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ARTICLE

Phytochemical Analysis, Antioxidant and Acetylcholinesterase Inhibitory Activities of Fruit Pulps of Selected Banana Varieties

Dedunu S. Senarathne[a], Anoja P. Attanayake[b] and Isurika R. Fernando[a*]

[a] *Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka.*
[b] *Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle 80000, Sri Lanka.*

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Abstract: This study focused on qualitative and quantitative analysis of phytochemicals and the study of antioxidant and acetylcholinesterase (AChE) inhibitory activities of fruit pulps of three abundant banana varieties grown in Sri Lanka. Aqueous, ethanol, ethyl acetate and hexane extracts of nearly three-month-old fruit pulps of *Musa accuminata* AAA, *Musa accuminata* AAB and *Musa balbisiana* ABB were prepared by shaking fresh, chopped banana pulps in the above-mentioned solvents separately. The standard spectrophotometric methods were used to quantify the alkaloids, flavonoids, condensed tannins and phenolic compounds contents of four solvent extracts of each banana variety. Among them, the ethanol extract of *M. accuminata* AAB possessed the highest total alkaloid content, while the ethanol extract of *M. balbisiana* ABB exhibited the highest total flavonoid content, total condensed tannin content and total phenolic content. The antioxidant and AChE inhibitory activities of each banana variety were determined using standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ellman's colorimetric assays, respectively. The ethanol extracts of *M. accuminata* AAB and *M. balbisiana* ABB demonstrated the highest antioxidant activity and AChE inhibitory activity, respectively. This reporting study revealed that *M. balbisiana* ABB and *M. accuminata* AAB could be recommended as promising sources of edible natural antioxidants and AChE inhibitors, respectively.

Keywords: Acetylcholinesterase inhibitory activity; Antioxidant activity; *Musa accuminata* AAA; *Musa accuminata* AAB; *Musa balbisiana* ABB.

Introduction

Banana is an economically important agricultural fruit crop that is grown in over 130 countries worldwide, mostly in the tropical and subtropical regions of the globe[1,2]. The evergreen perennial banana plant belongs to the family Musaceae of the genus *Musa* which constitutes one of the most popular edible raw fruits worldwide, sweet banana, and non-edible raw fruit which requires processing before consumption, plantain[1,3–5]. The edible *Musa* spp., sweet banana and plantain, also called dessert banana and cooking banana, respectively, are domesticated from the wild *Musa* spp.[4]. The various plant parts of edible and wild *Musa* spp. are enriched with phytochemicals that exhibit important biological activities, including antioxidant and acetylcholinesterase (AChE) inhibitory activities. Phytochemical screening of pseudostem of *M. acuminata* grown in Nigeria evidenced the presence of alkaloids, flavonoids, saponins, phenols and tannins[6]. Furthermore, Fatemeh et al.[7] analyzed the effect of ripening on the flavonoids and phenolic compounds in pulp and peel flour of two of the most popular

[*]**Corresponding Author:** Isurika R. Fernando **Email**: isurika.fernando@sjp.ac.lk

banana varieties in Asia, *M. acuminata* L, cv cavendshii and *M. acuminata* Colla. AAA, cv 'Berangan' along with an evaluation of their antioxidant properties[7]. Moreover, in 2016, Siji et al.[8] quantified alkaloids, tannins, flavonoids, phenolic compounds and saponins in the fruit pulp of five wild varieties of *M. accuminata* Colla and an edible variety, *M. accuminata* Cavendishii (AAA). In 2017, Ayoola et al.[9] reported the quantification of phenolic compounds and flavonoids, as well as the evaluation of antioxidant and AChE inhibitory activities of fruit pulp of a few varieties of *M. acuminata*. Among plantains, *M. paradisiaca*, which is commonly known as French plantain, is widely investigated for the phytoconstituents in various parts of the plant and their biological properties. Phytochemical screening, profiling and evaluation of the antioxidant potential of inflorescence of *M. paradisiaca* in four different solvents confirmed the presence of alkaloids, glycosides, steroids, saponins, tannins, flavonoids and terpenoids in various quantities with potent antioxidant activity[10]. Additionally, the presence of alkaloids, flavonoids, tannins, steroids and saponins in varying quantities was reported in the stalk and peel of *M. paradisiaca*[11,12]. Moreover, the fruit pulp of *M. paradisiaca* grown in India was reported to have alkaloids, tannins, flavonoids, phenolic compounds and saponins[8]. Most importantly, the fruit pulp of *M. paradisiaca* possesses antioxidant activity along with AChE inhibitory activity[13–15]. Among the different plant parts of various *Musa* spp. analyzed, the fruit pulps of bananas constitute important plant secondary metabolites that exhibit antioxidant activity along with the AChE inhibitory activity[9,13–15]. Therefore, the fruit pulps of bananas provide numerous health benefits to human beings while being a potential natural source to treat neurodegenerative diseases including Alzheimer's[15].

A non-communicable disease, Alzheimer's, is the most prevailing progressive and irreversible neurological brain disorder among the elderly community; it causes short-term memory loss leading to difficulties associated with the daily routine of patients[16,17]. Although the precise cause of Alzheimer's is uncertain, cholinergic hypothesis-based Alzheimer's management is focused on the inhibition of one of the over-activated key enzymes in neurotransmission, AChE[17,18]. The oxidative stress hypothesis-based management of Alzheimer's disease is emphasized on the inhibition of generation and existence of oxidative stress products which cause neurofibrillary tangles and consequently, brain neuronal cell destruction and memory loss[19,20]. Therefore, after considering the above facts, it is worthwhile to explore the edible sources of antioxidant-rich potential AChE inhibitors and incorporate those edible sources to the dietary regimes of the elderly community as a preventive measure for those who are at risk for developing Alzheimer's disease, and as a treatment for those who have Alzheimer's disease.

