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The value of the cocoa bean shell (hull) and the effect of various processing methods on the phyto-constituents and antioxidant activity of the nib and shell

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ABSTRACT

The cocoa bean shell is the major by-product of the cocoa bean during its processing into cocoa products. The cocoa bean shell makes about 10 – 12 % of the weight of the cocoa bean. Tons of the cocoa bean shell is disposed off as waste every year. Research over the years has been focused on cocoa polyphenols, especially the flavonoids, and its function as potent antioxidant in human health. In this study, the effect of various processing methods on the phytoconstituents (bioactive compounds) and the antioxidant activity of the nib and shell were assessed using the DPPH radical scavenging and total antioxidant capacity assays. All the cocoa samples showed the same trend in antioxidant activity for all the different processing methods. It was seen that micronizing before deshelling of the bean impacts on the nib quality in terms of antioxidant activity as compared to roasting the bean before deshelling. Again, the shell quality in terms of antioxidant activity was better when micronizing was done before deshelling. However, both processing methods still retains some antioxidant potency in the shell and the nib which, in the long term, protects the organism from oxidative damage leading to disease conditions.

Keywords: Cocoa shell, nib, cocoa polyphenols, antioxidants

INTRODUCTION

Cocoa is a very rich source of dietary flavonoids and reported of having higher flavonoids per serving than teas and red wine [1]. This invites the speculation that cocoa bean shell could provide a ready source of inexpensive polyphenol-rich dietary fibre [2]; for example, as a supplement to human foods [3]. Polyphenolic compounds belong to the secondary plant metabolites which have beneficial effects such as antioxidants, anti-carcinogenic, anti–microbial, anti – atherogenic, anti – ulcer, anti-thrombotic, anti – inflammatory, immune modulating, vasodilatory and analgesic [4,5,6,7]. They are involved in scavenging free radicals and thus protecting the organism from oxidative damage.

It is also reported [8] that the total dietary fibre content of cocoa hulls was extremely high- over 60 % of the dry matter. Dietary fibre is known to be beneficial for the human health and body function, thus a consumption of dietary fibre is associated with a reduced incidence of disorders and diseases common in developed countries such as chronic bowel disorders, obesity, diabetes, cardiovascular diseases and cancer [9, 10, 11, 12].

The cocoa bean shell is generated at the breaking and winnowing stage of cocoa bean processing [13, 14, 15]. The shell forms 10 – 12 % of the weight of the cocoa bean [16, 17]. Tons of the cocoa bean shell is disposed as waste every year [18]. According to [19], the cocoa bean shell is one of the industrial wastes generated by cocoa processing companies and most often disposed off at landfills. The presence of the shells at some landfills suggests that, there may be little value for it in the country. The existence of cocoa processing companies ensures the constant availability of the cocoa bean shells in commercial quantities. Therefore, research into the applications of the cocoa bean shells may help create a more profitable outlet for this by product. The knowledge gained from the research may also be used to create new industries, employment, generate income for the country (Ghana) and improve human health if medicinal components are found.

MATERIALS AND METHODS

Sample collection

Unroasted cocoa beans and cocoa beans after Infrared microniser were collected from Archer Daniels Midland Gh. Limited (Kumasi, Ghana). Similarly, roasted cocoa beans were also collected from BD Associates (Tema, Ghana).

Sample preparation

A knife was used to cut the roasted cocoa bean, unroasted cocoa bean and cocoa bean after Infra Red (IR) microniser to separate the nibs and the shells. The separated components were milled with a Philips blender (HR 2001) and kept in well labelled plastic containers (100 ml capacity). The samples were labelled as follows - Unroasted nib (URN), Nib after IR (NIR), Roasted nib (RN), Unroasted nib shell (URNS), Shell of nib after IR (SIR) and Roasted nib shell (RNS).

