Original Research Article

Evaluation of the total antioxidant capacity of three selected plants in Guyana

ABSTRACT

Aims: Phytochemicals have received much attention as novel sources of naturally occurring antioxidants. Therefore, the crude extracts of three commonly used medicinal plants from Guyana (Mustard, Antsbush, and Guava) were screened for total antioxidant capacity.

Study Design: Experimental-based study.

Place and Duration of Study: The experiments were performed at the chemistry laboratory in the Faculty of Natural Sciences and in the main laboratory at the College of Medical Sciences, at the University of Guyana during September 2022- August 2023.

Methodology: The leaves of these plants were collected, washed, and air-dried at 28^0C for approximately one month and then pulverized in a sterilized food processor. Plant extracts were then obtained by maceration with various solvents such as hexane, ethyl acetate, chloroform, and methanol, and then filtered using a sterilized filtration apparatus. The extracts obtained were subsequently reduced under pressure at 45^0C to produce the crude extracts, which were then subjected to total antioxidant quantification via the phosphomolybdate method using a spectrophotometer. Ascorbic acid was used as the standard and a calibration curve with a correlation coefficient (R^2) of 0.999 was obtained.

Results: Antioxidant activity was seen for all plant leaf extracts but in varying quantities. The ethanolic extracts showed the highest total antioxidant capacity (10.6 mg/ml for guava and 2.5 mg/ml for mustard leaves). The lowest total antioxidant capacity was observed for the hexane extracts. Of the three-plant leaf extracts tested; the most pronounced total antioxidant capacity was noted for the guava leaf extracts.

Conclusion: The results from this study suggest that mustard leaves, antsbush leaves, and guava leaves are sources of naturally produced antioxidants. The plant leaves especially the guava leaves have great potential for use as complementary and alternative medicines to

conventional treatments for the prevention and management of chronic diseases such as cardiovascular diseases, cancer, and diabetes in Guyana.

Keywords- antioxidants, phytochemicals, plant extracts, phosphomolybdate, ascorbic acid

1 INTRODUCTION

The association of free radicals in many diseases has been well studied. Free radicals are chemical species possessing an unpaired electron in the valence shell. They are highly reactive and unstable molecules and are mostly oxygenated or nitrogenated in nature. Examples of free radicals are superoxide, hydroxyl, peroxide, hydroperoxyl, nitric oxide, peroxynitrite, and nitrogen dioxide; among many others [1]. Free radicals are produced during metabolism and, when produced in excess, impair the important biological molecules in cells. They can oxidize lipids, DNA, carbohydrates, proteins, and enzymes, disrupting normal cell functions [2]. Free radicals may be the etiology of a wide variety of pathological conditions including cancer, diabetes mellitus, heart diseases, AIDS, degenerative diseases, and drug toxicity, among many others [3].

Antioxidants are compounds that can capture free radicals, thereby opposing oxidation. They function as free radical scavengers and reducing agents. They can also form complexes with pro-oxidant metals, and extinguish singlet oxygen formation [4]. Antioxidants possess the ability to reduce the oxidative damage linked with many infections and diseases. Antioxidants are therefore crucial for preventing and managing many diseases [5]. Over the last few decades, the interest in plants as natural sources of antioxidants has been explored rapidly [6].

Phytochemicals are defined as bioactive compounds produced by plants for their protection [7]. Studies have shown that extracts prepared from various plants contain a variety of phytochemicals such as alkaloids, saponins, tannins, flavonoids, phenolics, and steroids, among many others [8]. Studies have also shown that these phytochemicals have various properties such as antimicrobial, anticancer, antidiabetic, and antioxidant. These properties allow plants to have great medicinal value [9]. Plants contain phytochemicals like carotenoids, vitamins, and polyphenols that may possess antioxidant and free radical scavenging properties

[10]. Many investigations have been focusing on the phytochemical analysis of plants as a way of extracting, isolating, and identifying bioactive compounds that have medicinal value.

