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Nutritional, Antioxidant and Antimicrobial of the Fermented leaves of Kawal (Cassia obtusifolia) from two Sudanese Regions

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ABSTRACT

This study was conducted to investigate various nutrients and phytochemicals in kawal (Cassia obtusifolia) fermented leaves. Al-Genina Kawal (AK) had higher protein content, soluble protein fractions, protein digestibility, Ca, K, Mg, P, Mn, Fe, and Zn than Sinnar Kawal (SK). The most abundant minerals were Ca, K, and P. Both AK and SK were rich in essential fatty acid omega 6 (45.52% and 42.43%, respectively). Generally, AK and SK had low vitamin content except for vitamin C, with 90 and 67 mg/100g, respectively. Both AK and SK exhibited good antioxidant potential. AK had more phenolic content (290 mg GAE/100g) than SK (200 mg GAE/100g), however, the latter had higher flavonoids. Although the antimicrobial activity was found to be dose-dependent, SK had higher antimicrobial activity than AK. AK did not exhibit antifungal activity towards Candida albicans, while SK created a 10 mm inhibition zone, higher than reference antifungal Coltrimaxole (7.7 mm). Irrespective of the region, Kawal

had good nutritive value, and its leaf extracts might be a good source of natural antioxidant and antimicrobial agents.

Keywords: Kawal; Sudan; Meat; Nutrients; Phytochemicals.

INTRODUCTION

The increase in world population, combined with changes in socio-demographics, started to exert great pressure on the world's resources to provide an extra amount of diversified foods [1]. It became a known fact that animal protein production requires more water, more land and contributes to greenhouse gas emissions, like methane gas, resulting in a negative environmental impact. Therefore, calls by scholars for sustainable production of existing protein sources and seeking alternative sources for direct human consumption are justified [2]. Dietary proteins represent a vital issue for the future regarding worldwide food security. Besides animal sources, plant proteins represent an opportunity to contribute to protein demand mainly. According to FAO, 1/7 of the world population suffers from hunger or hidden hunger, about 1 billion people have inadequate protein intake [3]. The recognition of protein from plants' leaves is fast gaining prominence because of its availability, and perhaps it is the cheapest and most abundant potential source of protein [4].

Sickle pod (Cassia obtusifolia) is widely distributed in Africa and America. In Sudan, it is found mainly on the clay plains of the central rain lands and the southern regions known as kawal. Kawal leaves are prepared by a solid-state fermentation process and used as an ingredient of sauces destined for consumption with porridge. It imparts a savory and meaty flavour to the sauce [5]. It is used in relatively large quantities to prepare sauces as a meat substitute or meat extender by poor people and in small quantities as a spice by some urban rich people [6]. It has medicinal uses in folk medicine as anti-jaundice, anti-diabetic, anti-malarial, and other uses. Cassia obtusifolia is rich in calcium, iron, and phosphorous, an excellent source of vitamin B, minerals, riboflavin, and ascorbic acid. The value of kawal leaf protein seems to lie in the quality rather than in the quantity as it has high cysteine and methionine contents [7]. In light of the rising prices of all types of meats (red meat, poultry, and fish), kawal would be a reasonable substitute. In spite of its precious value during crises, particularly displacement, tribal conflicts, civil war, and times of food shortage, only a few studies are found about kawal in Sudan. Households and agribusinesses use soaking and fermentation [8]. Eastern Chad and Southern Sudanese people eat fermented Cassia obtusifolia leaves instead of meat. GC and GC/MC analyzed this legume's fermented leaves' methylene chloride extract for the first time. Thirty-three constituents. Hexanoic acid (27%), butyric acid (10.4%), and valeric acid (6.3%) are the main components, with p-ethylphenol (17.2%) and p-methylphenol (13%). Crude leaves had 20.2% protein and fermented leaves 12.9%. 10 g of fermented C. obtusifolia leaves contribute 13-25% of an adult's essential amino acid needs. This traditional food was rich in potassium and calcium [9].