The geographical diversity of Sri Lanka provides perfect soil and weather conditions for the growth of 29 different banana varieties which fruit abundantly all year long while making a range of banana varieties available to consumers at an affordable price throughout the year[21–23]. However, a systematic study on phytochemicals, nutritional and medicinal values of fruit pulps of *Musa* spp. as well as *Musa* varieties grown in Sri Lanka is still in its infancy. Therefore, this research project focused on qualitative and quantitative analysis of phytochemicals and the study of antioxidant activity and AChE inhibitory activity followed by a correlation analysis of four solvent extracts of the fruit pulps of three abundant sweet banana varieties grown in Sri Lanka; namely *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB, which are commonly known as Gros Michel banana, sour banana and sugar banana, respectively. This study is the first systematic study on qualitative and quantitative analysis of phytoconstituents along with the evaluation of antioxidant and AChE inhibitory activities of fruit pulps of the above-mentioned three banana varieties grown in Sri Lanka to date to the best of our knowledge. Subsequently, the findings of this reporting study provide a fundamental understanding and a proper direction for the bioassay-guided isolation of AChE inhibitors to treat Alzheimer's disease, thereby benefiting the food, pharmaceutical and nutraceutical industries.

Materials and Methods

Chemicals and equipment

All chemicals, reagents and solvents were of analytical grade and were used as received from chemical suppliers without any further purifica-

tion, unless otherwise mentioned. Water and ethanol were used after being distilled.

Thermostated water bath shaker table (Nickel-Electro Ltd., England), centrifuge machine (HERMLE Labortechnik, GmbH, Z 206 A), analytical balance (Precisa Instruments Ltd., max: 200 g, min: 0.0001 g), pH meter (Eutech Instruments, pH 700), rotary evaporator (Heidolph, Laborota 4000), freeze dryer (Ilshin Biobase) and stirrer hotplate (Labtech, LMS-1003) were used to carry out this research project. UV-visible spectrophotometer (Labomed Inc., UVD-2960) was used in the quantitative determination of phytochemicals and the evaluation of antioxidant activity, while UV-visible spectrophotometer (SHIMADZU, UV-1800) was used in the Ellman's colorimetric assay.

Collection of Banana Fruit Samples and Preparation of Solvent Extracts

Nearly three-month-old unripe fruits of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB were collected from geographical coordinates of 6°18'27.0"N 80°50'45.9"E, 6°18'20.7"N 80°50'48.1"E and 6°18'24.6"N 80°50'56.5"E, respectively, in Embilipitiya area, Sabaragamuwa province, Sri Lanka, during the month of August, 2019. Plant specimens of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB with voucher specimen numbers 2085, 2086 and 2087, respectively, were identified to their variety level by a research officer at the Division of Pharmaceutical Botany in Bandaranaike Memorial Ayurvedic Research Institute, Navinna, Western province, Sri Lanka.

Extraction of secondary metabolites was carried out as stated in the literature[24] with minor modifications as follows for fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB using water, ethanol, ethyl acetate and hexane as solvents separately.

The manually peeled-off fruits of banana were cut into small pieces and chopped using a mortar and pestle. A weight of approximately 30.0 g of chopped fruit pulp of banana was exhaustively extracted into a 90.0 mL of solvent by shaking in a thermostated water bath at a room temperature of 30 ^{o}C. After 16 h of shaking, the extract was decanted, and the decanted solution was centrifuged for 10 min. The resulting supernatant was carefully decanted. The decanted ethanol, ethyl acetate and hexane extracts were separately concentrated using a rotatory evaporator at 50 ^{o}C to obtain a semisolid substance with a constant weight. The decanted aqueous extract was lyophilized using a freeze drier. The completely dried solvent extracts of fruit pulps of bananas were stored at 4 ^{o}C until further use.

The experimental procedure explained above was repeated for the fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB using water, ethanol, ethyl acetate and hexane as solvents separately. This experiment was triplicated and the average of the extractable yield was reported.

Qualitative and Quantitative Analysis of Phytochemicals

A qualitative determination of phytochemicals was carried out for the aqueous, ethanol, ethyl acetate and hexane extracts of fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB according to literature procedures[25,26] with minor modifications. The observations of each test were compared and recorded with respect to the control sample.

The quantitative determination of total alkaloid content (TAC), total flavonoid content (TFC), total condensed tannin content (TCTC) and total phenolic content (TPC) was carried out using the standard spectrophotometric methods, bromocresol green, aluminium chloride, butanol-hydrochloric acid-iron and Folin-Ciocalteu methods, respectively[25,27–29] with minor modifications. Caffeine was used as the reference compound for the determination of TAC. Catechin was employed as the reference compound for the determination of TFC and TCTC. TPC was determined using gallic acid as the reference compound. Absorbance measurements for the determination of TAC, TFC, TCTC and TPC were recorded at 470 nm, 510 nm, 550 nm and 765 nm, respectively. Each absorbance measurement was triplicated and the mean value was taken.

