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The Comparative Suitability of Different Solvents Used in the Fractionation of Methanol Leaf Extract of *Persea americana* Mill: An *in Vitro* Studies

Ojochenemi E. Yakubu[1], Olawale Otitoju[2], Janya Danjuma[3], Abdulrashid Dauda Maianguwa[4], Isaac John Umaru[5], Gloria Omonefe Oladele[6]

[1,2,3,4,5]Federal University Wukari, Taraba State, Nigeria

[6]Federal Medical Centre Bida, Nigeria State, Nigeria

janyadanjuma2016@gmail.com

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Abstract

This research investigated the suitability of various solvents used in the fractionation of methanol leaf extract of *P. americana* to ascertain the flavonoid, vitamin and amino acid profile of the fractions. It has been demonstrated that a number of *Persea americana* plant components contain different phytochemicals associated with important biological function. Fresh leaves of *P. americana* were harvested from Baissa in Taraba State. The plant materials were air dried under shade at ambient temperature. The dried plant materials were pulverized to fine powder using mortar and pestle. The pulverized plant material was soaked in methanol for 72 hours for extraction. The extract was filtered using clean filter cloth and filter paper under reduced pressure. The filtrate was evaporated in in rotary evaporator and water bath to obtain the required concentrate. The extract was used to perform fractionation using separation funnel and solvents of different polarity such n-hexane, methanol and ethanol. Each of the fractions was used to analyze for flavonoid, vitamin and amino acid profile using HPLC. The result reveals that quercetin is present

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in each of the fractions. Caffeic acid phenyl ether was detected in diluted ethanol fraction, coumaric acid was identified in diluted n-hexane fraction, caffeic acid was identified in methanol fraction chlorogenic acid detected in n-hexane fraction. Vitamin K was identified in all the fractions, Vitamin B1 was identified in diluted ethanol fraction and n-hexane fraction while vitamin B9 was detected in diluted n-hexane fraction and n-hexane fraction. Vitamins B2 and B6 were identified in methanol fraction and diluted ethanol fraction respectively. The diluted ethanol fraction was seen to contain 8 different amino acids followed by the methanol fraction with 6, then the diluted n-hexane fraction with 3 and the n-hexane fraction with 2. This reveals that for isolation of amino acids, the ethanol fraction may be more suitable considering that the highest number of amino acids were found in it.

Keywords: Fractionation, Flavonoid, Phytochemicals, Extraction, Caffeic acid phenyl ether, Coumaric acid, Caffeic acid, Chlorogenic acid

INTRODUCTION

In the fight against both ancient and modern illnesses that have resisted numerous conventional pharmaceuticals, medicinal plants have given humanity hope. Mankind was given hope by medicinal plants in the fight against new and old illnesses that have resisted the effects of many conventional medications (Amaza *et al*., 2016). Because plant constituents including carbohydrates, lipids, protein, vitamins, and minerals are also components of body composition, medicinal plants are thought to be safer and superior to manufactured medications (Amaza *et al*., 2016). Eighty percent of Africans cure themselves with medicinal plants from traditional medicine due to cultural and economic factors (WHO, 2002; Kouamé *et al*., 2019).

In addition to their health advantages, there has been a false belief that these therapeutic herbs are completely risk-free and have no negative health effects (Larrey, 1994; Ernst, 2005). However, because to their unclear safety profiles and numerous well-publicized medicinal values that have not undergone scientific evaluation, they have been rejected (Ernst, 2005). Therefore, it is important to carry out safety evaluations on natural compounds for which specific medical applications have received scientific validation.

The tree under study, *Persea americana*, is native to Central and South America, although it is also grown in some parts of Asia, the United States, Tropical Africa, and Europe (Morton, 1987; Adisa *et al*., 2011). It belongs to the *Lauraceae* family, which comprises about 50

genera and roughly 3000 species, and the class Magnoliopsida (Schaffer *et al.*, 2013; Integrated Taxonomic Information System, 2018).

According to ethnobotanical surveys, several components of this plant, including the leaves, bark, fruit, and stone, are used either by themselves or in conjunction with other plants to treat ailments like headaches, rheumatism, toothaches, and skin conditions (Quattrocchi, 2012; Rosas-Piñón *et al.*, 2012). It has been demonstrated that a number of *Persea americana* plant components contain vasorelaxant qualities that lessen vasoconstriction, which lowers blood pressure (Owolabi et al., 2005; Ojowole *et al.*, 2007). *P. americana* is utilized in African traditional medicine to cure and manage a wide range of human conditions, such as epilepsy and convulsions in children (Ojewole and Amabeoku, 2006).

Traditional medicine practitioners from the Ibibio tribe in Southern Nigeria have stated that the leaves have effective anti-tussive, antidiabetic, and anti-arthritic properties. There have also been reports of the leaves' analgesic qualities (Anitia *et al.*, 2005). Aqueous and hydroethanolic extracts of *P. americana* leaves have been shown to exhibit antihypoglycemic effect on a type 1 diabetic Wistar rat model caused by streptozotocin and alloxan (Anitia *et al.*, 2005; Lima *et al.*, 2012). Because of a component fatty acid derivative known as persin, the leaves are toxic to animals when eaten raw (Oelrichs *et al.*, 1995; Brai *et al.*, 2014). It has been demonstrated that the leaves' persin is poisonous to silkworms and has anti-fungal qualities (Oelrichs *et al.*, 1995; Brai *et al.*, 2014).

People chew the leaves to treat pyorrhea, and the leaves' aqueous extract has a long-lasting hypotensive effect (Brai *et al.*, 2014). The fruit's leaves, bark, seeds, and skin are all poisonous, and the dried leaves retain their toxicity. Mammals fed dried leaves in an experiment had mammary gland cell death, a clinical result frequently seen in livestock (goats and calves) reported to have consumed sections of the avocado plant (Clipsham, 2007).

MATERIALS AND METHODS

Materials

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, Surgifield rotary evaporator (SM-5286A), Sykam HPLC (S3250 UV/visible detector).

Reagents/Chemicals

n-hexane, absolute ethanol, methanol, water. 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.

Fractionation of Methanol Leaf Extract

The methanol leaf extract of *Persea american* was be subjected to fractionation using separating funnel and solvent of different polarity. The extract was dissolved using methanol and poured into the separating funnel, thereafter, n-hexane was added and shaken until two clear layers were formed. The lower portion was the methanol fraction and was removed first through the tap while the n-hexane fraction was collected thereafter. The two fractions were collected in separate conical flasks and labelled appropriately. The same procedure was repeated with ethanol in place of n-hexane.

RESULTS

Table 1: Flavonoid profile for diluted ethanol fraction of methanol leaf extract of *P. americana*

Flavonoid	Amount (ppm)	Amount (%)	Retention time (min)
Quercetin	5.621	0.80	1.768
caffeic acid phenyl ether	716.923	99.20	2.277

The result in table 1 for diluted ethanol fraction of methanol extract of leaf of *P. americana* reveals that quercetin and caffeic acid phenyl ester were detected at 0.8% (5.62 ppm) and 99.2% (716.92 ppm) respectively.