Evaluate probiotic potential of isolated Bacillus species to develop starter cultures for kawal production. Bacillus strains were tested for protease, amylase, and phytase. Antimicrobial activity was tested in-vitro against Micrococcus luteus LMG3293, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 8739, and Salmonella spp. Biofilm synthesis, antibiotic sensitivity, and simulated gastric and intestinal juice tolerance were assessed. Most selected strains produced biofilms, amylase, protease, and phytase by clearing zones around colonies. Some strains survived better in simulated gastric and intestinal juice biochemical conditions than others. The strains were sensitive to a panel of antibiotics and resistant to penicillin G, oxacycline, and bacitracin. Some strains inhibited all indicator bacteria. These strains may be suitable microbial starters for controlled kawal fermentation [10]. Essential amino acids make them nutritionally valuable. Protein-rich kawal is a meat substitute. Mineral salts and carbohydrates abound. Kawal technology uses primitive equipment and uncontrolled fermentation.

Although traditional, this fermentation removes anti-nutritional factors from the leaves and improves nutritional value and aromatic compounds. It increases mineral bioavailability and reduces human deficiency. Bacillus and Lactobacillus in kawal help humans. Indeed, kawal can be a source of probiotic microorganisms when consumed without cooking after fermentation, keeping the probiotics alive and able to benefit health. This product's transformation generates income and helps value forest resources and food safety [11]. The compounds, quality, and major microorganisms of Kawal, fermented Senna obtusifolia leaves eaten in Chad as a meat substitute or appetizer. The pH ranged from 5.82 to 7.22, the acidity from 0.31 to 0.54, the dry matter from

90.00 to 92.46, the protein content varied from 15.52 to 22.06, and the ash from 17.12 to 19.3. Bacillus sp. had 3.7 × 108 and 9.1 × 109 CFU/g, lactic acid bacteria 1.1 × 102 and 2.8 × 106, Staphylococcus 1.5 × 103 and 3.7 × 107, and Micrococcus sp [12].Kawal protein contains essential and semi-essential amino acids in various concentrations. Leucine, valine, and lysine had maximum values of 208.56, 173, 46, and 205.58 mg/100 g (DM), respectively (DM). In fermented leaves, histidine, threonine, tyrosine, isoleucine, and phenylalanine are best at 157.36-397.93 mg/100 g (DM). Fermented leaves contain 121.96-1260.92 mg/100 g semi-essential amino acids (DM) [13]. Fourier Transform Infrared (FTIR) and atomic absorption spectrophotometer were used.

FTIR experiments used dried powder samples. After combustion for atomic absorption and inductively coupled plasma, the sample extracted minerals (K, P, Na, Mg, Ca, Zn and Fe). FTIR showed that C. obtusifolia (Kawal) fermented leaves contained alkyl halide, alkene, nitro, aromatic, carbonyl, alcohol, esters, ether, acid, and amides. Samples were mineralized. Ca-2.50%, Mg-0.66%, P-0.393%, Na-0.165%, and K-1.595%. The atomic absorption spectrophotometer was used to extract iron (Fe) and zinc (Zn) minerals from fermented cassia leaves. Zn was 0.665 mg/L and Fe 15.6411 mg/L [14]. Alkaline food fermentation increases traditional dietary diversity, reduces anti-nutritional components in raw plant materials, improves digestibility, produces vitamins, and may provide probiotic and post-biotic benefits to consumers [15]. Artichoke Italian Latent Virus (AILV) Alkaline fermentation reduces toxin levels like cyanhydric acid. Fermentation with beneficial Lactic Acid Bacteria (LAB) controlled spoilage (Pseudomonadaceae) and pathogenic bacteria (i.e. Entero bacteriaceae). Additionally, nutritional content is mproved

by increasing B-group vitamins, carotenoids, and reducing antinutrient and toxic compounds for certain AILVs. The fermentation processes also extend the shelf life of AILVs, proving that they are valorised [16]. Previous studies have dealt with the chemical composition, some aspects of nutritive value, and none of them studied phytochemicals that could be found in kawal. Therefore, the objective of this study was to evaluate chemical properties, the nutritional value of kawal, and its antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Fresh green leaves of the Cassia obtusifolia plant were collected and processed in Al-Genina (Location 13.484175 ″N, 22.508368 ″E), (western Sudan) and Sinnar (Location 13.549983 ″N, 33.617298 ″E) (central parts of Sudan).

