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In Vitro Antioxidative Activity of Indigenous Ghanaian Fruits and Vegetables †

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Abstract: Changes in human dietary patterns have contributed to the various forms of malnutrition and nutrition-related non-communicable diseases (NCDs) prevalent in the world today. Ghana, is not exempted from this epidemic as it is currently experiencing a rising prevalence of cardiovascular diseases and related NCDs. Recent studies report that underutilised crops, mostly fruits and vegetables may contain higher amounts of nutraceutical constituents such as: antioxidants, vitamins and minerals than conventional agricultural crops, signifying their potential to contribute to the elimination of these nutrient deficiencies and diet-related health problems. In view of this, we aimed to investigate the antioxidative potency of 7 indigenous edible fruits and vegetables collected from 3 different regions of Ghana. Methanolic extracts of samples were evaluated *in vitro* using the DPPH and ORAC assays. All fruit parts of *Chrysophyllum albidum*: epicarp, mesocarp and aril exhibited significant radical scavenging abilities in the ORAC assay (IC_{50} 58.13 ± 3.42 μg/ml; 18.54 ± 0.27 μg/ml; 37.34 ± 6.6 μg/ml respectively). The mesocarp of *C. subnudum*, however exhibited the highest antioxidative activity of IC_{50} 45.18 ± 8.61 μg/ml in the DPPH assay. The results suggest that these fruits may serve as effective antioxidants in the diets of Ghanaians and can be used in the food industry.

Keywords: Malnutrition; oxidative stress; antioxidant activity; underutilised crops; Western region of Ghana

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1. Introduction

Research have shown that antioxidants may help reduce the risks of non-communicable diseases (NCDs) by scavenging free radicals, mainly reactive oxygen species, produced during cell metabolism [1,2]. Changes in human dietary patterns have contributed to the various forms of malnutrition and nutrition-related NCDs such as: cardiovascular diseases, diabetes, obesity and certain types of cancer prevalent in the world today [3,4]. Ghana, is no exemption from this epidemic as latest data shows that the country is experiencing an increasing prevalence of cardiovascular diseases and related NCDs [5,6,7,8] with higher prevalence in urban than rural dwellers and significantly higher in women than men [6,9]. In view of this, sustainable and cost-effective measures are needed to mitigate this epidemic. Fruits and vegetables are known to be good sources of antioxidants hence eating recommended amounts can significantly reduce oxidative stress which is a precursor to oxidative damage and implicated in the pathogenesis of many NCDs [10,11].

The daily intake of fruits and vegetables increased the oxygen radical absorption capacity (ORAC) of blood, representing higher antioxidant defence of the body [10,12]. In recent years, it has been proven that underutilised crops may contain higher amounts of specific health-beneficial constituents such as antioxidants, vitamins and minerals than conventional agricultural crops, signifying their potential to contribute to the reduction of these nutrient deficiencies (hidden hunger) and diet-related health problems [13,14]. In sub-Saharan Africa and in Ghana, indigenous fruits and vegetables play a critical role in societies contributing to food, nutrition security and health [11,15]. Although most rural dwellers of Ghana utilise wild fruit and vegetable species to maintain and boost their health, the potential of these indigenous edible plant species to alleviate various NCDs prevailing in the country has not yet been exploited and studies that identify both the nutritional and functional properties of these plant species are limited [16,17]. Hence, data on their high phytochemical composition and bioactivities especially antioxidant activities is scant [18,19]. In the light of this, we aimed to investigate the antioxidative potency of some indigenous underutilised Ghanaian fruits and vegetables.

2. Materials and Methods

2.1 Plant material

A total of 7 plant species were selected based on ethnobotanical data obtained from literature and collaboration with the CSIR-Plant Genetic Resources Research Institute, Bunso-Ghana. They were collected from the wild in the Eastern (Begoro and Suhum-Koforidua), Western (Ankasa) and Western North (Bibiani and Juaboso) regions of Ghana in December 2020. Semi-structured interviews were conducted among a number of purposively sampled respondents to obtain data on the local names and traditional edible uses of plant species collected which consisted of: fruits, fruit parts and vegetables. Fresh plant material was air-dried in a shady place for several days and then stored in collecting bags and paper packs until use. Local botanist, Dr. S. K. Boateng authenticated the species collected. The ethnobotanical data including: scientific and local names of plant, family, area of collection, part used and traditional edible use(s) are summarised in Table 1.

