

Comparative determination of total antioxidant effects of ethanol extract of *Phyllanthus amarus* leaves

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Abstract. Yakubu OE, Abu MS, Akighir J, Onuche JI, Arabi A. 2021. Comparative determination of total antioxidant effects of ethanol extract of *Phyllanthus amarus* leaves. *Asian J Nat Prod Biochem* 19: 81-85. Some medicinal and therapeutic potency have been reported of *Phyllanthus* in traditional settings. Hence, the current study evaluated the total antioxidant potential, phenolics, and flavonoids of fractions of ethanol extract of *Phyllanthus amarus* leave obtained using column chromatography. Extraction was done using absolute ethanol; elution was performed using different organic solvents of varying polarities, from n-Hexane, Chloroform, Ethylacetate, Ethanol, Methanol, and finally, water. The results obtained from the study revealed that fraction 10b (83 mg/mL) has the highest concentration of total Antioxidant Capacity (TAC), the next being 1a, 5a, and 11b (48mg/mL). The reduction in the TAC of the fractions was in the order; of 3b>7a>6a>5b>4a> with F7b (13mg/mL) having the lowest TAC. The result for Total phenolic Content (TPC) shows that fraction 10b (430 mg/mL) has the highest concentration of TPC, just like in antioxidant activity, followed by F4b (428 mg/mL). Fraction 2a (9 mg/mL) has the lowest concentration of TPC. Total Flavonoid Content (TFC) showed that fraction 3b (201 mg/mL) has the most concentration of total flavonoids, followed by 4b (120mg/mL). Meanwhile, fractions 1a, 2b, and 9a have the same TFC. While fraction 2a showed the lowest flavonoid concentration (25mg/mL). The plant extract fractions were found to contain more both phenolics and flavonoids, which may have contributed to the antioxidant activity observed.

Keywords: Antioxidant, ethanol extract, flavonoids, phenolic, *Phyllanthus amarus*

INTRODUCTION

Phyllanthus amarus is of the *Euphorbiaceae* family, while the genus is *Phyllanthus*. The species is in *amarus* and can be readily found in tropical and subtropical countries of the world (Trease and Evans 2001). It is known in Nigeria among Yoruba as “eyin olobe,” Hausa as “geerontsuntsaaayee,” and Igbo as “Ite knwonwa nazu,” and in English as leaf flower or stone breaker. The plant is usually prepared as an herbal mixture and used by Nigerians for health purposes and is considered to have great economic importance (Umoh *et al.* 2013). Previously, the whole-plant extract has been used for the treatment of urinary disorders, liver disease, dyspepsia, anorexia, constipation, and dysentery (Samy *et al.* 2008), while many female anomalies like leucorrhoea, menorrhagia, and mammary abscess have been managed by this plant exudate (Samuel and Andrews, 2010). Again, fresh leaf paste prepared from *phyllanthus amarus* has the potential to alleviate white spots on the skin, manage diabetes, and reduce the incidence of jaundice (Ignacimuthu *et al.* 2008). Similarly, malaria has been successfully managed by *phyllanthus amarus* whole plant extract (Kuppusamy and Murugan 2010).

Methanolic extract of *Phyllanthus amarus* was found to have an antioxidant effect that inhibited lipid oxidation. It masked up reactive oxygen species *in vitro* (Meena *et al.* 2018), while ethanolic extract of *Phyllanthus amarus* hairy

roots showed antiproliferative activity against apoptosis initiated by increased intracellular reactive oxygen species (Abhyankar *et al.* 2010). The effect of nor-securinine, an alkaloid extracted from *Phyllanthus amarus*, was examined against spore growth of some fungi as well as powdery pea mildew (*E. pisi*) and was found to be potent (Meena *et al.*, 2018). *Phyllanthus amarus* ethanol, aqueous, and hexane extracts showed restriction of LPS-induced production of NO and PGE2 and decreased the LPS-induced secretion of Tumor necrosis (Kiemer *et al.* 2003). The combination of *Phyllanthus amarus* and *Andrographis paniculata* plant extracts demonstrated active snake anti-venom potential that could be useful against snakebites (Meena *et al.* 2018). Similarly, Sornakumar *et al.* (2014) reported that about 0.24 mg of di-herbal plant extracts comprising *phyllanthus amarus* effectively neutralized the cobra venom-induced toxicity.