Kawal preparation

Fresh green leaves of the Cassia obtusifolia plant were collected from Al-genina, Dar Fur State, and Sinnar State, Sudan. The two samples were processed following the traditional method of processing described by Dirar 5, completely dried kawal samples (at room temperature) were designated as Algenina Kawal (AK) and Sinnar Kawal (SK), kept separately in a sterile plastic sampling bag, transported immediately to the laboratory, and stored under refrigeration until analysis.

Proximate composition determination

1.0 g the proximate composition of the two kawal samples was performed according to the AOAC methods [17].

Fatty acid profile

A Shimadzu GC/MS-QP2010-Ultra equipped with a capillary column (Rtx-5ms-30 m*0.25 mm*0.25 μm) was used. The esterified sample was injected using a split mode. The carrier gas was helium with a flow rate of 1.61 mL/min. Injector temperature 300°C, ion source temperature 200°C, and the interface temperature 250°C for 24 min, with 10°C/min increase until reaching the maximum temperature. The fatty acid methyl esters were identified by comparing their retention times and mass fragmentation patterns with those available in the library [18].

Protein fractionation

Protein fractionation was determined according to Chagas and Santoro method [19]. Albumin, globulin, prolamine, and glutelin fractions were dissolved in water, salt, alcohol, and alkaline solvents. After that, each fraction was estimated using the micro Kjeldahl method.

In-vitro protein digestibility

Protein digestibility by pepsin in-vitro digestibility assessment was performed according to the method previously described by Mkandawire and Rose [20]. Briefly, 400 mg of sample was suspended in 70 mL of pepsin solution (P-

6887, Sigma-Aldrich, St. Louis, MO USA; 1.5 mg/mL) dissolved in 0.1 mol/L sodium phosphate buffer (pH 2.0) and incubated with magnetic stirring at 37°C for 2 h. After incubation, the mixture was neutralized with the addition of 3 mL of 2 mol/L NaOH and then centrifuged (4200 g for 10 min). The supernatant was discarded, and the residue was washed and centrifuged with 20 mL of 0.1 mol/L sodium phosphate buffer (pH 7.0). The washed residue was then dried overnight at 50°C and analyzed for nitrogen content using combustion (FP528 nitrogen/ protein determinator, LECO, St. Joseph, MI USA). The percent protein digestibility was calculated using the following equation:

Digestibility%=¼ [(initial N in sample – residual N)/initial N in sample]*100%

Mineral content determination

A 100ppm Na, K, Ca, Mg, Mn, Fe, and Zn were determined as described by Torrinha et al. [21] using a High-Resolution ContinuumSource Atomic Absorption Spectrometer (HR-CS-AAS, Analytik Jena ContrAA 700, Berlin, Germany) equipped with a xenon short-arc lamp XBO 301 (GLE, Berlin, Germany) operating in a hot-spot mode as a continuum radiation source,flame (AS 52 S), and graphite furnace (MPE60) autosamplers. While P was determined by the vanadate-molybdate-yellow method [22].

Vitamin content determination

Vitamin C was determined according to the method described by Adebooye (2008) [23]. Ascorbic acid was measured by titration with 2,6-dichlorophenol indophenol (DPIP). Kawal powder (0.2 g) was mixed with 4 mL of a buffer solution made up of 1 gm/L oxalic acid and 4 gm/L sodium acetate anhydrous. This was titrated against a solution containing 295 mg/L DPIP and 100mg/L sodium carbonate. The results were expressed as mg/100 g dry weight.Vitamin B group was determined according to Zhang and Hamauzu method [24]. Where 15 g of kawal powder was homogenized with 10 mL acetone at -20°C. The homogenate was filtered with four layers of cheesecloth. The residue was treated with acetone for three successive extractions until the green color could no longer be visually detected in the extract and residue.

The filtrate was combined and centrifuged at 4000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 µm advantec filter pore for HPLC analysis. Samples were separated on a Luna 5µ C18 column at 40°C by HPLC. The mobile phase consisted of acetonitrile:water (9:1) as solvent A and ethyl acetate as solvent B. The flow rate was 1.0 mL/min. Samples were detected at 450 nm. The carotenoids were expressed as IU/100 g. Analysis of A and E vitamins in samples was determined spectrophotometrically using ferrozine-Fe(II) complex as described by Jadoon and Aziz [25].