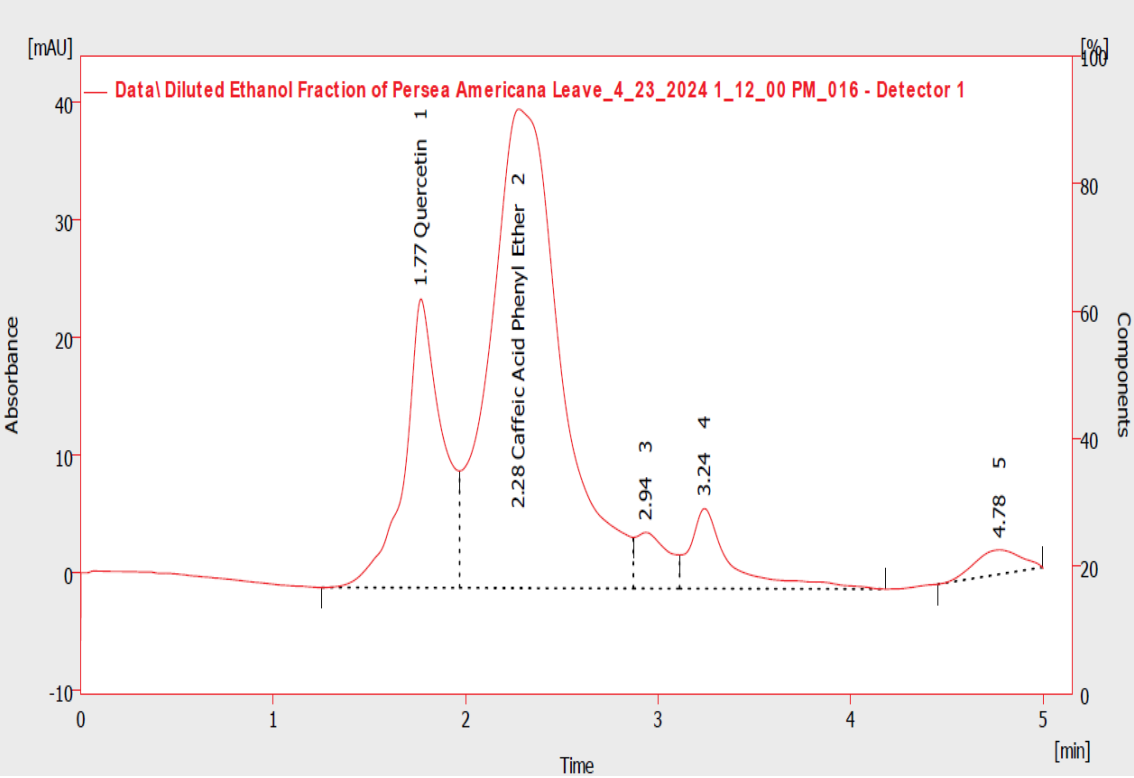

Figure 1: Chromatogram for diluted ethanol fraction of methanol leaf extract of *Persea americana* flavonoid profile

Table 2: Vitamin profile for diluted ethanol fraction of methanol leaf extract of *P. americana*

Vitamins	Amount (mg)	Amount (%)	Retention time (min)
Vitamin K	1.097	1	1.710
Vitamin B1	90.375	84.4	2.483
Vitamin B6	15.642	14.6	2.790

The result in table 2 reveals that vitamins K, B1 and B6 were shown to be present in the diluted ethanol fraction of methanol leaf extract of *P. americana*. The vitamin B1 detected was seen to be highest with 84.4% (90.38 mg) whereas vitamin K was least with 1.0% (1.10 mg).

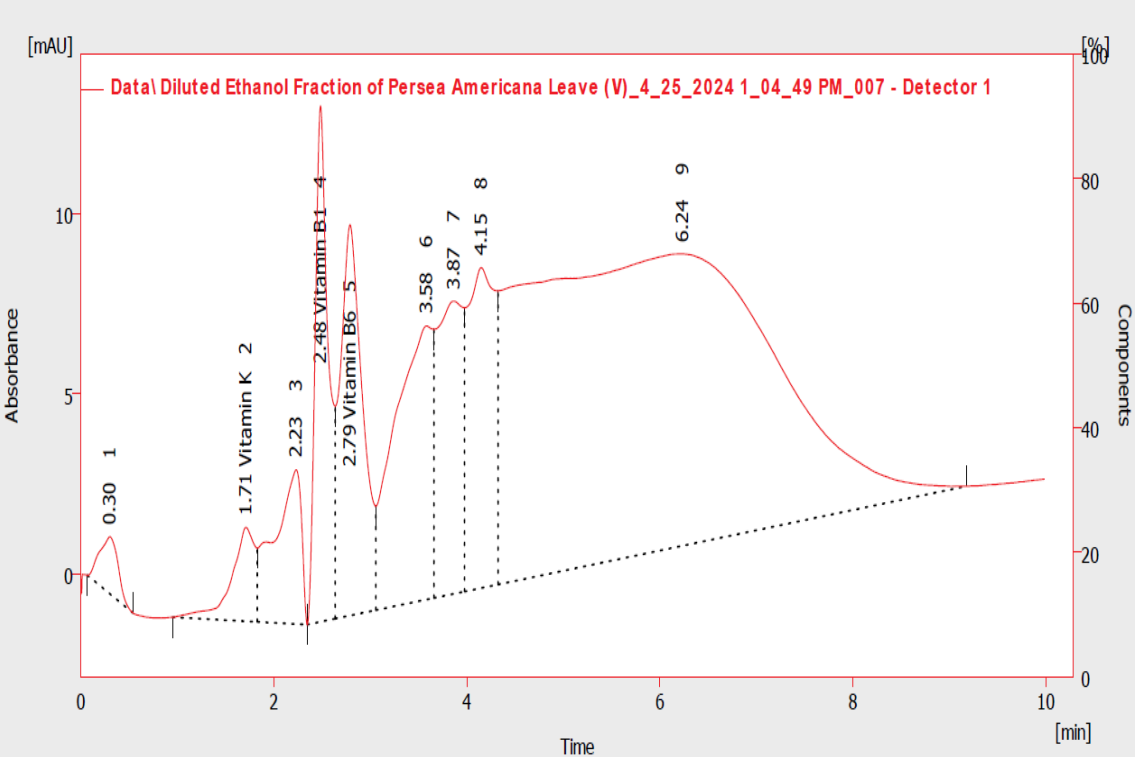

Figure 2: Chromatogram for diluted ethanol fraction of methanol leaf extract of *Persea americana* vitamin profile.