Antioxidant and Acetylcholinesterase Inhibitory Activity

Antioxidant activity of aqueous, ethanol, ethyl acetate and hexane extracts of fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB was separately estimated by carrying out the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric assay[30] using ascorbic acid as the standard compound. Each absorbance measurement recorded at 517

nm was triplicated and the mean absorbance value was taken. The percentage DPPH radical scavenging activity of each solution in the concentration series of standard and sample solutions was calculated according to Equation 1.

$$\%\text{DPPH Radical Scavenging Activity} = \frac{(A_{control} - A_{sample})}{(A_{control})} \times 100\% \quad (1)$$

$A_{control}$ is thereby the absorbance of the control and A_{sample} is the absorbance of the sample solution of fruit pulps of banana extract or that of reference compound.

AChE inhibitory activity of aqueous, ethanol, ethyl acetate and hexane extracts of fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB was estimated separately by performing the standard Ellman's colorimetric assay[31] using donepezil as the standard compound. Each absorbance measurement at 405 nm was triplicated and the mean absorbance value was taken. The percentage of AChE inhibitory activity of each solution in the concentration series of standard and sample solutions was calculated according to Equation 2. $A_{control}$ is thereby the absorbance of the control and A_{sample} is the absorbance of the sample solution of fruit pulps of banana extract or that of reference compound.

$$\%\text{AChE Inhibitory activity} = \frac{(A_{control} - A_{sample})}{(A_{control})} \times 100\% \quad (2)$$

Correlation between the antioxidant activity as well as the AChE inhibitory activity of aqueous, ethanol, ethyl acetate and hexane extracts of fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB with their corresponding TAC, TFC, TPC and TCTC was investigated separately by plotting $1/IC_{50}$ of DPPH assay or $1/IC_{50}$ of Ellman's assay *vs*. TAC, TFC, TPC or TCTC. Pearson's correlation coefficient (R) and linear regression (R^2) value of each plot were calculated using Origin 9.0 software.

Results and Discussion

The extractable yield of twelve solvent extracts compares the amounts of extractable secondary metabolites in each extract (Table 1). The extractable yield of the ethanol extracts of each banana variety is higher than those of aqueous, ethyl acetate and hexane extracts.

A qualitative analysis of phytochemicals provided a comparison of the presence or absence of secondary metabolites in the four different solvent extracts of the three selected banana varieties (Table 2). All solvent extracts demonstrated yellow and reddish-brown precipitates for Mayer's and Wagner's tests, respectively, while indicating the presence of alkaloids. A comparative analysis of the heights of the precipitates formed exhibited that the ethanol extract of *M. accuminata* AAB is constituted of the highest amount of alkaloids while the hexane extract of *M. accuminata* AAA is comprised of the lowest amount of alkaloids. The formation of red-color solutions for both cyanidin and proanthocyanidin tests indicated the presence of flavonoids and proanthocyanidins, respectively.

Table 1. Percentage extractable yields [% w/w] of four different solvent extracts of fruit pulps of three selected banana varieties.

Solvent	Percentage extractable yield [% w/w] of banana varieties		
	Musa accuminata AAA	*Musa accuminata* AAB	*Musa balbisiana* ABB
Water	7.68 ± 0.80	8.70 ± 0.46	4.43 ± 0.09
Ethanol	7.78 ± 0.79	9.67 ± 0.32	5.95 ± 0.16
Ethyl acetate	5.70 ± 0.12	7.99 ± 0.11	2.03 ± 0.11
Hexane	2.11 ± 0.48	6.07 ± 0.08	1.16 ± 0.42

Percentage extractable yield [% w/w] values are expressed as mean ± standard deviation of triplicates.

The ethanol extract of *M. balbisiana* ABB resulted in the most-intense, red-colored solutions in both the cyanidin and proanthocyanidin tests, indicating that this extract contained the highest amounts of flavonoids and proanthocyanidins. The hexane extract of *M. accuminata* AAA contained, on the other hand, the lowest amounts of flavonoids and proanthocyanidins.

The Liebermann-Burchard test demonstrated blue-green-color solutions with varying intensities for all solvent extracts analyzed, indicating the presence of sterols in different quantities. The ethanol extract of *M. accuminata* AAA contained thereby the largest amount of sterols, while the hexane extract of *M. balbisiana* ABB contained the least amount of sterols.

All solvent extracts subjected to the Salkowski test showed a cherry-red-color interface between the organic and aqueous layers with different intensities indicating the presence of terpenoids in varying amounts. The ethanol extract of *M. accuminata* AAA contained thereby the highest amount of terpenoids, while the hexane extract of *M. balbisiana* ABB contained the least amount of terpenoids.

The formation of a white-color precipitate with 1% gelatin solution containing 10% sodium chloride indicated the presence of tannins. A qualitative comparison of the heights of the white-color precipitates exhibited that the ethanol extract of *M. balbisiana* ABB possesses the highest amount of tannins, while the hexane extract of *M. accuminata* AAA is comprised of the least amount of tannins.

Phenolic compounds result in a deep violet color solution for the ferric chloride test. The most intense deep violet-color resulted from the ethanol extract of *M. balbisiana* ABB that is comprised of the highest amount of phenolic compounds. The least intense violet-color resulted from the hexane extract of *M. accuminata* AAA indicating thus the lowest amount of phenolic compounds.

The honey comb froth with different heights for froth test indicated the presence of saponins in the aqueous extracts of the three banana varieties studied. Among them, *M. accuminata* AAA contains a comparatively high amount of saponins.