Many plants have been studied for their various properties, such as antimicrobial properties. However, a review of the literature reveals limited information about the antioxidant capacity of plants such as guava (*Psidium guajava*), mustard (*Brassica juncea*), and antbush (*Struchium sparganophora*), all of which are indigenous to Guyana.

Brassica juncea is usually referred to as mustard greens or as the Chinese or Indian mustard. This plant belonging to the family of Brassicaceae is an important leafy vegetable widely used in Oriental dishes worldwide [11]. Mustard plants are found abundantly in the Caribbean regions, including Guyana. They are an economically important plant with great medicinal value. *Brassica juncea* is a source of a variety of micronutrients, antioxidants, β-carotenoids and vitamins like vitamin C and E.It is thought to be an environment friendly source of many conventional medicines designed to treat a variety of non-communicable illnesses [12]. Phytochemicals such as flavonoids, tannins, saponins, glycosides, steroids/triterpenoids, anthraquinone, and polyphenols have been found in *Brassica juncea* via phytochemical screening [13]. A few brassinosteroids such as castasterone, teasterone, typhasterol and 24-epibrassinolide has been identified in *B. juncea* via in-depth phytochemical analysis[14]. Mustard leaves have antioxidant compounds that lower lipid peroxidation, oxygen radicals' levels and manage the metabolism of glucose. Antioxidants in mustard leaves can limit the rate at which oxidative stress in diabetes occurs [11]. However, it is unclear whether the species in Guyana have substantial amounts of antioxidants.

Struchium sparganophora iscommonly called antbush in Guyana and water bitter leaf in Africa. It belongs to a large family of plants called Asteraceae [15]. This plant has been used extensively as herbal remedies to treat a variety of communicable and non-communicable conditions such as malaria, fungal infections, cancer, diabetes, gonorrhea, among others [16][17]. Antsbush can be found abundantly in Africa, Southeast Asia and South America [15][19]. It is particularly know for treating diaper rash, and yeast infections in the rural areas of Guyana [18]. Antbush is use as a herb in food preparation and a treatment for poisons, malaria and gonorrhea in Nigeria [19]. Phytochemicals such as alkaloids, cardiac glycosides,

flavonoids, and hydrolyzable tannins have been found to be present in Antsbush [20]. Antsbush have antioxidants that scavenge 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals and also inhibit the enzymatic activity of α - amylase and glucosidase [21]. However, it is unclear whether the species in Guyana have considerate quantity of antioxidants.

Psidium guajava, belonging to the *Myrtaceae* family, is commonly referred to as guava. The *Myrtaceae* is a family consists of dicotyledonous plants that are Angiosperms (flowering plants) [22]. Guava trees are mostly grown in tropical and subtropical parts of the world, such as some parts of Asia, the Caribbean, and South America. The *Psidium guajava* tree is a plant used around the world for its medicinal benefits, which incorporates the use of the fruit (guava) and the leaves. While the fruit (guava) is widely used in the food industries in the preparation of jam, jellies, fruit spreads and even in the beverage industries for the production of fresh juices, it is also used traditionally by some tribes to monitor high blood pressure levels in some parts of the world [23]. While the fruit is very beneficial with high concentrations of vitamin C, the leaves possess several bioactive metabolites. The *Psidium guajava* plant has phytochemicals like flavonoids, tannins, phenols, terpenoids, and glycosides, which have significantly influenced the development of the plants' colour, smell, and flavours [24]. However, it is unclear whether the species in Guyana have desirable antioxidants capacity.

The majority of the world's population (64%) utilises plants that contain phytochemicals as a substitute for synthetic medications, which are commonly used to treat chronic conditions [25]. These bioactive molecules are not considered essential nutrients and, hence, not consumed as food. However, they reveal significant medicinal values including substrates, cofactors and inhibitors of biochemical reactions, scavenger of toxic chemicals, compounds that improve the absorption of essential nutrients, growth factors for beneficial enteric organism, among others [26].

