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Stembark Methanol Extract of *P. americana*: Total Antioxidant Capacity, Total Flavonoids Content, Total Phenolics Content and Its Flavonoids, Vitamins and Amino Acids Profile

Ojochenemi E. Yakubu[1], Michael S. Abu[2], Janya Danjuma[3], Isaac John Umaru[4], Gloria Omonefe Oladele[5]

[1,2,3,4]Federal University Wukari, Taraba State, Nigeria

[5]Family Medicine Federal Medical Centre Bida Niger State, Nigeria

janyadanjuma2016@gmail.com

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Abstract

This research investigated the total antioxidant capacity, total flavonoids content, total phenolics content as well the flavonoids, vitamins and amino acids profile of stembark methanol extract of *P. americana*. Plant materials have been known be rich source of various medicinal active ingredients. These may include alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, and tannins as well as nutritional components including sugars, amino acids as well as proteins in addition to other vital components. The stembark of *P. americana* was harvested in its plant in Baissa, Taraba State, Nigeria. The plant material was air dried and pulverized and soaked in methanol for 72 hours before filtration. The filtrate was further concentrated using rotary evaporator and water bath. The concentrate was analyzed for total antioxidant capacity using DPPH, flavonoids content using aluminium chloride and phenolics content using folin ciocaltue. Flavonoids, vitamins and amin acids profile was determined using HPLC. The result total antioxidant capacity shows that inhibition increased with concentration with the 100µL/100mL had inhibition percentage of 87.88% whereas the lowest concentration of 31.25µL/100mLhad the inhibition percentage of 40.14%. The results for total flavonoid content and total phenolics content show significant levels with 42.21±0.23mg QE/100g and 33.65±3.02mg GAE/100g respectively. The

result for flavonoid and phenolics profile reveals that quercetin and caffeic acid phenyl ester were identified with 0.50% and 99.50% respectively. For vitamins, vitamin K was detected with 0.40%, vitamin B1 with 80.20% and vitamin B2 with 19.50% abundance. There seven different amino acids identified, these include asparagine, threonine, phenylalanine, aspartic acid, glutamic acid, leucine and arginine. The outcomes reveal that the plant material could possess important phytochemicals which could be used in disease treatment as well as vital macromolecules that can be used as supplements.

Keywords: Total antioxidant capacity, Total flavonoid content, Total phenolics content, Nutritional, Folin ciocaltue, Inhibition, Quercetin, Caffeic acid phenyl ester, Macromolecules, Supplements

INTRODUCTION

Bioactive compounds are a valuable resource for drug discovery and can be found in plants (Kumar *et al.*, 2018). The entire plant or some parts of it may have therapeutic activity. Pharmaceutical compositions using active ingredients sourced from plants are known as herbal drugs (Poreds, 2022). The whole plant or any part of it may be used to make the product. In addition, preparations derived from oils, gums, and other secretions of herbal plants are included in the field of herbal medicine (Doughari, 2012; Pandey and Tripathi, 2014; Azwanida, 2015).

Primary plant constituents are the principal nutrients found in plants, including proteins, amino acids, common sugars, and chlorophyll. These are not particularly therapeutic, if at all. Secondary metabolites, also referred to as alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, and tannins, are a group of plant constituents. Numerous biological and pharmacological processes are managed by these (Sofowora, 1980; Sasidharan *et al.*, 2011; Rungsung *et al.*, 2015).

One of the main sources of issues, especially in the food and health industries, is the free radical reaction. It is the source of numerous horrible illnesses, such as cancer (Salehi et al., 2020) and food oxidative rancidity (Velasco *et al.*, 2010). To reduce oxidative damages, the pharmaceutical and food sectors frequently use both natural and synthetic antioxidants (Lourenco *et al.*, 2010; Lobo *et al.*, 2015; Kumar *et al.*, 2019). Nevertheless, research has shown that synthetic antioxidants typically have negative side effects and may even be harmful (Xu *et al.*, 2021). Consequently, plant-based natural antioxidant substitutes are advised. Because they contain phytochemicals such terpenoids, flavonoids, phenolics, and alkaloids, a range of plant materials are naturally occurring sources of antioxidants (Salehi *et*

al., 2020). One potential source of novel and potent antioxidants is medicinal plants, particularly those with a long history of being used in folk medicine to treat oxidative stress and related conditions.

