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Bioactive constituents of waakye; a local Ghanaian dish prepared with *Sorghum bicolor* (L.) Moench leaf sheaths

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ABSTRACT

Rice and beans are widely consumed in most countries in the world. 'W*aakye*', a popular traditional dish widely consumed in Ghana, is prepared with *Sorghum bicolor* leaves to give it a distinctive colour. For the first time, the phytochemical properties of *waakye* prepared with *Sorghum bicolor* leaves are determined. Total polyphenols and flavonoid content were determined by Folin Ciocalteau and Aluminium chloride techniques respectively. The antioxidant activity of *waakye* was analyzed based on Ferric reducing antioxidant power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. Sorghum leaves showed high fibre (22.53%) and fat composition (8.86%). The leaves depicted high polyphenols (7.30–107.85 mg GAE/g), flavonoids (10.99–960.29 mg CE/g) and good antioxidant ability. Due perhaps to processing related losses, the polyphenols and antioxidant ability retained in the differently treated *waakye* products significantly reduced with exception of the *waakye* treated with saltpetre, which showed a higher ferric reducing ability than the aqueous extract of the leaves. *Waakye* (prepared with sorghum leaves) was therefore found to contain essential phytochemical constituents including antioxidants than without the sorghum leaves (control). The nutritional implication of these findings is that the consumption of ***w**aakye* could be a potential cheap source of essential fatty acids, antioxidant and polyphenols which could contribute to the health and biological activities.

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Introduction

Sorghum bicolor (L.) Moench (sorghum), also known as Guinea corn or sweet sorghum, botanically belongs to the family Poaceae (Grass). It is a cereal with many uses in the poultry and livestock industries, and also in the food industry, particularly the brewing [1–2]. It is a staple cereal crop consumed mainly in the Northern, Upper East and Upper West regions of Ghana, West Africa [3]. Most parts of the plant have been used by and large in the northern sectors of Ghana as herbs in folk medicine, usually as a haematinic, fever suppressant, colorant for foods such as millet porridge, dye and as feeds for livestock [3].

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Sorghum red leaves are usually employed as colouring additive in the preparation of *waakye*, a dish comprising rice and beans boiled together with the *Sorghum bicolor* (L.) Moench leaves to give the reddish-brown distinctive colour of the dish. Previous studies carried out on the bioactive and phytochemical components on *Sorghum bicolor* (L.) Moench leaves showed appreciable levels of ferulic, tannic, p-coumaric acids and flavonoids, which included luteolin, apigenin, catechin gallate and epigallocatechin [2–3].

These phytochemicals, especially antioxidants, the first line of defence against free radical damage are critical for maintaining optimum health and wellbeing [4]. They are defined as man-made or natural substances that may prevent or delay some types of cell damage [5]. The use of sorghum leaves in *waakye* preparation may potentially fortify the final product (*waakye*) with essential antioxidants to provide protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract [5–6].

However, the antioxidant levels and activity of plants, herbs, spices and other food products can be affected by cooking methods [6]. Previous studies reported decreases in antioxidants such as flavonoids due to heat treatment, while other antioxidants levels were elevated under these same conditions [6–8]. Though previous findings implicated sorghum leaves as a rich source of antioxidants, scientific studies regarding its phytochemical properties in *waakye* dish is nearly non-existent to the best of our knowledge. This study was carried out to investigate the phytochemical properties of *waakye* prepared with *Sorghum bicolor* leaves and the factors that may influence antioxidant transfer into the *waakye*.

Materials and methods

Source of materials

Dry *Sorghum bicolor* leaves, uncooked white rice (Fortune®, Vietnam) and black-eyed beans (*Aleŋfeade*) were bought from a local market in Ho (*Asiga me*) in the Volta region of Ghana and kept in a sterile sample bag for transport to study site. The chemicals and reagents used for analyzing the phytochemical and antioxidant compounds included Gallic acid, Catechin, Sodium Nitrate, Sodium Carbonate, Folin-Ciocalteu phenol reagent, Trichloroacetic acid, Sodium Nitrite, Aluminium Chloride and methanol. The 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4, 6-tripyridyl-S-triazine (TPTZ), $FeCl_3 \bullet 3H_2O$, Potassium persulphate, Sodium acetate, and Sodium Carbonate were supplied from Sigma-Aldrich, USA. Also, Catechin, Ascorbic acid and Gallic acid were also obtained from Zinabel Scientific Ltd, Ghana. All chemicals used were of analytical grade.