Total phenols and flavonoids

The cold extraction method was employed for the extraction. 10 g of the kawal powder was weighed into a conical

flask to which 90 mL of pure methanol was added and maintained for 72 h. The mixture was filtered, and the methanolic filtrate was concentrated under vacuum at 40°C and stored at 4°C for further use.

Determination of total phenols

The total phenols were measured by the Folin-Ciocalteu reagent assay according to Singleton and Lamuela [26]. 10 pl of samples or diluted gallic acid standard solution were added to 90 pl of water. Shaking added FolinCiocalteu reagent (10 frl). After 5 min, 100 1t"l of 7Vo (w/v) NarCO3 were added, and the solution was diluted with water to 250 pl, shaken, and incubated for 90 min at room temperature. A microplate spectrophotometer reader (Synergy HT multi-mode microplate reader, BioTek, Milano, Italy) measured absorbance at 1.750 nm and compared it to a gallic acid calibration curve (y: 0.002r+0.030, R2: 0.9997). Gallic acid represented total phenolic content. Three triplicate experiments are presented as means*standard deviations.

Determination of total flavonoids

Total flavonoid content was measured by the aluminum chloride colorimetric assay according to Kim, and Lee [27] by using a UV- Vis Spectrophotometer at 510 nm. The total flavonoid content of extracts was expressed as mg Catechin Equivalents (CE)/100 g fresh weights.

Rancidity test

Raw groundnuts oil heated to 80°C, aerated (compressed air, air bubbles 0.5kg/cm2) then treated with 15 mg/L kawal extract. The peroxide value of oil was measured at 30, 60, 90 and 120 min intervals [28].

Antimicrobial activity

Four bacterial isolates (Staphylococcus aureus, Salmonella paratyphi, E. coli, and Klebsiella pneumoniae) and fungi (Candidaalbicans) were obtained from the microbiological laboratory (University of Gezira, Wad Madani, Sudan). Twenty grams of ground kawal samples were extracted in a round bottom flask with 150 mL methanol at room temperature and loaded on an orbit shaker at speed of 120 rpm for 24 h. After that, the extract was filtered through filter paper and dried using a rotary evaporator.

Then the crude extract was dissolved in methanol (1:5 ratio) and stored in a refrigerator at 4°C for later use. The antimicrobial activity was evaluated using the disc diffusion method as described by Kilani et al. [29]. The pure young culture of the selected organisms on nutrient agar and Savoured dextrose agar was used for bacteria and fungi. The surface of the agar and savoured dextrose agar plate was streaked by using a sterile loop to make an even lawn. The impregnated filter paper discs with the sample extract were placed on the surface of the plate suitably spaced apart. Plates were incubated at 37°C for 18-24 h for bacteria and 30°C for 3 days for fungi, then examined for inhibition zones of bacterial and fungal growth around discs which indicates the organism's Susceptibility to the

extracts.

Statistical analysis

The data collected were subjected to analysis of variance. The mean and Standard Error (SE) was determined using SAS program. All experiments were conducted three times.

RESULTS AND DISCUSSION

Proximate composition

Obviously, the chemical composition of the two kawals differs significantly. Except for fiber and ash, all other contents were higher (P<0.05) in AK than SK (Figures 1a and 1b). Kawal plant origin and fermenting microorganisms' variances could account for such differences. Except for ash content, AK and SK had proximate contents that are quite comparable to those reported by Harper and Collins [30]. The result of the ash content of both AK and SK was about 2-3 times less than that reported by Mbaiguinam, et al. [5] and Harper and Collins [30]. Such discrepancy could be attributed to plant origin and soil type or varietal differences were significantly better when compared to PC (1.94 and 1.39, respectively). A maximum log reduction of 2.17 was achieved for all 3 MPS solutions at 8 hours (Figures 1a-1d).