Certainly, *Phyllanthus amarus* has been reported to have medicinal and therapeutic potency in traditional settings. These medicinal properties are mostly implicated in the presence of phytochemicals which are believed to exert some specific pharmacological effects on the body. Hence, the current study evaluated the total antioxidant capacity, phenolics, and flavonoids of GC-MS partially fractionated ethanol extract of *phyllanthus amarus* leaves.

MATERIALS AND METHOD

Plant material

Fresh, healthy-looking leaves of *Phyllanthus amarus* were collected within the premises of Federal University Wukari, Taraba State. *Phyllanthus amarus* was identified and authenticated by Dr. Yakubu J.O.E, a Pharmacologist in the Department of Biochemistry, Faculty of Pure and Applied Science Federal University Wukari, Taraba State. The leaves were air-dried in the laboratory at 25°C for one week, pulverized manually using laboratory mortar and pestle into a smooth particle, and stored in an air-tight container for further use.

Solvent Extraction

About 500 g of the pulverized plant leaves were soaked in 1.3 L of 97 % ethanol for 48 hours at 25°C with periodic shaking to ensure maximum extract yield, according to Yakubu et al. (2014). The crude extract was filtered using clean white sieving mesh and Whatman number 1 filter paper. The filtrate was evaporated to dryness with a rotary evaporator at low temperature under reduced pressure to obtain green crude extract.

Fractionation of Ethanol extract by Column Chromatography

The ethanolic green crude extract was subjected to column chromatography to separate the extract into its component fractions. Silica gel was used as the stationary phase, while different solvents of increasing polarity were used as the mobile phase. According, to Yakubu et al. (2014), the bottom part of the glass column was packed with glass wool with the help of a glass rod. Exactly 235 g of silica gel of mesh size 60-200 was dissolved in 225 mL absolute n-Hexane to make the slurry (activating silica gel). The chromatographic column (30 mm diameter by 40 mm height) was packed with silica gel, and the solvent was allowed to flow into a conical flask below freely. At the end of the packing process, all taps were locked, the setup was allowed to stand for 24 hours, and the clear solvent at the top of the silica gel was allowed to drain down the meniscus.

Elution

The Yakubu et al. (2014) method was adopted for the elution. Two grams of the ethanol extract of *Phyllanthus amarus* leaves was dissolved in 15 % absolute ethanol, and the solution was applied into the chromatography column (30 mm in diameter and 400 mm in height), followed by elution of the extract using n-hexane, chloroform, ethyl acetate, ethanol, methanol, and finally distilled water. The following ratios (v/v) in mL of solvent combinations were frequently used in the elution process: n-Hexane: chloroform 100:0, 50:50; Chloroform: ethylacetate 100:0, 50:50; Ethylacetate: ethanol 100:0, 50:50; Ethanol: methanol 100:0 50:50; Methanol: distilled water 100:0 50:50; Distilled water:100. A measured volume (200 mL) of each solvent combination was poured into the column using a separator funnel. The eluted fractions were collected in aliquots of 100 mL in conical flasks.

Determination of total antioxidant capacity (TAC) (Singleton et al. 1999)

The absorbance was measured in triplicate for each fraction. Total antioxidant capacity (TAC) was calculated as mg/mL of Trolox equivalent (TE) using the regression equation from the calibration curve. Exactly 0.05g of the extract was dissolved in 250 mL of methanol to prepare 0.2mm. One mL of methanol extract was added to 2mL of DPPH in each test tube, followed by 100 µL of the fraction to each test tube. The mixture was shaken vigorously and left in the dark at room temperature for 30minutes. The absorbance of the solution was measured immediately at 517 nm in a UV-Visible spectrophotometer. The experiment was performed in duplicate.

Determination of total flavonoids content (TFC) (chang et al. 2002)

Quercetin standard was used for derivation of the calibration curve. The total flavonoids were express as mg/mL quercetin equivalent (QE). A 10% aluminium chloride solution was prepared by dissolving 10 g of aluminium chloride (ALCL₃) in 100mL ethanol. Exactly 100 µL of diluted sample was added to the test tube containing 1.5 mL methanol followed by 100 µL of aluminium chloride (ALCL₃) and 100 µL potassium acetate solutions. The mixture was incubated at room temperature for 30minutes. The absorbance of the reaction mixture was measured at 765 nm wavelength using a UV-Visible spectrophotometer using distilled water as the blank.