Sample preparation

All samples were weighed using the OHAUS® electronic balance (S/N: B523020750) with a readability of 0.01 g. Dried samples of the matured red sorghum leaves were cut into smaller pieces, milled and sieved to pass through 25 µm mesh sieve to obtain a fine powder.

Cooking methods of waakye *(boiled beans and rice preparation)*

Four different cooking methods were used for the study. In the first method, 40 g of beans were boiled to half cooked in 800 ml of distilled water. Eighty grams (80 g) of rice were added and stirred to get a uniform mixture. The mixture was tightly covered and allowed to fully cook.

In the second method, 5 g of dried sorghum leaves were soaked in 800 ml of warm water with 5 g of crystalline KNO_3 (saltpetre) for 12 h to allow for leaching. Forty grams (40 g) of black-eyed beans were allowed to boil in the 800 ml solution of the leached sorghum water with the saltpetre until the beans were half cooked. This was followed by the addition of 80 g of rice and stirred to obtain a uniform mixture. The mixture was tightly covered and allowed to fully cook.

In the third method, 5 g of dried sorghum leaves were soaked in 800 ml of warm water without saltpetre for 12 h. Forty grams (40 g) of black-eyed beans were allowed to boil in 800 ml solution of the leached sorghum water without the saltpetre until the beans were half cooked. This was followed by the addition of 80 g of rice and stirred to obtain a uniform mixture. The mixture was tightly covered and allowed to fully cook.

In the fourth method, 40 g of beans were boiled to half cooked in 800 ml of distilled water. Eighty grams (80 g) of rice were added and stirred to get a uniform mixture. The system was then incubated with 5 g of previously sanitized sorghum leaves and kept under boiling temperature until fully cooked.

Samples from the various treatments (methods of cooking *waakye*) were taken, labelled and stored at 4 °C for further analysis.

Proximate analysis of the Sorghum bicolor *leaves*

Analysis of the *Sorghum bicolor* leaves was carried out as follows: Moisture, ash, fat, fibre and nitrogen content were determined according to the method of AOAC [9].

Sample extraction

Phenolic extraction of the various samples was determined by the method outlined by Mohammedi and Atik [10] and Abugri et al. [3] using ethanol (70%), methanol (70%), acetone (70%) and distilled water with some modifications. For each solvent, 1 g of dry *Sorghum bicolor* leaves powder and fresh cooked *waakye* was extracted with 25 mL solvent by thoroughly stirring at room temperature for 20 min. The mixtures were then vortexed for 5 min and the supernatant was collected and centrifuged for 5 min at 816 rcf. The supernatants were further filtered using 125 mm filter paper, transferred into 50 mL sample containers and stored at 4 °C in a refrigerator. The supernatants were then used to determine the total phenol content, total flavonoid and antioxidant screening.

Determination of total polyphenol content

The total polyphenol content was colorimetrically estimated using Folin-Ciocalteu reagent as described by International Organization for Standardization per ISO 14502–1 [11] and Nsor-Atindana et al. [12] with modifications. About 5 µL of each crude extract with volume adjusted to 1 ml was mixed with 0.2 mL Folin-Ciocalteu reagent in a 10 mL test tube. The mixture was allowed to rest for 1 min before the addition of 0.5 mL sodium carbonate (20%). The mixture was then incubated at room temperature for 10 min before reading the absorbance at 750 nm using a UV spectrophotometer. The total phenol content was quantified and expressed as Gallic Acid Equivalent (GAE) mg/g sample weight basis (dry weight), and the range of a calibration curve was from 0.001–0.025 $mgmL^{-1}$.

Determination of total flavonoid content

The total flavonoid (TF) content was determined by the Aluminium Chloride colorimetric assay as modified by Nsor-Atindana et al. [12]. An aliquot of 60 µL of the extracts were added to 10 mL test tube containing 2 mL of distilled water. About 150 µL of 5% $NaNO_2$ was added to each mixture and rested for 5 min before the addition of 0.75 mL $AlCl_3$ (10%). The resulting mixture was allowed to stay for 6 min before adding 2 mL of 1.0 M NaOH and vortexed for 10 s. The absorbance of the aliquot was measured against prepared reagent blank at 510 nm and the TF content was expressed as Catechin Equivalent (CE) mgg^{-1} on dry weight.