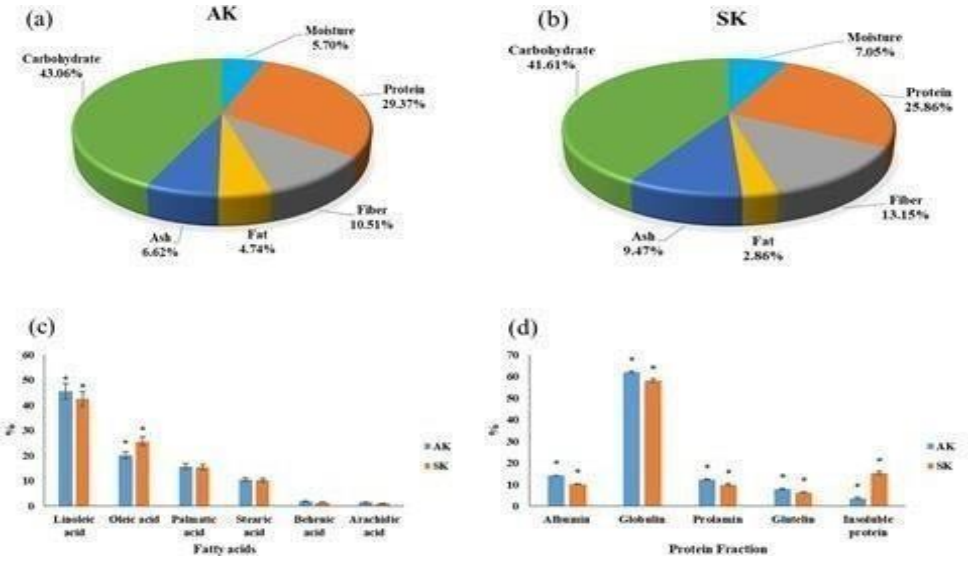

Figure 1: Proximate composition of AK **Note:** (a) and SK; (b) fatty acid composition of AK and SK; (c) protein fractions ofAK and SK; (d) values in the same column group bearing* are significantly different (P<0.05); AK=AlGenina kawal; SK=Sinnar kawal.

Fatty acid composition

AK was superior (P<0.05) to SK in linoleic acid (AK=45.52% and SK=42.43%) content, while the opposite was true for oleic acid (AK=20.18% and SK=25.68%) content (Figure 1c). The sample's origin and fermentation microorganisms could be accounted for the observed differences. Both AK and SK had similar (P>0.05) palmitic acid (15.69% and 15.39%) andstearic acid (10.55% and 10.22%) contents, respectively. Linoleic acid is a polyunsaturated omega 6 fatty acid that belongs to the essential fatty acids family necessary to human health [31]. indicated that the major fatty acids of sicklepod (Cassia obtusifolia) seed oil were linolenate (38.2%), linoleate (24.4%), palmitate (20.0%), and oleate (9.6%) [32] reported that soybean meal fermentation using Bacillus subtilis as a starter culture resulted in a significant increase in linoleate and oleate.

Protein fractions and in-vitro protein digestibility (IVPD)

Ak is superior (P<0.05) to Sk in all soluble protein fractions (Figure 1d). In both samples, globulin is the major protein fraction, which agrees with Osman et al. finding [6]. Albumin and globulin fractions are the major plant protein fractions. They are known to be rich in lysine and methionine Essential Amino Acids (EAAs), in addition to cysteine and arginine, which are conditionally EAAs. This is in general agreement with Rahim [7] finding that kawal had high methionine and cysteine contents. Figure 1d shows that AK had a significantly higher IVPD value (72.36%) than SK (58.09%). Microorganisms involved in fermentation could impart factors that improve IVPD, including proteolytic enzymes present in microbial cells and changes in pH, which may cause protein denaturation. The digestibility of AK and SK were 72.36 ± 0.28 and 58.09 ± 0.69 respectively. Both kawal samples had less digestibility when compared with that of beef meat treated with pepsin and trypsin enzymes which had an IVPD of 80% [33].

Minerals' content

Mineral contents of kawal samples are shown in Table 1. Ca, Mg, Mn, Fe, Zn, k, and P were higher in Ak, while Na was higher in Sk. Clear variations were noticed within this study and from previous ones attributed to different soil composition and time factors between different studies. Except for Ca and Mg, the AK and SK had mineral contents (Table 1) that are comparable to those reported by Harpe and Collins [30]. However, Ca, Mg, K, and Na contents of AK and SK were way less than that reported by Mbaiguinam et al. Such discrepancy could be attributed to plant origins, soil type, or varietal differences. Compared with beef and lamb meat from previous studies, both kawal samples had higher Ca, Mg, P, Zn, and Fe contents, while the latter had less Na and K contents (Table 1).