Determination of total phenolic content (TPC) (Yakuku et al. 2014)

The folin ciocalteu reagent was diluted (1:10 using distilled water). A volume of 100 µL of the crude sample was added to each test tube, and 2.5mL of Folin ciocalteu reagent was added to each test tube, followed by 2mL of Na₂CO₃ solution. The solution was incubated for 15minutes at 45°C. The absorbance was measured at 415 nm in a UV-Visible spectrophotometer using distilled water as the blank.

RESULTS AND DISCUSSION

Total antioxidant capacity (TAC)

Total Antioxidant Capacity (TAC)

Figure 1 shows the total antioxidant capacity (TAC) of fractions obtained from ethanolic extract of *Phyllanthus amarus* leaves. Fraction 10b (83 mg/mL) has the highest concentration of total antioxidant capacity, followed by F1a, F5a, and 11b, which all have similar TAC values (48 mg/mL). Other fractions, F4b, F9b, F10a are also of the same value (44 mg/mL) while fractions, 1b, 2a, 2b, 3a, 6a, 8b, and 9a (23 mg/mL) possess the same TAC. The decrease observed in the TAC of the fractions is in the order of F3b>F7a>F6a>F5b>F4a>, with F7b (13mg/mL) showing the lowest total antioxidant capacity.

Total Phenolic Content (TPC)

The result for total phenolic content of ethanol extract of *Phyllanthus amarus* leaves shows that fraction 10b (430 mg/mL) has the highest concentration of total phenolic content as it was in the case of antioxidant activity. This was followed by F4b (428 mg/mL), while fraction 2a (9 mg/mL) has the lowest concentration of TPC, as shown in figure 2.

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of ethanol extract of *Phyllanthus amarus* leaves revealed that fraction 3b (201 mg/mL) possesses the highest concentration of total flavonoid content, as depicted in Figure 3, followed by 4b (120 mg/mL). Fractions 1a, 2b, and 9a have the same TFC. The decrease observed in the TFC of the fractions is in the order of 8b>5a>5b>7a>11a>7b. Meanwhile, F2a has the lowest concentration (25 mg/mL).

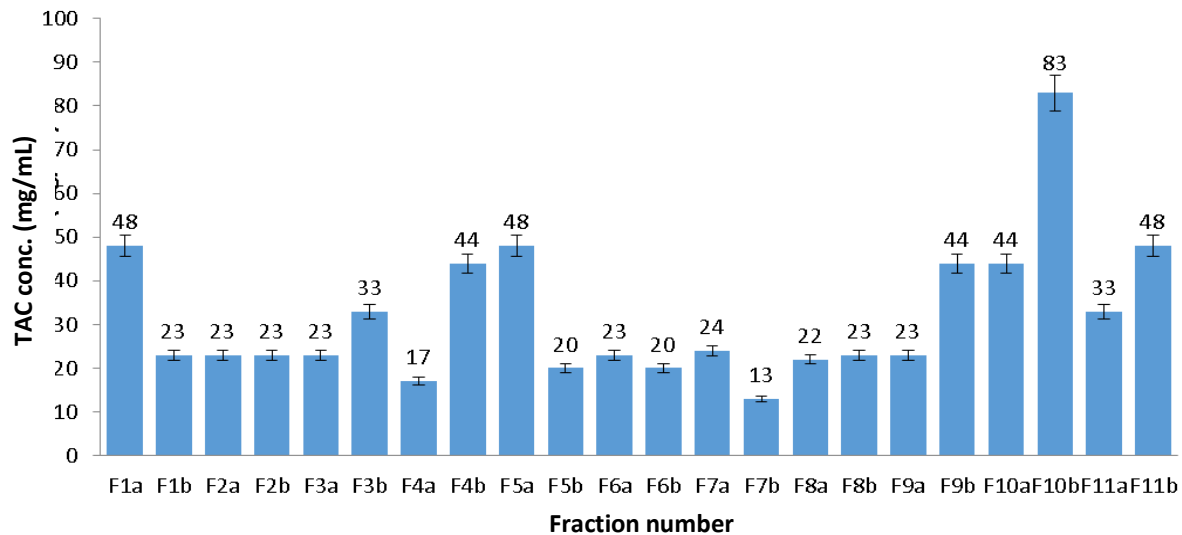


Figure 1. Total antioxidant capacity (TAC). F1: n-Hexane: (100:0), F2: n-Hexane/chloroform: (50:50), F3: Chloroform: (100:0), F4: Chloroform/ethyl acetate (50:50), F5: ethyl acetate: (100:0), F6: Ethyl acetate/ethanol (50:50), F7: Ethanol (100:0), F8: Ethanol/ Methanol: (50:50), F9: Methanol: (100:0), F10: Methanol/water (50:50), F11: Distilled water: (100:0)