2.2 Chemicals and reagents

2,2'-azobis (2-methylpropionamidine) dihydrochloride [AAPH], 2,2-diphenyl-1-picrylhydrazyl [DPPH], (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and fluorescein sodium salt were purchased from Sigma-Aldrich (Prague, Czech Republic). Methanol for preparation of extracts and antioxidant activity assay was acquired from Penta (Prague, Czech Republic). Inorganic salts K_2HPO_4 and KH_2PO_4 were obtained from Lach-Ner (Neratovice, Czech Republic).

2.3 Preparation of extracts

Air-dried plant material was pulverised using a Grindomix apparatus (GM100 Retsch, Haan, Germany). 5 g of each milled plant sample was extracted at room temperature in 150 ml of 99.8% methanol using an orbital laboratory shaker (GFL3005, GFL, Burgwedel, Germany) for 24 h. Each extract was filtered afterwards and concentrated to dryness using a rotary evaporator Hei-VAP Expert (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) in vacuum at 40°C. Dry residues were stored at room temperature until tested. A total of 11 extracts were prepared and the yield of dry residue (%) of each extract determined (Table 1).

2.4 *In vitro* antioxidant activity

2.4.1 DPPH assay

Method described by Sharma & Bhat [20] was slightly modified and used to evaluate the ability of extracts to inhibit the DPPH radical. Concentrations and volumes of samples, standard and reagent were adjusted to be used in a microplate format. A two-fold serial dilution of each extract in methanol was performed via the automated pipetting platform

Freedom EVO 100 (Tecan, Männedorf, Switzerland) in 96-well microtiter plates. Subsequently, 75 µl of methanol and 25 µl of freshly prepared 1 mM DPPH in methanol was manually added to each well using a multichannel pipette (Eppendorf, Hamburg, Germany) in order to start the radical antioxidant reaction. The final concentration range of each extract and Trolox (positive control) in microtiter plate was 0.125 – 256 µg/ml. Plates were incubated for 30 minutes at 25°C in an incubator IPP55plus (Memmert GmbH & Co. KG, Schwabach, Germany). Absorbance was spectrophotometrically measured at 517 nm using a Multimode Reader Cytation 3 (BioTek Instruments, Winooski, VT, USA). All tests were performed as three independent experiments each carried out in triplicate. Results were expressed as half maximal inhibitory concentration (IC_{50}) with standard deviation (±SD) in µg/ml.

2.4.2 ORAC assay

The ORAC assay was performed to determine samples' ability to protect fluorescein against oxidative degradation by AAPH using a protocol by Ou et al. [21] which was slightly modified. Stock solutions of AAPH radical (153 mM) and fluorescein (48 nM) were prepared in 75 mM phosphate buffer (pH 7.0). A two-fold serial dilution of each extract was prepared in phosphate buffer in black absorbance 96-well microtiter plates using the automated pipetting platform Freedom EVO 100 (Tecan, Mannedorf, Switzerland). Subsequently, 150 µL of fluorescein was added into each well and incubated for 10 min at 37°C. Afterwards, the reaction was started by application of 25 µL of AAPH and the plates were incubated for 90 min at 37°C in an incubator IF110plus (Memmert GmbH & Co. KG, Schwabach, Germany). The final concentration range of each sample tested was between 1 and 256 µg/ml. Outer wells of each microtiter plate were filled with 200 µl of distilled water, in order to provide better thermal mass stability. Blank 1 (fluorescein with AAPH in phosphate buffer) and blank 2 (fluorescein in phosphate buffer) were part of each microtiter plate. Trolox was used as a positive control. Fluorescence changes during the incubation were measured using the Multimode Reader Cytation 3 (BioTek Instruments, Winooski, VT, USA) in 1-min intervals with excitation and emission wavelengths set at 485 nm and 528 nm, respectively. All tests were performed as three independent experiments each carried out in triplicate. ORAC was expressed as mean values of IC_{50} ± SD in µg/ml.

Table 1. Ethnobotanical data on collected indigenous Ghanaian fruits and vegetables