Preparation of extracts for antioxidant activity

Each powdered sample was weighed into a conical flask (250 ml) and 100 ml of distilled water added. The content of the conical flask was boiled over a water bath for 30 minutes. The extracts were filtered with absorbent cotton plugged in a funnel over another cornical flask. Empty crucibles were weighed and known amounts of filtered extracts weighed. The crucibles and its contents were kept in an oven (Fisher scientific oven) for 2 days at 80 ^{o}C. The crucibles were cooled in a dessicator and weighed. The yields were calculated by dividing the weights of the content in the crucibles by the initial amounts of sample taken. The yields were – URNS (10.25 % $^{w}/_{w}$), RNS (13.60 % $^{w}/_{w}$), SIR (15.40 % $^{w}/_{w}$), NIR (9.24 % $^{w}/_{w}$), RN (3.52 % $^{w}/_{w}$) and URN (6.81 % $^{w}/_{w}$).

Preparation of different concentrations of extracts

0.025 g of each of the extract was weighed and dissolved in 50 ml of distilled water in a 50 ml volumetric flask to form a stock of 500 µg/ml. 20 ml each of 250, 125, 62.5 and 31.25 µg/ml concentrations were prepared from the stock solution by dilution with distilled water.

Antioxidant activity assays

Scavenging of 2, 2 – diphenyl – 1 – picrylhydrazyl (DPPH)

The free radical scavenging activity was determined as described by [20]. Five concentrations (500, 250, 125, 62.5 and 31.25 µg/ml) of the extracts in water were prepared.1 ml each of the extract concentration in triplicates were dispensed in a test tube using a pipette. 3 ml of methanolic solution of DPPH solution (20 mg/L) was added to each triplicate. The reaction mixture was kept at 25 ^{o}C for 30 minutes. The absorbance of DPPH was determined at 517 nm with a T90+ UV/VIS Spectrophotometer (by PG Instruments Ltd., USA). 1 ml of a mixture of methanol and distilled water (50:50) was added in a test tube and 3 ml of DPPH added and kept as same conditions as extracts. This was used for the control. Ascorbic acid with concentrations (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) were used as a standard free radical scavenger. The scavenging effect of the DPPH radical by the extract and standard antioxidant were calculated using the equation:

$$\% \text{ DPPH scavenging effect} = (1- At/Ab) \times 100$$

Where At = absorbance of sample at 517 nm
Ab = absorbance of control at 517 nm

EC_{50} value which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50% [21] was determined from the scavenging activity against the Log of concentration of extract/standard antioxidant.

Total antioxidant capacity (TAC) assay

The assay was based on method used by [20]. Molybdenum is reduced from Mo^{+6} to Mo^{+5} by the extracts and subsequent formation of a green phosphate molybdate (Mo^{+5}) complex at acidic pH [22]. Test tubes containing 1 ml each of the extract in triplicates (500 µg/ml) and 3 ml of the reagent solution (0.6 M sulfuric acid, 28 mM, disodium phosphate and 4 mM ammonium molybdate) were incubated at 95 ^{o}C for 90 minutes in an oven (Fisher scientific oven). After the solutions have cooled to room temperature, the absorbances of the solutions were measured at 695 nm with T90+ UV/VIS Spectrophotometer (by PG Instruments Ltd., UK). Six concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg/ml) of ascorbic acid (AA) each in triplicates were used to construct a calibration curve. The antioxidant capacity was expressed as *mg of ascorbic acid equivalent (AAE) per g of extract.*

Phytochemical Screening

Qualitative tests to determine the presence of tannins, saponins, steroids, terpenoids, glycosides, flavonoids, purine alkaloids, etc. were done according to the methods described by [23, 24, 25]

Statistical analysis of data

Microsoft excels 2007 and GraphPad Prism 5 [26] were used in drawing and analyzing results. EC_{50} was determined using the iterative computer least square method, with the following nonlinear regression equation;

$$Y = \frac{a+(b-a)}{1+10^{(Log\ EC50-X)}}$$

Where, x is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

RESULTS AND DISCUSSION

Cocoa and its derived products contain varied polyphenols contents and possess different levels of antioxidant activities [13]. The antioxidant activities of extracts are attributed to their phenolic content and other constituents. Due to this, the extracts were subjected to the DPPH radical scavenging and total antioxidant capacity assays to determine the antioxidant potency of the extracts and the effect of temperature or the processing methods on these activities.