Table 3: Amino acid profile for diluted ethanol fraction of methanol leaf extract of *P. americana*

Amino acids	Amount (µL)	Amount (%)	Retention time (min)
Asparagine	11.054	0.6	0.323
Threonine	114.257	6	1.948
Serine	21.12	1.1	2.488
Alanine	207.467	10.9	2.867
Cysteine	33.601	1.8	3.578
Leucine	66.544	3.5	3.857
Isoleucine	66.174	3.5	4.138
Glutamine	1390.261	72.8	6.125

The result in table 3 above for diluted ethanol fraction of methanol leaf extract of *P. americana* reveals that there are eight different amino acids present in the fraction. These are asparagine, threonine, serine, alanine, cysteine, leucine, isoleucine and glutamine at different percentage abundance. Glutamine is seen to have the highest percentage of abundance with 72.8% (1390.26µL) while asparagine is having least abundance with 0.6% (11.05µL).

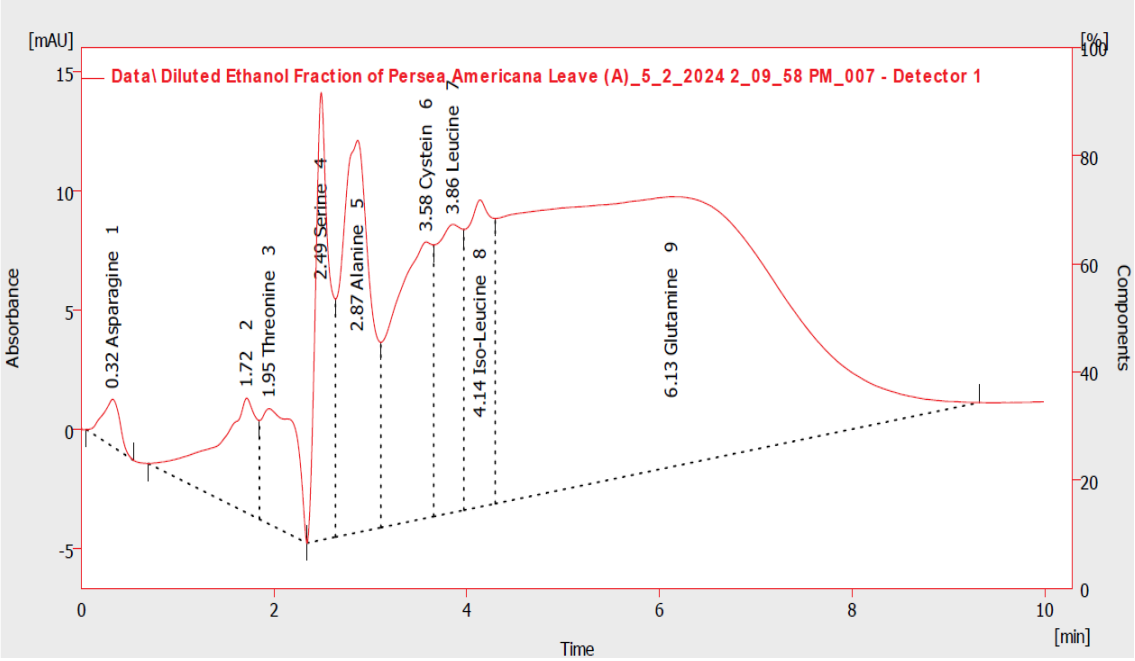

Figure 3: Chromatogram for diluted ethanol fraction of methanol leaf extract of *Persea americana* amino acid profile

Table 4: Flavonoid profile for diluted n-hexane fraction of methanol leaf extract of *P. americana*

Flavonoid	Amount (ppm)	Amount (%)	Retention time (min)
Quercetin	1.925	1.80	1.767
Coumaric acid	102.221	98.20	2.387

The result in table 4 for diluted n-hexane fraction of methanol extract leaf of *P. americana* reveals that quercetin and coumaric acid were detected at 1.86% 1.93 ppm) and 98.2% (102.22 ppm) respectively.

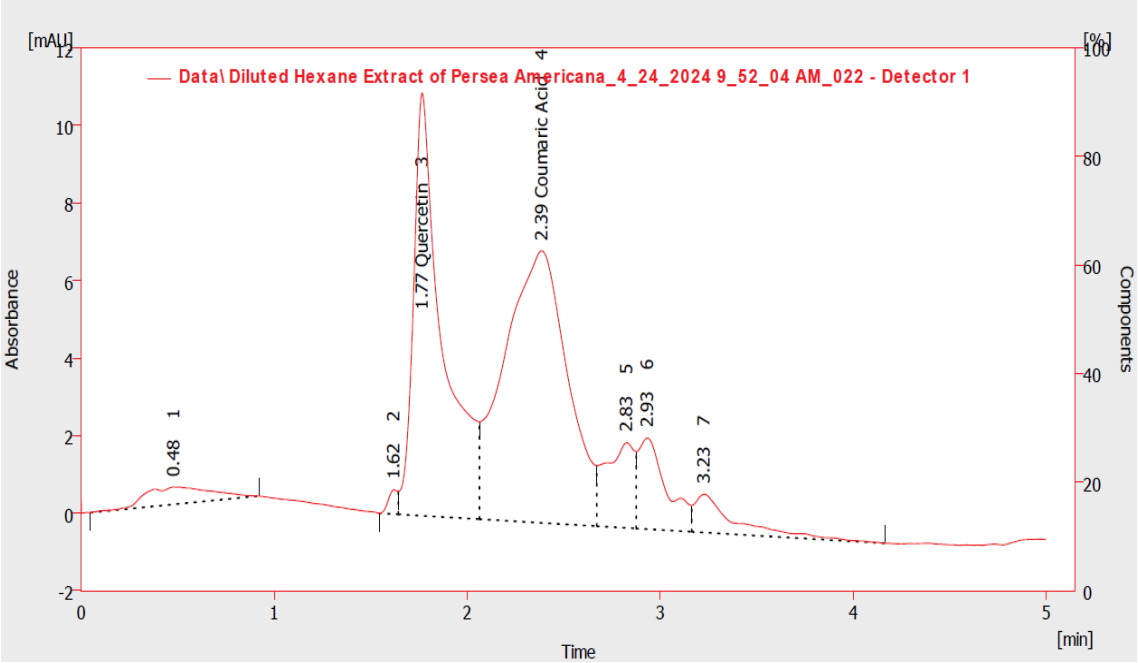

Figure 4: Chromatogram for diluted n-hexane fraction of methanol leaf extract of *Persea americana* flavonoid profile

Table 5: Vitamin profile for diluted n-hexane fraction of methanol leaf extract of *P. americana*

Vitamins	Amount (mg)	Amount (%)	Retention time (min)
Vitamin K	2.9	10.20	1.848
Vitamin B9	25.527	89.80	2.892

The result in table 5 reveals that vitamins K, B1 and B3 were shown to be present in the diluted n-hexane fraction of methanol leaf extract of *P. americana*. The vitamin B9 detected was seen to be higher with 89.8% (25.53 mg) whereas vitamin K was least with 10.2% (2.90 mg).

