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The Total Antioxidant Levels, Amino Acids, Vitamins, Flavonoids and Phenolics Profile of *Persea americana* Using HPLC

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Abstract

This research investigated the antioxidant levels, flavonoids and phenolics, amino acid and vitamin profile of *P. americana* seed methanol extract. Fresh fruits of *P. americana* were procured from Baissa in Taraba State. The fruits were cut open and the seed removed, washed and sliced. The plant material was air dried at room temperature after which it was pulverized and extraction was carried out using methanol for 72 hours. Extract was subjected to total antioxidant test using DPPH by serial dilution. Different portions of the extract were used to analyze for total flavonoid content and total phenolics content using aluminium chloride and folin-ciocalteu respectively. Furthermore, a portion of the extract was used to analyze for amino acid, vitamin and flavonoids and phenolics profile. The result reveals that the portion antioxidant activity increase with concentration. The portion with 1000µg/1000mL had over 80% which was the highest inhibition percentage in the series. Total flavonoid was found to be 39.88 ± 0.44 while the total phenolic content was seen to be 35.84 ± 4.30. Quercetin and chlorogenic acid were seen to be present for HPLC analysis of flavonoids and phenolics profile to be 15.8% and 84.2% respectively. The result for vitamin profile reveals that three vitamins were detected: vitamins K, B1 and B3 at 0.1%, 20.4% and

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79.5% respectively. The amino acid present include asparagine, threonine, serine and aspartic acid with 1.1%, 13.9%, 10.4 and 74.6% abundance respectively. This shows how rich how this plant material can be and also reveals the hidden potentials inherent in it. This could be used to supplement diets and meet various nutritional and needs of individuals.

Keywords: Antioxidant, Flavonoids, Phenolics, Pulverized, Extraction, Inhibition, Quercetin, Chlorogenic acid, Asparagine, Threonine, Serine, Aspartic acid

INTRODUCTION

Since the dawn of human civilization, plants have served multiple purposes, including food, feed, fiber, and medicine. Notably, various plant parts (such as roots, leaves, fruits, and bark) contain unique chemicals with diverse medicinal properties. For instance, phenolic compounds play a crucial role in antioxidant activity (Kousar *et al.*, 2023). The statement can be rephrased as follows: "Each plant possesses unique biochemical compounds with specific therapeutic effects" (Bouafia and Laouini, 2021). As an illustration, oxygen's heightened reactivity leads to the production of free radicals, which play a role in various disorders (Mythri *et al.*, 2020). The impact of oxidative stress caused by free radicals on body tissues significantly contributes to various pathological conditions, including cellular aging, infections, and effects within medicinal and biological systems (Lai *et al.*, 2001; Akbari *et al.*, 2022). As per Seelinger and colleagues (2012), the ongoing need for drug discovery remains critical in biomedical research, and documentation highlights related issues in Nigeria's clinical progress. Herbal medicines share similarities with conventional drugs in their mechanisms of action. *Persea americana* (avocado) is a plant widely used by indigenous communities for its nutritional benefits and to address various health issues (Tene Tcheghebe *et al.*, 2016).

"Avocado (*Persea americana* Mill. var. Hass) belongs to the Lauraceae family and originates from Central America and Mexico. It is cultivated in various regions of Nigeria, including Northern states (Kaduna, Plateau, Nasarawa, Taraba, Kogi, Kwara, Niger), as well as the South-East, South-West, and South-South regions. Avocado is valued for its economic significance and abundant nutritional and medicinal components. Different parts of the plant contain polyphenols, flavonoids, alkaloids, and vitamins (Nyakang'I *et al.*, 2023). The

seed of this fruit is an underutilized resource, containing abundant phytochemicals with potential medicinal and therapeutic properties (Mohammed, 2023). When compared to other parts of the fruit, the seed exhibits the highest antioxidant capacities, phenolic content, and procyanidins (Wang *et al.*, 2010; Setyawan *et al.*, 2021).