In biological systems, reactive oxygen species (ROS) formed by free radicals processes are involved in both initiation and promotion of carcinogenesis by causing heritable DNA damage [27]. The ability of an extract to scavenge the DPPH radical suggests that it is capable of preventing cancer, inflammatory and neurodegenerative diseases in humans.

The free radical scavenging of the extract was compared to vitamin C, a standard antioxidant. The extracts showed a concentration dependent radical scavenging activity (Figure 1). The rank order of antioxidant potency, measured by the EC_{50} (Table 1) is as follows;

AA > URN > RN > NIR > URNS > SIR > RNS

The extracts - Unroasted nib (URN), Roasted nib (RN),Nib after IR (NIR), Unroasted nib shell (URNS), shell of nib after IR (SIR) and Roasted nib shell (RNS) are 1.09x, 3.19x, 4.78x, 11.14x, 14.72x and 18.55x respectively less potent than vitamin C, used as the standard antioxidant.

Table 1: EC_{50} values of ascorbic acid (AA) and extracts

Sample	AA	URN	RN	NIR	URNS	SIR	RNS
EC_{50}	23.75	25.86	75.65	113.60	264.50	349.50	440.60

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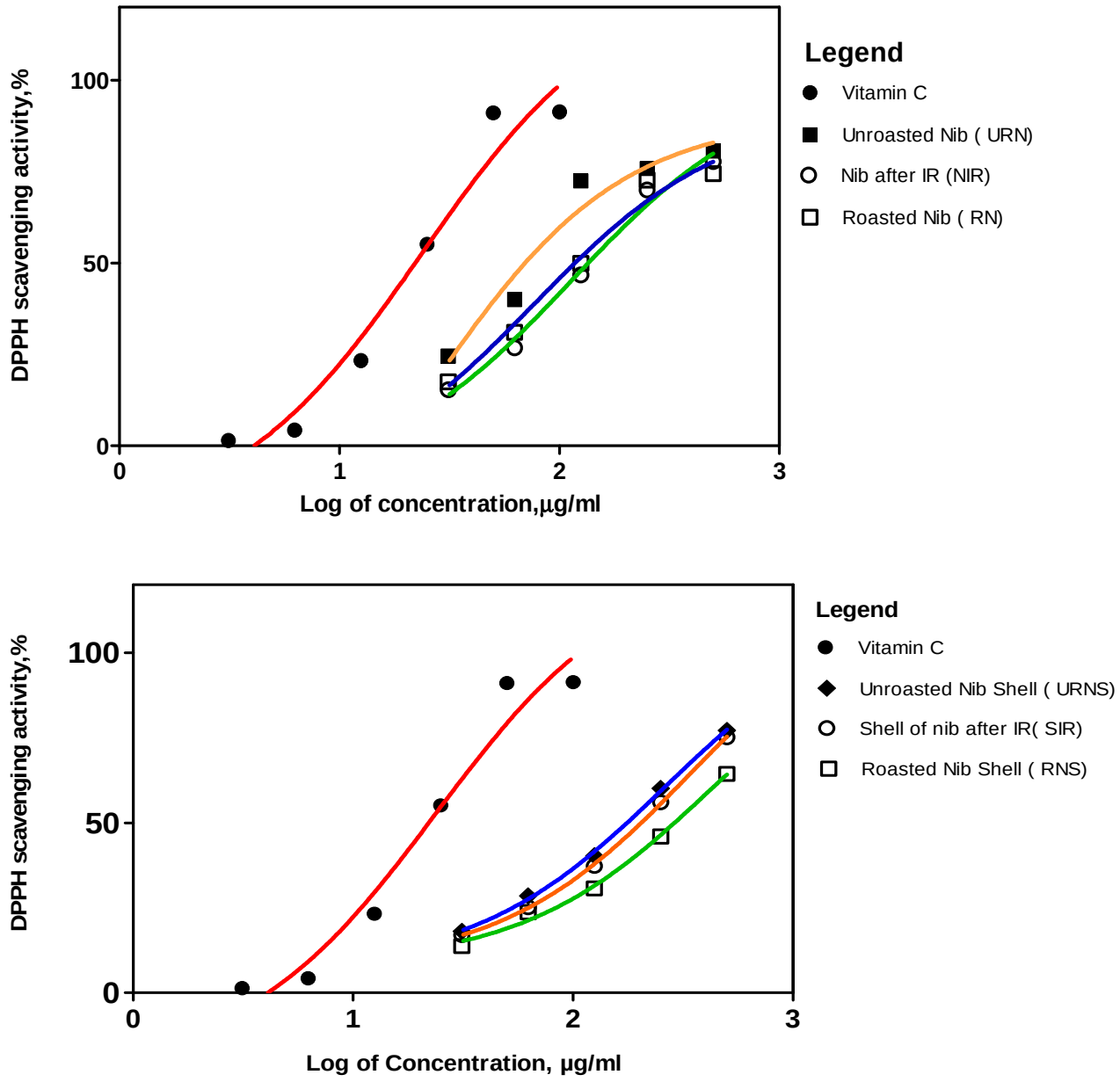