Table 1: Minerals' content of AK and SK.

Minerals (mg/100g)	AK	SK
Ca	304.29 ± 5.25*	277.40 ± 0.42*

Mg	44.54 ± 0.87*	30.74 ± 1.27*
Mn	12.93 ± 0.77*	3.53 ± 0.16*
Fe	6.22 ± 0.18*	2.58 ± 0.08*
Zn	6.72 ± 0.59*	3.29 ± 0.06*
K	263.10 ± 2.14*	187.33 ± 0.99*
Na	23.87 ± 0.39*	25.50 ± 1.45*
P	254.54 ± 8.99*	179.29 ± 6.02*
Note: n=3, values are means ± SE, means in the same row bearing* are significantly different (P<0.05) AK=AlGenina kawal; SK=Sinnar kawal - = No data		

Vitamin's content

Both AK and SK contained an appreciable amount of vitamin C (Table 2). AK had higher (P<0.05) vitamin C content than SK (90.23 vs. 67.50 mg/100 g, respectively). The two kawals had similar content of A, B complex, and E vitamin (Table 2). Vitamin A content of both kawal samples were about 10 IU/100 g, i.e., about 6 mcg/100 g beta-carotene and 3 mcg retinoids, yet higher than that of beef and lamb meat (<5 and 8.0 mcg, respectively) reported by Williams[34]. Vitamin A in AK or SK was way less than that in kawal plant seed (213.6 IU/100 g) reported by Ingweye and Umoren [35]. Both kawal samples were poor in vitamin B1, B2, B3, B5, and B6 compared with the lamb and beef meat (Table 2), as reported by Williams [34]. This is generally in agreement with the fact that meat is the major source of vitamin B group except for riboflavin, which is also available in plant sources, especially green leaves. Vitamin E content was 0.13 and 0.08 mg/kg for AK and Sk, respectively (Table 2). Such values are about 50 to 80 times less than that of beef and lamb meats. Vitamin E content equals 6.3 and 4.4 mg/kg. These results are somewhat in line with the findings of Duke [36] who mentioned that sicklepod leaves are the richest leaves in riboflavin among thirty legume leaves, second with respect to beta- carotene and third in ascorbic acid except for riboflavin content. Generally, fermentation is known to increase riboflavin, thiamin, and niacin. Also, B. subtilis is known to form riboflavin during fermentation [37]. No data showed vitamin content in fermented kawal and kawal leaves, but Ingweye et al. 35 reported vitamin content of kawal plant seed as 0.1, 0.6, 1.85, 11.88 mg/100 g for riboflavin, thiamin, niacin, and ascorbic acid, respectively. These results were comparable with the results of this study except that of ascorbic acid, and it is known that leafy vegetables and citrus are rich in ascorbic acid (Table 2)

Table 2: Vitamin content of AK and SK fermented kawal leaves.

Vitamin	AK	SK
A (IU/100g)	10	9.9

B1 (mg/kg)	0.24	0.21
B2 (mg/kg)	0.23	0.2
B3 (mg/kg)	0.22	0.19
B5 (mg/kg)	0.15	0.13
B6 (mg/kg)	0.026	0.022
B7 (mg/kg)	0.084	0.072
B9 (mg/kg)	0.13	0.11
E (mg/kg)	0.13	0.08
C (mg/100g)	90.23*	67.50*
Note: values in the same row bearing* are significantly different (P<0.05); AK=AlGenina kawal; SK=Sinnar kawal		

Antioxidant activity

The Peroxide Value (PV) of the control sample increased progressively with time to reach 20.49% (Figure 2a) at the end of the reaction time (120 min). On the other hand, PV of the treated samples decreased substantially by 84.06% and 80.09% for samples treated with AK and SK extracts, respectively. This reduction in peroxide value could have been initiated by the phenolic and flavonoids compounds present in the kawals extract (Figure 2b). Ingweye et al. and Vadivel, and Biesalski [35, 38] evaluated the antioxidant potential of kawal leaves and seeds concluded that the kawal plant has antioxidant potential (Figures 2a, 2b).