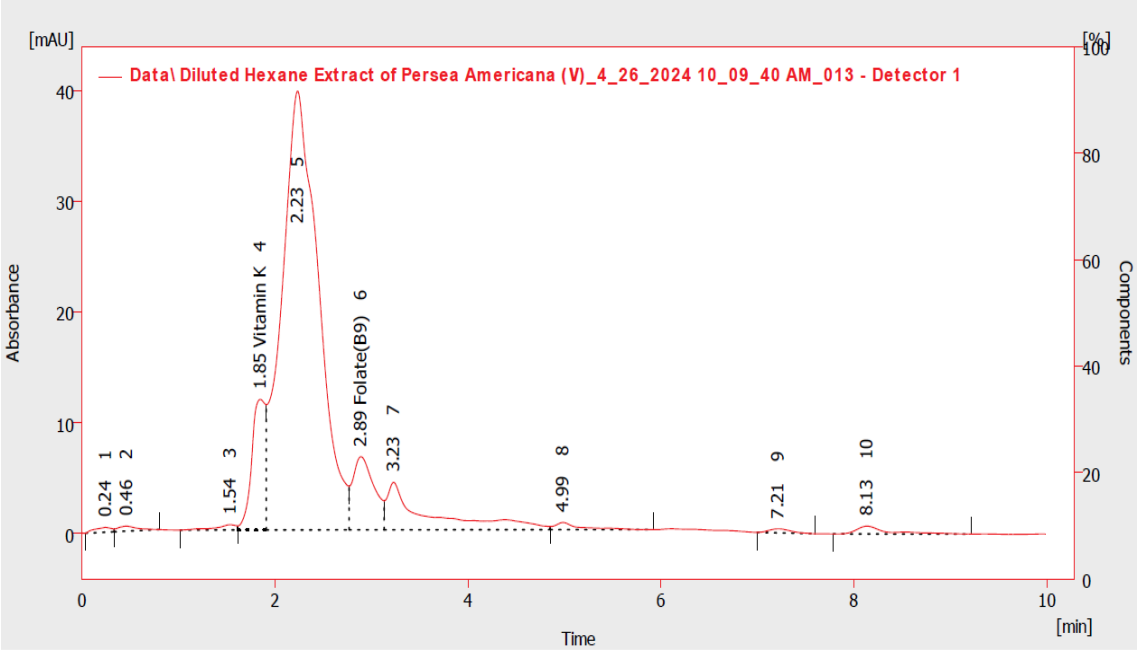

Figure 5: Chromatogram for diluted n-hexane fraction of methanol leaf extract of *Persea americana* vitamin profile

Table 6: Amino acid profile for diluted n-hexane fraction of methanol leaf extract of *P. americana*

Amino acids	Amount (µL)	Amount (%)	Retention time (min)
Methionine	4.221	4	1.990
Serine	73.312	69	2.452
Tyrosine	28.719	27	2.882

The result in table 6 above for diluted n-hexane fraction of methanol leaf extract of *P. americana* reveals that there are three different amino acids present in the fraction. These are methionine, serine and tyrosine in different percentage. Serine is seen to have the highest percentage of abundance with 69.0% (73.31µL) while asparagine is having least abundance with 4.0% (4.22µL).

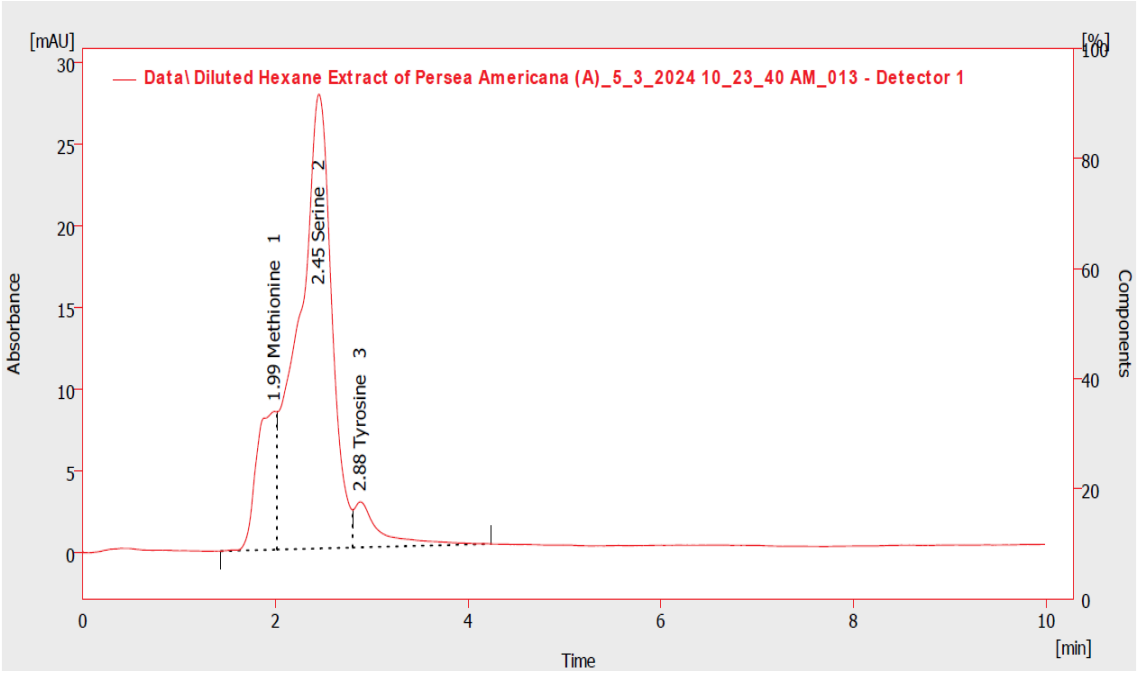

Figure 6: Chromatogram for diluted n-hexane fraction of methanol leaf extract of *Persea americana* amino acid profile

Table 7: Flavonoid profile for methanol fraction of methanol leaf extract of *P. americana*

Flavonoid	Amount (ppm)	Amount (%)	Retention time (min)
Quercetin	4.677	23.00	1.770
Caffeic acid	15.615	77.00	2.253

The result in table 7 for methanol fraction of methanol leaf extract of *P. americana* reveals that quercetin and caffeic acid were detected at 23.0% (4.68 ppm) and 77.0% (15.62 ppm) respectively.