Figure 1: % DPPH scavenging activity versus Log of concentration of extracts

The total antioxidant capacity, which refers to a full spectrum of all measureable antioxidants in a mixture, was also evaluated. A standard calibration curve was constructed (Figure 2) for the standard antioxidant (ascorbic acid) from which the total antioxidant capacity of the extracts were calculated from the calibration equation. The ascorbic acid showed a good linearity with variance [r^2] value of 0.9895.

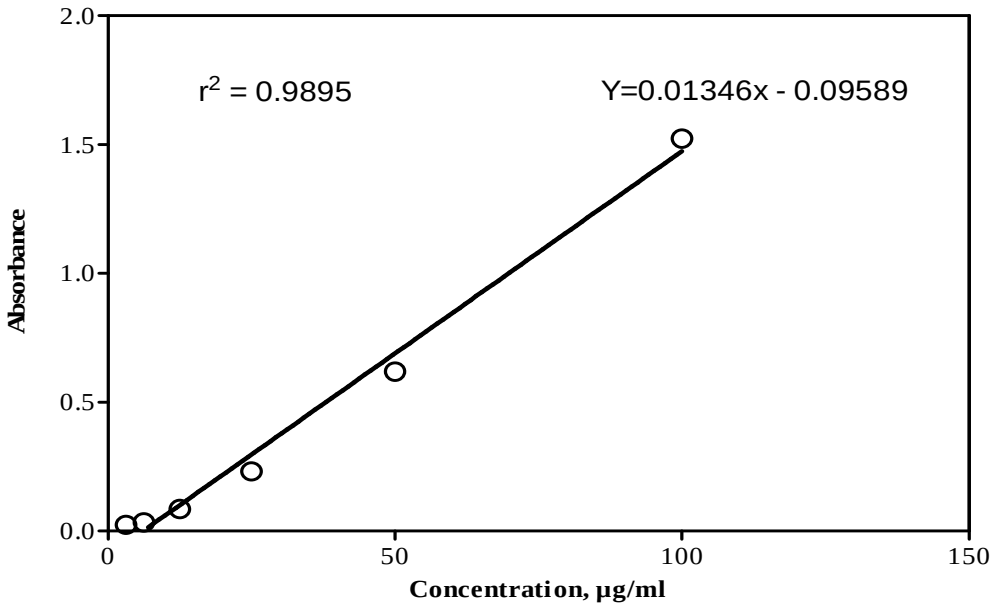

Figure 2: Calibration curve of ascorbic acid at various concentrations

The total antioxidant capacity of the extracts was measured in *mg Ascorbic Acid Equivalent per gram of extract (mg AAE / g extract)*. The rank order of antioxidant capacity potency is URN > RN > NIR > URNS > SIR > RNS (Table 2; Figure 3). All the extracts showed the same trend in both the DPPH scavenging activity and total antioxidant capacity assay. That is, the antioxidant activities of the unroasted nib (URN) and the unroasted nib shell were higher than those roasted or subjected to IR. This suggests that as the beans undergo different temperature treatments the phenolic content and other thermolabile antioxidant constituents are destroyed. According to [28] high processing temperatures and or longer processing time reduce the amount of cocoa polyphenols.