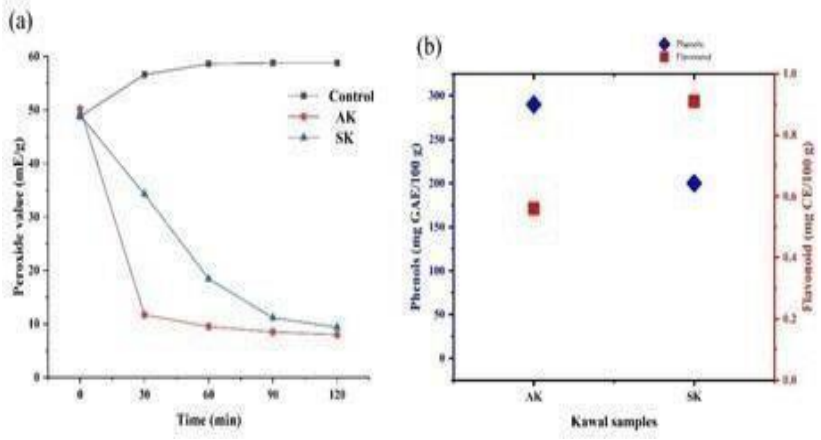

Figure 2: peroxide value of AK, SK and control sample. Note: (a) total phenols and flavonoids of AK and SK (b); AK=AlGenina kawal; SK=Sinnar kawal.

With the thiosulphate, the number of moles reacted with caffeine can be determined. But in this research, the caffeine levels in the energy drink samples were determined by calculating the volume difference between the blank titration and the average volume of each titration, and hence, calculating the concentration by equivalence. The results of caffeine determination in each sample of energy drink by iodometric back titration are presented in Tables1 and 2. The caffeine levels in drink samples assayed were in the range of 40-165 mg of caffeine per serving volume. The study showed that the estimated caffeine levels for Bullet (BU) drink was 57.50 mg/serving can, Fearless (FE) was 165.00 mg/serving bottle, Predator (PD) was 160.00 mg/serving bottle, Power Horse (PH) was 117.15 mg/serving can, and that of Passion (PA) drink was 40.00 mg/sachet dissolved in 200 mL of water. The presence of other compounds in the energy drinks studied can be an interference resulting in the addition reaction with caffeine, and also in iodometric back titration, the properties of iodine solution show that it is volatile. Therefore, some iodine can be lost during the experimental process, thus, contributing to titration errors. Comparison of the results from the two methods show significant differences in caffeine levels in Fearless and Predator energy drinks, but the results of the other drinks were in close range.

Total phenols and flavonoids

Total phenols of methanolic extract of kawal samples were 290 and 200 mg GAE/100 g for AK and SK, respectively (Figure 2b). Vadivel et al. [38] found phenolic compounds of seed extract to be 13.33 g CE/100 g. Researchers believe that many factors, such as water, air, soil, elevation (asl), differences between species, extraction methods, and antioxidant measurements affect the number of secondary metabolites in plants, including phenol and flavonoids. Researchers [39,40] claim that flavonoids have a positive relation with antimicrobial activity. Flavonoid content of kawal or fresh leaves were not reported previously. Total flavonoids of AK (0.56 mg CE/100 g) were lower than SK (0.91 mg CE/100 g). SK had better antibacterial and antifungal activity than AK, as evidence for the previous claim. In addition, the quality of phenols and flavonoids affect their antioxidant and antimicrobial activity.

Antimicrobial activity

Anti-Bacterial Activity (ABA) results are shown in (Table 3) as inhibition zones of some microorganisms created by kawal extracts, while inhibition zones created by standard antibiotics are shown in (Table 4). Sk methanolic extract (50 µL) exhibited ABA against S. aureus, Salmonella paratyphi, E. coli, and K. pneumoniae. At the same time, (25 µL) dose showed no inhibition activity against S. aureus. Antibacterial activity may be attributed to the presence of phytochemical compounds such as phenolic and flavonoid compounds. The phenolic compounds in spices herbs could play a major role in their antimicrobial effects [41]. A systematic investigation of the relationship between bacterial inhibition and total phenolic content of spices herbs needs further attention. At a 50 µL dose, AK extract showed less antibacterial activity against S. aureus, Salmonella paratyphi, Klebsiella pneumoniae and no activity against E. coli. The antibacterial effect of AK extract on K. pneumonia is dose-dependent. Compared with standard antibiotics, inhibition zones created by kawal extract of both AK and SK are good, especially in the case of K.

