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Characterization and Spatial Influence of Protein Content and Phytochemical Properties of *Allanblackia parviflora* Kernel and Seed Cakes from Ghana

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Abstract

Seed or kernel cakes (meals) are by-products obtained after the oil has been extracted from plant seeds. Assessing the properties of seed cakes has, over the years, helped in finding an appropriate use for these seed by-products. This study sought to evaluate the protein content, total phenolic compounds (TPC), individual phenolic acids, and the phenolic profiles of *Allanblackia parviflora* seed and kernel cakes. Both seed and kernel cakes were bulk samples from different trees, representing their respective communities within three (3) ecological zones. The spatial variation of the seed and kernel cake characteristics was therefore evaluated. Protein content was determined by determining the total nitrogen percentage. TPC was determined using the Folin-Ciocalteau method. Individual phenolic acids were determined using both HPLC and LC-MS methods of analysis. The results obtained for kernel protein ranged from 7.8% to 11.4%, with a mean of 10.4%. The seed cake protein content ranged between 3.7% and 4.8%, with a mean of 4.3%. The total phenolic content (TPC) ranged from 35.6 - 61.1 mg GAE/g for kernel cake with a mean value of 53.3 mg GAE/g. Concerning seed cakes, TPC ranged from 22.9 - 44.8 mg GAE/g with a mean value of 35.7 mg GAE/g. Kernel protein and total phenolic content were higher than their respective seed cake protein and total phenolic content. The common individual phenolic acids used as standards were found not to be present in both seed and kernel cakes. However, two prominent peaks suspected to be phenolics appeared after our last standard had eluted. Results from this study suggest that *Allanblackia parviflora* kernel and seed

cakes can be used to feed ruminants.

Keywords

Allanblackia parviflora, Protein, Total Phenolics, Seed Cakes, Kernel Cakes, Spatial Variation, Ecological Zones

1. Introduction

The by-product after oil extraction from oilseed is generally referred to as "oilseed cake" [1]. Increasing demands and struggling supplies of feedstuffs are a major challenge to livestock farmers. These have resulted in an inadequate supply of nutrients to animals, making animal rearing an expensive and capital-intensive activity globally [2]. Constant efforts are being made by animal nutritionists to find alternative feed sources, and more and more attention is being paid to the use of by-products from food processing waste and under-exploited forest products [3]. Copra cake, palm kernel cake, and soybean meal (deoiled soy cake) are by-products from food processing that are commonly added to poultry feed. Additionally, due to challenges associated with disposing of oilseed residues by industries, more investigations of possible applications for these by-products from agricultural and industrial sources have been identified [4]. The identification of the usefulness of seed cakes from different plant seeds also represents promising sources of compounds with technological and nutritional interest [4].

Little commercial use has been reported in the literature for the by-products of *Allanblackia* seeds. Abbiw [5] mentioned the usefulness of the *Allanblackia* species seedcakes in feeding cattle. Milled kernels of *Allanblackia parviflora* have been reported to contain carbohydrate, 17.32%, protein, 4.27%, and fiber, 5.70% [6] indicating that it has potential as an ingredient in animal feed. In Ghana, feeding animals with *Allanblackia* cakes is sometimes unintentionally carried out at the subsistence level, irrespective of their potential in animal feeding. To date, evidence of the commercial utilization of *Allanblackia* cakes as animal feed is lacking. This may be due to the little evidence available on the chemical composition and effect of feeding seedcake to livestock. No literature was sighted characterising the kernel or seed cakes of *Allanblackia* species. Therefore, there is a need to investigate the composition of *Allanblackia parviflora* cakes to determine the nutritional composition of the cakes and their nutritional potential for animal feeding.

Phytochemicals are biologically active chemical compounds in plants that provide colour, aroma, and flavour and can also serve as a natural defence system for host plants [7] [8]. Most phytochemicals have antioxidant properties that promote good health when consumed by people. Such compounds are mostly present in fruits, vegetables, common grains, beverages, oils, nuts, marine products, and medicinal and herbal plants [9]-[12]. For *Allanblackia* species, different studies have generally isolated xanthones, flavonoids, and alkaloids from the stem bark, seeds, leaves, and roots of the plants [13]-[17].