The total antioxidant capacity of the unroasted nib shell was only 1.3 times less potent than the unroasted nib (Table 2). Thus the cocoa shell contains antioxidant compounds, comparable to the nib, which may have other biological functions. This is supported by the results of the phytochemical screening. Tannins, steroids, glycosides, terpenoids, purine alkaloids and flavonoids were found in all the samples (Table 3). These metabolites have been found to have pharmacological activities such as anti- oxidant, anti-cancer, anti - diabetic, anti- inflammatory etc. For example, purine alkaloids (theobromine and caffeine) have stimulating effect. Theobromine and its derivatives act as smooth muscle relaxant, coronary vasodilator, a diuretic and cardiac stimulant [29]. Flavonoids have antioxidant activity which promotes cardiovascular health [3, 30]. Therefore, the presence of these metabolites in both the nib and shell samples gives a positive indication of promoting human health, thus value addition to the tons of cocoa bean shell discarded in landfills by cocoa processing companies.

Table 2: Total Antioxidant Capacity (TAC) of extracts

Extract	Mean TAC ± SEM, mg AAE/g extract
URN (Unroasted nib)	72.921 ± 0.877
RN (Roasted nib)	59.421 ± 1.620
NIR (Nib after IR)	55.005 ± 2.424
URNS (Unroasted nib shell)	55.260 ± 0.724
RNS (Roasted nib shell)	45.581 ± 0.848
SIR (Shell after IR)	52.289 ± 1.340

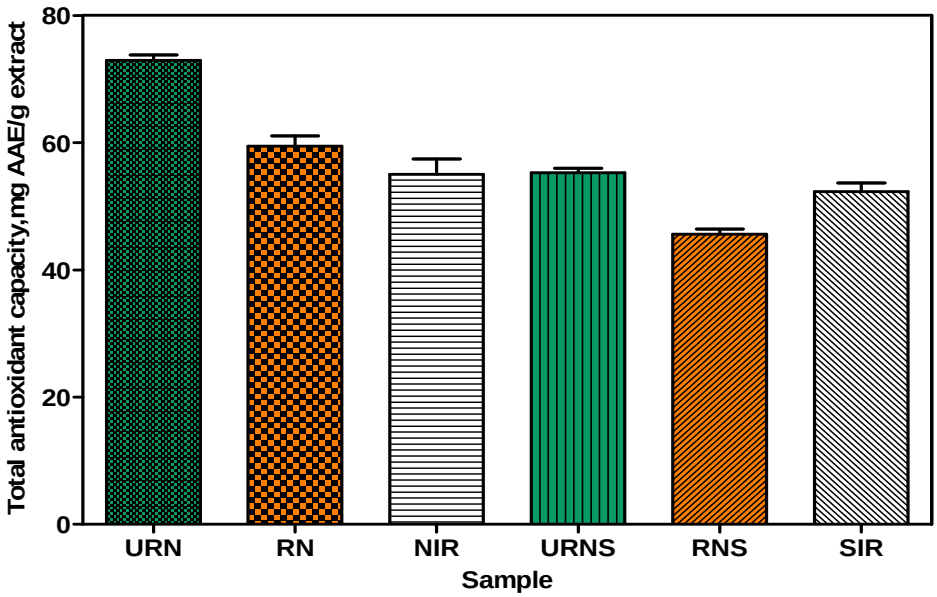

Figure 3: Bar graph showing the total antioxidant capacity of extracts

Table 3: Phytochemical screening of samples

Phytochemical	RNS	RN	URN	URNS	NIR	SIR
Tannins	+	+	+	+	+	+
Saponins	-	-	-	-	-	-
Steroids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Purine Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+

Present (+), absent (-)

CONCLUSION

The phytochemical screening and antioxidant activity of the shells has shown that, the cocoa bean shell contain plant metabolites that have the potential of mopping up free radical from the body. The results of the antioxidant activities has also confirm work done by other researchers about the degrading effect of plant metabolites at high processing temperatures.

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