OPEN ACCESS
Freely Available Online

JOURNAL OF ANTIOXIDANT ACTIVITY

ISSN NO: 2471-2140

Research Article

DOI: 10.14302/issn.2471-2140.jaa-20-3478

Antioxidant Activity of Surinamese Medicinal Plants with Adaptogenic Properties and Correlation with Total Phenolic Contents

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Abstract

Plant-based preparations are commonly used in Suriname (South America) as adaptogens. In this study, fifteen alleged adaptogenic Surinamese plants have been assessed for their antioxidant activity (AA), total phenolic contents (TPC), and total flavonoid contents (TFC). The investigated plants were *Anacardium occidentale*, *Spondias dulcis*, *Annona muricata*, *Euterpe oleracea*, *Oenocarpus bacaba*, *Luffa acutangula*, *Punica granatum*, *Malpighia emarginata*, *Syzygium aqueum*, *Syzygium cumini*, *Averrhoa carambola*, and *Renealmia alpinia* (fruit); *Hibiscus sabdariffa* (calyx); as well as *Aloe vera* and *Cestrum latifolium* (leaf). Aqueous extracts (1 - 3,000 µg/ mL) were prepared. AA was determined by the FRAP and the DPPH assay. TPC and TFC were determined by the Folin-Ciocalteu's and an $AlCl_3$ colorimetric method, respectively, using gallic acid (GA) and rutin (R), respectively, as standards. Data are means ± SDs ($n \geq 3$; $P < 0.05$). FRAP values and DPPH-scavenging activities correlated positively with each other and with TPC but not with TFC. The preparations from *M. emarginata*, *A. carambola*, *A. occidentale*, *O. bacaba*, *C. latifolium*, and *H. sabdariffa* displayed the highest FRAP values (54 ± 14 to 412 ± 30 µM Fe^{2+}/100 µg), DPPH-scavenging activities (IC_{50} values of 33 ± 14 to 250 ± 50 µg/mL), and TPC (51 ± 4 to 280 ± 78 µM GAE/100 µg). TFC of all samples were ≤ 10 ± 3 RE/100 µg. The adaptogenic properties of these plants may (partially) be attributed to their high content of antioxidant phenolic compounds and may make them candidates of novel sources of health-promoting antioxidants.

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Running title: Antioxidant activity and total phenolic contents of adaptogenic plants

Keywords: Suriname, medicinal plants, adaptogenic properties, antioxidant activity, total phenolic content, total flavonoid content

Received: Jul 08, 2020 **Accepted:** Jul 16, 2020 **Published:** Jul 17, 2020

Editor: Jie Yin, Institute of Subtropical Agriculture & University of Chinese Academy of Sciences, China.

Introduction

The dependence of humans on oxygen for their metabolism leads to the continuous formation of reactive oxygen-derived species (ROS) in the body as by-products of reactions involving oxygen [1]. ROS can be generated from either endogenous or exogenous sources. Endogenous sources of ROS are cellular organelles where oxygen consumption is high, such as mitochondria, peroxisomes, and endoplasmic reticulum [2]. Exogenous sources of ROS are hazardous environmental chemicals which, as shown for the alkylating antineoplastic agent cyclophosphamide, produce free radicals during their metabolic conversion (see, for instance [3]). Furthermore, in individuals with inherited erythrocyte glucose-6-phosphate dehydrogenase deficiency [4], the red blood cells provide insufficient NADPH in response to the rate of ROS formation, resulting in the accumulation of ROS, damage to the red blood cells, and hemolytic anemia (see, for instance, [5]).

Examples of ROS are superoxide radical anion, hydrogen peroxide, peroxyl radicals, and hydroxyl [1]. These species play important roles in key physiological functions such as cell signaling and homeostasis [6, 7]. However, ROS can also attack cellular macromolecules like the nuclear DNA and plasma membrane lipids causing cellular injury [8]. Fortunately, the body has both enzymatic antioxidant systems (for instance, superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic mechanisms (for instance, bilirubin and albumin) to help mitigate this damage [9]. However, when ROS overwhelm these physiological defenses, oxidative stress occurs [10]. Oxidative stress can lead to lipid peroxidation, cell and tissues toxicity, and several types of genetic damage that eventually could cause genotoxicity, mutagenicity, secondary cancers, and even cell death (see, for instance [11]). The resulting homeostatic disruption of multiple metabolic processes may eventually result in the development of neoplastic, cardiovascular, diabetic, neurodegenerative, age-related, and inflammatory ailments [10, 12].

In addition to innate defense systems, exogenous antioxidants provided through the diet and/or nutritional supplements may help protect the body from oxidative stress [13]. Indeed, several studies have suggested that the consumption of compounds rich in antioxidants decreases the risk of developing the above-mentioned diseases [14, 15]. An important class of plant-derived antioxidants is represented by phenolic compounds, secondary plant metabolites made up of one or more aromatic ring(s) coupled to one or more hydroxyl group(s) [16]. Phenolic compounds also help protect plants from pathogens, animal and insect attack, as well as ultraviolet radiation; provide plants their characteristic colors; and contribute to the organoleptic properties of plants [17]. There are tens of thousands of plant phenolic compounds including the main dietary constituents flavonoids, phenolic acids, and tannins, in addition to coumarins, naphthoquinones, stilbenes, anthraquinones, and lignans [13, 16]. Their mitigating effect on oxidative stress has been attributed to their ability to eliminate potentially harmful oxidizing free radical species by acting as reducing agents, hydrogen donors, quenchers of singlet oxygen, or chelators of metal ions that catalyze oxidation reactions (13, 16].

Not surprisingly, the interest in plant-derived phenolic compounds with antioxidant activity is on the rise, and many of these compounds are promoted for preventing and treating illnesses and maintaining general well-being (see, for instance, [18]). Compounds used for the latter purpose have been called adaptogens, an unofficial term that refers to herbal substances that would help fight stress and fatigue and stimulate well-being by increasing the body's ability to adapt and survive [19]. When considering the importance of antioxidants to human health [13-15, 17, 18] and the capacity of plant phenolic compounds to act as antioxidants [13, 16], these phytochemicals may well constitute important ingredients of adaptogens.