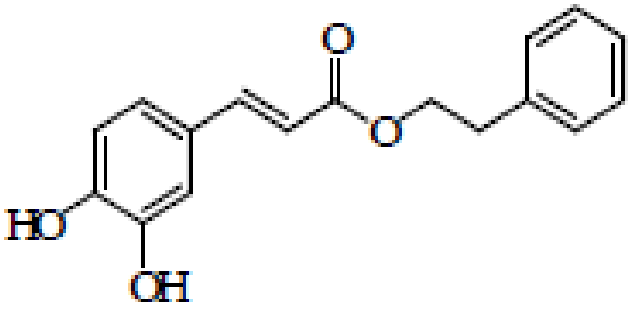
Persea americana is a native tree of the Lauraceae family, native to both Americans and Mexicans. It is commonly grown as evergreen leaves in tropical and subtropical regions of the world, with an average height of 8 to 9 meters (Lu *et al*., 2005; Obute and Adubor, 2007; Egwaoje *et al*., 2017). In Mexican and African traditional medicine, avocado seed dicots are a powerful treatment for conditions including diabetes, menstrual irregularities, and muscular aches (Adeboye *et al*., 1999; Adeyemi *et al*., 2002; Egwaoje *et al*., 2017). According to recent reports, avocados are not harmful to people (Egwaoje *et al*., 2017). However, several animals, including cats, dogs, calves, goats, birds, fish, and horses, are poisoned by its leaves, fruits, and skin (Ozolua *et al*., 2009). Alkaloids, tannins, flavonoids, triperpenes, carotenoids, phenols, cellulose, steroids, polyuronoids, beta-galactoside, and volatile oil are among the phytochemicals found in avocado seeds, leaves, and fruits (Kawagishi *et al*., 2001; Ding *et al*., 2007; Olaete *et al*., 2007). Nutrient-dense bursts found in avocados include dietary fiber, monounsaturated fatty acids, potassium, calcium, magnesium, iron, and vitamins B, E, and K (Naveh *et al*., 2007).

The plant extracts also contained flavonoids, which are super antioxidants and free radical scavengers that shield cells from oxidative damage and have anticancer properties. Among other things, flavonoids protect against cancer, bacteria, ulcers, viruses, allergies, inflammations, and platelet aggregation (Oboh *et al*., 2016). Plant extracts from *P. americana* may be used as antispasmodic, antifungal, and antibacterial drugs since they contain flavonoids. This explains why these herbs are used by the natives to cure microbiological infections, diarrhea, and spasmodic bronchitis (Umar *et al*., 2018). The presence of tannins in *P. americana* extracts may account for its widespread use in herbal medicine to treat burns to heal, hemorrhoids, ulcers, and frostbite injuries (Okigbo *et al*., 2019). Tannins are metal chelators that can react with macromolecules to form compounds. This process depletes the essential substrates, co-factors, and enzymes of microorganisms, leading to cell death. Tannins also have astringent properties that aid in the healing of wounds and irritated mucous membranes (Okigbo *et al*., 2019). The presence of saponins is strong evidence that *P. americana* has cytotoxic effects, such as intestinal permeabilization (Oboh *et al*., 2016). Saponins are thought to be accountable for the hemolytic properties of the cell, which permit harmful chemicals to enter and crucial components to exit (Raquel, 2017). Steroids

have been found to have antibacterial properties, and their interactions with sex hormones make them extremely significant substances (Rao and Sung, 2015; Okwu, 2017). Additionally, it has been shown that steroids bind in the digestive system, inhibiting the growth of cancer cells and lowering blood cholesterol levels (Nyarko and Addy, 2016).