pneumonia, where a 17 mm zone was obtained. Such finding is comparable with that of Doughari, et al [42], who examined fresh Senna obtusifolia leaf extract in Nigeria using different solvents extracts against S. aureus and E. coli; the largest zone was less than 10 mm created by acetone extract against E. coli. Coltrimaxole (30 mcg) as standard antifungal gave an inhibition zone of 7.7 mm for Candida albicans. The SK extracts at 25 and 50 µL doses gave inhibition zones of 8.0 mm and 10.0 mm, respectively, using Candida albicans as a testing organism. So, SK may be a promising natural antifungal, while AK had no antifungal activity. This may be due to the absence of one or more phytochemical compounds responsible for antifungal activity in AK extract. In conclusion, AK and SK had considerable protein content with good digestibility in addition to appreciable vitamin C and omega 6 fatty acids contents. Kawal origins and different environmental conditions may lead to significant variations in chemical composition, photochemistry, and nutritional value. Kawal antimicrobial and antioxidant activities propose it as a functional additive to preserve food against microbial growth and oxidation (Tables 3,4).

Table3: Inhibition zones (mm) of some microorganisms created by methanolic extract at two doses of AK and SK

Microorganism	Dose	
	25 µL	**50 µL**
AK		
Staphylococcus aureus	$7.7 \pm 0.3^{*}$	$9.9 \pm 0.9^{*}$
Salmonella paratyphi	$7.6 \pm 0.4^{*}$	$10.10 \pm 0.6^{*}$
E.coli	-	-
K.pneumoniae	-	$12.11 \pm 0.01^{*}$
C. albicans	-	-
SK		
Staphylococcus aureus	-	$10.11 \pm 0.22^{*}$
Salmonella paratyphi	$9.9 \pm 0.22^{*}$	$11.12 \pm 0.04^{*}$
E.coli	$7.8 \pm 0.14^{*}$	$10.10 \pm 0.06^{*}$
K.pneumoniae	$9{,}10 \pm 0.19^{*}$	$17{,}15 \pm 0.23^{*}$
C. albicans	$8.0 \pm 0.33^{*}$	$10.0 \pm 0.21^{*}$
Note: n=3, values are means ± SE, means in the same row bearing * are significantly different (P<0.05) AK=AlGenina kawal; SK=Sinnar kawal		

Table 4: Inhibition zones (mm) of some microorganisms created by standard antibiotics

Antibiotic**	*S. paratyphe*	*S. aureus*	*E. coli*	*K. pneumoniae*	*C. albicans*

AS (20mcg)	14	-	13	25	-
BA (25mcg)	7	17	17	26	-
CF (30mcg)	7	-	14	-	-
TZf (10 mcg)	19	19	ND	27	-
CH (30mcg)	13	26	ND	26	-
CP (5mcg)	-	25	25	25	-
RO (15mcg)	10	25	ND	27	-
TE (30mcg)	12	12	19	ND	-
OF (5mcg)	-	29	ND	28	-
GM (10m Cg)	-	28	20	27	-
AM (30mcg)	-	29	ND	ND	-
LE (5mcg)	-	28	8	ND	-
CL (30mcg)	-	-	-	-	7.7

Note: *values are average of three replications; **AS=Ampicilin/Sulbactam, BA=Co-trimoxazole, CF=Cefotazime, TZF=Tazobactum/peperacilin, CH=Chloranfinicol, CP=Ceporoloxacin, RO=Roxithromycin, TE=Tetracyclin, OF=Ofloxancin, GM=Gentamycin, AM=Amikacin, LE=Levoflocxacin, CL=Clotrimaxole.

CONCLUSION

In conclusion, both kawal samples showed considerable protein content with good digestibility, large calcium, potassium, and phosphorus contents, in addition to appreciable omega 6 fatty acids content. The vitamin content of both kawal samples was relatively low except for vitamin C, which showed high content. Another important finding of this study is that kawal origins and different environmental conditions may lead to significant variations in chemical composition, phytochemistry, and nutritional value. Finally, kawal leaf extract showed good antimicrobial and antioxidant potentials that can propose it as an excellent functional additive to preserve food against microbial growth and oxidation.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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