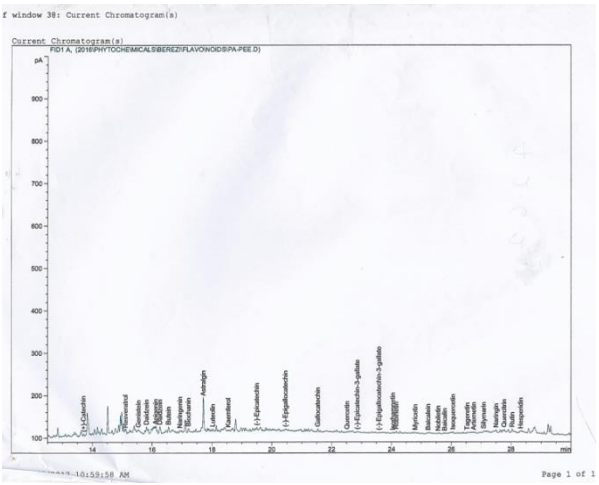


Fig 4B: Spectra of Pet. Ether extract of Flavonoids

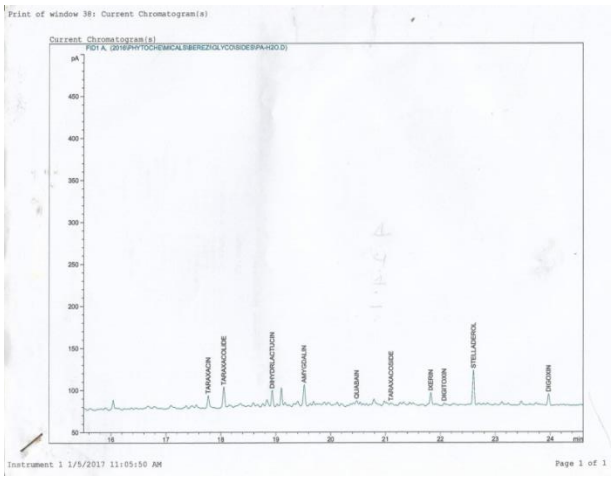


Fig 5A: Spectra of Aqueous extract of Glycosides

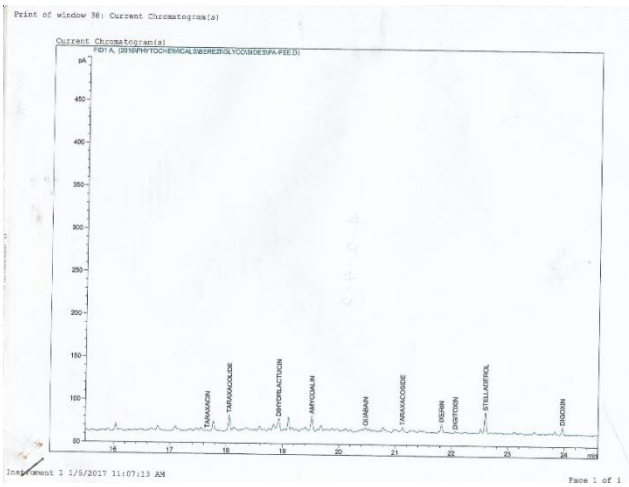


Fig 5B: Spectra of Pet Ether extract of Glycosides

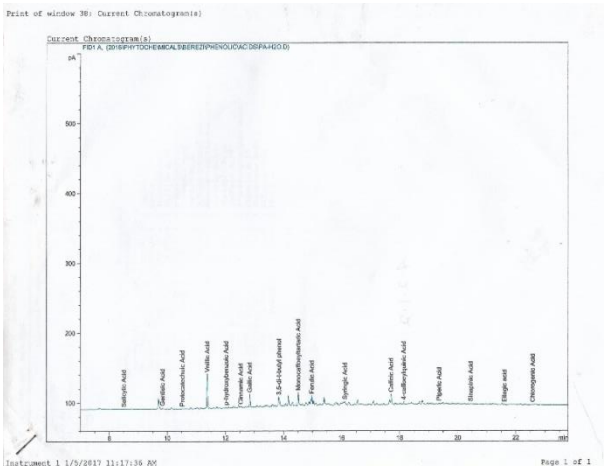


Fig 6A: Spectra of Aqueous extract of Phenols

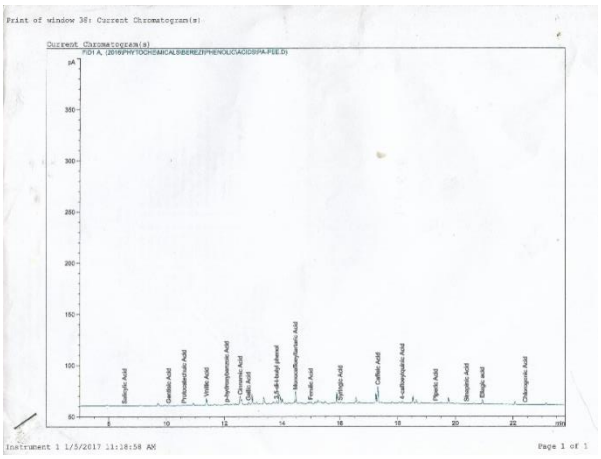


Fig 6B: Spectra of Pet. Ether extract of Phenols

Discussion

The presence of bioactive compounds in plants for medicinal purpose had been variouslyreported[23]. Aqueous and petroleum ether leave extracts of *Phyllanthus amarus*was found to contain Flavonoids such as catechin, astralgin, rutin, kaemferol etc. in different concentrations (Figures 4a & 4b). Flavonoids had been reported to act as antioxidants and free-radical scavenger[24]. Kaemferols effect as an anti-cancer agent is under consideration [25], Rutin in Flavonoid had been reported to be a major contributor in its anti-microbial and anti-cancer property [26, 27]. The high concentration of rutin in this extracts is in support of report from Shitlana *et al,* (2016) of its anti-malaria activity.Phyllantene, Phyllantidine, Norsecurininc are some of the pharmacologically active components of alkaloid found in the leave extracts of *Phyllanthus amarus*. Alkaloids have been reported to exert biological activities such as anti-malaria, anti-inflammatory and anti-microbial [28,29] functions. Alkaloids have been used as pain killers (morphine) muscle relaxers (cocaine), Tranquilizers (curare), stimulants (caffine) [30]. The higher concentrations of the alkaloid (Figs.1a&1b) in the