Natural substances with antioxidant and anti-inflammatory qualities, caffeic acid (3,4-dihydroxycinnamic acid) and caffeic acid phenethyl ester (CAPE) are present in many plants, such as coffee, fruits, and vegetables (Murtaza *et al.*, 2014; Espíndola *et al.*, 2019). The bioactivities of anti-oxidation (Sud'ina *et al.*, 1993; Pascal *et al.*, 1994), anti-tumor (Lee *et al.*, 2000; Chen *et al.*, 2001), anti-inflammatory (Krol *et al.*, 1996; Michaluart *et al.*, 1999), immunological modulation (Park and Kahng, 1999; Zhang *et al.*, 2014), and other bioactivities were demonstrated to be significantly influenced by CAPE. Furthermore, according to Michaluart and Mirzoeva (Mirzoeva and Calder, 1996; Zhang *et al.*, 2014), CAPE may impede the synthesis of prostaglandin (PG) and leukotriene. The studies discovered that CAPE decreased the cytotoxicity induced by cadmium chloride ($CdCl_2$) through the upregulation of circulatory RNAs, which are involved in the activation of apoptosis and the inhibition of autophagy. Hepatoma carcinoma cell lines (HepG2 cells), which are frequently employed in toxicological investigations, benefit from these processes by lessening the harmful effects of cadmium (Hao *et al.*, 2020; Arzumanian, *et al.*, 2021; Hao *et al.*, 2021). According to Hao *et al.* (2021), this is corroborated by the discovery that CAPE treatment reduces oxidative stress and inflammation mediators such TNF-α, IL-6, and IL-1β by blocking the PI3K/Akt/mTOR pathway and apoptosis-induced indicators.

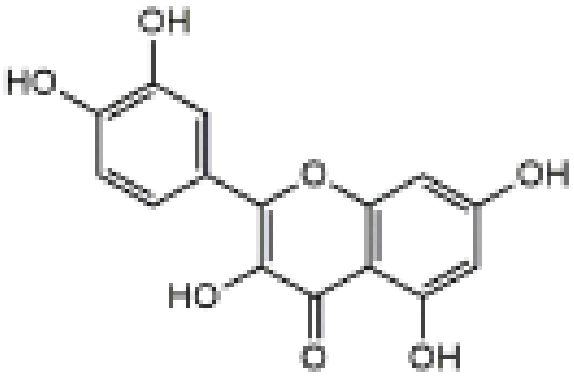
Moreover, the application of CAPE has demonstrated protective effects against CCl4-induced hepatotoxicity. In animal models of CCl4 toxicity, CAPE acts to lower the level of the Fas/FasL protein, which is responsible for inducing apoptosis, and diminishes the elimination effect of CCl4 on antioxidant enzymes like catalase, superoxide dismutase (SOD), and GST. (Kus *et al.*, 2004; Lee *et al.*, 2008) Additionally, there was evidence that CAPE was effective in protecting against renal injury in the CCL4-induced nephrotoxicity model. In contrast to the group that did not receive the preventive dose of CAPE, the Ogeturk *et al.* (2005) study found that the use of CAPE (10 micromol/kg, i.p.) reduced the amount of MDA and histological alterations, including glomerular, tubular, and interstitial tissue damage.



Chemical structure of caffeic acid phenethyl ester (CAPE).

Plant pigment quercetin is widely distributed in various ethnic plants, including onions and tea, so one can eat enough of it every day (Manach *et al.*, 2005). It's a special kind of bioflavonoid that scientists have investigated for a long time. Due to its use as an antioxidant, anticancer agent, and neuroprotectant, quercetin is significant in the field of ethnopharmacology (Dajas, 2012). According to reports, it is a potent antioxidant and free radical scavenger (Ferry *et al.*, 1996). According to Jan *et al.* (2010), quercetin has demonstrated an inhibitory effect on tyrosine kinase in phase I clinical trials, indicating potential therapeutic benefits against cancer.

Multifaceted and possessing a wide range of pharmacological qualities, quercetin is an antioxidant, neuroprotective, antiviral, anticancer, cardiovascular, antibacterial, anti-inflammatory, hepatoprotective, and anti-obesity drug (Maalik *et al.*, 2014).



Chemical Structure of Quercetin

MATERIALS AND METHODS

Materials

Digital analytical weighing balance (Ohaus: PA-1000), Beakers, Whatman number 1 filter paper, Conical flask, Spatula, Measuring cylinder, Aluminum foil, Sample bottles, Retort stand, Separating funnel, Plastic funnels, Thermostatic water cabinet (Model:HH-W420), Spectrophotometer (UV-Visible light), Micro pipette, Agile autohaematology analyzer (S-30), Surgifield rotary evaporator (SM-5286A), Liston classic centrifuge (C2204), Sykam HPLC (S3250 UV/visible detector).

Reagents/Chemicals

Aluminum chloride, formalin, chloroform, n-hexane, absolute ethanol, methanol, water, follin-ciocalteu reagent, sodium carbonate, trichloroacetic acid (TCA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), normal saline. The rest of the chemicals were of analytical grade.