From a quantitative point of view, the ethanol extracts demonstrated the highest TAC, TFC, TCTC and TPC for the fruit pulps of the three selected banana varieties, while those of aqueous, ethyl acetate and hexane extracts exhibited a decreasing order (Table 3). This observation corroborates with several investigations carried out on the effect of solvent polarity on the extraction of different classes of phytochemicals[32].

Alkaloids represent a structurally diverse group of low molecular weight, nitrogen-containing plant secondary metabolites that form comparatively stable-yellow color complexes with an acid dye, bromocresol green[25,33]. The comparatively less time-consuming bromocresol green spectrophotometric method, demonstrated high sensitivity over the other methods such as gravimetric and titrimetric methods which are used to quantify alkaloids[25]. In this study, the TAC values of all the samples (Table 3) are expressed as milligrams of caffeine equivalent / 100 g fresh weight of plant material (mg CFE / 100 g FW) based on the calibration curve that has a R^2 value of 0.9990; $y = 0.0053\,x - 0.0051$. In each solvent extract analyzed, the highest TAC was exhibited by *M. accuminata* AAB while TAC of *M. balbisiana* ABB and *M. accuminata* AAA follow, consecutively. Therefore, the ethanol extract of *M. accuminata* AAB exhibited the highest TAC, while the hexane extract of *M. accuminata* AAA demonstrated the lowest TAC. A recent study on the gravimetric analysis of TAC of fruit pulps of eight banana varieties carried out by Siji et al.[8] reported that some of the edible banana varieties including *M. paradisiaca* L. (AAB) [Nendran] and *M. accuminata* Cavendshii (AAA) [Robusta] contain 3.76% and 2.77% of TAC for fresh weight of plant material, respectively, being significantly higher than the TAC of the three banana varieties analyzed in this study.

Flavonoids are one of the most common groups of phenolic compounds among the plant secondary metabolites that demonstrate a significant antioxidant activity[33]. The aluminum chloride colorimetric assay employed in the determination of TFC is based on the nitration of an aromatic ring among the three rings in the flavonoid structure that bears a catechol group in its third or fourth unsubstituted or sterically unhindered positions[27]. The sequential addition of aluminum chloride and sodium hydroxide turns the yellow-color nitrated flavonoids immediately to a red-color solution which exerts maximum absorbance at 510 nm wavelength[27]. The TFC of all extracts (Table 3) subjected to the analysis was determined using the calibration

Table 2. Qualitative analysis of phytochemicals of four different solvent extracts of fruit pulps of three selected banana varieties.

Solvent	Banana variety	Alkaloids		Flavonoids and Proanthocyanidins		Sterols	Terpenoids	Tannins	Phenolic compounds	Saponins
		Mayer's test	Wagner's test	Cyanidin test	Proanthocyanidin test	Liebermann-Burchard test	Salkowski test	Gelatin test	Ferric chloride test	Froth test
Water	*Musa accuminata* AAA	+	+	+	+	+++	+++	++	++	++++
	Musa accuminata AAB	+++	+++	++	++	++	++	+++	+++	+++
	Musa balbisiana ABB	++	++	+++	+++	++	++	++++	++++	+++
Ethanol	*Musa accuminata* AAA	++	++	++	++	++++	++++	++	++	NA
	Musa accuminata AAB	++++	++++	+++	+++	++++	++++	+++	+++	NA
	Musa balbisiana ABB	+++	+++	++++	++++	+++	+++	++++	++++	NA
Ethyl acetate	*Musa accuminata* AAA	+	+	++	++	++++	++++	+	+	NA
	Musa accuminata AAB	+++	+++	+++	+++	+++	+++	++	++	NA
	Musa balbisiana ABB	++	++	++++	++++	+++	+++	+++	+++	NA
Hexane	*Musa accuminata* AAA	+	+	+	+	+++	+++	+	+	NA
	Musa accuminata AAB	++	++	++	++	++	++	++	++	NA
	Musa balbisiana ABB	+	+	+++	+++	++	++	+++	+++	NA

+, slight amounts ; ++, moderate amounts ; +++, large amounts ; ++++, very large amounts and NA, not applicable.

Table 3. Quantitative analysis of secondary metabolites of four different solvent extracts of fruit pulps of three selected banana varieties.