The limited information sourced in the literature on the phytochemical components of *Allanblackia* plants was mostly on leaf extracts [18], seeds [17], roots [19], and stem bark [13] [14]. Ayoola [18] undertook phytochemical screening and investigated free radical scavenging activities, discovering high phenolic content (65 and 12 mg GAE/g) of powdered fruits and leaves respectively for *Allanblackia floribunda*. The only report on total phenolic content (TPC) in oilseed cakes was conducted by Boudjeko [20], who reported 54.39 mg FAE/g of total phenolic content (TPC) in *Allanblackia floribunda* kernel cakes. The report also indicated that the kernel cake of *Allanblackia floribunda* has antioxidants that can influence blood glucose levels.

To date, there have been no reports on the phytochemical analysis of *Allanblackia parviflora* kernels or seed cakes. The little literature cited on *Allanblackia parviflora* phytochemical screening was on the oil [6] and stem bark [21]. These two studies on *Allanblackia parviflora* qualitatively revealed the absence of phytochemicals (total phenolics, saponins, carotenoids, and tannins) in the kernel oils and the presence of alkaloids, tannins, flavonoids, cardiac glycosides, reducing sugar, triterpenoids, anthraquinones, saponins, and phytosterols in the stem bark of *Allanblackia parviflora.* It can therefore be hypothesized that the seeds may contain these phytochemicals and may be present in the cake and the oil-based on the seed processing method adopted. Considering this, the objective of this study was to characterise the protein and phytochemicals of the kernel and seed cakes and to determine whether variations due to protein content and phytochemical characteristics occur spatially.

2. Materials and Methods

2.1. Study Area

The study was conducted in the three (3) ecological zones in Ghana described by Peprah [22] as the distribution range of *Allanblackia parviflora* in Ghana. These ecological zones included the semi-deciduous forest zone (SD) covering 66000 km^2; the moist evergreen forest zone (ME) and the wet evergreen forest zone (W) both covering about 9500 km^2. The zones differed from one another based on their average annual rainfalls (1250 - 1500 mm for SD; ME 1500 - 1750 mm; and W > 1750 mm) [22]. To ensure maximum coverage, a total of 157 trees were sampled from 16 communities across these ecological zones (eight communities from SD because of its wider coverage, and four each from ME and W) (see **Table 1**).

2.2. Tree Selection and Harvesting

For each tree, the location (latitude, longitude, and altitude) was determined by Garmin Etrex 10 GPS. Selection and fruit collection for *Allanblackia* trees occurred between December 2014 and April 2015. In each community, a maximum of 10 trees, each spaced at least 100 m apart (and no more than two per farm property), were selected. Selected trees conformed to a healthy status (not heavily

infested with mistletoes, free from fungal infection, without wilting, dead or broken branches, and with healthy fruits), and of sufficient maturity (trees of at least 10 cm diameter at breast height (DBH)). Individual trees were visited at least four times during the fruiting season, and recently fallen fruits were collected to avoid the possibility of harvesting immature fruits or collecting rotten fruits and seeds. Harvested fruits were kept for 4 days in nylon sacks to enhance fermentation. The period of fermentation softens the fruit pulp to facilitate seed extraction.

Kernel and seed cakes were obtained after oil extraction using the manual screw press. The *Allanblackia parviflora* kernel and seed cakes from each tree were kept in transparent zipped plastic bags, placed in boxes, and transported from Ghana to Western Australia by Air and stored in a refrigerator (4.5˚C) at Edith Cowan University (ECU) Chemistry laboratory until analysed. The cakes (kernels and seeds separately) were prepared for further analysis by grinding the cakes from each tree to a fine powder using a mortar and pestle. The kernel and seed cakes of 10 trees in each community (16 communities) were then bulked and thoroughly mixed to ensure homogeneity. Protein content determination, total phenolic contents, and individual phenolic identification were the chemical analyses performed on each cake for each community.