Collection and Preparation of Plant Materials

Stembark of *P. americana* were plucked from its tree located in Baissa, Taraba State. The stembark was allowed to air dry under shade at room temperature for five days. The dried stembark plant materials were pulverized in a clean mortar and pestle until fine powder of it was formed. The pulverized sample was set aside for extraction.

Crude Extraction with Methanol

The crude extraction was carried out in accordance with the method reported by Yakubu *et al.* (2014) and Ayodele *et al.* (2022). Exactly 500 grams of each of the pulverized sample was soaked in about 2L of methanol in the ratio 1:4 (w/v) for 72hours. The extract was filtered using a clean filter cloth of which the filtrate obtained were further filtered under reduced pressure using Whatmann No. 1 filter paper, to obtain the final filtrate. The filtrate was concentrated using rotary evaporator, the concentrated extracts were then placed in a water bath at 45^{o}C to obtain the desired concentrate.

Determination of Total Phenolic Contents (TPC)

The Folin Ciocalteu (FC) method reported by Lachman *et al.* (2000), was used with slight modification as reported by Yakubu *et al.*, (2014). During the experiments, the reagents and sample solutions were prepared as follows: the FC reagent was diluted in ratio 1:10 with distilled water just before the experiment. Sodium carbonate (7.5% w/v) was also prepared in distilled water. Exactly 1ml sample was added to test tube containing 0.5ml Follin

reagent. About 1.5ml Sodium carbonate solution was added and the volume made up to 10ml using methanol water. The reactions were conducted in triplicates and absorbance of the samples were measured against blank, i.e., distilled water. The results were expressed as garlic acid equivalent (GAE).

Estimation of Total Flavonoids Content (TFC)

Flavonoids was determined using the aluminum chloride colorimetric method of Chang *et al.* (2002) as reported in Yakubu *et al.* (2014). Quercetin was used for derivation of the calibration curve. About 0.5 ml of the diluted sample was taken into test tube containing 1.5ml methanol. About 0.1ml of 10% aluminum chloride solution and 0.1 ml potassium acetate then added. After incubation at room temperature for 30 minutes, the reactions were conducted in triplicates and absorbance of the reaction mixtures were measured at 750nm and the concentration of flavonoids in the sample estimated from the calibration curve. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Total flavonoid was expressed as mg/ml quercetin equivalent (QE).

Determination of Total Antioxidant Activity Capacity (TAC)

The scavenging action of the plant extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined spectrophotometrically at 517nm using Trolox as standard according to the method described by Singleton *et al.* (2002) as reported by Yakubu *et al.* (2014). About 39.4mg of DPPH will be dissolved in 1L 80% Methanol to make a 0.1Mm. Serial dilution of the sample was carried out at 1000µg/1000ml, 500µg/1000ml, 250µg/1000ml, 125µg/1000ml, 62.5µg/1000ml and 31.25µg/1000ml. Exactly 2ml of DPPH solution was pipette into a cuvette followed by 1ml of sample. It was then mixed thoroughly for 30second and incubated in the dark at 37^{o}C for 20 minutes. The absorbance was measured for each sample against the blank. Total antioxidant activity capacity (TAC) was calculated as mg/ml of Trolox equivalent (TE) using the regression equation from the calibration curve.

HPLC Determination of Amino Acid, Vitamin, and Flavonoid and Polyphenol Profile

The Determination of amino acids profile, vitamins profile, and flavonoid and phenolic profile of *P. americana* seed extract was carried out using Sykam HPLC (S3250 UV/visible detector).

RESULTS

Table 1: Result for total flavonoid content and total phenolic content of stembark methanol extract of *P. americana*

Phytochemicals	Stembark
TFC (mg QE/100g)	42.21±0.23
TPC (mg GAE/100g)	33.65±3.02

Results are expressed mean ± standard of results obtained (n = 3).

The result in table 1 shows the total flavonoid content and total phenolic content of the stembark extracts of *P. americana*. The flavonoid content shows to be 42.21±0.23while the total phenolic content was seen to be 33.65±3.02.