Solvent	Banana variety	Total Alkaloid Content [mg CFE / 100 g FW[a]]	Total Flavonoid Content [mg CTE / 100 g FW[b]]	Total Condensed Tannin Content [mg CTE / 100 g FW[b]]	Total Phenolic Content [mg GAE / 100 g FW[c]]	Antioxidant Activity IC_{50} [mg L^{-1}]	Acetylcholinesterase Inhibitory Activity IC_{50} [µg mL^{-1}]
Water	*Musa accuminata* AAA	6.45 ± 0.05	162.86 ± 6.05	374.70 ± 13.57	12.03 ± 0.37	$2.70 \times 10^6 \pm 9.7 \times 10^3$	173.60 ± 0.40
	Musa accuminata AAB	9.48 ± 0.24	241.68 ± 8.23	461.20 ± 12.62	22.83 ± 0.41	$3.82 \times 10^5 \pm 2.3 \times 10^3$	140.24 ± 0.13
	Musa balbisiana ABB	7.10 ± 0.12	364.91 ± 20.19	747.29 ± 19.55	41.19 ± 0.40	$3.20 \times 10^3 \pm 13.89$	124.12 ± 0.40
Ethanol	*Musa accuminata* AAA	8.35 ± 0.22	274.15 ± 5.91	593.55 ± 6.57	32.84 ± 0.36	579.19 ± 8.95	143.57 ± 0.06
	Musa accuminata AAB	11.71 ± 0.35	353.83 ± 10.22	762.88 ± 19.55	36.57 ± 0.34	176.07 ± 0.84	75.01 ± 0.11
	Musa balbisiana ABB	9.93 ± 0.38	549.38 ± 11.59	863.48 ± 20.94	65.85 ± 0.35	35.25 ± 1.99	101.93 ± 0.11
Ethyl acetate	*Musa accuminata* AAA	4.82 ± 0.02	77.15 ± 8.53	44.89 ± 0.53	5.43 ± 0.37	2737.46 ± 55.34	121.65 ± 0.41
	Musa accuminata AAB	8.30 ± 0.36	132.61 ± 3.83	141.26 ± 1.34	7.73 ± 0.66	880.71 ± 9.81	90.39 ± 0.31
	Musa balbisiana ABB	5.49 ± 0.24	203.74 ± 13.44	171.55 ± 10.09	17.51 ± 0.60	1729.04 ± 20.60	108.65 ± 0.07
Hexane	*Musa accuminata* AAA	1.86 ± 0.06	39.94 ± 2.23	34.45 ± 1.29	3.83 ± 0.08	5437.78 ± 80.50	81.79 ± 0.17
	Musa accuminata AAB	2.95 ± 0.06	104.17 ± 4.98	139.06 ± 8.29	6.91± 0.37	1397.80 ± 6.31	226.03 ± 0.27
	Musa balbisiana ABB	2.03 ± 0.06	149.83 ± 10.37	156.97 ± 7.38	9.01 ± 0.48	2939.57 ± 29.48	280.93 ± 0.18

Values are expressed as mean ± standard deviation of replicates.
[a] milligrams of caffeine equivalent / 100 g fresh weight of plant material.
[b] milligrams of catechin equivalent / 100 g fresh weight of plant material.
[c] milligrams of gallic acid equivalent / 100 g fresh weight of plant material.

curve with an R^2 value of 0.9995; $y = 2.828\times10^{-4}$ x + 0.0070 and the values are expressed as milligrams of catechin equivalent per 100 g fresh weight of plant material (mg CTE / 100 g FW). In each solvent extract, *M. balbisiana* ABB exhibited the highest TFC while *M. accuminata* AAA exhibited the lowest TFC. Therefore, among the total of twelve different extracts of the three banana varieties analyzed, the ethanol extract of *M. balbisiana* ABB exhibited the highest TFC, while the hexane extract of *M. accuminata* AAA exhibited the lowest TFC. In 2016, Ayoola et al.[9] reported the TFC of fruit pulps of *M. accuminata* AAB grown in Nigeria as ~ 6.5 mg quercetin equivalent / 1 g of dry weight of plant material. Moreover, Fatemeh et al.[7] reported the TFC of fruit pulp flour of edible species *M. acuminata* L, cv cavendshii on dry matter basis using catechin as a reference. Their study reported the TFC of 281.18 ± 1.88 mg CTE / 100 g of dry matter for unripe fruit pulp flour of *M. acuminata* L, cv cavendshii that decreased to 196.45 ± 0.89 mg CTE / 100 g of dry matter in ripe fruit pulp flour of the same banana variety. However, the differences in reference compounds, quercetin *vs*. catechin and basis of the weight, dry weight *vs*. fresh weight make the results reported by Ayoola et al.[9] and Fatemeh et al.[7] incomparable with the findings of this reporting study.

Condensed tannins or proanthocyanidins are flavonoid polymers formed from the condensation of flavans[33]. In acid–butanol assay, acid-catalyzed oxidative depolymerization of condensed tannins occurs through the cleavage of interflavan bond of condensed tannins to yield red-color anthocyanidins[33]. The TCTC of all the samples (Table 3) analyzed was determined using the calibration curve with an R^2 of 0.9981; $y = 3.568\times10^{-4}$ x – 0.1101 and the values are expressed as mg CTE / 100 g FW. Among the banana varieties analyzed, *M. balbisiana* ABB and *M. accuminata* AAA demonstrated the highest and the lowest TCTC, respectively. The highest TCTC was exhibited by ethanol extracts of *M. balbisiana* ABB, whereas the lowest TCTC was represented by hexane extracts of *M. accuminata* AAA. Siji et al.[8] from India in the year of 2016 reported the TCTC of eight banana varieties that vary from the highest TCTC of 4.40 milligrams of gallic acid equivalent per 100 g fresh weight of plant material (mg GAE / 100 g FW) in an edible species of *M. paradisiaca* L. (AAB) the lowest TCTC of 1.66 mg GAE / 100 g FW in a wild species of *M. accuminata* Colla (AAA) cv. 'Red Dacca'. Although Siji et al.[8] determined the TCTC of several banana varieties, the difference between the standard compounds, gallic acid in the study of Siji et al.[8] and catechin in this reporting study limits the comparison of TCTC of fruit pulps of different banana varieties.