The extraction of phytochemicals from herbal plants is involves a series of steps. A few techniques have well been documented. Some of the most established ones used are maceration, soxhlet, percolation and decoction, among a few others [9]. However, the most common and simplest method involved is maceration. Maceration involves socking of dried ground particles with solvents for several hours under occasional shaking [27]. Several solvents

may be utilised in the extraction of phytochemicals. Solvents that are polar, non-polar, and semi-polar are frequently used so as to extract a wide range of phytochemicals [28] [29]. Some common solvents used include acetone, ethyl acetate, ethanol, methanol, hexane, etc. Polar solvents can be used to extract polar phytochemicals, semi-polar solvents can be used to extract semi-polar phytochemicals, and non-polar can be used to extract non-polar phytochemicals [28] [29]. Studies have shown that methanol, ethanol, ethyl acetate, acetone, and hexane are some of the best solvents used to extract phytochemicals [7]. A handful of techniques were developed for evaluating antioxidant capacity in the laboratory. These include assays such as the DPPH, 2, 2- azino- bis- (3- ethylbenzothiazoline- 6- sulfonic acid) (ABTS), ferric reducing the antioxidant power (FRAP) test, and phosphomolybdate test [30].

The DPPH assay is the most commonly used technique for antioxidant activity. It assesses the ability of substance to provide hydrogen ions or scavenge free radicals. This technique utilizes a commercially available reagent called 1, 1-diphenyl-1-picrylhydrazil (DPPH) [31]. The antioxidants in the substance of interest would reduce the DPPH reagent (a purple-coloured compound) to phenyl picrylhydrazine (a yellow-coloured compound). The rate of this reaction depends on the ability of the substance of interest to donate hydrogen ions [32]. When the substance accepts an electron, it becomes a stable compound in methanol and generates a maximum absorption value at a wavelength of 517nm [33].

The FRAP assay is associated with the reducing potential of iron. Reducing potency is directly linked to the presence of reductones. Reductones breaks the free radical chains by providing electrons [34]. They limit the formation of peroxides by reacting with specific peroxide precursors. Reductants facilitate the donation of electrons, which leads to the reduction of ferric to ferrous ions. The quantity of ferrous complex formed is determined by the formation of Perl's Prussian blue at a wavelength of 700nm. Exponential rise in absorbance values indicated a rise in the reducing ability of iron. Iron is said to be a transition metal that is crucial for the transport of oxygen, enzyme activity and respiration. Furthermore, iron is a reactive metal that can produce free radicals from peroxides by Fenton's reaction, and has thus been implicated in cardiovascular and other diseases. Ferrous iron is also said to be associated

with the generation of oxyradicals and peroxidation of lipid. A reduction of ferrous levels in the Fenton's reaction thereby hinders oxidative stress [34].

The phosphomolybdate method is a spectrophotometric method used to assess total antioxidant capacity. It was developed by Prieto et al. in 1999. In this assay, Molybdenum (VI) is reduced to Molybdenum (V) by the substance in the sample. Consequently, a green phosphate Molybdenum (V) complex is formed at an acidic pH [35]. This assay has been maximized to reflect linearity and reproducibility for the evaluating the concentration quite a few antioxidants. The spectrophotometric assay is usually carried out in vitro for the estimation of total antioxidant activity of plants and vitamin E in seeds and beans, etc.

Studies suggest that phenolic compounds and flavonoids are useful antioxidants [36] [37]. Evaluation of total phenolic content (TPC) and total flavonoid content (TFC) is therefore useful in antioxidant studies as well. TPC can be evaluated via the Folin-Ciocalteu method, while the TFC can be evaluated by the aluminium chloride colourimetric estimation. The antioxidant capacity of phenolic compounds is mostly associated with their redox potential [31]. Phenolics behave as singlet oxygen quenchers, reducing agents, and metal chelators and electron donors. Because of this, they are considered powerful free radicals scavengers and lipid peroxidation inhibitors. The TPC of plants extracts were widely investigated for their involvement in antioxidant activities. Studies have shown the association of TPC of plants extracts to their antioxidant activity [34] [38]. Phytochemicals such as phenols and flavonoids in plant extracts can be screened for according to standard testing procedures [39].