2.3. Reagents and Solvents

All chemicals were analytical reagents (AR), HPLC, or LC/MS grade unless otherwise stated. Acetonitrile, methanol, toluene, and ethyl acetate were obtained from Fisher Chemicals, Australia; BHT (99.0%), ascorbic acid, vanillin, phosphomolybdic acid, and methylene chloride were purchased from Sigma chemicals, Australia; Folin-Ciocalteau reagent from Sigma Aldrich (Merck); sodium carbonate from BDH chemicals; H_2SO_4 (98.08%), ethanol (96%) and formic acid from Univar, Australia; Acetic acid from Chem-Supply; and dichloromethane from Scharlau chemicals, Australia. The following standards were purchased in Australia from Sigma chemicals with a percentage purity greater than 99%: phloroglucinol, gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, chlorogenic acid, ellagic acid, caffeic acid, vanillic acid, p-coumaric acid, ferulic acid, sinapic acid, m-coumaric acid, quercetin, cinnamic acid, and rutin.

2.4. Determination of Crude Protein Content

Bulked samples were prepared for every community by mixing equal quantities of each tree kernel or seed cake. Sub-samples of both kernel and seed cakes from the 16 communities were analysed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Laboratory, Western Australia for percentage total nitrogen determination. Finely ground samples were analysed for total nitrogen using the Dumas high-temperature combustion (LECO analyzer, Truspec CN, St. Joseph, Michigan, USA). The samples were loaded into a combustion tube at 950˚C and flushed with oxygen. Gases generated from this process were measured using a Thermal conductivity cell for nitrogen [23]. The percentage of crude

protein content was determined by multiplying the percentage of total nitrogen by a conversion factor of 5.30 for oilseeds [24].

2.5. Extraction and Analysis of Total Phenolic Content (TPC)

The method described by Teh [25] was adopted with some modifications. Approximately 0.1 g, accurately weighed, of each bulked cake sample was transferred to a separate centrifuge tube (15 mL) and extracted with 5.0 mL of solvent (aqueous methanol, 80% v/v) over ice, while being agitated, for 10 minutes. The sample was centrifuged for 10 minutes at 4000 rpm and the supernatant was pipetted into a separate labeled centrifuge tube. The seedcake residue was extracted a second time (10 minutes) and then centrifuged as before. The supernatant was taken and combined with the supernatant from the first extraction. The combined supernatant was diluted 1 in 10 with 80% aqueous methanol.

A range of gallic acid standards were prepared in aqueous methanol-water (80:20) and in the concentration range 0.005 - 0.1 ml/mL (0.005, 0.01, 0.025, 0.05, 0.08 and 0.1). The total phenolic content of the diluted cake extracts, the blank, and the gallic acid standards was determined according to a method described by Ayoola [18].

An aliquot (0.50 mL) of each diluted extract/blank/standard was transferred into separate labeled centrifuge tubes (15 mL). In the fumehood, Folin-Ciocalteau reagent (diluted 1 in 10; 2.5 mL) was added to each tube and mixed. Then 2.0 mL of sodium carbonate solution (saturated, 35% w/v) was added and mixed thoroughly. The solution was left to stand for 60 minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant was transferred to UV/Vis cuvettes for analysis. The absorbance of the blank, standards, and samples were read at 760 nm using a spectrophotometer (UV mini-1240, UV-Vis Spectrophotometer, Shimadzu, China). Total phenolic contents were obtained from the regression equation of the calibration curve of gallic acid.