Simple phenols, phenolic acids, coumarins, flavonoids, hydrolysable and condensed tannins, lignans and lignins are among the phenolic compounds found in plants[33]. The Folin–Ciocalteu phenol reagent used in the determination of TPC is a redox reagent that consists of a mixture of sodium molybdate and sodium tungstate (Na_2MoO_4/Na_2WO_4) with molybdenum and tungsten at +6 oxidation state[29]. The phenolic compounds in the banana fruit pulp extracts undergo a redox reaction with the Folin–Ciocalteu phenol reagent to yield mixtures of molybdenum blue and tungsten blue at +4 oxidation state that show maximum absorbance at 765 nm[25]. The TPC of each solvent extract of three banana varieties (Table 3) was determined using the calibration curve with an R^2 of 0.9980; y = 0.0082 x + 0.0093, and the values are expressed as mg GAE / 100 g FW. Among the fruit pulps of the three banana varieties analyzed, *M. balbisiana* ABB exhibited the highest TPC, whereas the TPC of *M. accuminata* AAB and *M. accuminata* AAA follow consecutively, regardless of the extracting solvent. Additionally, the ethanol extract of *M. balbisiana* ABB exhibited the highest TPC, while the hexane extracts of *M. accuminata* AAA constituted the least TPC. A collaborative research work carried out by Ayoola et al.[9] reported that the fruit pulps of *M. accuminata* AAB grown in Nigeria constituted of ~25.0 mg GAE / 1 g of dry weight of plant material. Fatemeh et al.[7] from Indonesia in 2012 exhibited that the TPC of 373.88 ± 0.92 mg GAE / 100 g of dry matter in unripe fruit pulp flour of an edible species *M. acuminata* L, cv cavendshii decreased to 230.21 ± 1.19 mg GAE / 100 g of dry matter in ripe fruit pulp flour. Fatemeh et al.[7] and Ayoola et al.[9] expressed the TPC of fruit pulps of banana varieties on dry matter basis, while in this reporting study, the TPC of fruit pulps of three selected banana varieties was expressed on a fresh weight basis. Therefore, this difference restrained the comparison of the TPC of this reporting study with the findings of Fatemeh et al.[7] and Ayoola et al.[9]

The antioxidant activity was evaluated by the DPPH assay. The deep violet-colored DPPH radicals abstract hydrogens from the antioxidants in banana fruit pulp extracts leading to the disappearance of the color of DPPH radicals that show maximum absorbance at 517 nm[29,30]. The plots of percentage DPPH radical scavenging activity against the concentration of samples or the reference yielded the antioxidant activity of each sample (Table 3) that was expressed as IC_{50} values in mg L^{-1}, the concentration of the sample or that of the reference where the percentage radical scavenging activity is reduced to half of its initial value. The standard antioxidant compound used in this study, ascorbic acid, represented antioxidant activities of 2.22 ± 0.15 mg L^{-1} in water and 0.64 ± 0.01 mg L^{-1} in ethanol. The highest antioxidant activity was observed for the ethanol extract of *M. balbisiana* ABB, but was lower than that of ascorbic acid. Moreover, the ethanol extracts of *M. accuminata* AAB and aqueous extracts of *M. accuminata* AAA exhibited the highest and the lowest antioxidant activities, respectively. A collaborative research work of Fatemeh et al.[7] demonstrated that the ripe fruit pulp flour of an edible species *M. acuminata* L, cv cavendshii has a DPPH radical scavenging activity of 29.38 ± 0.70 %, which is lower than that of unripe fruit pulp flour, 35.21 ± 0.98 %.

This reporting study indicated that the TFC, TCTC, TPC and antioxidant activity of aqueous and ethanol extracts of the three banana varieties follow the same descending order of *M. balbisiana* ABB, *M. accuminata* AAB to *M. accuminata* AAA. This obvious relationship among the antioxidant activity, on one side, and the TFC, TCTC and TPC contents, on the other side, confirmed that the concentration of plant phenolic compounds, including flavonoids and condensed tannins, is proportional to the antioxidant activity. Also, the polar phenolic compounds, including flavonoids and condensed tannins, readily extract into polar solvents, ethanol and water, than into the moderately polar ethyl acetate and the nonpolar hexane. Eventually, these results confirmed that *M. balbisiana* ABB that constitutes the highest TFC, TPC and TCTC contents, together with the highest antioxidant activity, could be used as a potential source of natural antioxidants.

Following the cholinergic hypothesis of Alzheimer's disease which explains that the inhibition of AChE enzyme, which catalyzes the hydrolysis of acetylcholine neurotransmitter, increases the levels of acetylcholine in the neuromuscular junctions, thus improving cholinergic functions of Alzheimer's patients[15,17,18], the AChE inhibitory activity was assessed using the Ellman's colorimetric assay in which the AChE enzyme hydrolyzes the substrate acetylcholine iodide to thiocholine and acetic acid[31]. The resulting thiocholine reacts with the coloring agent, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB) while developing a yellow-colored compound which exerts its maximum absorption at 405 nm wavelength. The AChE inhibitory activity is expressed as the IC_{50} value, that is the concentration of the sample where the percentage AChE activity is reduced by 50%. The IC_{50} values (Table 3) of standard compounds and the fruit pulp extracts in different solvents were obtained using the corresponding plots of percentage AChE inhibitory activity against the concentration of the standard or fruit pulp extracts. In this study, the reference compound, donepezil exerted the highest AChE inhibitory activity of 16.43 ± 0.53 µg mL^{-1}. The highest AChE inhibitory activity was exerted by the ethanol extract of *M. accuminata* AAB, while the lowest AChE inhibitory activity was represented by the hexane extract of *M. balbisiana* ABB. A recent study entitled 'Screening for cholinesterase inhibitors in selected fruits and vegetables'[14] reported that the aqueous extracts of immature and mellow fruits of *M. paradisiaca* exhibit AChE inhibitory activity of 3.60 ± 0.65 eserine µmol L^{-1} and 1.78 ± 1.16 eserine µmol L^{-1}, respectively, using eserine as the reference compound. In addition, the aqueous extracts of several edible fruits, wild strawberry; *Fragaria vesca* L. (Rosaceae), apple; *Malus domestica* Borkh. var. Idared (Rosaceae) and peach; *Prunus persica* L. (Rosaceae) expressed comparatively high AChE inhibitory activities of 6.10 ± 1.13 eserine µmol L^{-1}, 6.10 ± 0.62 eserine µmol L^{-1} and 6.10 ± 0.58 eserine µmol L^{-1}, respectively[14]. In contrast, the aqueous extracts of fruits of grapes; *Vitis vinifera* L. (Vitaceae) and garden strawberry; *Fragaria* x *ananassa* Duch. (Rosaceae) exhibited comparatively low AChE inhibitory activities of 0.98 ± 0.91 eserine µmol L^{-1} and 0.07 ± 0.07 eserine µmol L^{-1}, respectively[14].