Avocado contains various bioactive compounds, including phenolic compounds (such as hydroxycinnamic acids, flavonoids, and proanthocyanins), acetogenins, phytosterols, carotenoids, and alkaloids (Salazar-López *et al.*, 2020). Plant bioactive compounds, also known as secondary metabolites, are chemically active compounds produced by plants in response to stress. These compounds exhibit complex structures and are more restricted in distribution compared to primary metabolites (such as carbohydrates, proteins, lipids, and fats) (Kumari, 2017). Exploring secondary metabolism in plants is crucial for identifying bioactive compounds with diverse applications. Many of today's therapeutic lead compounds are derived from natural products (Mustafa *et al.*, 2017). The majority of secondary metabolites exhibit diverse therapeutic effects and directly engage with receptors, cell membranes, and nucleic acids (Velu *et al.*, 2018). Bioactive compounds (secondary metabolites) can influence metabolic processes and demonstrate antioxidant effects, receptor activity modulation, enzyme inhibition or induction, and regulation of gene expression (Carbonell-Capella *et al.*, 2014). Various secondary metabolites, such as phenols, flavonoids, tannins, alkaloids, steroids, and saponins, exhibit bioactivity. For instance, avocado seed extract induces apoptosis in LNCaP cells and reduces NFκB nuclear translocation. Additionally, avocado phytochemicals like Persin cause G2/M phase arrest in breast cancer cell lines, while Quercetin and its derivatives induce G2/M arrest in multiple cell types, including lung cancer and prostatic carcinoma cells (Ding *et al.*, 2007).

Albert Szent Gyorgyi, a Nobel Prize laureate, first identified dietary flavonoids in 1936. These compounds are well-known for their potential health benefits. Quercetin, a major flavonoid found in our daily diet, is estimated to be consumed in amounts ranging from 5 to 40 mg per day (Hertog *et al.*, 1995). Following absorption, quercetin undergoes significant metabolism in the intestine and liver. Typically, its plasma concentration remains in the nanomolar range, but consumption of quercetin-rich foods can elevate it to the micromolar range (Russo *et al.*, 2012).

Quercetin possesses three rings and five hydroxyl groups (depicted in Figure 1). It predominantly exists as glycosides and ethers, with sulfates occurring to a lesser extent

(Williams and Grayer, 2004). Glycosylation enhances quercetin's hydrophilicity, stability, and bioavailability (He et al., 2006). Quercetin, extensively researched among flavonoids, demonstrates antioxidant, antiviral, antibacterial, anti-inflammatory, and anticarcinogenic effects (Duthie and Dobson, 1999; Ramos *et al.*, 2006; Boots *et al.*, 2008; Zandi *et al.*, 2011).

Prior treatment with isoquercetin elevated ROS-scavenging enzymes (SOD, CAT, and GPx) and decreased lipid peroxidation in PC12 cells exposed to 6-OHDA. Similarly, quercetin reduced protein carbonyl content and lipid hydroperoxide levels in the striatum of 6-OHDA-treated rats (Haleagrahara *et al.*, 2011). Quercetin, a multifaceted molecule, possesses various pharmacological properties such as antioxidative, neuroprotective, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, hepatoprotective, and anti-obesity effects (Maalik *et al.*, 2014). Quercetin can neutralize reactive oxygen species, and its antioxidant properties arise from this ability to scavenge free radicals (Jan *et al.*, 2010).

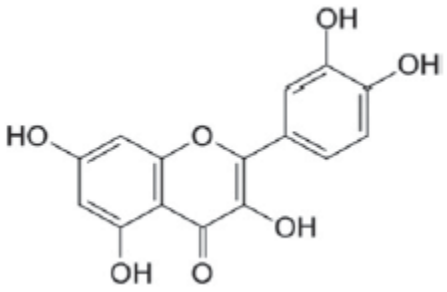

Figure 1: Chemical structure of quercetin

Chlorogenic acid (CGA) and its family members are prevalent phenolic acid compounds in plants. They form by linking the hydroxy group of quinic acid with the carboxyl group of caffeic acid as the foundational structure (Nguyen *et al.*, 2024). The CGA family comprises several members, including 1L-(−)-quinic acid, caffeic acid (CA), ferulic acid, and the p-coumaric acid (p-CoQA) group. This group encompasses p-CoQAs, caffeoylquinic acids (CQAs), and feruloylquinic acids (FQAs) (Stalmach *et al.*, 2010; Clifford *et al.*, 2017; Li *et al.*, 2020; Xue *et al.*, 2023). The CGA family exhibits protective effects by mitigating chronic inflammatory and age-related disorders through anti-inflammatory, antioxidative, and metabolic homeostasis-modulating actions (Stalmach *et al.*, 2010; Clifford *et al.*, 2017; Li *et al.*, 2020; Xue *et al.*, 2023). Despite its health benefits, CGA has restricted bioavailability in plant-based foods due to its binding with cell wall components like proteins, lignin, and

cellulose ((Liu, 2013)). To enhance release, proper food processing methods are necessary (Dewanto *et al.*, 2002).