Antioxidant activity

FRAP assay

The ferric reducing antioxidant power (FRAP) assay was performed as described by Benzie and Strain [13] with some modifications. A 30 ml freshly prepared FRAP reagent was warmed to 37 °C and a reagent blank reading was taken at 593 nm; 100–150 µL of each sample was then added and volume made up to a total volume of 1 ml with distilled water. Absorbance (A) readings were taken with a Jenway Vis Spectrophotometer 6305 after 0 s and 4 min thereafter, and the change in absorbance (ΔA593 nm) between the final reading selected and the blank reading was calculated for each sample and related to ΔA593 nm of a Fe^{II} standard solution tested in parallel. The 4 min readings were selected for calculation of FRAP values.

DPPH radical scavenging activity assay

This was determined as per protocol described by Lewis [14] and Mohammedi and Atik [10] with modifications. The working DPPH reagent was prepared by adding 10 mL of the stock (0.6 mM) to 45 mL of methanol. For each extract, 1 mL methanol DPPH was added to 30–200 µL extract (at different concentrations). Absorbance was determined after 30 min at 517 nm using a spectrophotometer (Jenway 6305). The percentage inhibition activity was calculated as follows:

{[(Ac-At)/Ac] x100},

where:

Ac is the absorbance of the control and

At is the absorbance of the extract.

The inhibition curves (IC) were prepared; and IC_{50} values defined as the amount of antioxidant necessary to decrease the initial $DPPH^+$ concentration by 50% was determined [10].

Data analysis

The experimental results for chemical composition and functional properties were expressed as the mean ± standard deviation (SD) of triplicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA), and Fisher's least significant difference (LSD) tests using Minitab 16 software were carried out to ascertain significant effects at $p < 0.05$ level of significance among treatments.

Table 1
Proximate compositions of *Sorghum bicolor* leaves on dry weight basis.

Composition	Percentage (%)
Moisture	11.10 ± 0.00
Crude fibre	22.53 ± 0.07
Fat	8.89 ± 0.10
Ash	7.04 ± 0.04
Protein	4.14 ± 0.03

Table 2
Total phenolic and flavonoid content of *Sorghum bicolor* leaves extract.

Solvent	Phenolic content (mg GAE/g)	Flavonoid content (mg CE/g)	FRAP (mol Fe^{2+}/L)
70% Ethanol	$107.85^{a} \pm 2.61$	$960.29^{a} \pm 252.14$	$2.452^{a} \pm 0.042$
70% Acetone	$100.63^{a} \pm 16.42$	$756.08^{ab} \pm 5.30$	$2.363^{a} \pm 0.018$
70% Methanol	$93.17^{a} \pm 3.42$	$602.94^{b} \pm 28.73$	$2.166^{a} \pm 0.551$
Water	$7.30^{b} \pm 0.26$	$10.99^{c} \pm 0.62$	$0.957^{b} \pm 0.088$

Values are means of triplicate ± SD. Means that do not share a letter within the same column are significantly different at $p < 0.05$.

Results and discussion

Proximate analysis of the Sorghum bicolor *leaves*

Proximate analysis was an essential technique in this study for the categorization of *Sorghum bicolor* L. Moench (Sorghum) leaves into its nutritional and non-nutritional constituent. From Table 1, the major constituent of the sorghum leaves was fibre (22.53%), followed by fat (8.89%) of which majority as reported by Abugri et al. [2,3], may be comprised of omega-3 and omega-6 fatty acids in the ratio of 1:2. This is the recommended level required for optimum nutritional and anti-inflammatory benefits.

Total phenolic, flavonoid content and FRAP of Sorghum bicolor *leaves extract*

Total phenolic content

The total phenolic content of *Sorghum bicolor* extracts in different solvent expressed as Gallic acid equivalent per gram of sample (mg $GAEg^{-1}$) on a dry weight basis can be seen in Table 2.