2.6. Identification of Free Phenolics Using HPLC-UV and LC-MS

The method of Obied [26] and Teh [25] was adopted with slight modifications. Seed and kernel cake samples (approximately 0.1 g, accurately weighed) were transferred to separate centrifuge tubes (15 mL) and extracted with 5.0 mL of solvent (LC-MS graded methanol, 80%: water, 20%, v/v) for 30 minutes using a shaker (KS125 Basic, IKA Labortechnik) at 800 rpm. The samples were centrifuged for 10 minutes at 4000 rpm using a centrifuge (Thermo Scientific, Heraeus Megafuge 8, China), and the supernatants were pipetted into clean, labeled centrifuge tubes. The seedcake residues were extracted a second and third time with solvent (15 min) and then centrifuged as before the combined extracts were diluted 1 in 5 using mobile phase (acidified water, 90%: methanol, 10%) for HPLC-UV analysis and 1 in 100 using mobile phase (95% water; 5% acetonitrile 0.1% formic acid) for LC-MS analysis. The standard mix of phenolics was prepared by adding 0.01 g of each phenolic to 5 mL of the mobile phase solvent. A mixture of

common phenolic compounds used were gallic acid, protocatechuic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric, sinapic acid, ferulic acid, m-coumaric, o-coumaric, ellagic acid, cinnamic acid, quercetin.

The diluted cake extracts, blank, and standard phenolic mixture were analysed using a Thermo Scientific Ultimate 3000 liquid chromatograph fitted to a Thermo Scientific Ultimate 3000 Ultraviolet Detector, Germany. Separation was achieved on an Alltech C18 Apollo column (150 mm × 4.6 mm with 5 μm particles) using a mobile phase consisting of methanol (A) and water acidified with 2% formic acid (B) and a flow rate of 1.5 mL per minute. Initial conditions were 10% A and 90% B held for 2 minutes, and then the composition was changed linearly to 80% A over 22 minutes. The mobile phase was then increased to 100% A over 0.4 minutes and held for 2.1 minutes. Over 0.5 minutes, the mobile phase was returned to starting conditions and equilibrated for 3 minutes before sample injection. The UV detector set to record at 254 nm, 280 nm, 320 nm, and 360 nm was used to detect the phenolics.

Diluted cake extracts were also analysed on a Thermo Scientific Ultimate 3000 liquid chromatography system fitted to a Thermo Fisher High-Resolution Q Exactive Focus Mass Spectrometer. The extracts were separated on a Waters C18 column (100 mm × 2.1 mm, particle size 1.7 μm). The flow rate was 0.4 mL/min, and the temperature of the column was 30˚C. The initial mobile phase conditions were 95% water with 0.1% formic acid (A) and 5% acetonitrile with 0.1% formic acid (B). These conditions were held for 2 minutes before the B phase was increased linearly to 60% over 9 minutes and held for 2 minutes. The mobile phase was then changed back to initial conditions and equilibrated for 4 minutes.

The mass spectrometer was operated in both full scan and MS/MS modes. The resolution was 35,000 Hz for both modes, the scan range was 120 - 1000 m/z, and the collision energy was 20 kV. The MS was operated in negative polarity mode, with a spray voltage of −3 kV. The capillary temperature was 350˚C, and the auxiliary heater was set at 400˚C. The sheath and auxiliary gas flows were 40 and 10, respectively.

3. Statistical Analysis

The statistical analysis was performed at two spatial levels: ecological zones and communities. Untransformed data were used for the descriptive statistics (maximum and minimum ranges; means) to show the differences between communities (means, minimum, maximum, and standard deviations) for the protein and total phenolic contents. The data were tested for normality and then analysed for correlation between protein and total phenolic contents (Pearson). One-sample t-test was used to determine whether differences existed between samples from different communities, while the independent t-test was used to determine differences between kernel and seed cake samples. A 5% level of significance was set for all statistical tests, and all were conducted using SPSS (version 23) [27].

4. Results and Discussion

4.1. Protein Content of *Allanblackia parviflora* Kernel and Seed Cakes

The protein contents of kernel and seed cakes of *Allanblackia parviflora* were analysed to determine their usefulness in animal production. As shown in **Table 1**, the kernel protein for all (16) samples ranged from 7.85% - 11.4% with a mean value of 10.4%. Concerning seed protein, the protein content ranged from 3.7% - 4.8% with a mean seed protein of 4.3%.