The traditional use of plants and plant-based preparations is deeply rooted in the Republic of Suriname (South America), despite the nationwide availability of affordable and accessible allopathic forms of medicine [20]. Many traditional preparations are also used for promoting general health, to fight stress, and to obtain extra health benefits [21-30], and can therefore be regarded as adaptogens. Thus, these substances may display unusually high antioxidant activity and contain

unusually high amounts of phenolic compounds. So far, studies dealing with these subjects have not been carried out. Therefore, it was decided to assess a number of Surinamese plant preparations that may qualify as adaptogens for their antioxidant activity *in vitro* and their total phenolic content. As flavonoids represent an important class of plant phenolics that are also able to scavenge free radicals and inactivate catalytic metal ions [31], the plant samples have also been evaluated for their total flavonoid content. The results obtained may provide scientific substantiation for the adaptogenic properties of the plants and may help identify novel natural sources of antioxidants.

Materials and Methods

Plant selection and preparation of Plant Extracts

The plants evaluated in the current study are mentioned in Table 1. They have been selected on the basis of the number of times they have been dealt with in well-known publications on the use of medicinal plants in Suriname [21-30]. The plants have been collected in the period between September and November 2019 in rural areas around Suriname's capital city Paramaribo that had been free from herbicidal or pesticidal use for at least the preceding six months. The collected plants have been authenticated by staff members from the National Herbarium of Suriname. The plant parts of interest (Table 1) were washed with distilled water, air-dried, washed again, macerated, and extracted with distilled water. This was based on the traditional custom to prepare herbal medicinal teas, infusions, and decoctions by extracting, brewing, or boiling leaves, fruits, stembark, roots, or other plant parts with water [21-30]. The extracts were filtered, concentrated by freeze-drying, and the material obtained was divided in aliquots of 0.2 g which were stored at -20 ^{o}C and tested shortly thereafter.

Drugs and Chemicals

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Folin-Ciocalteu reagent, gallic acid, aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), rutin, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was from Applichem GmbH (Darmstadt, Germany), sodium carbonate (Na_2CO_3) from Merck, (Darmstadt, Germany), and sodium acetate (CH_3COONa) from BDH Laboratory Supplies (Poole, UK). All other chemicals used were from our laboratory stock and were of the highest grade available.

Determination of Antioxidant Activity of Plant Extracts by the Ferric Reducing/Antioxidant Power Assay

The antioxidant activity of the plant extracts was determined by a spectrophotometric method based on the ability of an antioxidant to reduce a ferric (Fe^{3+}) ion from the Fe^{3+}-TPTZ complex to the ferrous (Fe^{2+}) ion from a Fe^{2+}-tripyridyltriazine complex through the donation of an electron at low pH [32]. The reactions were spectrophotometrically monitored by measuring the change from the colorless Fe^{3+}-TPTZ complex to the intensely blue-colored Fe^{2+}-tripyridyltriazine complex at a wavelength at 593 nm. Thus, 3 mL freshly prepared ferric reducing/antioxidant power (FRAP) reagent was mixed with 100 µL of a plant extract and 1 mL distilled water. The FRAP reagent consisted of TPTZ 10 mM in HCl, $FeCl_3 \cdot 6H_2O$ 20 mM, and acetate buffer 300 mM pH 3.6 in the proportion of 1/1/10 (*v/v/v*).

After thorough mixing and incubation for 30 min in the dark and at room temperature, the absorbance at 593 nm was recorded against a blank consisting of samples where the plant extract was substituted by distilled water. The change in absorbance was directly related to the total reducing power of the electron-donating antioxidants present in the plant extracts. These were estimated from a calibration curve constructed from the absorbance of different concentrations of $FeSO_4$ at 593 nm and expressed as µm Fe^{2+} equivalents reduced per 100 µg lyophilized plant extract.

Determination of Antioxidant Activity of Plant Extracts by the 1,1-diphenyl-2-picrylhydrazyl Assay

The plant extracts were also assessed for antioxidant activity using a DPPH free radical scavenging assay [33]. This assay is based on the ability of an antioxidant to inactivate the stable DPPH cation free radical following donation of an electron or hydrogen atom. During his process, the violet colored DPPH molecule becomes colorless to pale yellow, which can spectroscopically be monitored at a wavelength 517 nm.

Table 1. Plants investigated in the current study, plant part used and method of processing, as well as the most common traditional medical uses in Suriname

Plant family		**Plant part used and method of processing**	**Most common traditional adaptogenic uses (references)**
Anacardiaceae	*Anacardium occidentale* L. (cashew; kasyu)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Throat infections [23, 24. 26, 28]
Anacardiaceae	*Spondias dulcis* L. (ambarella; pommesitère)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Fever, cough, wounds, sores, burns [22, 25]
Annonaceae	*Annona muricata* L. (soursop; zuurzak)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Insomnia, tension, anxiety, exam stress, bedwetting [24, 26, 28, 29]
Arecaceae	*Euterpe oleracea* Mart. (açai; podosiri)	Fruit; pulp around seeds removed, macerated, and extracted with distilled water at room temperature, filtered, and freeze-dried	Anemia, low blood pressure [29, 30]
Arecaceae	*Oenocarpus bacaba* Mart. (turu palm; kumbu)	Fruit; pulp around seeds removed, macerated, and extracted with distilled water at room temperature, filtered, and freeze-dried	Anemia, low blood pressure (28, 29]
Asphodelaceae	*Aloe vera* (L.) Burm.f. (aloe; aloë)	Inner leaves; squeezed, and gel diluted with distilled water at room temperature, filtered, and freeze-dried	Burns, scars, wound infections, skin rash, scars, hair loss, dandruff, fever, headache [24, 26, 28-30]
Cucurbitaceae	*Luffa acutangula* (L.) Roxb. (ridged gourd; sukwa)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Gall bladder functioning [24]

Lythraceae	*Punica granatum* L. (pomegranate; granaatappel)	Fruit; seed pulps removed, macerated, and extracted with distilled water at room temperature, filtered, and freeze-dried	General health tonic, bleeding gums, lower respiratory tract complaints, diarrhoea [21, 24, 28]
Malpighiaceae	*Malpighia emarginata* DC. (1753) (acerola; Westindische kers)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Flu, sore throat, pimples (28, 29]
Malvaceae	*Hibiscus sabdariffa* L. (roselle; syuru)	Calyces; macerated, and infusion prepared, filtered, and freeze-dried	Coughing, microbial infections, skin and hair care [30]
Myrtaceae	*Syzygium aqueum* (Burm.f.) Alston (watery rose apple; pommerak)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Tonic to improve liver and brain functioning [22, 25]
Myrtaceae	*Syzygium cumini* (L.) Skeels. (jambolan; dyamun)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Anemia, abdominal pain, diarrhoea, coughing up of blood [23, 24, 26, 28]
Oxalidaceae	*Averrhoa carambola* L. (star fruit; birambi)	Fruit; squeezed, and juice collected at room temperature, filtered, and freeze-dried	Fever, respiratory tract complaints, fungal skin infections [28]
Solanaceae	*Cestrum latifolium* Lam. (bitter greens; bitawiwiri)	Leaves; macerated and extracted with water for 1 h at 70 °C, filtered, and freeze-dried	Anemia, migraine, stress, flu, eye inflammation, sore throat, pimples, itching [24, 283, 29]
Zingiberaceae	*Renealmia alpinia* (Rottb.) Maas (ink plant; masusa)	Fruit; pulp extracted at room temperature, filtered, and freeze-dried	Genital steam baths [27]