A correlation study was carried out to investigate the relationship of TAC, TFC, TCTC and TPC with the antioxidant and AChE inhibitory activities of aqueous, ethanol, ethyl acetate and hexane extracts of fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB separately. The plots of $1/IC_{50}$ of DPPH assay of the three banana varieties against the corresponding TAC exhibited the strongest positive correlation for the ethanol extract (Figure 1a) which has an R value of 0.9968 with an R^2 value of 0.9926. Similarly, regarding the $1/IC_{50}$ of DPPH assay, the TFC of ethanol extracts (Figure 1b), TCTC of ethyl acetate extracts (Figure 1c) and TPC of aqueous extracts (Figure 1d) demonstrated a strong positive linear correlation which is evidenced by R and R^2 values of 0.9036 and 0.7903; 0.9748 and 0.9431; and 0.9716 and 0.9361, respectively. The R and R^2 values of 0.9722 and 0.9373; 0.9656 and 0.9228; 0.9113 and 0.8059; and 0.9658 0.9231 obtained for the correlation of corresponding $1/IC_{50}$ of Ellman's assay against the TAC of ethanol extracts (Figure 2a), TFC (Figure 2b), TCTC (Figure 2c) and TPC (Figure 2d) of aqueous extracts, respectively demonstrated a strong positive linear relationship. The above-mentioned strong linear positive relationship obtained for antioxidant or AChE inhibitory activity against the corresponding TAC or TFC, TPC, TCTC of different solvent extracts of the three selected banana varieties confirms that they could be used as a valuable source of information for the bioassay-guided isolation of phytochemicals from the three studied banana varieties to treat Alzheimer's disease by addressing oxidative stress and/or cholinergic hypotheses.