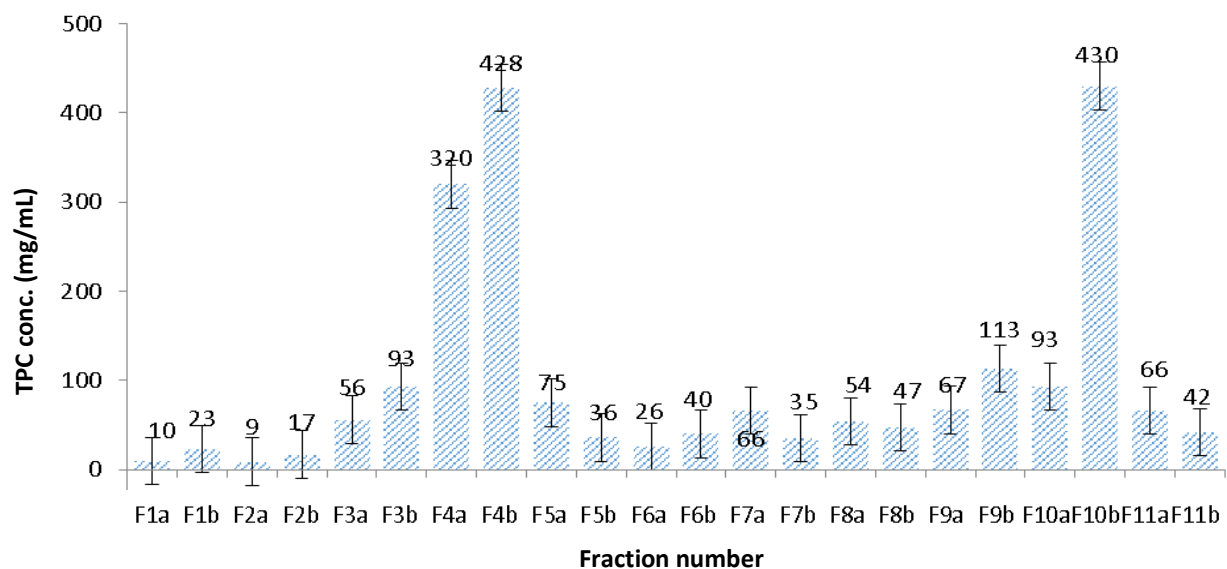


Figure 2. Total phenolic content (TPC). F1: n-Hexane: (100:0), F2: n-Hexane/chloroform: (50:50), F3: Chloroform: (100:0), F4: Chloroform/ethyl acetate (50:50), F5: Ethyl acetate: (100:0), F6=Ethyl acetate/ethanol (50:50), F7=Ethanol (100:0), F 8: Ethanol/ Methanol: (50:50), F9: Methanol: (100:0), F10: Methanol/water (50:50), F11: Distilled water: (100:0)