Thus, for each plant extract, seven serial dilutions between 1 and 3,000 µg/mL were prepared, and 0.3 mL of each dilution was mixed with 3 mL absolute ethanol and 0.5 mL DPPH solution of 0.5 mM in ethanol. After 90 min in the dark and at room temperature, the absorbance of the solutions was measured at 517 nm against a mixture of 3.3 mL ethanol and 0.5 mL sample as a blank. The control solution consisted of 3.5 mL ethanol and 0.3 mL DPPH solution.

The percentage antioxidant activity (AA %) of each dilution of each plant extract was determined using the formula:

$$AA\ \% = 100 - \left| \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \right|$$

where Abs_{sample} is the absorbance of the plant extract, Abs_{blank} the absorbance of the blank, and $Abs_{control}$ the absorbance of the control. For each plant extract, the absorbance values of the dilutions were plotted against the corresponding concentrations. From the resulting dose-response curve, IC_{50} values were derived, *i.e.*, the concentrations of the plant extracts (in µg/mL) accomplishing a 50% decrease in absorbance when compared to untreated controls. The lower the IC_{50} value, the higher the antioxidant activity.

Determination of Total Phenolic Content of Plant Extracts

The total phenolic content of the extracts was determined using the Folin-Ciocalteu's method [34]. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate, and the method is based on the transfer of electrons in alkaline medium from phenolic compounds to the phosphomolybdate/phosphotungstate complex to form a blue chromophore that is spectrophotometrically detectable. Thus, each plant extract was dissolved in distilled water to a concentration of 100 µg/mL. Of each extract, an aliquot of 1.0 mL was added to 0.1 mL Folin-Ciocalteu reagent 1 N, after which 0.9 mL distilled water was added. The mixture was shaken and allowed to react for 5 min at room temperature. Then, 1.0 mL of Na_2CO_3 7% (*w/v*) was added. This solution was adjusted with distilled water to a final volume of 3.4 mL and thoroughly mixed. After incubation for 30 min in the dark, the absorbance was read at 765 nm with respect to a blank containing only Folin-Ciocalteu reagent 1 N and Na_2CO_3 7% (*w/v*). The total phenolic content of the plant extracts was calculated from the linear equation of a standard curve prepared with gallic acid (1 to 200 µg/mL) and expressed as µM gallic acid equivalents (GAE) per 100 g lyophilized plant extract.

Determination of Total Flavonoid Content of Plant Extracts

Total flavonoid content of the plant extracts was determined using a previously described aluminum chloride ($AlCl_3$) colorimetric method [35]. This method is based on the formation of acid-stable complexes between $AlCl_3$ and the hydroxyl groups of flavones and flavonols. Thus, each plant extract was dissolved in distilled water to give samples of 100 µg/mL. A volume of 0.5 mL $AlCl_3$ 2% (*w/v*) in absolute ethanol was added to 0.5 mL aliquots of each sample, after which 0.5 mL 1 M potassium acetate and 0.5 mL 1 M HCL were added. The mixture was incubated for 10 min at room temperature and the absorbance was measured at 425 nm against a blank of distilled water. A yellow color indicated the presence of flavonoids. Total flavonoid content of the plant extracts was calculated by intrapolation into a standard curve of rutin prepared from serial dilutions of this compound between 0 and 200 µg/L. Data were expressed as mg rutin equivalents (RE) per 100 g lyophilized plant extract.

Data Processing and Statistics

All experiments have been carried out at least three times in triplicate. Based on the degree of antioxidant activity found, the samples have been classified into those with high, intermediate, and low antioxidant activity. Data (means ± SDs) have been compared using Student's t test. The relationship between FRAP values and DPPH free radical-scavenging activities, and between FRAP values or DPPH free radical-scavenging activities and total phenolic contents or total flavonoid contents, were explored using two-tailed analysis of bivariate correlation. In all cases, P values < 0.05 were taken to indicate statistically significant differences.

Results

Relationships Between FRAP Values and DPPH free Radical-scavenging Activities, and Between Antioxidant Activities and Phytochemical Contents of the Plant Samples

In the current study, fifteen plant extracts that are used in Suriname as adaptogens have been assessed for their antioxidant activity, total phenolic content, and total flavonoid content. Using linear regression analysis, a significant positive correlation (p value < 0.001) was found between FRAP values and DPPH free radical-scavenging activities (a correlation coefficient R^2 of about 0.30; Figure 1).

Particularly FRAP values of the preparations correlated well with their total phenolic contents (correlation coefficient R^2 of about 0.91; Figure 2a), those with higher activity having a relatively high phenolic content and those with low activity a relatively low phenolic content (p value < 0.001). Such a good correlation was not found between DPPH free radical-scavenging activities and total phenolic contents, but there was still a significant positive relationship (p value < 0.001) between both parameters (a correlation coefficient R^2 of about 0.25; Figure 2b).

On the other hand, FRAP values and DPPH free radical-scavenging activities did not correlate well with total flavonoid contents. Correlation coefficients R^2 were 0.0012 and 0.0092, respectively (Figures 3a and 3b, respectively), indicating a poor correlation between antioxidant activities and total flavonoid contents (p values of 0.904 and 0.594, respectively).

FRAP Values and DPPH Free Radical-scavenging Activities, and Total Phenolic and Flavonoid Contents of the Plant Samples

Based on the degree of antioxidant activity found, the samples have been classified into those with high, intermediate, and low antioxidant activity (Table 2).

