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Determination of the Total Phenolics, Flavonoids, Phenolics and Flavonoids Profile, Vitamins and Amino Acids Profile Using HPLC and DPPH Free Scavenging Radical Acitivities of the Methanol Leaf Extract of *Persea americana*

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Abstract

This research examined the total phenolics, flavonoids, flavonoids and phenolics profile, vitamins and amino acids profile uing HPLC as well as the DPPH free scavenging activity of methanol leaf extract of *P. americana*. Plants have shown to possess phytochemicals which are beneficial to man including therapeutic and disease prevention. *P. americana* leaves was harvested from its tree in Baissa, Taraba State. The leaves were air dried under shade at room temperature after which it was pulverized using clean mortar and pestle. The powdered sample was extracted using methanol for 72 hours. Extract was filtered using whatmann no. 1 filter paper and evaporated using rotary evaporator and water bath to obtain the required concentrate. The concentrated extract was subjected to total flavonoid and total phenolics content using aluminium chloride folin ciocaltue respectively. Total antioxidant

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capacity of the leaf extract was carried out using DPPH. The amino acids, vitamins, flavonoids and phenolics profiles were evaluated. The result reveals that there is a significant level of flavonoid and phenolics in the extract which accounted for 43.32±0.15 mg GAE/100g and 32.84±2.13 mg CE/100g respectively. The antioxidant capacity showed a promising inhibition of DPPH free radical scavenging activity with increase in dose. The amino acids that were found to be present in the extract include asparagine, threonine, histidine, phenylalanine and aspartic acid with different percentage. Vitamins K, B1 and B3 were detected significantly in the leaf extract. The various phytochemicals detected have shown according to research to have beneficial to the biological system, especially in scavenging free radicals and mitigation of oxidative stress. Vitamins are essential for normal functions and metabolism in the biological system, their deficiency could have deleterious effects. Amino acids are necessary for protein synthesis and are required for other biological processes. The extract has shown to be a rich source of various phytochemicals and have shown a promising presence of various vitamins and amino acids

Keywords: Phenolics, Flavonoids, Scavenging, Phytochemicals, Antioxidant, Inhibition, Oxidative Stress, Free Radicals

INTRODUCTION

Plants and herbs have historically been essential as both food sources and folk remedies. They have also been the basis for creating synthetic drugs. The World Health Organization (WHO) states that over 80% of the global population depends on traditional medicine for primary healthcare (Bharti *et al.*, 2012). This reliance is particularly significant in developing countries, where traditional medicine remains a primary healthcare provider for over 80% of the population (Srinivasan *et al.*, 2015). Yakubu and colleagues (2023) emphasize that medicinal plants, due to their affordability and accessibility, now play a crucial role in delivering healthcare in rural regions, especially in African countries. The rising demand for safer and more cost-effective plant-based treatments aims to address issues related to drug resistance and pharmaceutical side effects.

Avocado (*Persea americana Mill*) is a tropical tree belonging to the Lauraceae family. Known for its complete nutritive profile (Ramdhan and Yusuf, 2023), avocados are rich in monounsaturated fatty acids, including palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid (Sunil *et al*., 2015; Segovia *et al*., 2018). Additionally, avocados contain carotenoids, vitamins, minerals, lecithin, B-sitosterol, polyphenols, and flavonoids. Research indicates that avocado extract exhibits antioxidant, sunscreen, and antimicrobial properties (De Oliveira, 2013; Ramadan, 2019). Yasir and colleagues (2010) highlight that numerous drugs in current use originate from herbs due to their safety, quality, and effectiveness.

The leaves of *P. americana* are widely used in Turkey for treating kidney stones and urinary tract infections (Demirkol, 1995; Gruenwald *et al*., 2004; Sharma *et al*., 2009; Sargin, 2015). Internally, they serve as an infusion for various conditions, including diarrhea, stomachaches, headaches, wounds, and sore throats. Interestingly, in Mexico, they are believed to ward off bad spirits, while in Caribbean islands, a decoction is used for managing high blood pressure (Josabad *et al*., 2012; Clement *et al*., 2015). Fresh leaves of *P. americana* have been traditionally used as aqueous infusions or decoctions for various purposes, including treating influenza, bronchitis, menstrual pain, diabetes, and rheumatism. Externally, they serve as a hair tonic in Ecuador. Additionally, these leaves have been explored for their antimalarial properties in Nigeria (Tene *et al*., 2007; Dike *et al*., 2012). The presence of essential oils and flavonoids in *Phytolacca americana* leaves has been documented by several studies, including those by De Almeida *et al.* (1998), Ogunbinu *et al.* (2007), Larijani *et al.* (2010), Owolabi *et al.* (2010), and Niogret *et al.* (2013). Avocado leaves contain persin, a toxin harmful to lactating livestock (Oelrichs *et al*., 1995). Meanwhile, *P. americana* leaves exhibit various pharmacological properties, including analgesic, anti-inflammatory, anticonvulsant, antihyperlipidemic, hypocholesterolemic, hypoglycemic, and hypotensive effects (Adeboye *et al*., 1999; Adeyemi *et al*., 2002; Ojewole *et al*., 2006; Brai *et al*., 2007; Kolawole *et al*., 2012).