Table 2: Results for percentage inhibition of DPPH scavenging activities of methanol stembark extract of *P. americana*

Concentration (µg/ml)	% Inhibition
1000	87.88
500	86.08
250	78.66
125	60.47
62.5	41.78
31.25	40.14

The result in table 2 shows the percentage inhibition in DPPH scavenging activities with concentration difference in the extracts. The result shows that as concentration increases, percentage inhibition increases. However, the result for methanol stembark extract of *P. americana* shows a significant inhibition effect on DPPH scavenging activities.

Table 3: Phytochemical profile for methanol extract of stembark of *P. americana*

Phytochemical	Amount (ppm)	Amount (%)	Retention time (min)
Quercetin	22.601	0.50	1.853
Caffeic acid phenyl ether	4928.038	99.50	2.257

The result in table 3 for methanol stembark extract reveals that quercetin and Caffeic acid phenyl ether were detected at 0.50% (22.60ppm) and 99.50% (4928.04 ppm) respectively.

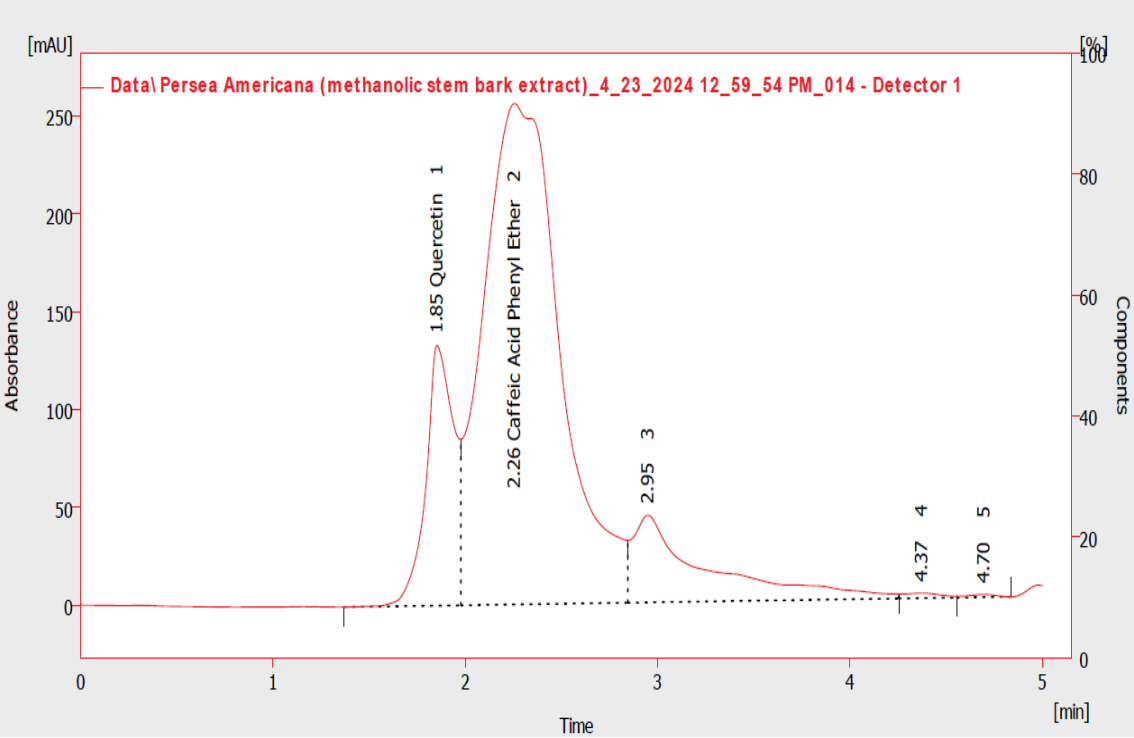


Figure 1: Chromatogram of flavonoids and phenolics profile for methanol stembark extract of *Persea americana*

Table 4: Vitamin profile for methanol extract of stembark of *P. americana*

Vitamins	Amount (mg)	Amount (%)	Retention time (min)
Vitamin K	3.540	0.40	1.815
Vitamin B1	803.695	80.20	2.368
Vitamin B2	195.408	19.50	2.942

The result in table reveals that vitamins K, B1 and B2 were shown to be present in the methanol stembark extract. The vitamin B1 detected was seen to be highest with 80.2% (803.695 mg) whereas vitamin K was least with 0.4% (3.54 mg).