Plant Extracts with high Antioxidant Activity

The *Malpighia emarginata* DC fruit extract exhibited the highest antioxidant activity of the 15 plant samples evaluated, *i.e.*, a FRAP value of µM Fe^{2+} equivalents reduced per 100 µg lyophilized material and a DPPH free radical-scavenging activity at an IC_{50} value of 33 ± 14 µg/mL (Table 2). This preparation also had the highest total phenol content, namely 280 ± 78 µM GAE per 100 µg lyophilized plant material (Table 2). However, its total flavonoid content was relatively low (3 ± 0 RE per 100 µg lyophilized material; Table 2). Thus, the relatively high antioxidant activity of the *M. emarginata* preparation correlated well with its relatively high total phenolic content but not with its relatively low total flavonoid content.

Plant Extracts with an Intermediate Antioxidant Activity

The extracts from *Averrhoa carambola* L., *Anacardium occidentale* L., and *Oenocarpus bacaba* Mart. fruit as well as those from *Hibiscus sabdariffa* L calyx and *Cestrum latifolium* Lam. leaf had intermediate to high FRAP values (54 ± 14 to 165 ± 29 µM Fe^{2+} equivalent reduced per 100 µg lyophilized material, respectively; Table 2), and relatively high DPPH free radical-scavenging activities (IC_{50} values of 78 ± 17 to 250 ± 50 µg/mL; Table 2). When compared to the *M. emarginata* sample, these preparations had the second highest total phenolic content, namely 51 ± 4 to 83 ± 10 µM GAE per 100 µg lyophilized plant material (Table 2). Their total flavonoid contents ranged from were 3 ± 1 to 10 ± 3 µM RE per 100 µg lyophilized plant material (Table 2). Thus, the fairly high antioxidant activity of these preparations correlated reasonably well with their intermediate to high total phenolic content but not with their total flavonoid content.

Plant Extracts with a Low Antioxidant Activity

The extracts from *Euterpe oleracea* Mart., *Aloe vera* (L.) Burm.f., *Punica granatum* L., *Syzygium cumini* L., *Renealmia alpinia* (Rottb.), *Spondias dulcis* L., *Annona muricata* L., *Luffa acutangula* (L.) Roxb, and *Syzygium aqueum* (Burm.f.) exhibited very low to intermediate FRAP values (0 to 59 ± 12 µM Fe^{2+} equivalents reduced per 100 µg lyophilized material; Table 2) and very low to high DPPH free radical-scavenging activities (IC_{50} values of > 3000 to 308 ± 8 µg/mL; Table 2). Their total phenolic content was on the lower side (9 ± 3 to 25 ± 4 GAE per 100 µg lyophilized plant material; Table 2). Their total flavonoid content ranged from 3 ± 0 to 6 ± 2 µM RE per 100 µg lyophilized material; Table 2). Apparently, the

Table 2. FRAP values, DPPH-scavenging activities, total phenolic contents, and total flavonoid contents of the plant extracts investigated in the current study

Plant species	FRAP activity (μm Fe^{2+} equivalents reduced per 100 μg lyophilized plant extract)	DPPH activity (IC_{50} in μg/mL)	Total phenolic content (μM GAE per 100 μg lyophilized plant extract)	Total flavonoid content (μM RE per 100 μg lyophilized plant extract)
High antioxidant activity				
M. emarginata	412 ± 30	33 ± 14	280 ± 78	3 ± 0
Intermediate antioxidant activity				
O. bacaba	165 ± 29	78 ± 17	53 ± 2	4 ± 2
A. carambola	123 ± 13	133 ± 14	89 ± 1	10 ± 3
C. latifolium	87 ± 17	150 ± 0	83 ± 10	3 ± 1
H. sabdariffa	63 ± 9	183 ± 29	51 ± 4	4 ± 2
A. occidentale	54 ± 14	250 ± 50	61 ± 4	10± 4
Low antioxidant activity				
E. oleracea	59 ± 12	617 ± 29	25 ± 2	3 ± 0
P. granatum	53 ± 4	400 ± 0	22 ± 2	3 ± 0
S. aqueum	36 ± 8	2,533 ± 351	13 ± 2	6 ± 2
R. alpinia	25 ± 5	> 3,000	23 ± 4	4 ± 1
S. dulcis	10 ± 3	> 3,000	12 ± 2	5 ± 1
S. cumini	0	308 ± 8	25 ± 4	3 ± 0
A. vera	0	1,850 ± 650	22 ± 10	3 ± 0
L. acutangula	0	> 3,000	9 ± 3	4 ± 1
A. muricata	0	> 3,000	13 ± 5	4 ± 1