Avocado leaves, beyond their nutritional value, have therapeutic properties and have been widely used for centuries in ancient cultures and folk medicine. They are commonly employed as infusions and are associated with benefits such as blood sugar control, reduced inflammation, kidney protection, and cholesterol reduction (Hurtado-Fernandez *et al*., 2017), the avocado leaves have been traditionally used for their biological properties in managing conditions such as anemia, diabetes, gastritis, and bronchitis (Ross, 2001).

In the context of leaf extract treatments, studies have demonstrated their effectiveness in various ways: reducing hypertension (Adeboye *et al.*, 1999; Owolabi *et al.*, 2005), lowering blood sugar levels (Antia et al., 2005), and providing analgesic and anti-inflammatory effects (Adeyemi *et al.*, 2002). According to Yasir and colleagues (2010), the aqueous extract of *Phytolacca americana* leaves significantly inhibits control writhes in a dose-dependent manner. Beyond its hypotensive, analgesic, and anti-inflammatory properties, this plant leaf is associated with various essential biological functions. These functions include anticonvulsant effects, antiviral activity, wound healing, antiulcer effects, antihepatotoxic properties, antioxidant activity, hypoglycemic effects, and a significant impact on weight. Researchers have indicated that avocado leaf extract has vasorelaxant effects on isolated rat aorta. This effect is dependent on the release of endothelium-derived relaxing factors (EDRFs) and prostanoids, ultimately inhibiting calcium influx through calcium channels (Yasir *et al.*, 2010; Ngbolua *et al.*, 2019). Researchers have found that the leaves of *Persea americana* (avocado) contain beneficial phytochemicals, including flavonoids and phenolics. These compounds contribute to the plant's antioxidant properties and potential health benefits. Notably, avocado hydroalcoholic extracts, particularly from leaves, exhibit intriguing *in vitro* anti-inflammatory effects, making them relevant for human health applications (Ovalle-Marin *et al.* 2020).

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 leaves of *P. americana* were harvested from its tree located in Baissa, Taraba State. The leaves were allowed to air dry under shade at room temperature for five days. The dried leaf 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 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 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 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°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 leaf methanol extract of *P. americana*

Phytochemicals	Leaf
TFC (mg GAE/100g)	43.32±0.15
TPC (mg QE/100g)	32.84±2.13

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 leaf extracts of *P. americana*. The flavonoid content shows to be 43.32 ± 0.15 while the total phenolic content was seen to be 32.84 ± 2.13.

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

CONCENTRATION (µg/ml)	% inhibition
1000	76.12
500	70.37
250	31.81
125	10.77
62.5	7.20
31.25	5.78

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

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

Phytochemical	Amount (ppm)	Amount (%)	Retention time (min)
Quercetin	6.148	15.60	1.773
Chlorogenic acid	33.273	84.40	2.400

The result in table 3 for methanol leaf extract reveals that quercetin and chlorogenic acid were detected at 15.6% (6.15 ppm) and 84.4% (33.27 ppm) respectively.

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

Vitamins	Amount (mg)	Amount (%)	Retention time (min)
Vitamin K	4.244	0.2	1.818
Vitamin B1	854.517	45.1	2.407
Vitamin B3	1035.337	54.7	2.953

The result in table reveals that vitamins K, B1 and B3 were shown to be present in the methanol leaf extract. The vitamin B3 detected was seen to be highest with 54.7% (1035.34 mg) whereas vitamin K was least with 0.2% (4.24 mg).

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

Amino acids	Amount (µL)	Amount (%)	Retention time (min)
Asparagine	1.339	0.5	0.373
Threonine	41.678	14.3	1.797
Histidine	80.082	27.5	2.282
Phenylalanine	44.831	15.4	2.400
Aspartic acid	123.168	42.3	2.93

The result in table 5 above for methanol leaf extract of *P. americana* reveals that there are five different amino acids present in the extract. These are asparagine, threonine, histidine, phenylalanine and aspartic acid in different percentage. Aspartic acid is seen to have the highest percentage of abundance with 42.3% (123.17µL) while asparagine is having least abundance with 0.5% (1.34µL).

DISCUSSION

There are very ancient references for utilization of plants in clinical treatments. This information of using plants parts for clinical treatment is very well known to common people living in rural areas of developing countries. These people are using plants traditionally, for the treatment of many sicknesses. Medicinal plants have the ability to synthesize a wide variety of chemical compounds which are used to perform biological functions and to defend against attack from predators. Medicinal plants have recently

become a focus of interest because it is said that they play key roles in the treatment of a majority of diseases with minimal or no side effects. (Tene Tcheghebe *et al.*, 2016).

The result in table 1 for total flavonoid and total phenolics content reveals that both phytochemicals are present in a significant amount. A variety of plant materials are natural sources of antioxidants due to the phytochemicals such as alkaloids, flavonoids, phenolics, and terpenoids they contain (Salehi *et al.*, 2020). The presence of these phytochemicals signifies a huge potential related to various biological functions as they are known to be vital and beneficial activities to man. This include free radical scavenging activity and mitigation of reactive oxygen species.