Scientific name [a]	Family name [a]	Local name [b]	Area of collection	Part used	Traditional edible use(s) [b]	EY [c] (%)
Chrysophyllum albidum G.Don	Sapotaceae	Adasaa/Adesema	Maanfe-Nkwanta (Koforidua-Suhum)	epicarp	chewed as a gum	29.2
				meso-carp	eaten fresh and can be chewed as a gum	30.8
				aril	eaten fresh	28.0
Chrysophyllum perpulchrum Mildbr. ex Hutch. & Dalziel		Asaa/Atabene	Juaboso-Nkwanta	meso-carp	eaten fresh and can be chewed as a gum	16.0
				aril	eaten fresh	39.4
Chrysophyllum subnudum Baker		Aningene/Akasa		meso-carp	chewed as a gum	21.8
Delpydora gracilis A. Chev.	Apocynaceae	Agyinamoa hwoa	Ankasa	aril	eaten fresh	52.2
Landolphia dulcis var. *barteri* (Stapf) Pichon		Osono kotodwe	Bibiani-Anwiaso	aril		43.6
Morinda morindoides (Baker) Milne-Redh.	Rubiaceae	Kyeremabua/Agyinamowa aniwa	Juaboso-Nkwanta/Begoro Nature Reserve	aril		55.4
Sterculia tragacantha Lindl.	Malvaceae	Foto/sɔfɔ	Begoro Nature Reserve	seeds	can be roasted and eaten whole like peanuts/pounded and then cooked with vegetables	17.2
				leaves	cooked as a vegetable/used in wrapping kenkey	16.2

[a] Scientific names of the species and families are given according to WFO (2021): World Flora Online, http://www.worldfloraonline.org/; [b] ethnobotanical knowledge; [c] EY extract yield

3. Results

The mesocarp of *C. subnudum* exhibited the best scavenging activity against the DPPH radical with a remarkable IC_{50} ± SD value of 45.18 ± 8.61 µg/ml but showed a moderate activity of 75.53 ± 8.12 µg/ml in the ORAC assay. Contrastingly, mesocarp of *C. albidum* demonstrated the best antioxidative activity of 18.54 ± 0.27 µg/ml among the samples in the ORAC assay but recorded a moderate activity of 95.63 ± 13.24 µg/ml in the DPPH assay. The aril and epicarp of *C. albidum* also recorded significant activities of 37.34 ± 6.6 µg/ml and 58.13 ± 3.42 µg/ml respectively in the ORAC assay. Weak and no antioxidative activities were observed in the DPPH and ORAC assays for the remaining samples. The complete data of the results is presented in Table 2.

Table 2. Antioxidative activity of collected indigenous Ghanaian fruits and vegetables

Species	Part tested	Assay [IC_{50} ± SD (µg/ml)]	
		DPPH	ORAC
Chrysophyllum albidum	epicarp	135.20 ± 6.13	58.13 ± 3.42
	mesocarp	95.63 ± 13.24	18.54 ± 0.27
	aril	184.48 ± 6.41	37.34 ± 6.6
Chrysophyllum perpulchrum	mesocarp	>256	>256
Chrysophyllum subnudum	aril	>256	>256
	mesocarp	45.18 ± 8.61	75.53 ± 8.12
Delpydora gracilis	aril	>256	153.27 ± 8.89
Landolphia dulcis var. *barteri*	aril	>256	175.79 ± 8.38
Morinda morindoides	aril	>256	>256
Sterculia tragacantha	seeds	>256	>256
	leaves	>256	>256
Trolox		9.28 ± 0.18	17.33 ± 0.26

IC_{50}: half maximal inhibitory concentration, ± SD: standard deviation of three independent experiments each in triplicate, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ORAC: oxygen radical absorbance capacity

4. Discussion and Conclusion

In the present study, all fruit parts of *C. albidum* showed antioxidative activities in both assays performed with a higher activity recorded in the ORAC as compared to the DPPH. Furthermore, the mesocarp extract exhibited the highest activity among all samples tested for the ORAC assay, showing an activity comparable with Trolox (IC_{50} 17.33 ± 0.26 µg/ml), the water-soluble analogue of a well-known antioxidant vitamin E. Previous studies conducted by Oloyede & Oloyede [22]; Oboh et al. [23]; Arueya & Ugwu [24] confirms that this indigenous African fruit is rich in antioxidants, total phenols and flavonoids, thus a potential fruit to combat oxidative stress. This is well corresponding with results of our study. To the best of our knowledge, this is the first report on any antioxidant activity of *C. subnudum* mesocarp and aril, *D. gracilis* and *L. dulcis* var. *barteri* arils. *C. subnudum* mesocarp produced the highest activity in the DPPH assay among all samples tested. Remaining plants did not show any inhibition against the DPPH and AAPH radicals even at the highest concentration tested.

In summary, the findings from this study suggests some degree of radical specificity in the scavenging abilities of potent antioxidative fruit parts and provides evidence that the fruit parts of *C. albidum* and mesocarp of *C. subnudum* exhibit significant antioxidant properties when evaluated using both electron and hydrogen atom transfer methods hence

they may serve as effective components of diets as well as antioxidants which can be used in the food industry.

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