Although no significant ($p > 0.05$) differences were observed from the solvents used in extracting the total phenolic content from the sorghum leaves with the exception of water, ethanol recorded the highest phenol. Extraction of polyphenols from plants' tissues is greatly influenced by the polarity and other chemical properties of the extraction medium [15,16]. Ethanol, methanol, acetone and water have different chemical properties and therefore interact differently with polyphenolic compounds in plant tissues during extraction of such bioactive compounds.

In the current study, 70% formulations of the three organic solvents used, yielded greater amounts of polyphenol compounds than water from the sorghum leaves. The fact that water medium yielded the least amount of polyphenols from sorghum suggests that the polyphenolic compounds in the leaves are less polar and therefore have a weak interaction with the pure water molecules (most polar medium) during extraction [15]. However, when 70% of ethanol, methanol and acetone were used, the levels of polyphenolic yields from sorghum were in the order of increasing polarity of the three organic solvents as ethanol > acetone > methanol, though no significance existed amount the three organic solvents.

The results of this study disagree with findings of Abugri et al. [2,3] who reported the highest yield of polyphenols in methanol extract of sorghum leaves. This may be due to differences in the quality and composition of the soils, agricultural practices, plant physiological response to stress and processing and storage conditions as suggested by Butryee et al. [17].

Total flavonoid content

The flavonoid content of the sorghum leaves varied according to the treatments as seen in Table 2. Presumably, each of the solvent used had different extraction potentials due to their variation in polarity and other chemical properties, which define their distinctive association with the bioactive phyto-compounds during extraction. Furthermore, Mariya et al. [18] reported the concentration of the solvent, methods of extraction, acidification, temperature and purification as the limiting factors in extracting a higher amount of flavonoids from tea leaves. A possible explanation for 70% ethanol extract of the sorghum leaves having the highest flavonoid content could be attributed to the possibility of flavonoids and their glycosides being thought to be efficiently extracted from plant materials by ethanol [3,19,20]. Abugri et al. [3] reported flavonoid values of the leaves to be 0.20–0.36 mg/g of catechin hydroxide equivalent which varied greatly compared to the present study's report of 10.99–960.29 mg CEg^{-1} (Table 2); rather their values were similar to the total phenolic content

Table 3
Effect of sorghum leaf treatment on total phenolic content and ferric reducing ability from water extract of freshly prepared *waakye*.

Sample	Concentration (mg GAE/g) of phenolic content	Amount retained (%)	FRAP in *waakye* (μmol Fe^{2+}/L)	Amount retained (μmol Fe^{2+}/L)	% contributed	Water extract (μmol Fe^{2+}/L)
*SBLM	–	–	–	–	–	957.60
A	$0.09^{b} \pm 0.02$	–	527.8	0	0	–
B	$0.41^{a} \pm 0.10$	4	1388.3	860.5	90	1388.3
C	$0.15^{b} \pm 0.02$	1	678.1	150.3	16	678.1
D	$0.21^{b} \pm 0.07$	2	776.0	248.2	26	776.0

Values are means of triplicate ± SD. Means that do not share a letter within the same column are significantly different at $p < 0.05$.
*SBLM = Only Sorghum leaves.
A = *waakye* without SBLM leaves (Rice and beans only).
B = *waakye* + water from soaked SBLM leaves with saltpetre.
C = *waakye* + water from soaked SBLM leaves without saltpetre.
D = SBLM leaves added to *waakye* halfway through cooking.
(Percentages are results of sample values- control sample).

in the differently treated *waakye* (Table 3). The decrease in flavonoid content retained from the leaves into the *waakye,* however, concurred with previous similar investigations [6,7,20].

Ferric reducing activity based on FRAP assay

The ferric reducing ability of the *Sorghum bicolor* leaves extract in a different solvent, which may serve as a reflection of its antioxidant activity determined using a modified Fe^{3+} to Fe^{2+} reduction assay, as represented in Table 2. The highest ferric reducing ability was in the 70% ethanol extract. Water extract recorded the lowest reducing ability, 957.60 μmol Fe^{2+} L^{-1}, which was significantly different ($p < 0.05$) from the three organic solvents used.

Effect of sorghum leaf treatment on total phenolic content and ferric reducing ability

Practically, *waakye* is usually prepared by adding the *Sorghum bicolor* leaves directly to the half-way cooked rice and beans and allowed until fully cooked. Alternatively, water extracts solution of *Sorghum bicolor* leaves could be used to cook the rice and beans to get the distinctive colour of the *waakye*. Table 3 presents the phenolic contents in mg $GAEg^{-1}$ on a wet weight basis of the *waakye* using the various methods of preparation.