The result in table 2 reveals the ability of the *P. americana* leaf to inhibit DPPH free radicals which was presented in percentage. This is probably due to the presence of various phytochemicals and flavonoids, particularly the quercetin and chlorogenic acid as seen in table 1. Boots *et al.* (2008) and Maalik *et al.* (2014) had reported that quercetin is capable of scavenging reactive oxygen species, this was seen to be in tandem with this research work. The free radical scavenging activity *P. americana* could be attributed to the presence of quercetin. Furthermore, quercetin acting as free radical scavengers was shown to exert a protective effect in reperfusion ischemic tissue damage (Fraga *et al.*, 1987; Halliwell, 1994; Santos *et al.*, 1998; Lakhanpal and Rai, 2007). Quercetin prevents free radical induced tissue injury by various ways. One way is the direct scavenging of free radicals. By scavenging free radicals, Flavonoid; particularly quercetin can inhibit LDL oxidation in vitro (Kerry *et al.*, 1997; Lakhanpal and Rai, 2007). This action protects against atherosclerosis. In addition, polyphenolic compounds in general (including chlorogenic and caffeic acid) are reputed to inhibit carcinogenicity by scavenging and trapping potentially DNA-damaging electrophiles, free radicals, and toxic metals (Friedman and Smith, 1984; Tanaka *et al.*, 1993; Tanaka, 1994; Friedman, 1997).

The result in table 3 shows the richness of *P. americana* leaf with flavonoid and phenolic compound which are vital for various for maintaining cellular wellbeing. The result reveals that quercetin and chlorogenic acid. Quercetin has shown to be anticancer because it inhibits cell proliferation and cellular senescence (Russo *et al.*, 1999; Lamson and Brignall, 2000; Zamin *et al.*, 2009). This could imply that *P. americana* leaf could be considered for treating cancerous cells since it is rich in this important flavonoid. Furthermore, quercetin can induce cell death and cell cycle arrest in cancer cells through the downregulation of

oncogenes, including Mcl-1, Bcl-2, Ras, MEK, and PI3K, or the upregulation of tumor suppressor genes, including p53 and p21 (Iwao and Tsukamoto, 1999; Sharma *et al.*, 2005; Lim *et al.*, 2007; Spagnuolo *et al.*, 2011). This further speaks on cancer reversing ability of quercetin which is present in the extract of the plant material of discourse. Quercetin has been said to have pharmacological potentials in antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, hepatoprotective, protective of the reproductive and ant-obesity effect (Maalik *et al.*, 2014).

One other phytochemical detected in a high amount is the chlorogenic acid. This particular is known for its various biological importance. The presence of this phenolic speaks to the ability of this plant material to treat disease that chlorogenic acid can treat. However, this is in no way claiming that the whole plant material can this. It is also worthy to note that this material may contain other chemical substance that may be detrimental to health which is a subject of further research. According to Nguyen *et al.* (2024), the pharmacological effects of chlorogenic acid may include the following anti-inflammatory and anti-oxidation, glucose and lipid metabolic homeostasis modulation, cardiovascular protective effect, mitigative effects on diabetes mellitus, hepatoprotection, neuroprotection, anticancer effect, skin protection, antiviral and antimicrobial effects among others.

The table 4 result shows various vitamins present in the methanol leaf extract of *P. americana*. The result reveals that three vitamins are present; these are vitamin K, vitamin B1 (Thiamine) and vitamin B3 (Niacin). 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. Though can be synthesized from tryptophan in the body, vitamin B3 deficiency could cause a disease condition known as pellagra. The deficiency of the various vitamins could be detrimental and lead to life-threatening conditions. There arises the need to supplement and the plant extract has shown to be an important source of these vitamins.

The result for amino acid profile in table 5 reveals that five amino acids are present in the leaf methanol extract of *P. americana*. These are asparagine, threonine, histidine,

phenylalanine and aspartic acid. Out of the five amino acids, histidine and threonine are all considered essential, that is, they are not synthesized within the body of man and must be supplied in diet. The other two can be synthesized within the body and are considered non-essential. Since these important amino acids are rich in this plant material, it is imperative to consider using this plant material as a source of the essential amino acids in supplements.

CONCLUSION

The research investigated the total flavonoid, total phenolics, free radical scavenging activities of the methanol leaf extract of *P. americana* as well as the flavonoids and phenolics profile, vitamin profile and amino acid profile. The result obtained showed a significant amount of each of the parameters. The flavonoid and phenolics contents show a significant amount of each which was indicative of the suitability of the extract in performing various functions associated with such compounds. The DPPH scavenging activity shows a progressive increase in the plant extract in the inhibition of DPPH activity as concentration of the extract was increased. The flavonoid and phenolics profiles revealed that quercetin and chlorogenic acid were present confirming the effects seen in the DPPH inhibition. The vitamin profile was seen to be significant for vitamin K, B1 and B3. The amino acid profile revealed that various essential amino acids were present, this is indicative of the effectiveness and potential of this plant extract to serve as a functional food.

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