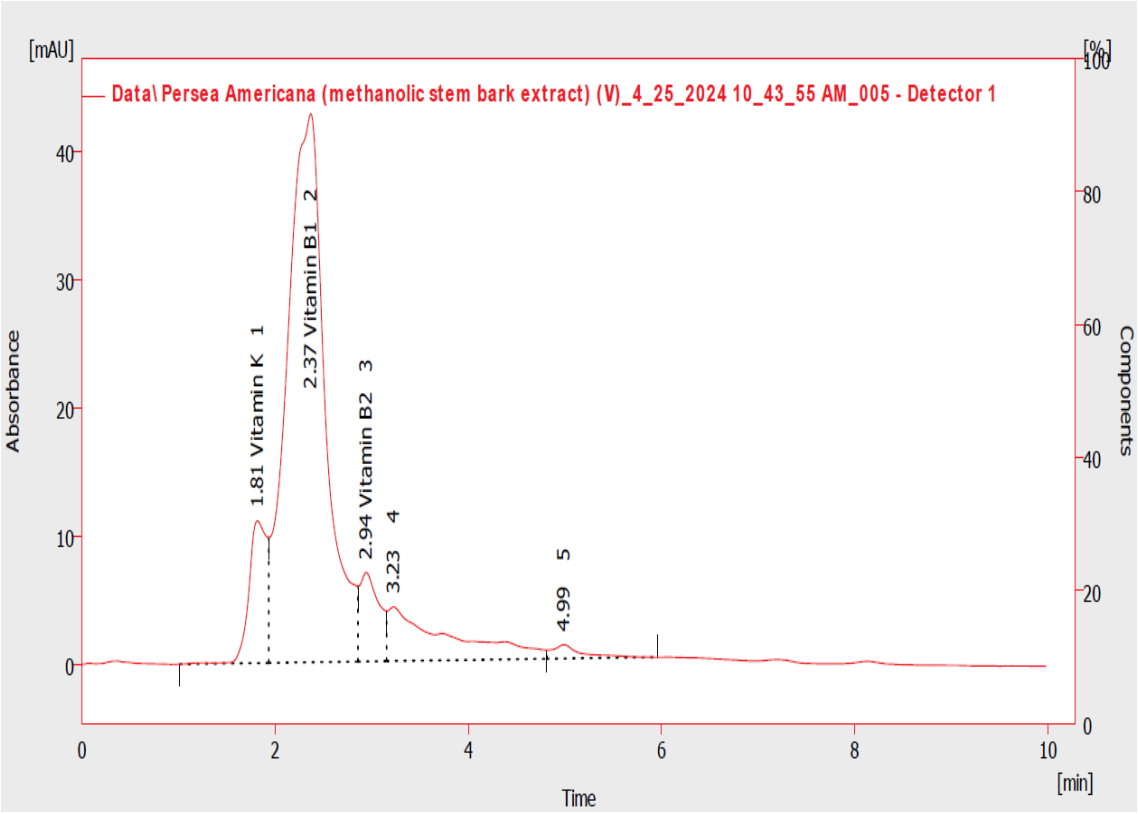


Figure 2: Chromatogram of vitamins profile for methanol stembark extract of *Persea americana*

Table 5: Amino acid profile for methanol extract of stembark of *P. americana*

Amino acids	Amount (µL)	Amount (%)	Retention time (min)
Asparagine	3.788	0.6	0.398
Threonine	185.423	27.1	1.825
Phenylalanine	284.956	41.6	2.383
Aspartic acid	104.415	15.2	2.932
Glutamic acid	56.657	8.3	3.252
Leucine	43.599	6.4	3.753
Arginine	6.126	0.9	5.022

The result in table 5 above for methanol stembark extract of *P. americana* reveals that there are seven different amino acids present in the extract. These are asparagine, threonine, phenylalanine, aspartic acid, glutamic acid, leucine and arginine in different percentage. phenylalanine is seen to have the highest percentage abundance with 41.6% (284.96 µL) while asparagine is having least abundance with 0.6% (3.79 µL).