The results showed that *waakye* prepared with water from leaves soaked with saltpetre had the highest phenol content of 0.41 mg $GAEg^{-1}$. This was statistically different ($p < 0.05$) from the phenolic content obtained from the other preparation methods. Although saltpetre (Potassium Nitrate) is used to cure meat and also used to make boiled beans tender within a short period of time, consumers who patronise *waakye* most often complain of stomach upset which is as a result of a possible overdose. Therefore, should be used sparingly during *waakye* preparation. The lowest phenolic content of 0.09 mg $GAEg^{-1}$ was recorded in *waakye* prepared without the *Sorghum bicolor* leaves (rice and beans only). No significant difference ($p > 0.05$) was observed by adding the *Sorghum bicolor* leaves to the half-way cooked rice and beans and *waakye* cooked with water from soaked *Sorghum bicolor* leaves (SBLM) without saltpetre.

Pokorný [6] and Duodu [21] established that the amount of phenols and antioxidants present in food changes during food processing such as boiling, frying and smoking, depending upon the type of food product and the degree and length of the processing method. Consequently, the amount of phenols retained in the *waakye* from the water extract of the leaves showed expected decreases as shown in Table 3; which may be due to the degradation of the phenols by the boiling process. Boiling seemed to cause more phenolic loss as compared to other processing methods as supported by Rothwell et al. [22]. The phenol content retained in the food decreased in line with several other similar studies [6,8,22,23]. The amount of phenols retained in the *waakye* from the water extract of the leaves are 4% for *waakye* with water from soaked sorghum leaves with saltpetre (sample B), 1% for *waakye* with water from soaked sorghum leaves without saltpetre (sample C) and 2% for sorghum leaves added to *waakye* halfway through cooking (sample D) as depicted in Table 3. *Waakye* prepared with water from the leaves soaked with saltpetre (sample B) had the highest reducing ability (1388.30 μmol Fe^{2+}/L) than the control and the other methods of *waakye* preparation (Table 3). Interestingly, a closer look at the total ferric reducing ability of the *waakye* sample shows that *waakye* prepared with water from SBLM leaves soaked with saltpetre (sample B), exhibited the higher ferric reducing ability than the water extract from the sorghum leaves without the addition of saltpetre. This could be attributed to the ability of saltpetre (potassium nitrate) to stimulate the release of phenolic compounds and antioxidant activity [24].

Correlations coefficient among antioxidant activity evaluation indices

There was a higher positive correlation coefficient between total phenol content and FRAP value than the total flavonoid content and FRAP value in the sorghum leaves (Table 4). However, in the *waakye*, there was a positive correlation coefficient between the total phenol content and FRAP value; but the correlation coefficient between total flavonoid content and FRAP

Table 4
Correlations coefficient among Antioxidant Activity Evaluation Indices, Total Phenolic Content (TP) and Total Flavonoid Content (TF).

	SBLM				*Waakye*			
	FRAP	p-value	DPPH	p-value	FRAP	p-value	DPPH	p-value
TP	0.999	0.001	−0.475	0.234	0.996	0.004	0.567	0.135
TF	0.979	0.021	−0.685	0.061	0.871	0.129	0.515	0.192

Values are significantly different at $p < 0.05$.
Scavenging activity on DPPH radical.