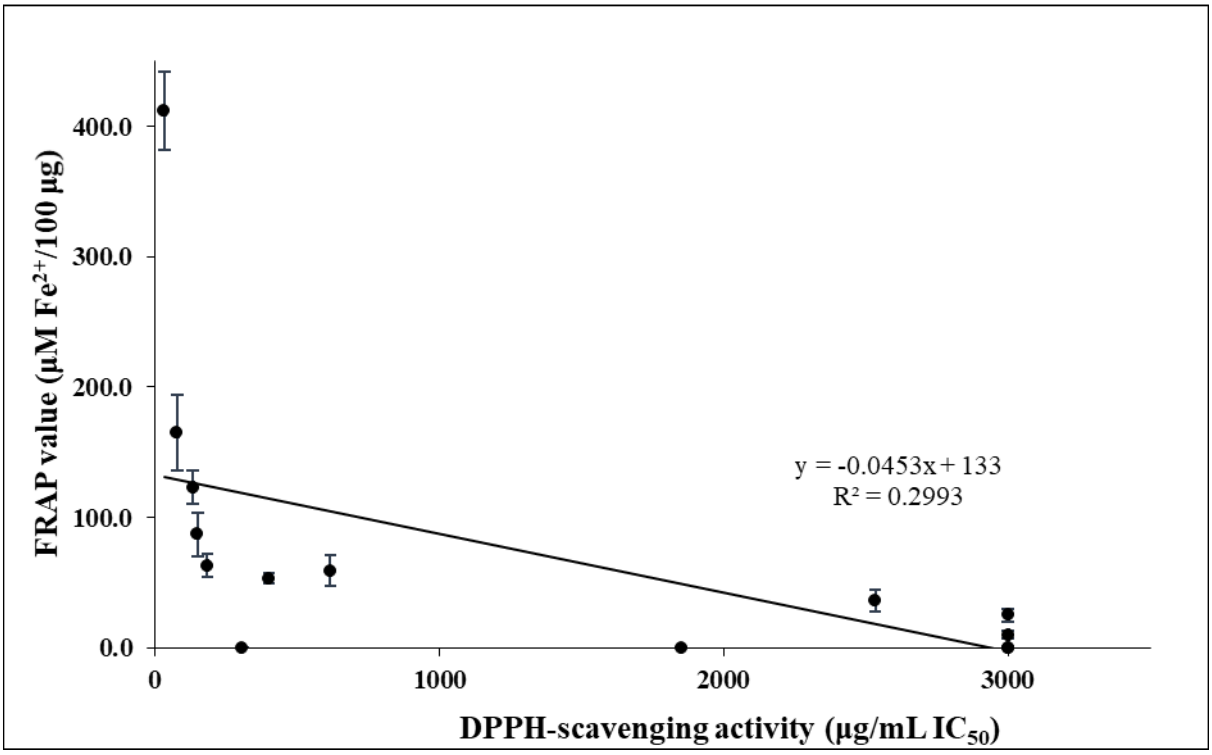

Figure 1. Relationship between DPPH-scavenging activities and FRAP values in the plant extracts

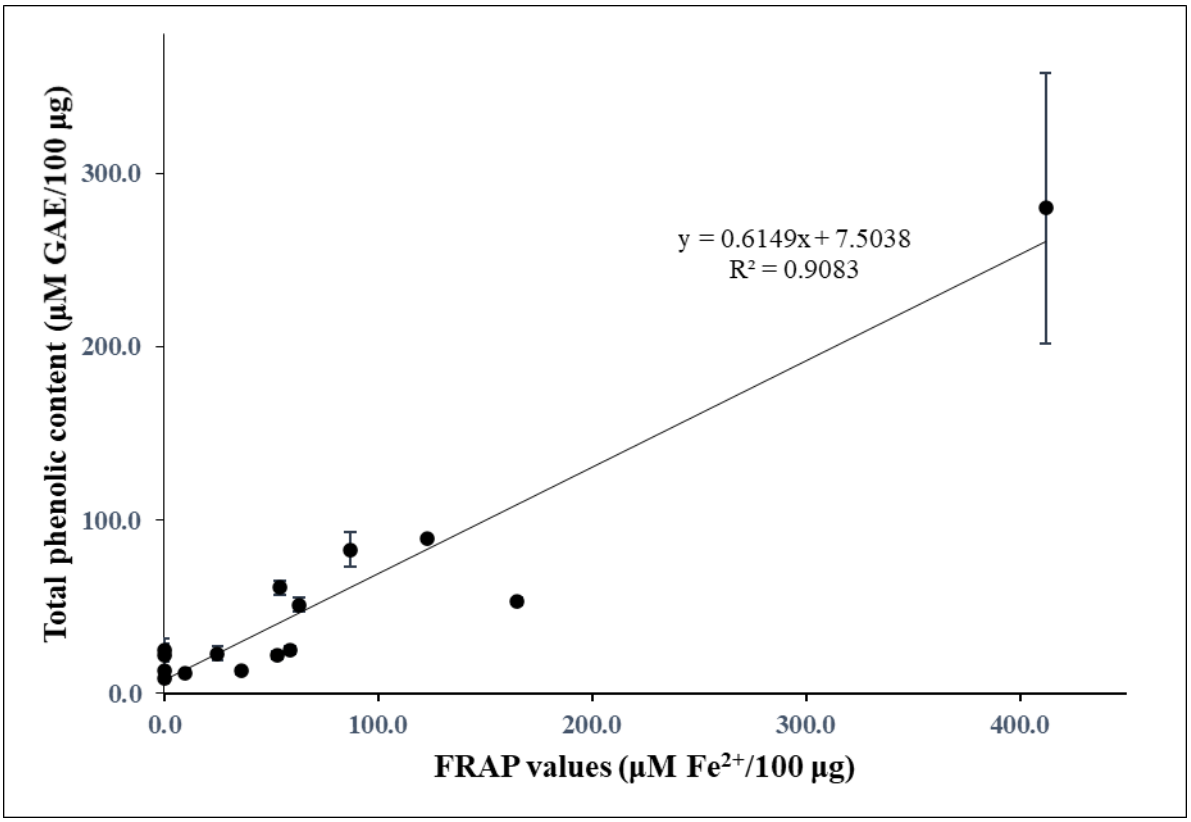

Figure 2a. Relationship between total phenolic contents and FRAP values in the plant extracts