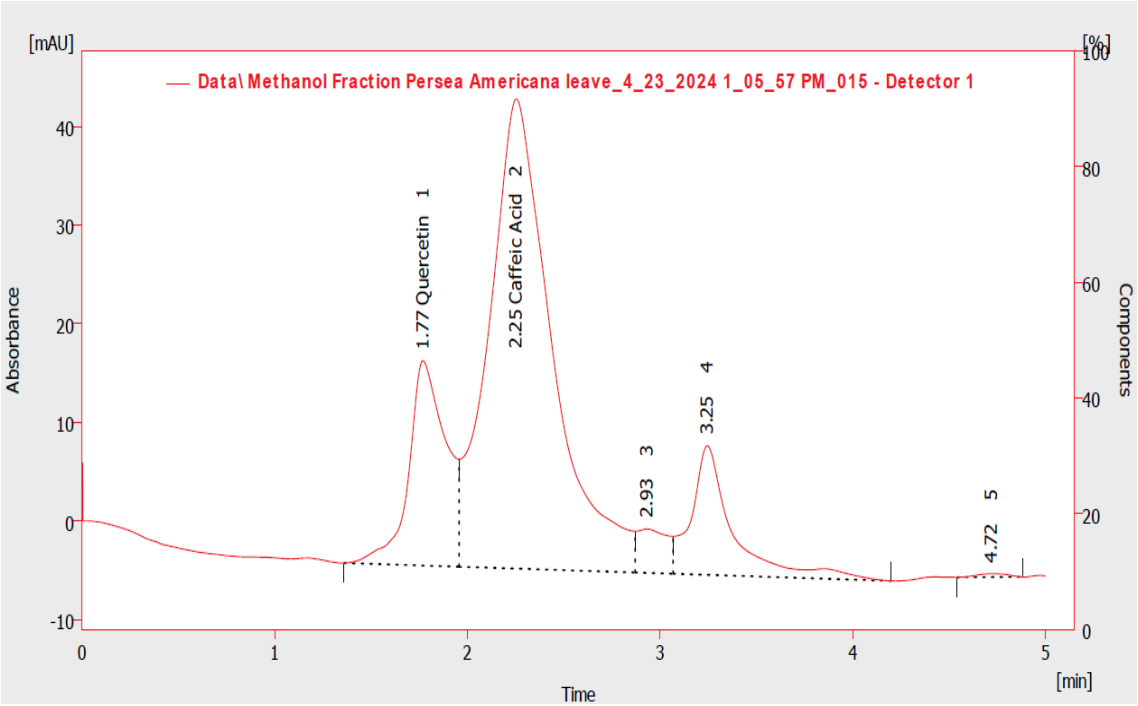

Figure 7: Chromatogram for methanol fraction of methanol leaf extract of *Persea americana* flavonoid profile

Table 8: Vitamin profile for methanol fraction of methanol leaf extract of *P. americana*

Vitamins	Amount (mg)	Amount (%)	Retention time (min)
Vitamin K	9.555	2.50	1.810
Vitamin B2	371.614	97.50	2.937

The result in table reveals that vitamins K and B2 were shown to be present in the methanol fraction of methanol leaf extract of *P. americana*. The vitamin B2 detected was seen to be highest with 97.5% (371.61 mg) whereas vitamin K was least with 2.5% (9.56 mg).

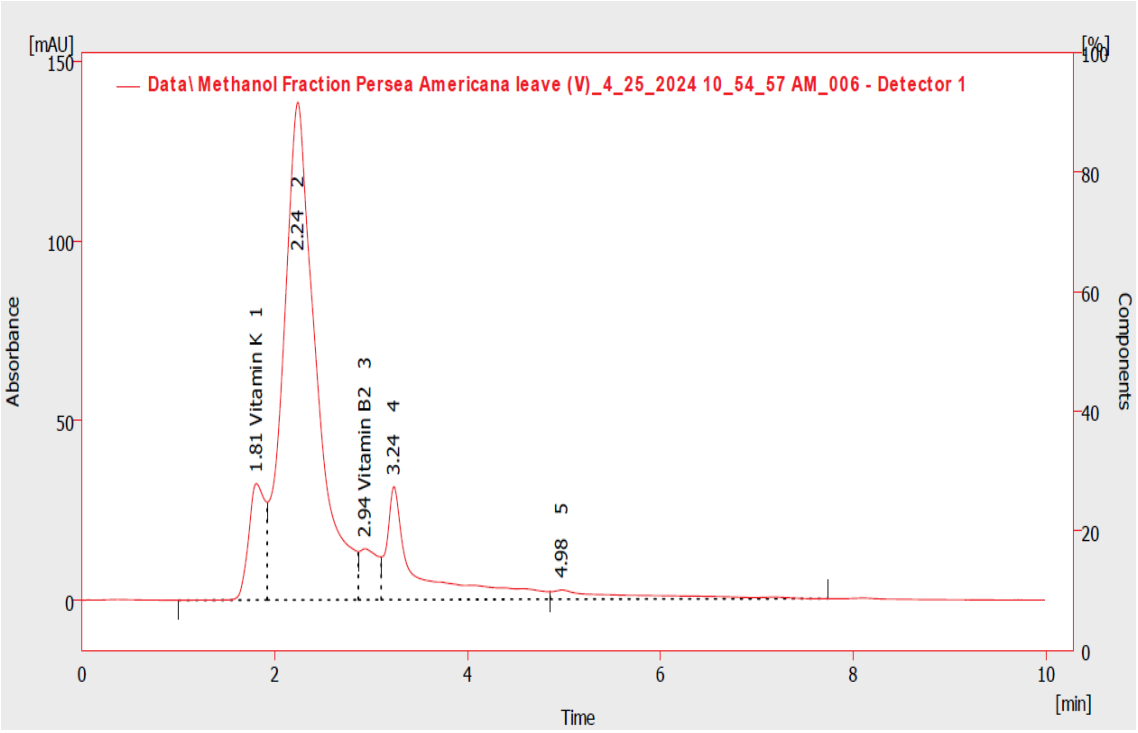

Figure 8: Chromatogram for methanol fraction of methanol leaf extract of *Persea americana* vitamin profile

Table 9: Amino acid profile for methanol fraction of methanol leaf extract of *P. americana*

Amino acids	Amount (µL)	Amount (%)	Retention time (min)
Asparagine	2.768	0.1	0.398
Threonine	478.803	19.2	1.805
Histidine	1427.328	57.2	2.252
Aspartic acid	204.168	8.2	2.947
Glutamic acid	254.297	10.2	3.253
Isoleucine	45.611	1.8	4.078
Glycine	53.182	2.1	4.618
Arginine	28.793	1.2	5.013

The result in table 9 above for methanol fraction of methanol leaf extract of *P. americana* reveals that there are eight different amino acids present in the extract. These are asparagine, threonine, histidine, aspartic acid, glutamic acid, isoleucine, glycine and arginine in different percentage. Histidine is seen to have the highest percentage of abundance with 57.2% (1427.33µL) while asparagine is having least abundance with 0.1% (2.77µL).