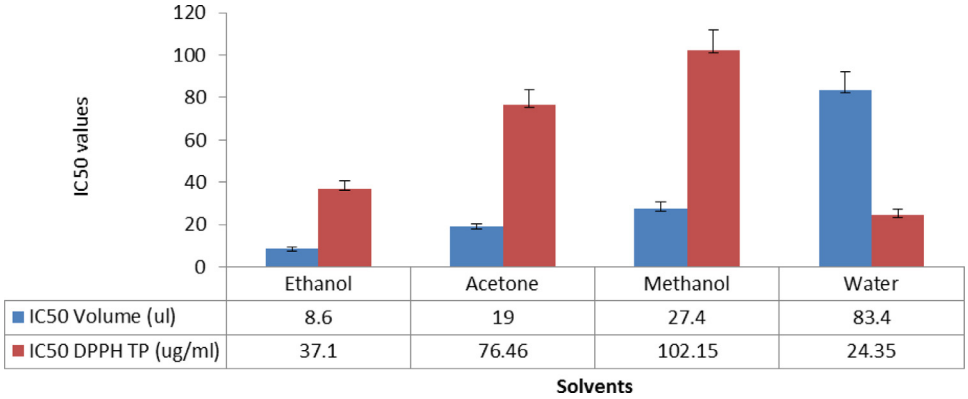

Fig. 1. Free Radical scavenging activity of Sorghum leaves extract on DPPH.
IC50 vol (µl) = Volume of sample necessary to decrease the initial DPPH by 50%.
IC50 DPPH TP (µg/ml) = Concentration of Total phenolics (TP) necessary to decrease the initial DPPH by 50%.

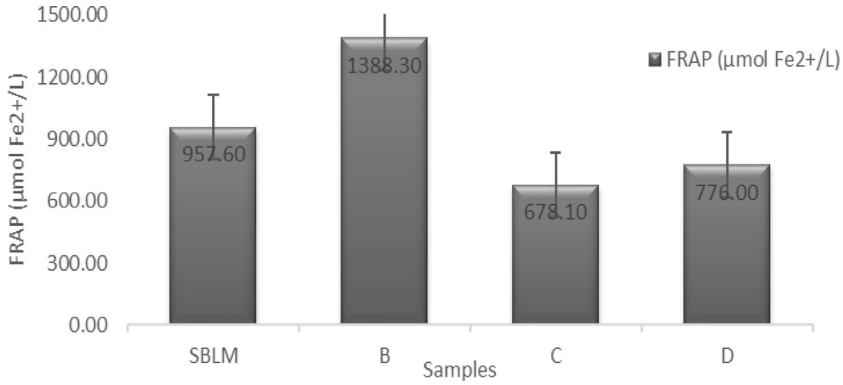

Fig. 2. Comparative effect of sorghum leaf treatment on ferric reducing ability (µmol Fe2+ /L) of freshly prepared 'waakye' and water extract of sorghum leaves.
SBLM = Sorghum leaves.
B = 'waakye' + Water from soaked Sorghum leaves with Saltpetre.
C = 'waakye' + Water from soaked Sorghum leaves without Saltpetre.
D = Sorghum leaves added to 'waakye' halfway through cooking.

value was not significant. This was in accordance with the published findings of Nwaichi and Anyanwu [7]. Further analyses of these results, as seen in Table 4, showed a negative correlation between phenol content and IC_{50} vol (−0.475, $p = 0.234$) and between flavonoid content and DPPH value (−0.685, $p = 0.061$) in the sorghum leaves. However, there was a positive correlation between phenol content and DPPH value (0.576, $p = 0.135$) and between flavonoid content and DPPH value (0.515, $p = 0.192$) in the *waakye* extracts.

The scavenging activity of the *Sorghum bicolor* leaves extract on DPPH in different solvents is illustrated in Fig. 1. It was found that the 70% ethanol extract required the lowest volume necessary to decrease the initial DPPH concentration by 50% with water requiring the highest volume.

The scavenging activity of the differently prepared *waakye* extracts on DPPH is shown in Fig. 2. The results showed that the *waakye* prepared using water from the leaves soaked with saltpetre (sample B) required the lowest volume necessary to decrease the initial DPPH concentration by 50%. This was followed by the control sample A (rice and beans without the sorghum leaves), then *waakye* prepared using water from the leaves soaked without saltpetre (sample C) and *waakye* prepared by halfway addition of sorghum leaves (sample D), respectively.

Conclusion

Our results demonstrated that *Sorghum bicolor* (L. Moench) leaves together with the rice and beans contained appreciable amounts of antioxidants as evidenced in the various tests used. Different cooking methods of *waakye* were discovered to influence the quantity of polyphenols and antioxidant retained in the *waakye* after it has been prepared with the *Sorghum bicolor* leaves. Saltpetre used in the preparation of *waakye* also altered the phytochemical quantities found. The nutritional implication of these findings is that the consumption of *waakye* could be a potential cheap source of essential fatty acids, fibre, antioxidant and polyphenols; which could contribute to beneficial health and biological activities.

Conflict of interest

There is no conflict of interest among the authors.

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