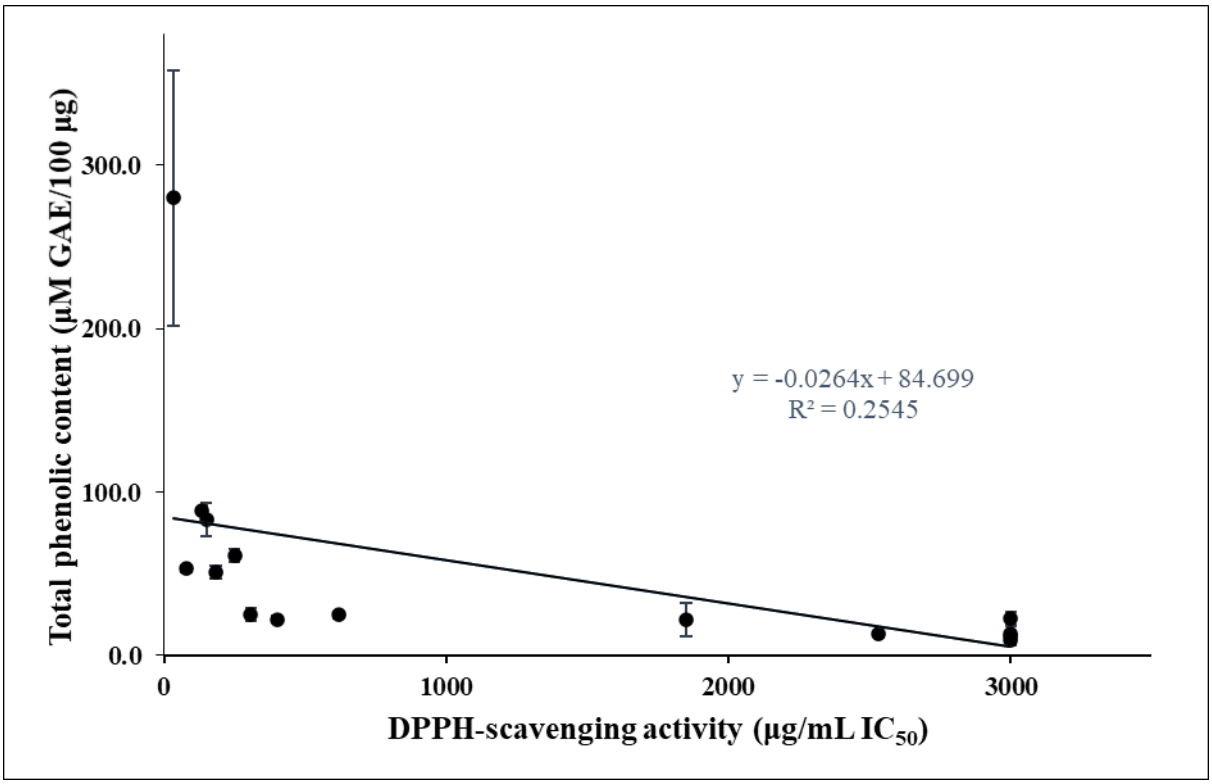

Figure 2b. Relationship between total phenolic contents and DPPH-scavenging activities in the plant extracts

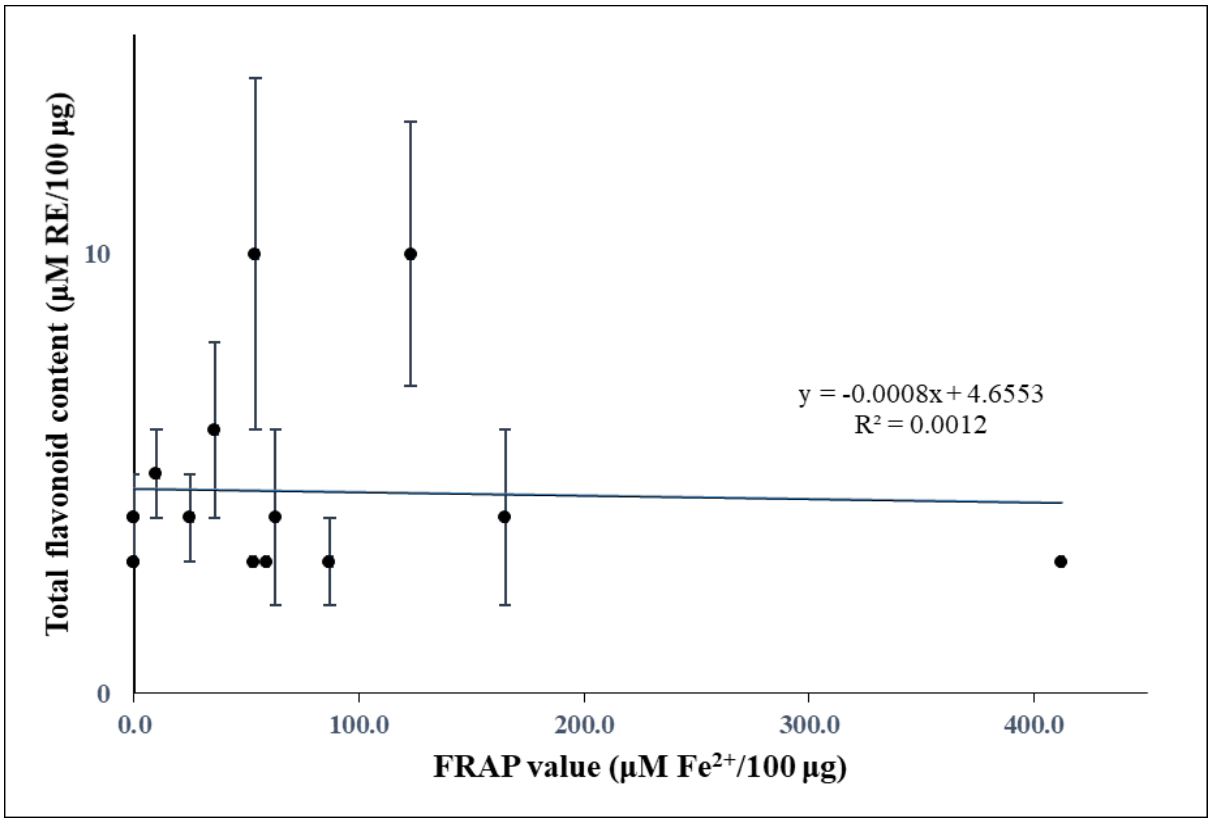

Figure 3a. Relationship between total flavonoid contents and FRAP values in the plant extracts

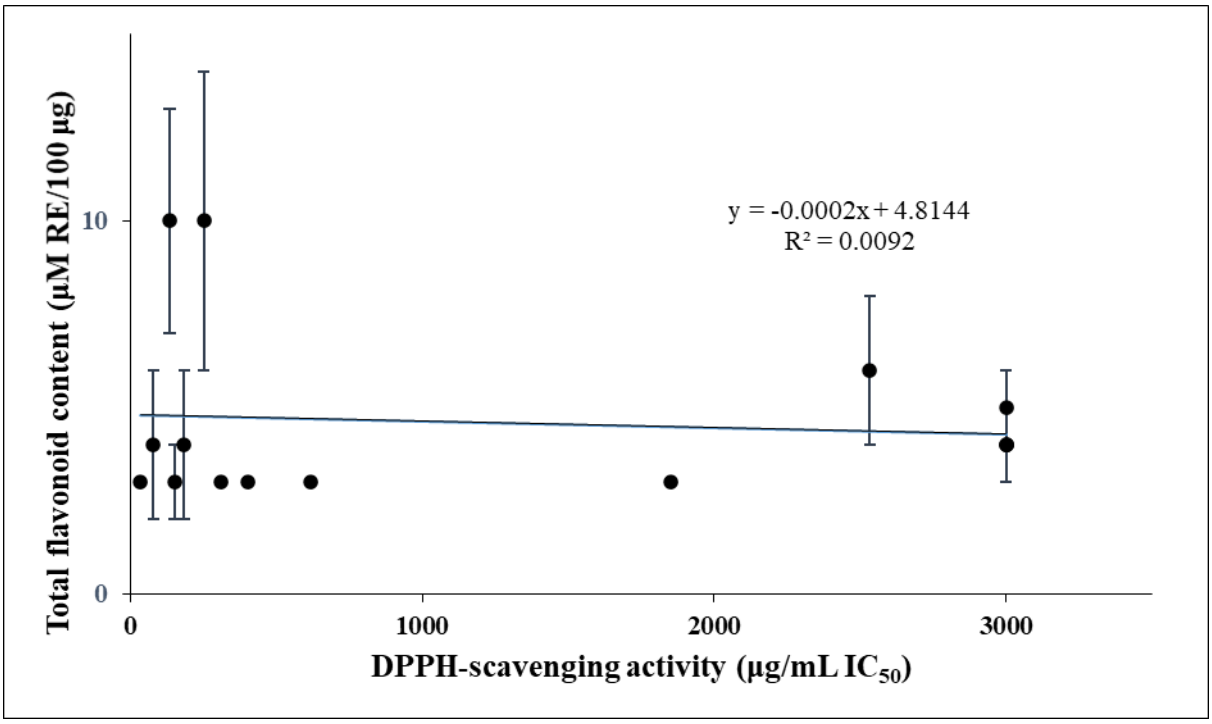

Figure 3b. Relationship between total flavonoid contents and DPPH-scavenging activities in the plant extracts