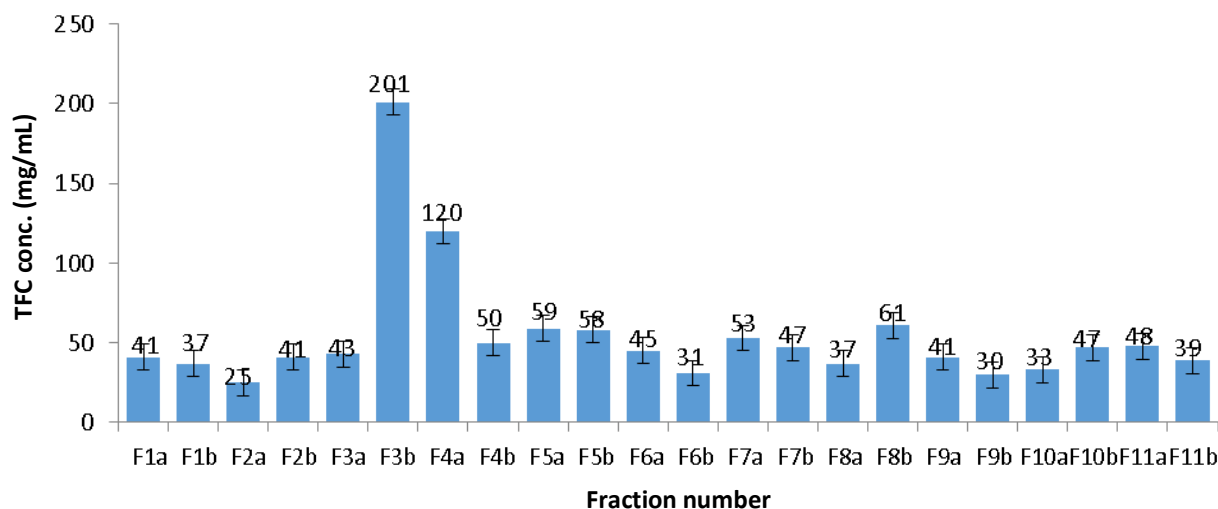


Figure 3. Total flavonoid content (TFC). F1: n-Hexane: (100:0), F2: n-Hexane/chloroform: (50:50), F3: Chloroform: (100:0), F4: Chloroform/ethyl acetate (50:50), F5: Ethyl acetate: (100:0), F6: Ethyl acetate/ethanol (50:50), F7: Ethanol (100:0), F8: Ethanol/Methanol: (50:50), F9: Methanol: (100:0), F10: Methanol/water (50:50), F11: Distilled water: (100:0)

Discussion

Phyllanthus amarus extract has attracted several researchers over the decades because of its potent pharmaceutical uses. Every country has its traditional use of *Phyllanthus amarus*, but the way of curing disease is almost common everywhere (Meena et al. 2018). Antioxidant compounds usually deactivate free radicals and inhibit the process of lipid peroxidation, which is one of the major reasons for the deterioration of food and biological cells (Halliwell, 2010).

In this study, a considerable high level of antioxidant activity was observed in the ethanol extract of *Phyllanthus amarus* leaves. The TAC revealed that fractions 10b (84 mg/mL), 1a, and 5a (48 mg/mL), possessed higher antioxidant capacity, implying that the fractions have higher antioxidant values when compared with the other fractions. This observation was substantiated by the positive correlation that was seen between the TAC and the concentration of phenolic compounds present in the fractions; since it has been demonstrated by several studies that the higher the phenolic content, the higher the TAC. The result also indicated that methanol-water (50:50) is the most efficient solvent for elution of antioxidant activity of ethanolic extract of *Phyllanthus amarus* leaves, as reported by Yakubu et al. (2018). However, the low antioxidant capacity observed in fractions 4a (17mg/mL) and 7b (13mg/mL) could be attributed to the fact that antioxidant activity depends on the type, polarity of the extracting solvent, and purity of active compounds (Demiray et al. 2009).

Plants have diverse phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids, which have radical scavenging power (Olamide et al., 2017). The result obtained for the TPC shows that fraction 10b (430 mg/mL) has the highest concentration of total phenolic content. Yakubu et al. (2019b) stated that phenolic compounds are plants' most effective and abundant natural

antioxidants. They are very important plant constituents because their hydroxyl groups confer scavenging ability (Motawi et al., 2011). Thus, it can be inferred with certainty that these phenolics are responsible for the marked antioxidant activity of these fractions, which is compatible with several studies reported on the relationships between phenolic content and antioxidant activity (Yakubu et al., 2018).

In conclusion, different methods employed in the study revealed that the ethanolic extract of *Phyllanthus amarus* leaves is rich in phenolic and flavonoid compounds, demonstrated good antioxidant activity, and can be useful in masking up free radicals as assayed through various *in vitro* models in this study. Thus, preventing free-radical related disorders respectively. There appears to be no correlation between antioxidant activity and flavonoid content, implying that the plant extract contains several phytochemicals other than flavonoids which may contribute to the antioxidant capacity. Hence, supporting the use of this plant as a good source of natural antioxidants should be encouraged.

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