In Guyana, the antioxidant properties of mustard, antsbush, and guava have never been explored. This is a borderline study, which will provide valuable insights into whether or not these plants have the potential to be used as complementary and alternative medicine for chronic conditions. The objective is to measure the total antioxidant capacity of the leaves of these three plants. We specifically sought to find out which of the three plant leaf extracts has the highest antioxidant capacity and which solvent used in the extraction process is associated with the most antioxidant activity.

2 METHOD

This was an observational study carried out at the Chemistry Laboratory in Natural Sciences and the Medical science Laboratory in the College of Medical Sciences at the University of Guyana. Three traditional medicinal plants in Guyana were evaluated in this study. They were Mustard leaves (*Brassica juncea)*, Antbush leaves (*Struchium sparganophora*), and Guava leaves (*Psidium guajava*) (Fig.1). The collection and preparation of the plant leaf extracts for all three indigenous plants was performedduringthe period 2022-2023.

A-Mustard Leaves **B-Antsbush** **C-Guava leaves**

Fig. 1. Leaves of the plants investigated in this study

2.1 Collection of plant materials

Authentic *Brassica juncea* seeds were purchased from a certified botanist in Region 6 and were grown to obtain healthy mustard leaves. Guava leaves were collected from villages in Regions 3 and 4. Antbush leaves were collected from the National Agricultural Research & Extension Institute (NAREI) in Region 4 and a site in Kimbia, Berbice River (Region 6). The plants' leaves were identified and authenticated by the Centre for Study of Biological Diversity, University of Guyana. Leaves that showed no sign of deterioration were used. The plant leaves were processed separately but similarly.

2.2 Preparation of plant leaf extracts

The crude extracts of these plants were prepared based on a method outlined by Ede et al (2023) [40]. The leaves of the different plants were washed thoroughly with purified water and then air-dried at 28^{o}C for a period of 2-4 weeks. A sterilized food mixer was used pulverized

the dried leaves. The pulverized mustard leaves were macerated separately using three different solvents namely ethanol, ethyl acetate and hexane. The pulverized antsbush leaves were macerated separately using two different solvents namely ethyl acetate and chloroform. The pulverized guava leaves were macerated separately using four different solvents namely 95% ethanol, methanol, ethyl acetate and hexane. A variety of solvents with different polarities were selected in hope of extracting a wide range of compounds with antioxidant properties. The extracts were then filtered using standard filtration apparatus and subsequently reduced under pressure at 45^0C to produce the crude extracts. All extracts were stored at 4°C in the dark until needed.

2.3 Antioxidant testing

The total antioxidant capacity of the three different plant leaf extracts was performed spectrophotometrically via the phosphomolybdenum assay [35]. Extracts containing antioxidant compounds were reduced by the phosphomolybdenum reagent by producing a blue colour at an acidic pH. Antioxidant testing was carried out over two days with freshly prepared phosphomolybdenum reagents. The total antioxidant capacity of the plant leaf extracts was estimated from an ascorbic acid calibration curve ranging from 0.05-5 mg/ml (R^2=0.9905). The phosphomolybdate assay is quantitative in nature and the total antioxidant capacity (TAC) of extracts is usually expressed as ascorbic acid equivalents. The phosphomolybdate method was chosen because the reagents were readily available and cheaper when compared to the other methods.

2.3.1 Calibration curve of Ascorbic acid

A calibration curve of ascorbic acid was prepared by adding 0.1 ml aliquots of 0.0010, 0.0100, and 0.1000 mg/ml ascorbic acid solution to 0.1 ml of the freshly prepared phosphomolybdenum reagent. The samples were mixed well and incubated at 95^OC for 90 minutes. After incubation, the quantitative antioxidant estimation was performed at a wavelength of 695 nm against a reagent blank. The absorbance value for each of the concentrations of ascorbic acid, including the blank, was taken at 695nm and recorded.