Chlorogenic acid (CGA) plays diverse roles in various pathological conditions. It mitigates inflammation, reduces oxidative stress, modulates glucose and lipid homeostasis, protects the cardiovascular system, kidneys, and liver, aids neurological recovery (including neurodegenerative disorders and diabetic peripheral neuropathy), inhibits tumor cell growth and migration, improves skin health, exhibits anti-pathogen effects, and contributes to anti-aging effects (Nguyen *et al.*, 2024).

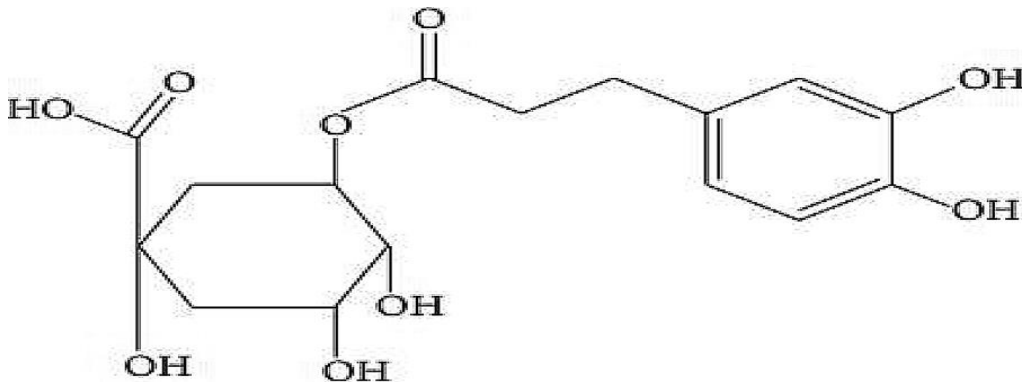

Figure 2: The chemical structure of chlorogenic acid (CGA).

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

Fresh fruits of *P. americana* were purchased from the local market. in Baissa. The fruits were cut open to remove seeds and seeds sliced into pieces. The sliced seeds obtained from the fruit of the *P. americana* were air dried until they become crispy, after which they were pulverized in a clean and dry mortar and pestle.

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 extracts were 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°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 used by Yakubu *et al.*, (2014). During the experiments, the reagents and sample solutions were prepared as follows: The FC reagent will be diluted to 1:10 with distilled water just before the experiment. Sodium carbonate (7.5% w/v) will also be 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 sample was 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 mixture was 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 flavonoids was expressed as mg/ml quercetin equivalent (QE).

Determination of Total Antioxidant Activity Capacity (TAC)

The scavenging action of the plant extracts 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°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 Flavonoids and phenolics Profile

The Determination of amino acids profile, vitamins profile, and flavonoids 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 seed methanol extract of *P. americana*

Phytochemicals	Seed extract
TFC (mg GAE/100g)	39.88±0.44
TPC (mg QE/100g)	35.84±4.30

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 seed extracts of *P. americana*. The flavonoid content shows to be 39.88 ± 0.44 while the total phenolic content was seen to be 35.84 ± 4.30.

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

Concentration (µg/ml)	% Inhibition
1000	81.52
500	71.19
250	55.91
125	38.85
62.5	33.10
31.25	36.10

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 methanol seed extract of *P. americana* shows to have good inhibition effect on DPPH scavenging activities.

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

Phytochemical	Amount (ppm)	Amount (%)	Retention time (min)
Quercetin	1.149	15.80	1.745
Chlorogenic acid	6.137	84.20	2.427

The result in table 3 for methanol seed extract reveals that quercetin and chlorogenic acid were detected at 15.8% (1.15 ppm) and 84.2% (6.14 ppm) respectively.

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

Vitamins	Amount (mg)	Amount (%)	Retention time (min)
Vitamin K	2.631	0.1	1.790
Vitamin B1	617.586	20.4	2.413
Vitamin B3	2400.219	79.5	2.947

The result in table reveals that vitamins K, B1 and B3 were shown to be present in the methanol seed extract. The vitamin B3 detected was seen to be highest with 79.5% (2400.22 mg) whereas vitamin K was least with 0.1% (2.63 mg).

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

Amino acids	Amount (µL)	Amount (%)	Retention time (min)
Asparagine	2.526	1.1	0.390
Threonine	33.1	13.9	1.775
Serine	24.81	10.4	2.430
Aspartic acid	177.394	74.6	2.955

The result in table 5 above for methanol seed extract of *P. americana* reveals that there are four different amino acids present in the extract. These are asparagine, threonine, serine and aspartic acid in different percentage. Aspartic acid is seen to have the highest percentage of abundance with 74.6% (177.39µL) while asparagine is having least abundance with 1.1% (2.53µL).