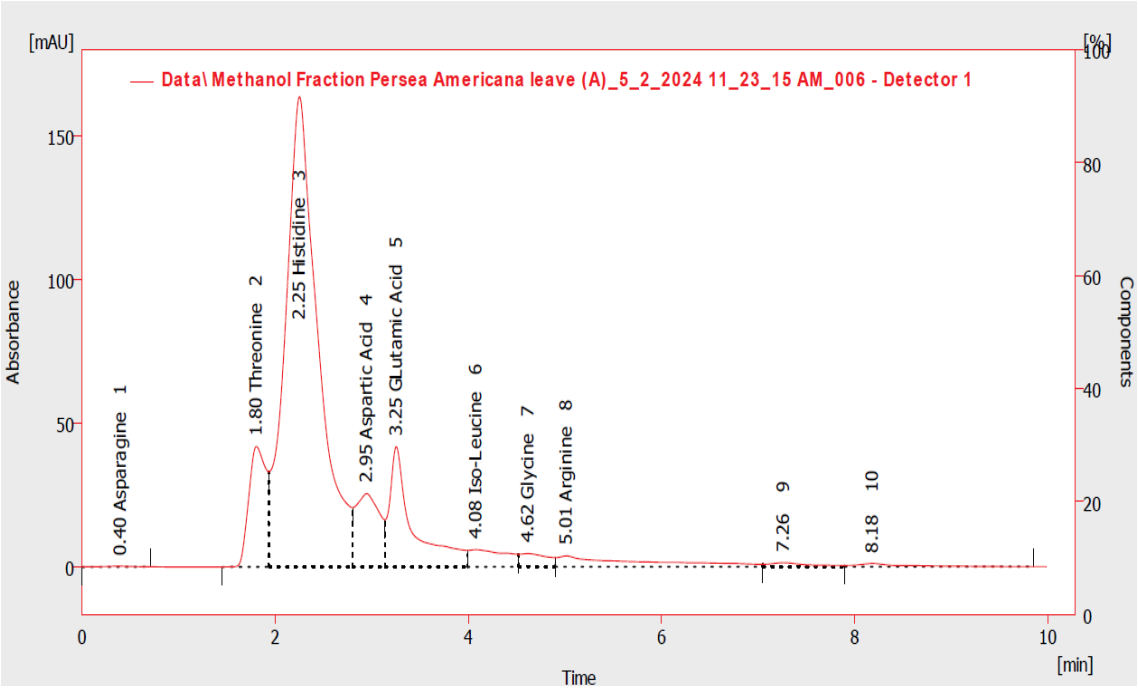

Figure 9: Chromatogram for methanol fraction of methanol leaf extract of *Persea americana* amino acid profile

Table 10: Flavonoid profile for n-hexane fraction of methanol leaf extract of *P. americana*

Flavonoid	Amount (ppm)	Amount (%)	Retention time (min)
Quercetin	2.22	16.10	0.372
Chlorogenic acid	11.57	83.10	2.390

The result in table 10 for n-hexane fraction of methanol extract of leaf of *P. americana* reveals that quercetin and chlorogenic acid were detected at 16.1% (2.22 ppm) and 83.1% (11.57 ppm) respectively.

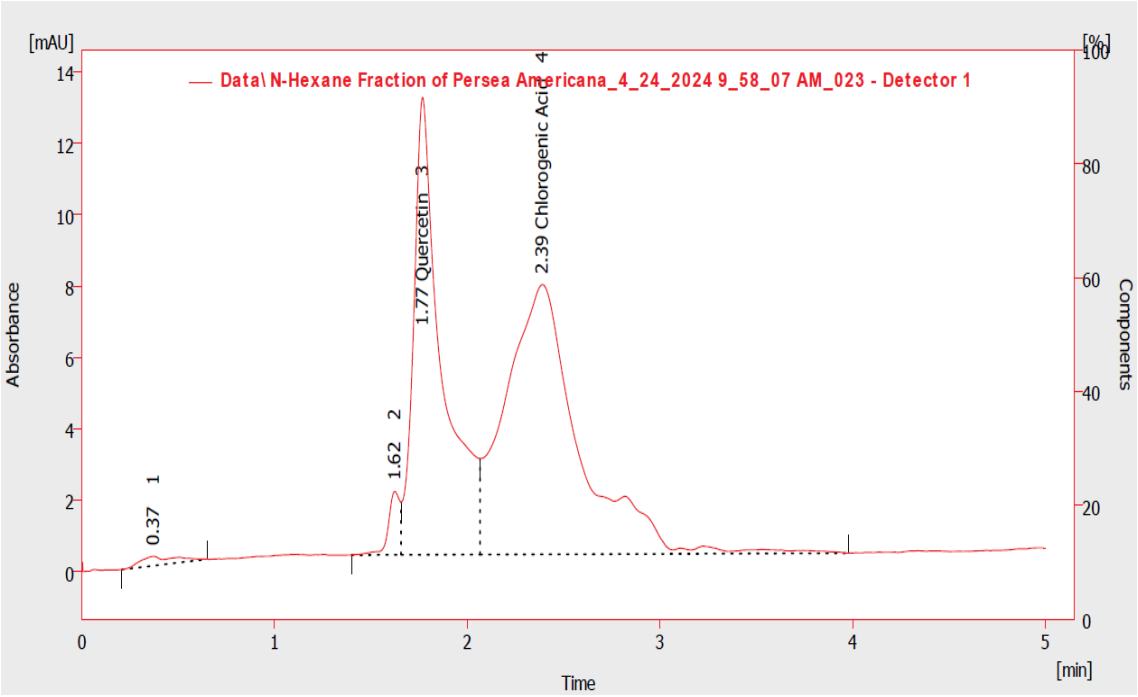

Figure 10: Chromatogram for n-hexane fraction of methanol leaf extract of *Persea americana* flavonoid profile

Table 11: Vitamin profile for n-hexane fraction of methanol leaf extract of P. *americana*

Vitamins	Amount (mg)	Amount (%)	Retention time (min)
Vitamin K	0.188	0.50	1.788
Vitamin B1	17.159	47.70	2.475
Vitamin B9	18.648	51.80	2.897

The result in table11 reveals that vitamins K, B1 and B9 were shown to be present in the n-hexane fraction of methanol extract of leaf of *P. americana*. The vitamin B9 detected was seen to be highest with 51.8% (18.65 mg) whereas vitamin K was least relatively with 0.5% (0.19 mg).