petroleum ether extract indicate that this is a better extracting solvent for alkaloids. Tannic acid was identified in *P.amarus*leaves (Figs.2a & 2b) in considerable concentrations. The somewhat bitter taste of *P.amarus* leaves can be attributed to the presence of tannins, as they are known to be astringent naturally. Tannic acid is reported to possess anti-hypertensive, anti-diarrheal, anti-diabetic, anti-inflamatory [31] and hepatoprotective [32] properties.

Saponins (Figs.3a&3b) are widely distributed in plants,their biological importance include anti-oxidant activity anti-microbials anti-inflamtory, anti-helminitic and hemolytic properties [33] coupled to their insecticidal activity which have been widely employed in pharmaceutical, food and cosmetic industries.It hasalso been reported to have play an important role in soil remediation [34].

Phenol and most phenolic derivatives (Figs.6a & 6b)have been found to possess analgesic, anti-inflammatory, anti-microbial, anti-oxidant, anti-convulsant, anti-cancer and anti-septic activities [35 36].

Conclusion

The main objective of this article is to compare with the use of GC-FID, the phytochemical constituents of aqueous and petroleum ether leave extracts of *Phyllanthus amarus*. Data obtained from the study showed the presence and high concentration of some vital secondary metabolites such as; Alkaloids, Tannins, Saponins, Flavonoids, Phenols and Glycosides in the leave extracts of *P.amarus*. It can therefore, be inferred that *Phyllanthus amarus* possess significant pharmaceutical andtherapeutical potentials for the benefit of mankind.

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