2.3.2 Processing of the leaf extracts

The crude samples of the three plant leaf extracts were performed simultaneously. An aliquot of 0.1 ml crude extracts was added to 0.1 ml of the freshly prepared phosphomolybdenum. The samples were carefully mixed and then incubated at 95^{O}C for one and a half hours. After incubation, the quantitative antioxidant estimation was performed at wavelength of 695 nm against a reagent blank. The absorbance for each plant leaf extract including the reagent blank was taken and recorded.

2.4 Phytochemical screening

We identified the plant that had the highest total antioxidant activity and performed phytochemical screening for two essential antioxidants, namely phenols and flavonoids, according to standard testing procedures [39].

2.4.1 Test for Flavonoids

Screening for flavonoids was done by adding about 3 pieces of magnesium metal ribbon to 1 mL of the plant leaf extracts. Next, concentrated hydrochloric acid was added in a drop-manner. A positive test was revealed by the formation of a pinkish red colour [39].

2.4.2 Test for phenol

Screening for phenolic compounds was done by dissolving ferric chloride solution in 0.20g of the plant leaf extracts. A positive test was revealed by the formation of a green or dirty green precipitate [39].

3 Results

The total antioxidant capacity of various solvent fractions of the mustard leaves decreased in this order: hexane > ethyl acetate > ethanol. The total antioxidant capacity of various solvent fractions of the antsbush leaves decreased in this order: chloroform > ethyl acetate. The total antioxidant capacity of various solvent fractions of the guava leaves decrease in this order: hexane > methanol > ethyl acetate > ethanol (Table 1). Antioxidant activity was seen for all plant leaf extracts but in varying quantities. The ethanolic extracts showed the highest total antioxidant capacity (10.6 mg/ml for Guava and 2.5 mg/ml for mustard leaves). The lowest total antioxidant capacity was observed for the hexane extracts (0.24 mg/ml for mustard leaves and 0.33 mg/ml for guava leaves). Of the three plant leaf extracts evaluated, the most pronounced antioxidant activity was noted for the Guava leaf extracts.

Table 1- Total antioxidant capacity of the plant leaf extracts.

Plant leaves	**Type of extracts**	**TAC (mg/ml)**
Brassica juncea	**Hexane**	**0.24**
	Ethanol	**2.50**
	Ethyl acetate	**1.33**
	Mean	**1.36**
Struchium sparganophora	**Ethyl acetate**	**0.92**
	Chloroform	**0.51**
	Mean	**0.90**
Psidium guajava	**Ethyl acetate**	**5.84**
	Ethanol	**10.6**
	Methanol	**4.19**
	Hexane	**0.33**
	Mean	**5.24**

Figure 2 shows an ascorbic acid calibration curve with a correlation coefficient close to 1 (R^2=0.9999). This indicates a strong linear relationship between absorbance and concentrations and validates the readings obtain for the plant leaf extracts.