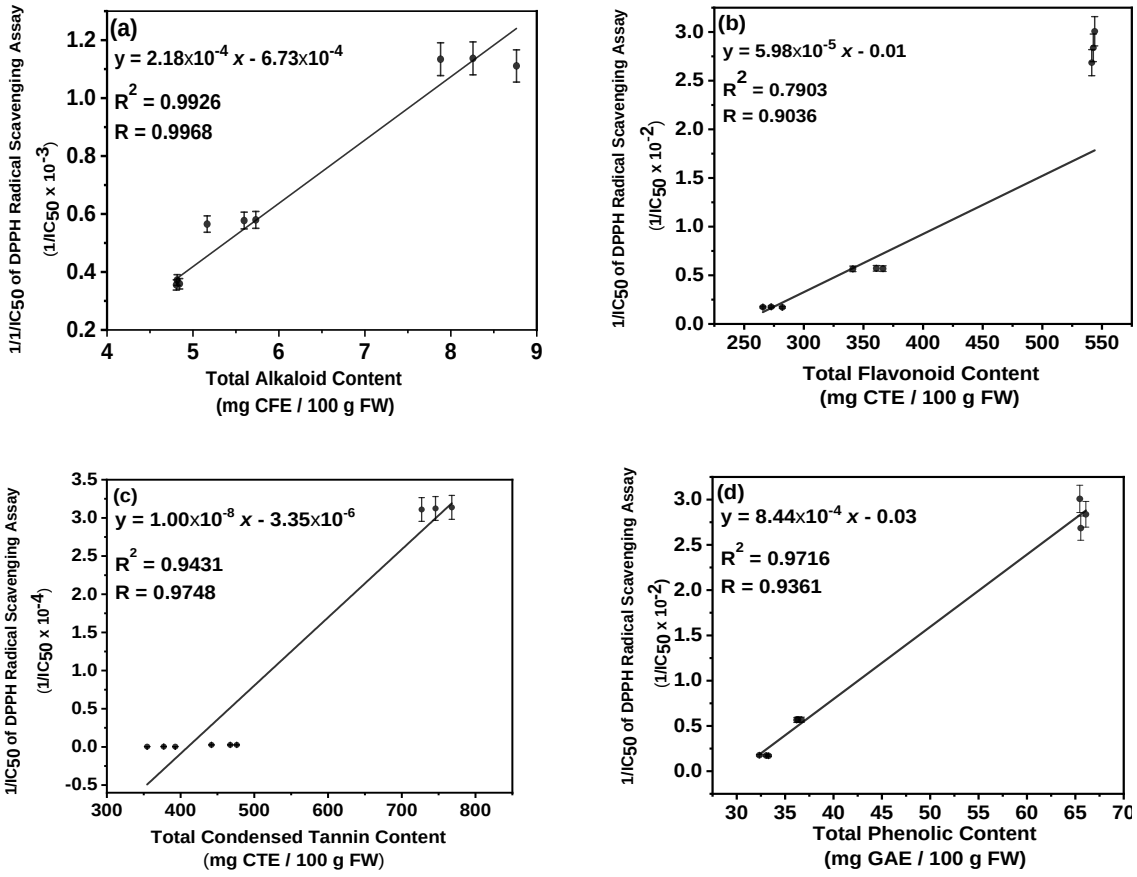

Figure 1. Correlation between the $1/IC_{50}$ of DPPH assay and the corresponding (a) total alkaloid content in ethanol, (b) total flavonoid content in ethanol, (c) total condensed tannin content in ethyl acetate and (d) total phenolic content in water.

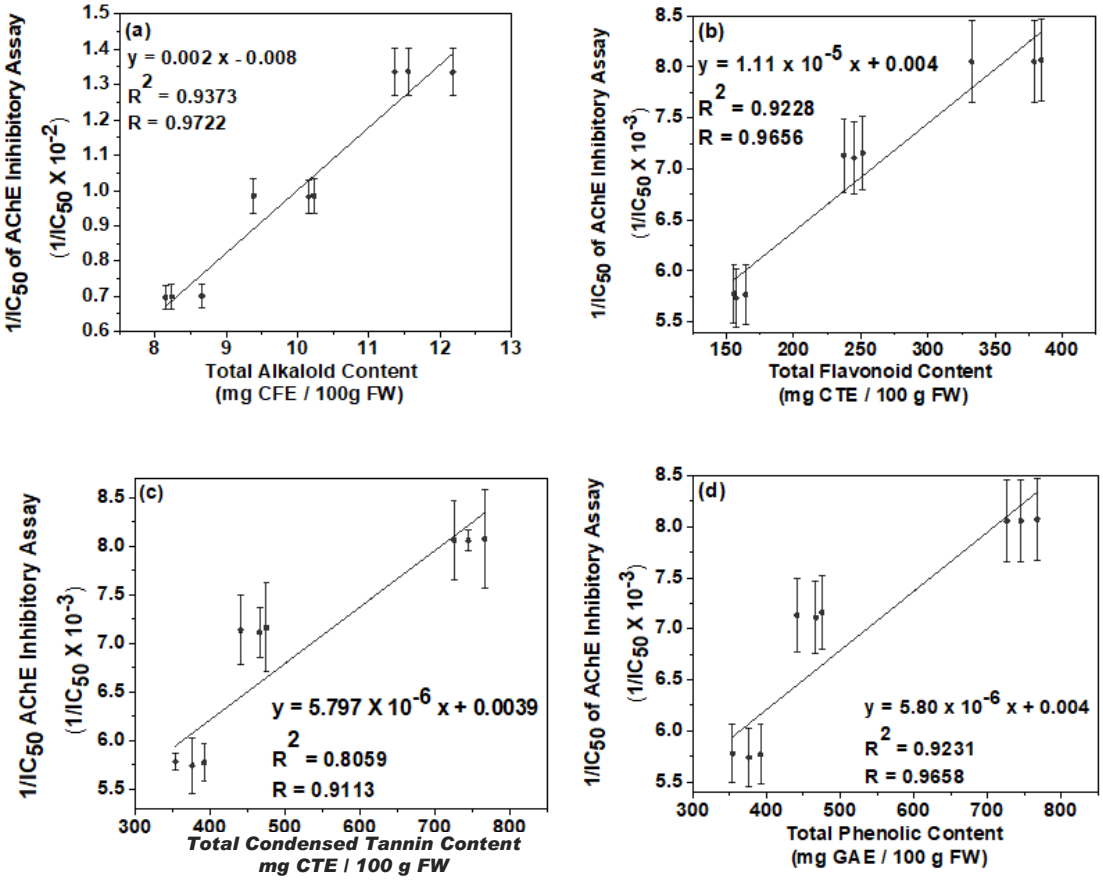

Figure 2. Correlation between the $1/IC_{50}$ of Ellman's assay and the corresponding (a) total alkaloid content in ethanol, (b) total flavonoid content in water, (c) total condensed tannin content in water and (d) total phenolic content in water.

Conclusion

Four different solvent extracts of nearly three-month-old edible fruit pulps of *M. accuminata* AAA, *M. accuminata* AAB and *M. balbisiana* ABB were subjected to analysis. The ethanol extract of each studied banana variety exhibited the highest extractable yield, while fruit pulps of *M. accuminata* AAB yielded the highest extractable yield in each solvent. Each solvent extract of fruit pulps of the three banana varieties was constituted of several plant secondary metabolites, alkaloids, flavonoids, proanthocyanidins, sterols, terpenoids, tannins and phenolic compounds, while the aqueous extracts of the above-mentioned banana varieties contained saponins in different quantities. The ethanol extract of fruit pulp of *M. balbisiana* ABB possessed the highest TFC, TCTC and TPC along with the highest antioxidant activity. The ethanol extract of fruit pulp of *M. accuminata* AAB constituted the highest TAC and the highest AChE inhibitory activity. Therefore, *M. balbisiana* ABB and *M. accuminata* AAB could be used as promising sources of edible natural antioxidants and AChE inhibitors, respectively.

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