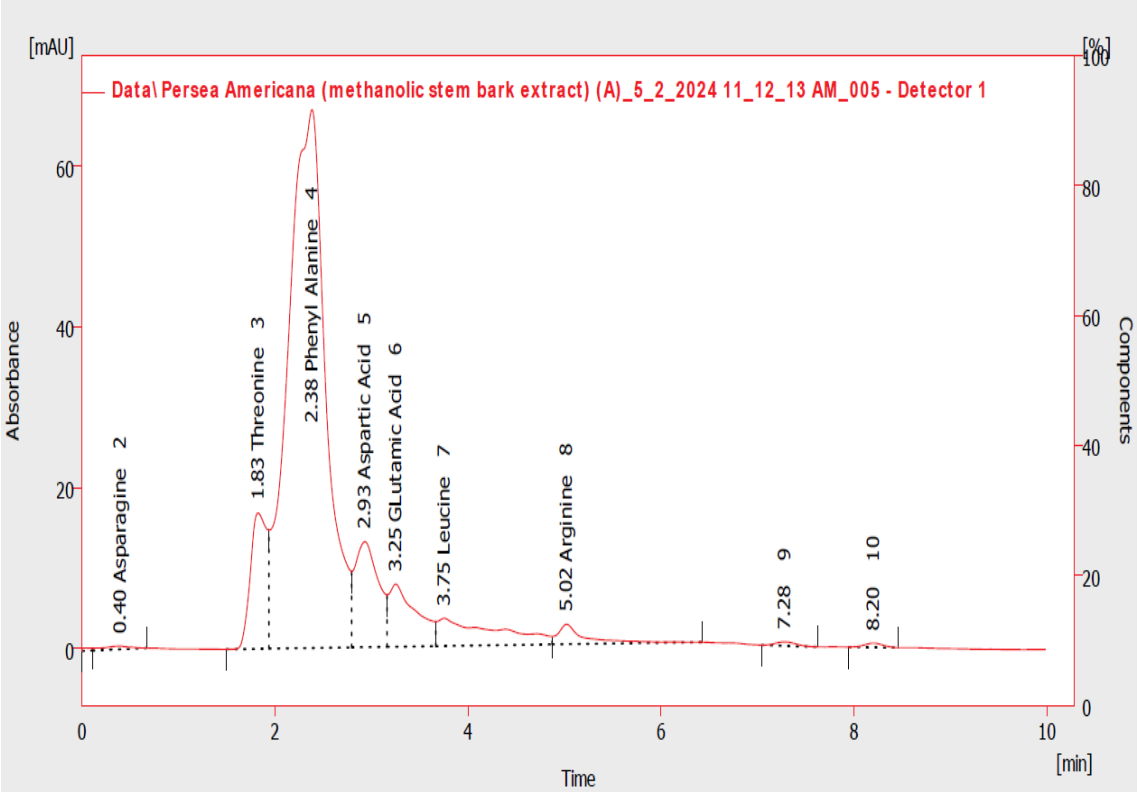


Figure 3: Chromatogram of amino acids profile for methanol stembark extract of *Persea americana*

DISCUSSION

The result in table 1 shows the total flavonoid and total phenolics levels in the stembark methanol extract of *P. americana*. The result show significant levels of each of the phytochemicals. Oboh *et al.* (2016) asserted flavonoids, among other things, protect against allergies, inflammations, platelet aggregation, bacteria, ulcers, viruses, and cancer. This could imply that the plant extracts could be used for defense in the body against conditions such as allergies, ulcerations, viral infections, bacterial infections among others. Furthermore, as result of the presence of flavonoids, plant extracts from *P. americana* might be employed as antispasmodic, antifungal, and antibacterial medications. This explains why the local communities often make use of this important plant material in the treatment of disease conditions such as diarrhea, spasmodic bronchitis, and other microbial illnesses (Umar *et al.*, 2018).

Polyphenols are strong antioxidants that complement and add to the functions of antioxidant vitamins and enzymes as a defense against oxidative stress caused by excess

reactive oxygen species (ROS) (Williams *et al.*, 2004; Dong *et al.*, 2009). From the result in table 1, it can be seen that the stembark of *P. americana* can be a good source of phenolics. According to Kumar *et al.* (2018), the free radical scavenging activity of phenolics could be due to the presence of hydroxyl (-OH) group in the structure. This perhaps further explains why the rural dwellers prefer the use of plant materials, since they are rich sources important phytochemicals.