Fig.2. Calibration curve for ascorbic acid

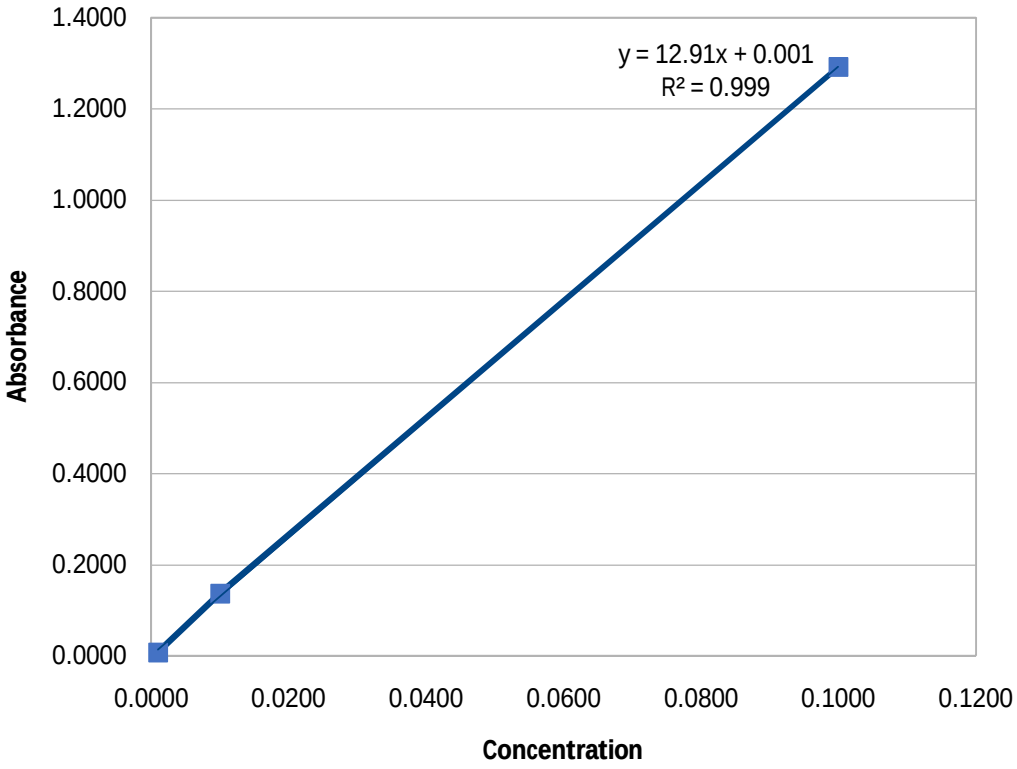

Table 2 shows that the ethanolic and methanolic extracts for *P. guajava* leaf extracts were positive for phenols, while all the extracts were positive for flavonoids (Table 2).

Table 2 - Flavonoids and phenols screening of *P. guajava* leaf extracts

Phytochemicals	Flavonoids	Phenols
Ethanol extract	+	+
Methanolic extract	+	+
Ethyl acetate extract	+	-
Hexane extract	+	-

+ Presence, - Absence

4 DISCUSSIONS

Antioxidants are compounds that prevent the oxidation of biological molecules by reducing the oxidising agents. They can destroy free radicals thereby minimizing oxidative stress associated with redox reactions. Essentially, they help prevent oxidative damage in humansand plants [5]. Antioxidant activity in plants is critical in protecting the human body from many deadly conditions, including cardiovascular diseases, cancer, and diabetes [41]. The antioxidant capacity of plants is solely related to the bioactive compounds present in them. The composition and concentration of these compounds in plants tends to vary from plants to plant [42]. Variation bioactive compounds are also seen in different plant parts. In this current study, one sample, that is the leaves from 3 different plants indigenous to Guyana, was collected and investigated separately using the phosphomolybdate method. This method evaluates the total antioxidant capacity in regard to the reductive activity of phenolics and non-phenolic compounds such as vitamin C and E.

Two to four different fractions of the crude extracts (100% concentrations) were used for comparison of their TAC. The *P. guajava leaf extracts yielded the highest TAC,* whereas the *S. sparganophora* gave the lowest. The polar ethanolic fractions give the highest TAC, whilst the non-polar hexane extracts gave the least. Our study showed that polar solvents may be used to extract antioxidant compounds in higher amounts. Similar results were noted for a study done

by [7] and [28]. Solvents such as methanol and ethanol have high polarities and are used to extract polar compounds. Examples of polar compounds include phenols and flavonoids and these are thought to be potent antioxidants [37]. Simamora et al said that flavonoids are the most important phytochemical found in the ethanol guava leaf extract, which in turn are the key component associated with antioxidant activity in their study [43]. Antioxidant activity is also said to be related to the presence of polyphenols. These polyphenols are thought to increases antioxidant activity [30].Our study showed that flavonoids and phenols were present in both the ethanolic and methanolic guava leaf extracts. A methanolic crude extract was only investigated for *P.guajava* leaf extracts. Surprisingly, this particular fraction did not yield a high TAC value as the ethanolic fraction. However, since we quantified TAC and screened for phenols and flavonoids but did not quantify specific antioxidants, including phenolics and flavonoids, the statement made by Altemimin & Choudhary (2013) may still stand. We postulate that the ethanolic fraction may have had large quantities of phenols and flavonoids along with other antioxidants.