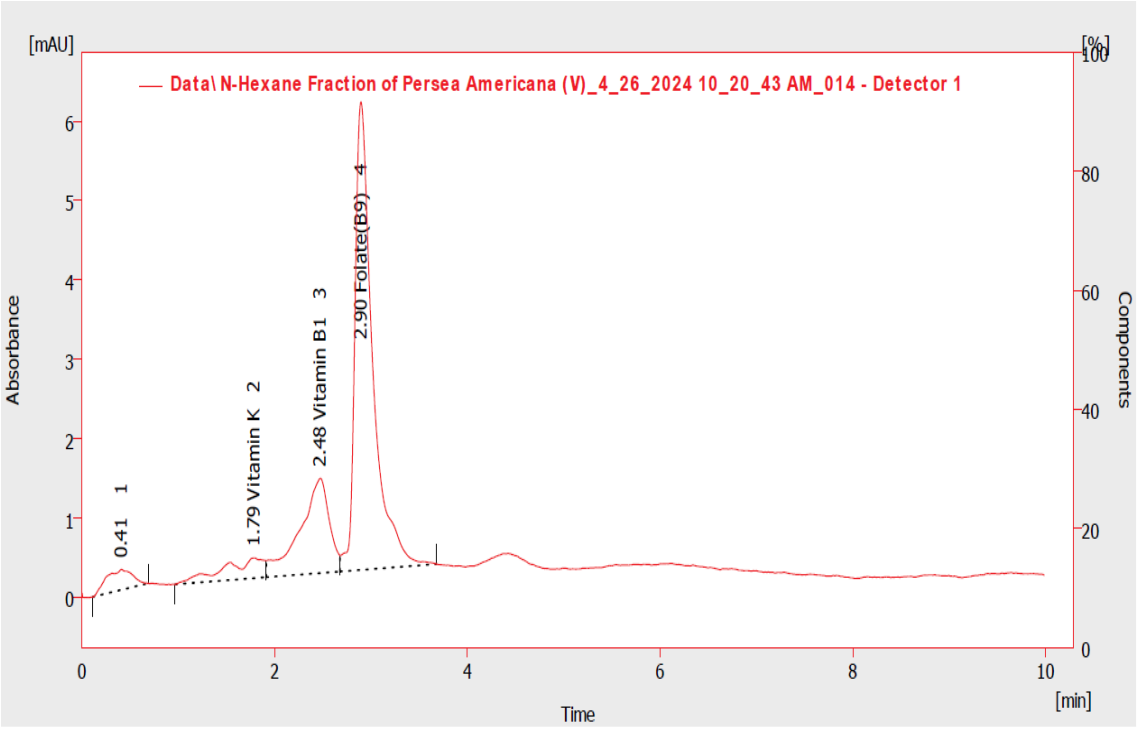

Figure 11: Chromatogram for n-hexane fraction of methanol leaf extract of *Persea americana* vitamin profile

Table 12: Amino acid profile for n-hexane fraction of methanol extract leaf of *P. americana*

Amino acids	Amount (µL)	Amount (%)	Retention time (min)
Serine	1.022	6.5	2.488
Tyrosine	14.633	93.5	2.888

The result in table 12 above for n-hexane fraction of methanol extract of leaf of *P. americana* reveals that there are two different amino acids present in the extract. These are serine and tyrosine in different percentage. Tyrosine is seen to have the higher percentage of abundance with 93.5% (14.63µL) while asparagine is having relatively lower abundance with 6.5% (1.02µL).

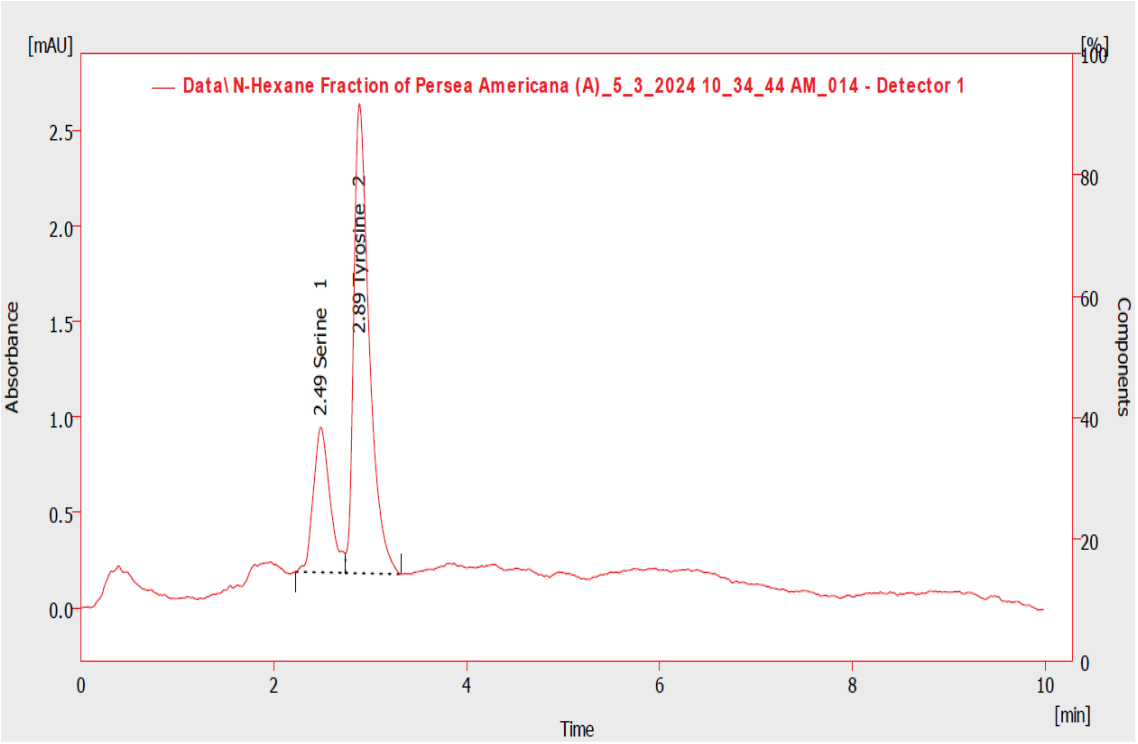

Figure 12: Chromatogram for n-hexane fraction of methanol leaf extract of *Persea americana* amino acid profile

DISCUSSION

The flavonoid profile for diluted ethanol fraction of methanol leaf extract of *P. americana* reveals that quercetin and caffeic acid phenyl ether (CAPE) as shown in table 1 and figure 1. When compared to other results, such as the ones seen in tables 4, 7 and 10 as well as figures 4, 7 and 10, coumaric acid, caffeic and chlorogenic acid were identified respectively in place of CAPE. Quercetin being common to all shows to have variations in the levels of abundance, diluted ethanol fraction shows to possess higher concentration of the phytochemical with 5.62ppm. Methanol fraction, n-hexane fraction and diluted n-hexane fraction trail with 4.68, 2.22 and 1.93ppm respectively.

The CAPE, coumaric acid, caffeic acid and chlorogenic acid have shown to have some relationship, particularly in their synthesis. Caffeic acid (3,4-dihydroxycinnamic acid) and caffeic acid phenethyl ester (CAPE) are natural compounds with antioxidant and anti-inflammatory properties, found in a variety of plants, including coffee, fruits, and vegetables (Murtaza *et al.*, 2014; Espíndola *et al.*, 2019). According to Espíndola *et al.* (2019), the biosynthesis and metabolism of these compounds have been extensively studied for

their potential health benefits. Caffeic acid is produced through the phenylpropanoid pathway in plants, starting with phenylalanine as the key precursor. Phenylalanine is converted to cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL). Cinnamic acid is then converted enzymatically converted to p-coumaric acid by cinnamate 4-hydroxylase (C4H), then p-coumaric acid is converted to caffeic acid by the action of the enzyme 4-coumarate CoA ligase (4CL) (Lin and Yan, 2015). CAPE is not the major component of propolis, but it is one of the most potent antioxidants supplied by the caffeic acid moiety, which has a higher antioxidant capacity than related phenylpropanoids such as ferulic acid and p-coumaric acid (Rice-Evans *et al*., 1996).