DISCUSSION

Different parts of the plant are rich in polyphenols, flavonoids, alkaloids and vitamins (Nyakang'I *et al.*, 2023). The result in table 1 shows the relatively abundance of flavonoids in the seed extract. However, the the phenolics though lower than the flavonoids was seen to be abundant as well. The presence of these phytochemicals could account for nephroprotective and anti-inflammatory potential of the aqueous seed extract of *P. americana* reported by Osukoya *et al.* (2021). Flavonoids and phenolics, among other phytochemicals have also been reported for *P. americana* seed (Setyawan *et al.*, 2021) and it was also noted to possess antioxidant and anti-inflammatory properties (Bangar *et al.*, 2022). These may have contributed to its amelioration of acetaminophen toxicity (Ogbonnaya *et al.*, 2024). Compared with other parts of the fruit, the seed contain the highest antioxidant capacities, phenolic content, and procyanidins (Wang *et al.*, 2010; Setyawan *et al.*, 2021).

The result of antioxidant capacity of the methanol seed extract of *P. americana* in table 2 reveals its free radical scavenging activities. The percentage inhibition of DPPH effect was quite high, the 1000µg/1000mL shows over 80% inhibition (table 2). This is quite in agreement with El-Magd and colleagues who stated that owing to the documented antioxidant activity of avocado seeds extracts, oral administration of the extracts ameliorated cyclosporin-A induced hepatoxicity via inhibition of oxidative stress and ER stress (El-Magd *et al.*, 2022). This could be partly due to the presence of quercetin in the

seed, Jan and others (2010) stated that quercetin has the potential of scavenging reactive oxygen species and its antioxidant potential is attributed to this free radical scavenging activity.

In table 3, the flavonoids and phenolics profile of the methanol seed extract of *P. americana* reveals the presence of quercetin and chlorogenic acid, both of which are important phytochemicals capable of playing vital roles in the biological system. Hertog *et al.* (1995) had reported that quercetin is the major flavonoid in the human diet with daily intake ranges between 5 and 40mg. According to Maalik *et al.* (2014), quercetin is a multipurpose biomolecule having unique pharmacological properties which may include antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, hepatoprotective, protective of the reproductive system and anti-obesity agent.

On the other hand, chlorogenic acid has also shown to possess beneficial effects including protective effects on ameliorating some chronic inflammatory and disorders related to aging by applying the essential effect of anti-inflammation, antioxidation, and metabolic homeostasis modulation (Stalmach *et al.*, 2010; Clifford *et al.*, 2017; Li *et al.*, 2020; Xue *et al.*, 2023). The result shows the abundance of this polyphenol which is in excess of 80% to be high relative to the quercetin with about 15%.

Table 4 shows the results of vitamins detected in milligram and percentage by HPLC. The result shows a relative abundance of various vitamins, particularly vitamin K (phylloquinone), vitamin B1 (Thiamine) and vitamin B3 (Niacin) which are both essential for normal functioning and metabolism in the body. Vitamin K is essential for blood clotting and as such are need to avoid unnecessary loss of blood when there is physical injury. However, this seed can be supplemented when need arises as a source of vitamin K. Thiamine pyrophosphate is involved in carbohydrate metabolism. Thiamine pyrophosphate is also involved in the hexose monophosphate shunt. It is a neuro-protective agent (Ikeda *et al.*, 2016). Its deficiency leads to a disease known as beriberi. n essential to the human diet, but can be synthesized in the body from tryptophan. Its deficiency leads to a condition known as pellagra (Akram *et al.*, 2020).

The result in table 5 shows that four different amino acids were detected. Amino acids being building block of proteins are essential for repair of worn out tissue and synthesis of various proteins, both structural and regulatory. The various amino acids detected include

asparagine, threonine, serine and aspartic acid. Of the four amino acids detected, only threonine is considered essential, that is it must be supplied in diet or other supplements.

CONCLUSION

The methanol seed extract of *P. americana* has shown to be a rich source of various phytochemicals needed in the body. There arises a need to look for alternative ways to solve this problem of both chronic disease and diet supplement. With this work, the ameliorative work of phytochemicals, amino acids and vitamins detected has gone a long way to reveal the hidden treasure in what is often considered waste. Considerations can be made on how this plant seed can be used in supplentation in diet, especially to the haemophiliacs considering that it can be a source of vitamin K and many more needed for normal metabolism.

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