4.1 B. juncea leaf extracts

Our study evaluated the TAC of mustard leaf extracts using three solvents (ethanol, ethyl acetate, and hexane) via the phosphomolybdenum assay. The crude extracts exhibited TAC ranging from 0.2- 2.5 mg/ml. Of the three solvents used, the most effective one was the polar solvent ethanol, followed by the semi-polar solvent ethyl acetate. The least effective solvent was the non-polar hexane. A similar finding was observed in a study done by Moirangthem & Praveen (2020), where the polar methanolic extract was most effective, followed by the semi-polar ethyl acetate extracts. The least effective solvent was the non-polar chloroform extract [44]. However, Moirangthem & Praveen (2020) employed the DPPH method. We could not find any study in the literature that examined the TAC of mustard leaf extracts via the phosphomolybdenum method. However, Aziz et al., (2020) evaluated the antioxidant capacity of Mustard seed extracts using the DPPH and ABTS assays using 30% ethanolic extracts and water extracts. They found that the antioxidant activity using the DPPH assay with IC50 value ranged from 0.170- 0.390 mg/ml, and the ABTS assay with inhibition

percent values revealed 69-76% [45]. The results from our study were much higher when compared to the DPPH values obtained by Aziz et al. (2020), however, it must be noted that the DPPH method tests for radical scavenging activity only while the phosphomolybdenum method considers the TAC.

4.2 S. sparganophora leaf extracts

Our study investigated the TAC of *S. sparganophora* leaf extracts using different solvents, namely ethyl acetate and chloroform, via the phosphomolybdenum assay. The crude extracts exhibited TAC ranging from 0.5-0.1 mg/ml. Of the two solvents used, the most effective one was the semi-polar ethyl acetate. We could not find any study in the literature that examined the TAC of *S. sparganophora* leaf extracts via the phosphomolybdenum method. However, Oboh (2006) investigated the antioxidant properties of the ethanolic extract of antsbush leaves and found that it had a strong antioxidant capacity as characterized by its high TPC (5.4 g/100 g), reducing power (2.50), and free radical scavenging ability (65.2%) [46]. Oboh (2012) later revealed that antsbush leaf extract destroyed DPPH radicals in a dose-dependent pattern (0 – 1.25 mg/ml) [21]. The results from our study were much higher when compared to the DPPH values obtained by Oboh (2012), however, it must be noted that the DPPH method tests for radical scavenging activity only while the phosphomolybdenum assay measures the TAC of the extracts.

4.3 P.guajava leaf extracts

Our study investigated the TAC of *P.guajava* leaf extracts using different solvents, namely ethanol, methanol, ethyl acetate, and hexane, via the phosphomolybdenum assay, and the most effective solvent was found to be ethanol since the highest concentration of the TAC was noted for the ethanolic extract. The next effective solvent was ethyl acetate, followed by methanol. The least effective solvent seemed to be hexane. Tachakittirungrod et al. (2007) did a similar investigation of *P.guajava* leaf extracts using different solvents, namely hexane, ethyl acetate, butanol, and methanol via the ABTS and FRAP assays, and found that the methanolic extracts possessed the highest antioxidant activity [47], which was different from our finding.

We postulate that if Tachakittirungrod et al. (2007) had investigated the ethanolic extract in their study, a similar result would have been found. Tachakittirungrod et al. (2007) also showed that the next effective solvent was butanol and ethyl acetate fractions, respectively, and the hexane fraction showed the least antioxidant activity, which corroborates our findings. Another study showed that ethanolic extract had high radical scavenging and TPC activities, but the authors investigated the guava peels [48]. However, Simamora et al (2018) in their study investigated TAC using the phosphomolybdenum assay and revealed that the leaf extracts showed stronger TAC than the fruit extracts [43].