Caffeic acid can be metabolized by a number of different mechanisms in the body. The chemical is conjugated with glucuronic acid by the enzyme UDP-glucuronosyltransferase (UGT), and then eliminated in the urine. This is one important mechanism. Sulfation is a different route that results in the molecule being excreted in the urine after being conjugated with sulfate by sulfotransferases (SULTs) (Ehtiati *et al*., 2023). In addition, cytochrome P450 enzymes (CYPs) can break down caffeine into a variety of metabolites, some of which have been demonstrated to have antioxidant and anti-inflammatory properties (Ehtiati *et al*., 2023). CAPE is a by-product of the caffeic acid present in propolis, a resinous product of bees. The enzyme caffeate O-methyltransferase (COMT) catalyzes the esterification of caffeic acid and phenethyl alcohol to produce CAPE (Ehtiati *et al*., 2023). Similar to caffeic acid, CAPE can be metabolized through glucuronidation and sulfation pathways in the body (Gülçin *et al*., 2016). However, CAPE has been shown to be more resistant to metabolism than caffeic acid, with a longer half-life in the body (Wang *et al*., 2009; Islam *et al*., 2016).

The results for vitamins profile of the various fractions used in this study are shown in tables 2, 5, 8 and 11 as well as figures 2, 5, 8 and 11. The result for diluted methanol fraction of methanol leaf extract of *P. americana* shows that vitamin K, vitamin B1 and vitamin B6. Table 5 on the other hand reveals the vitamin profile of the diluted n-hexane fraction of the same extract. It shows that vitamin K and vitamin B9 were seen to be present. In addition, the vitamin profile of the methanol fraction of the extract in table 8 reveals that vitamin K and vitamin B2. The n-hexane fraction of the extract in table 11 shows that vitamins K, B1 and B9. Vitamins are essential substances for the normal functioning and development of the body (Akram *et al*., 2022). Vitamins are also referred as a group of organic nutrients of various nature required in small quantities for multiple

biochemical reactions for the growth, survival and reproduction of the organism, and which, generally, cannot be synthesized by the body and must therefore be supplied by diet. The must prominent function of the vitamins is to serve as coenzyme (or prosthetic group) for enzymatic reactions (Aleksandrova and Rudko, 2016).

Vitamin K which according to Aleksandrova and Rudko (2016) is considered fat soluble if common to all the fractions. The methanol fraction of the methanol leaf extract of *P. americana* shows to possess the highest concentration of vitamin K with 9.56ppm. on the other hand, diluted n-hexane fraction, diluted ethanol fraction and n-hexane fraction of the extract have 2.90. 1.10 and 0.19ppm. The major function of vitamin K is the prothrombin formation in the liver along with other vitamin K dependent clotting factors such as VII, IX, X, protein C and S which are essential for normal blood coagulation or blood clotting. Vitamin K deficiency can result to generalized bleeding, the development of hemorrhagic disease of the newborn, and prolonged clotting time in adults (Akram *et al*., 2022).

Other vitamins found in various fractions were of vitamin B family. Diluted ethanol fraction of the extract was seen to contain vitamin B1 and B6 in addition to vitamin K. In addition, the diluted n-hexane fraction of the extract was seen to contain vitamin B9 beside the vitamin K. The methanol fraction on the other hand was seen to be rich in vitamin B2 in addition to the vitamin K. Furthermore, the n-hexane fraction of the extract was seen to comprise of vitamin B1 and vitamin B9 in addition to vitamin K. Beside the vitamin K, all other vitamins are considered water soluble and are known to exert important biological functions.

Thiamine or vitamin B1 acts as coenzyme known as thiamine pyrophosphate. 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 (Akram *et al*., 2022). Vitamin B2 or riboflavin It is involved in tissue respiration. Its derivatives are FAD (flavin adenine dinucleotide in its oxidized state) and FADH2 (FAD in its reduced form). Ariboflavinosis is characterized by cheilosis (textured desquamation of the skin around the mouth), glossitis (sparkly red and sore tongue), soreness of the lips, eye disturbances and photophobia (light sensibility), oily skin the nose, scrotal dermatitis (Henriques *et al*., 2010), Vitamin B6 or pyridoxine, pyridoxal or pyridoxamine functions as part of coenzyme pyridoxal phosphate in transamination and decarboxylation of amino acids and glycogen

phosphorylase. Deficiency may result to dermatitis of the eyes, nose and mouth (Aleksandrova and Rudko, 2016). Following conversion, vitamin B9 or folate becomes tetrahydrofolate, its active form. It is an essential molecule in the synthesis of nucleic acids (DNA and RNA). Folate deficiency can lead to neural tube defects, thus pregnant women should receive folate supplementations as a preventive method. Folate deficiency can also cause megaloblastic anemia, a type of macrocytic anemia, and prompts a differential diagnosis with vitamin B12 deficiency, which also causes megaloblastic anemia (Ankar and Kumar 2019; Lykstad and Sharma, 2019).

The results for the amino acids profile are shown on tables 3, 6, 9 and 12, the figures 3, 6, 9 and 12 also convey similar information on the various fractions. The amino acid profile of the diluted ethanol fraction reveals that eight different amino acids were detected. These amino acids are asparagine, threonine, serine, alanine, cysteine, leucine, isoleucine and glutamine. Of these amino acids, threonine, leucine and isoleucine are considered essential while asparagine, serine, alanine, cysteine and glutamine are known as non-essential amino acids. These reveals that the amino acids found in the extract are a blend of both essential and non-essential. The result for n-hexane fraction of methanol leaf extract of *P. americana* in table 6 and figure 6 reveals that three different amino acids are present. These amino acids are methionine, serine and tyrosine. With exception of methionine, the other two amino acids are regarded as non-essential. On the other hand, the result for methanol fraction of the extract reveals that six different amino acids are present in the fraction as seen in table 9 and figure 9. These amino acids include asparagine, threonine, histidine, aspartic acid, glutamic acid and isoleucine. Threonine, histidine isoleucine are all indispensable to the human system, hence they are regarded as essential amino acids whereas asparagine, aspartic acid and glutamic acid are all non-essential. Finally, table 12 and figure 12 all show the amino acids profile for n-hexane fraction of the extract. The result reveals that 2 amino acid is present in the fraction as shown in table 12. These amino acids are serine and tyrosine. Both tyrosine and serine are considered non-essential amino acids.

CONCLUSION

The research reveals that various solvents could be used to abstract various components/fractions of a plant extract with different effectiveness. The solvents used in the fractionation process include n-hexane, ethanol, methanol and water which is used for dilution. The result shows that quercetin can be fractionated out with all the solvents used in this research. On the other hand, different other phytochemicals were seen to be fractionated out by different solvents. Diluted ethanol was seen to isolate caffeic acid phenyl ester, diluted n-hexane fractionated out coumaric acid, caffeic acid was detected in methanol fraction and chlorogenic acid was present in n-hexane fraction. Vitamin K was identified in all the fractions, Vitamin B1 was identified in diluted ethanol fraction and n-hexane fraction while vitamin B9 was detected in diluted n-hexane fraction and n-hexane fraction. Vitamins B2 and B6 were identified in methanol fraction and diluted ethanol fraction respectively. The diluted ethanol fraction was seen to contain 8 different amino acids followed by the methanol fraction with 6, then the diluted n-hexane fraction with 3 and the n-hexane fraction with 2. This reveals that for isolation of amino acids, the ethanol fraction may be more suitable considering that the highest number of amino acids were found in it.

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