antioxidant activity of these samples partially correlated with their total phenolic content but not very well with their total flavonoid content.

Discussion

Preparations from *A. occidentale*, *S. dulcis*, *A. muricata*, *E. oleracea*, *O. bacaba*, *L. acutangula*, *P. granatum*, *M. emarginata*, *S. aqueum*, *S. cumini*, *A. carambola*, and *R. alpinia* fruit; *H. sabdariffa* calyx; as well as *A. vera* and *C. latifolium* leaf are extensively used in Suriname for their presumed adaptogenic properties [21-30]. In this study, the possibility that a relatively high antioxidant activity and phenolic content are involved in the alleged health-promoting properties of the plants has been investigated using FRAP and DPPH assays as well as Folin-Ciocalteu's and $AlCl_3$ colorimetric methods. The results obtained showed a good correlation between FRAP values and DPPH free radical-scavenging activities of the samples, as well as linear relationships between antioxidant activities and total phenolic content. Such a relationship was not found with flavonoid content. Furthermore, the samples from *M. emarginata*, *A. carambola*, *A. occidentale*, and *O. bacaba* fruit as well as *C. latifolium* leaf and *H. sabdariffa* calyx displayed both intermediate to high antioxidant activities and intermediate to high phenolic contents. These findings may account, at least partially, for the presumed adaptogenic properties of these plants. This did not seem to hold true for the samples of *S. dulcis*, *A. muricata*, *E. oleracea*, *L. acutangula*, *P. granatum*, *S. aqueum*, *S. cumini*, and *R. alpinia* fruit as well as that from *A. vera* leaf. Thus, these plants either cannot be considered 'genuine' adoptogens, or their adoptogenic qualities may be attributable to properties other than the capacity to eliminate free radicals.

The reasonable correlation between FRAP values and DPPH free radical-scavenging activity suggested that both activities were to some degree consistent with each other. This is in accordance with the comparable principles of these assays: the FRAP assay is based on the ability of an antioxidant to reduce Fe^{3+} ions to Fe^{2+} ions by donating a hydrogen atom [32], the DPPH assay on the capacity of an antioxidant to inactivate the stable DPPH cation radical by donating a hydrogen atom or electron [33]. Therefore, it can be suggested that the antioxidant ingredients in the different plant samples may have some structural and/or biochemical characteristics in common. Indeed, the possibility of

structure-activity-relationships accounting for these observations has been mentioned before (see, for instance [36]).

The statistically significant positive relationship of both FRAP values and DPPH free radical-scavenging activities with total phenolic contents suggests that phenolics played an important role in the antioxidant activity of the plant samples. This is in accordance with data from many previous studies (see, for instance, references [37, 38]) suggesting that the antioxidant activities of plant samples were to a considerable extent determined by their phenolic content. On the other hand, the absence of a significant positive relationship of either FRAP values and DPPH free radical-scavenging activities with total flavonoid contents suggests that these ingredients were not major contributors to the antioxidant activities of the plant extracts. Of note, such a poor correlation between antioxidant activity and total flavonoid content has been reported before [39, 40].

The highest total phenolic content and the highest antioxidant activity among the fifteen plants investigated was found for the *M. emarginata* fruit extract. This finding is in agreement with previous reports mentioning that an aqueous extract of *M. emarginata* fruit displayed very potent *in vitro* antioxidant activity [41, 42] and that its antioxidant activity correlated positively with its total phenolic content [43, 44]. Furthermore, a methanol extract of *M. emarginata* fruit had the highest antioxidant activity among ten other underutilized fruits of Andaman Islands (India) [43] and the highest phenolic content among eleven fruits from Ranong Province and local markets in Bangkok (Thailand) [44]. The antioxidant activity has been associated with, among others, phenolic compounds such as benzoic acid derivatives, phenylpropanoid derivatives, flavonoids, and anthocyanins, in addition to several carotenoids and an abundant amount of ascorbic acid [42].

The antioxidant activities of the extracts from *A. carambola*, *A. occidentale*, and *O. bacaba* fruit as well as that of *H. sabdariffa* calyx were in the intermediate to high range and these samples - along with that from *C. latifolium* leaf - had the second highest total phenolic content when compared to *M. emarginata* fruit. These observations are in accordance with the strong positive correlation found between total phenolic content and antioxidant activity for *A. carambola* fruit [45]. The current findings are also in agreement with the high antioxidant activity reported for *A. carambola*, *A. occidentale*, and *O. bacaba* fruit as well as *H. sabdariffa* calyx [45-48]. For the A. carambola sample, this was probably attributable to polyphenolic compounds such as gallic acid, syringic acid, p-coumaric acid, epicatechin, isoquercetin, and procyanidin B2 in addition to ascorbic acid [49]; for A. occidentale fruit preparations to proanthocyanidins, flavonoids, anthocyanins, tannins as well as ascorbic acid [50]; for *O. bacaba* fruit to various phenolic compounds including flavonoids and anthocyanins [51]; and for (methanol extracts of) *H. sabdariffa* calyx mainly to flavonoids, anthocyanins, phenylpropanoids, and carotenoids [52].