Although our study only quantified TAC, Morais-Braga et al (2017), identified the main phenolic compounds in guava leaf extracts as catechins, gallic acid, chlorogenic acid, caffeic acid, epicatechin, rutin, quercetin, kaempferol, and luteolin [49]. A study showed that ethanol guava leaf extract showed the strongest antioxidant activity with the Trolex equivalent antioxidant capacity (TEAC) value of 4.91 ± 0.050 mM trolox equivalents/mg extract [47], which coincides with our results for guava leaves, where an average value of 5.24 mg/ml was found . Qian (2004) claimed that *P.guajava* leaf extracts comprise a valuable possible source of natural antioxidants [50]. Simamora et al. (2018) investigated the antioxidant activity of Guava leaf and fruit extracts using four methods, including the phosphomolybdate assay. They found that both the leaf and fruit extracts destroyed DPPH radicals, with IC50 of 74.77 and 843.84 µg/mL, respectively, and both extracts showed low chelating activity for ferrous ion with IC50 of 147.07 and 2105.05 µg/mL respectively [43]. The reducing power assay results increased with increasing concentrations of the leaf extracts (50 to 200 µg/mL), which implied an increase in reducing ability, but the fruit extract did not show considerable reducing activity. The phosphomolybdate test showed that the extracts and standards show evidence of an increase in absorbance, suggesting an increased reduction of Mo (VI) to Mo (V) by the antioxidant compounds in the leaf extracts [43].

According to the World Health Organization (WHO), Cancer is a leading cause of death globally. It accounts for approximately 10 million deaths and about one in six deaths in 2020 [51]. Cancer is one of the most common causes of death in Guyana, as mentioned by the Health

Minister [52]. Guyana is rated amongst the highest in the world for Cervical Cancer incidence and deaths, [53] and Breast cancer remains the number one cancer in Guyana [54]. Cancer treatments such as chemotherapy and radiography are usually expensive and have many detrimental side effects [55]. In addition, Diabetes mellitus, a chronic metabolic disorder, is the 8th leading cause of death and disability combined in the world [56]. Approximately 460 million persons across every country and age category were living with this disorder in 2019. The age-standardized death rate due to this metabolic condition was estimated at 20.9 deaths per 100,000 populations. The age-standardised death rate from Diabetes is high in Guyana with about 82.6 deaths per 100,000 populations [57]. Many of the currently used antidiabetic therapies have serious side effects, which may lead to hypoglycemia and cardiovascular and metabolic alterations [58]. Furthermore, cardiovascular diseases (CVDs) are deemed as another leading cause of death worldwide. About 17.9 million people died from CVDs in 2019, accounting for 32% of all global fatalities [59]. CVD is the most common non-communicable disease in Guyana. It accounts for 526 deaths per 100,000 individuals per year [60]. In 2017, the primary causes of death in Guyana were Ischemic heart disease (IHD) and stroke.

Cancer, Diabetes, and Cardiovascular diseases are prevalent and leading causes of death globally and regionally. The search for cheaper complementary and alternative medicines to supplement conventional therapy without much toxicity and effects is necessary. Cancer, Diabetes, and Cardiovascular diseases lead to oxidative stress, and therefore, antioxidants have great potential for both adjunct and alternative therapies.

5 CONCLUSION AND RECOMMENDATIONS

Our study shows mustard leaves, antsbush leaves, and guava leaves can be potential sources of natural antioxidants. However, guava leaves possess the highest concentration of antioxidants and would be the preferred choice for preventing and managing many diseases. It may reduce the oxidative damage associated with cancer, diabetes, cardiovascular diseases, and many other conditions. These plants are easily accessible, widely available, and cost-effective and can therefore be used to supplement conventional therapy in the form of dietary supplements, or herbal decoctions and concoctions. There is also potential for their use as

alternative therapies. Further testing should to be done to investigate the toxicity levels of these plant leaves and to determine if they would be safe to be used as adjuncts or alternative therapies for Cancer, Diabetes, and Cardiovascular diseases.

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