The result in table 2 shows the ability of the plant extract to scavenge DPPH free radicals. The free radical reaction is one of the major causes of problems, particularly in the health and food industries. It causes many deadly diseases such as cancer (Salehi *et al.*, 2020) and oxidative rancidity of foods (Velasco *et al.*, 2010). The result shows a progression in percentage inhibition with increases in extracts concentration. This due to the significant levels of antioxidant in the stembark extract of *P. americana*. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997; Brighente *et al.*, 2007). Extracts of plants are allowed to react with the stable radical, DPPH, in methanol solution (Brighente *et al.*, 2007). The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm, induced by an antioxidant (AH) after 20 min, as follows:

$DPPH^{\cdot} + AH \longrightarrow DPPH\text{-}H + A^{\cdot}$ (Brighente *et al.*, 2007)

The phytochemical profile of the stembark methanol extract of *P. americana* is shown in table 3 and the HPLC chromatogram in figure 1. The result reveals that quercetin was detected as well as caffeic acid phenyl ester (CAPE). According to research, quercetin has been shown to possess certain ethnopharmacological activities such as its use in antioxidant, anticancer and neuroprotection (Dajas, 2012). Due to its antioxidant ability, it has been shown to be effective in free radical scavenging (Ferry *et al.*, 1996). Quercetin is a useful biomolecule possessing numerous pharmacological activities such as antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, hepatoprotective, protective of the reproductive system and anti-obesity agent (Maalik *et al.*, 2014). This shows that the plant material used in this research could be a important in treating disease conditions including neurological, cancer, bacterial infections, viral infections, liver and reproductive health challenges among others, if properly harnessed.

Furthermore, the result in table shows the richness of the phenolic, CAPE in the stembark methanol extracts. A wide range of pharmacological properties has been identified in CA and CAPE, such as antioxidant, anti-inflammatory, anti-cancer, antiviral, anticarcinogenic, antitoxic, and neuroprotective (Chuu *et al.*, 2012; Ehtiati *et al.*, 2023). Modern medical research showed that the main antioxidant groups of propolis were CAPE, caffeic acid, quercetin, kaempferol, galangal and cinnamic acid ester. The antioxidant effect of propolis which contained CAPE was stronger than that without CAPE, and the antioxidant effect of CAPE was stronger 0than that of galanga, which proved that CAPE played an important role in the antioxidant activity (Sud'ina *et al.*, 1993). Propolis has been for a long time used to treat burns (Zhang *et al.*, 2014).

The result in table 4 shows the vitamins profile of the stembark methanol extract of *P. americana*. The result reveals that three vitamins were identified in the extract. These include vitamin K, vitamin B1 and vitamin B2. Figure 2 shows the chromatogram of the stembark methanol extract of *P. americana*. The primary function of vitamin K is the formation of prothrombin in the liver along with other vitamin K dependent clotting factors which may include VII, IX and X (Akram *et al.*, 2020). Vitamin K deficiency can result to generalized bleeding, the development of hemorrhagic disease of the newborn, and prolonged clotting time in adults. Vitamin B1 on other hand is required for carbohydrate metabolism and its coenzyme is required in the hexose monophosphate shunt (Akram *et al.*, 2020). Deficiency of this important micronutrient may lead to a condition known as beriberi. Vitamin B2, also known as riboflavin they play key roles in energy metabolism since they are precursor for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

The result in table 5 and figure 3 show the amino acid profile of the stembark methanol extract of *P. americana*. The result shows that seven different amino acids were identified. The amino acids include asparagine, threonine, phenylalanine, aspartic acid, glutamic acid, leucine and arginine. Amino acids being building block of proteins are essential for repair of worn out tissue and synthesis of various proteins, both structural and regulatory. Asparagine, aspartic acid and glutamic acid are considered non-essential or dispensable whereas threonine, phenylalanine, leucine and arginine are regarded as essential or indispensable amino acids. The ratio of essential amino acids to non-essential amino acids is remarkable. This shows that the *P. americana* could be a good source of these amino acids and can be used in supplements.

CONCLUSION

This research work reveals in no small way how rich the stembark of *P. americana* is in times of various phytochemicals, especially the ones needed for their antioxidant activities. The result from this research brings to the fore the effectiveness of the plant extract to scavenge free radicals by inhibition DPPH free radicals. This is perhaps due to the presence of flavonoids such as quercetin which has been credited with that activity and phenolics like caffeic acid phenyl ester (CAPE) which has shown from research that it can be effective in same. The plant extract has shown to possess important molecules needed in the body such as vitamins, amino acids and proteins.

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