As mentioned above, the *C. latifolium* leaf sample also displayed an intermediate to high antioxidant activity and an intermediate total phenolic content in the current study. Unfortunately, to the best of our knowledge, there are no literature data available for comparison with our findings. However, leaf extracts from other *Cestrum* species such as the purple cestrum *C. elegans*, the red cestrum *C. fasicilatum*, the green cestrum *C. parqui*, and the night-blooming cestrum *C. nocturnum* elicited, comparably to that of *C. latifolium* in the current study, notable antioxidant activity [53, 54]. Phytochemical analyses revealed that *C. elegans*, *C. fasicilatum*, and *C. parqui* leaves were negative for phenolic compounds but positive for relatively high amounts of flavonoids [54], whereas methanol extracts of various parts of *C. nocturnum* contained substantial amounts of both flavonoids and phenols and exhibited notable free radical-scavenging properties [53]. Thus, the precise involvement of phenolics and flavonoids in the antioxidant activity of the *C. latifolium* leaf sample remains to be determined.

The extracts from *E. oleracea*, *P. granatum*, and *S. cumini* fruit displayed an intermediate to high antioxidant activity but a low total phenolic content in the current study. These findings are not in accordance with literature data mentioning that these parts of the plants had high antioxidant activity. Indeed, several investigators reported substantial antioxidant activity of, and considerable quantities of phenolics, - particularly

anthocyanins - in *E. olearacea* fruit pulp [55, 56]; appreciable antioxidant activity and a relatively high phenolic content of *P. granatum* juice that included, among others, gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, epicatechin, quercetin and rutin [57, 58]; and meaningful antioxidant activity and significant amounts of phenolics, - particularly anthocyanins and tannins - as well as carotenoids and antioxidant vitamins in the fruit of *S. cumini* [59, 60].

The discrepancy between the relatively low total phenolic contents found for the *E. oleracea*, *P. granatum*, and *S. cumini* samples in the current study, and values reported in the literature, could possibly be ascribed, at least in part, to differences in the extraction methods applied. For instance, samples of *P. granatum* peel, seed, and seed coat displayed much higher antioxidant activities and phenolic contents upon extraction with 0.1 M HCl: ethanol when compared to those extracted with distilled water [61]. The inconsistency between the intermediate to high antioxidant activity of the samples and their relatively low total phenolic contents suggests that phenolics were not the only or the major contributors to their antioxidant activity, and that other secondary metabolites might be involved in this activity. Markedly, for *E. oleracea* fruit pulp, the two major anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) reportedly contributed for about 10% to its overall antioxidant activity, signifying that unidentified substances were responsible for the largest part of activity [62]. Of note, non-phenolic antioxidant secondary metabolites such as volatile oils, carotenoids, polyunsaturated fatty acids, polysaccharides, and vitamins have also been found to be mainly responsible for the antioxidant activities of certain algae [63].

The extracts from *R. alpinia* fruit and *A. vera* leaves displayed (very) low antioxidant activity and total phenolic contents in the current study. These findings are partially in line with the relatively low antioxidant activity reported for *R. alpinia* fruit pulp [64] despite the presence of phenolic compounds, flavonoids, carotenoids, anthocyanins, and vitamins in this part of the plant, some of which are responsible for the yellow color of the pulp and the red-purple color of its peel [64]. It is possible that these compounds, similarly to those addressed in the preceding paragraph [61-63], did not possess major antioxidant activity, but this supposition must be verified in future studies.

The very low antioxidant activity of the *A. vera* leaf preparation seen in the current study is at variance with studies reporting high antioxidant activity of a leaf extract of the plant [65, 66]. This has been ascribed to, among others, flavonoids, tannins, β-carotene, as well as vitamins C and E [65, 66]. The dissimilarities between the results from the current study and those mentioned in the literature could be due to the often described variability in biological activity of *A. vera* samples caused by differences in the state of maturity and genotype; conditions of cultivation, harvest time, climatic factors, and the method for harvesting [44, 67], and/or the method of extraction and the solvent used for extraction [68].

The samples from *S. dulcis*, *A. muricata*, *L. acutangula*, and *S. aqueum* fruit had the lowest antioxidant activity and total phenolic content. For the preparations of *S. dulcis* and *A. muricata* fruit, these findings are in line with previous observations indicating that the ethanol extracts of *S. dulcis* and *S. cumini* fruit indeed displayed a relatively low DPPH radical scavenging activity and total phenolic content in a study with eleven cheap Bangladeshi fruits [69]. Furthermore, although *A. muricata* fruit contains phenolic compounds, flavonoids, ascorbic acid, carotenoids, as well as acetogenins with antioxidant activity [70], an ethanolic extract of Sri Lankan *A. muricata* fruit pulp displayed only a moderate antioxidant activity and total phenolic content when compared to, for instance, the Italian *A. cherimola* as well as pomegranate and mango fruits [71].

The current findings with the *L. acutangula* and *S. dulcis* samples could tentatively be explained by the dependence of their antioxidant activity and phenolic content on the polarity of the solvent used, extractions with more polar solvents yielding less activity and phenolics [72, 73]. In this respect, a methanol extract of *L. acutangula* fruit and several derived apolar fractions displayed appreciable antioxidant activities (which, however, did not correlate with phenolic and flavonoid contents) while the residual aqueous fraction did not [73]. And *S. aqueum* fruit reportedly displayed

notable antioxidant activity [43] and represented a rich source of phenolics and flavonoids [74] including anthocyanidines [75] but yielded less antioxidant activity and phenolic compounds when extracted with distilled water instead of methanol [43].

Conclusions

The results from the current study showed that preparations from *M. emarginata*, *A. carambola*, *A. occidentale*, and *O. bacaba* fruit as well as *C. latifolium* leaf and *H. sabdariffa* calyx displayed relatively high antioxidant activity that correlated well with a high phenolic content. These observations may qualify these plants as 'genuine' adaptogens and may help account for some of their claimed medicinal properties [21-30]. Importantly, these plants may represent novel natural sources of antioxidants and bioactive health-promoting phytochemicals. The samples from *S. dulcis*, *A. muricata*, *E. oleracea*, *L. acutangula*, *P. granatum*, *S. aqueum*, *S. cumini*, and *R. alpinia* fruit as well as that from *A. vera* leaf displayed relatively low antioxidant activities and phenolic contents. This suggests that these plants should not be considered 'genuine' adoptogens. However, the possibility exists that their adoptogenic qualities are attributable to compounds other than phenolic antioxidants such as carotenoids and/or vitamins C and E. It is also possible that the method of extraction - using distilled water instead of, for instance, methanol - was insufficiently efficient to produce phenolic antioxidants. Studies to assess these possibilities in our laboratories are currently in preparation.

Funding

This study was partially supported by the Suriname Conservation Foundation [project number SCF. 2012.005).

Declaration of Interest

The authors declare that they do not have competing interests.

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