The mean kernel protein percentage obtained (10.4%) is more than double the figure (4.27%) reported by Adubofour [6] and was also higher compared to Dike & Asuquo [17], who recorded kernel (not cake) protein content of 1.22% for *Allanblackia floribunda*. The differences in percentage protein contents between the present and previous studies could be because this is the first time that the protein content for kernel cake has been reported. The variation may also be due to differences in the methodology used; some may have used the micro-Kjeldahl approach to determine protein content, whilst in this study, the determination was based on the Dumas high-temperature combustion method. Additionally, the sample size (157 trees) was also different from the previous studies, whose analysis was on bulked milled kernel samples. Another possibility is that varietal and provenance differences could be the reason for the lower protein content found for *Allanblackia floribunda.*

The mean percentage protein content for *Allanblackia parviflora* obtained for the seed (entire seed) cakes was lower compared to kernel cakes. To the best of our knowledge, this is the first documented evidence on the protein content of *Allanblackia parviflora* seed (entire seeds without dehulling) cakes and indeed that for any other species of *Allanblackia*. The lower protein content in kernel and seed cakes has been explored in species other than *Allanblackia* and similar results were reported. Previous reports have suggested that the presence of hull reduces the percentage of protein content as seed husks do not contribute to protein. Beski [28] have demonstrated that the crude protein content of soybean cake or meal is about 40% - 48% depending on the number of seed shells (hulls) removed and the oil extraction method. Another study by Kadanthottu [29] has revealed 0.65% and 31.76% of shell and kernel proteins respectively in African mangosteen, which is also an oilseed crop. Kadanthottu [29] research has shown that seed shells (endocarp) have relatively lower protein compared to kernels. Indeed, the work of Liu [30] on structural soybean seed parts has measured the protein content in the kernel (43.0%), whole seed (40.0%), and shell (9.0%) and highlighted the low protein content in whole seeds and shell. Our work is the first to compare the protein content in kernel and seed cakes (as a by-product of oil extraction) of *Allanblackia parviflora* and evidently shows that protein is more concentrated in the kernels than shells.

Soybean cake/meal (35.0% - 48.0% protein) is the major source of protein in the animal feed industry. Other sources of protein include copra (15.0% - 25.0%

protein) and palm kernel (12.9% - 21.0% protein) cakes [30]-[34]. According to Alimon [35], palm kernel cake (PKC) contains some amount of protein and can be best categorised as an energy feed because of its relatively low protein content (16% - 18%), which would exclude it as a protein feed. Nonetheless, the protein content of PKC is considered adequate to meet the requirements of most ruminants. For instance, the protein requirement for both non-lactating and lactating cows is around 7.0% and 9.6% respectively [36].

Table 1. Protein, total phenolic content of seeds and kernels, with their means and standard deviations for communities' bulked samples within 3 ecological zones in Ghana.

Ecological zone (code)	Community (code)	Kernel Protein (%)	Seed Protein (%)	Kernel TPC (mg/g GAE)	Seed TPC (mg/g GAE)
The moist semi-deciduous forest zone (SD)	Adansi Akrofuom (SD-AA)	10.1	4.4	53.0	44.8
	Afosu (SD-AF)	8.4	4.0	59.0	29.7
	Akoase (SD-AK)	10.9	4.5	58.6	38.3
	Anwona (SD-AN)	10.9	4.4	49.7	36.4
	Atwereboana (SD-AT)	11.2	4.7	60.3	34.0
	Fenaso (SD-F)	7.8	3.9	35.6	33.5
	New Edubease (SD-NE)	8.8	3.7	53.6	43.0
	Wassa Akropong (SD-WA)	11.2	4.2	53.8	33.3
Moist evergreen forest zone (ME)	Benso (ME-B)	11.4	4.5	54.5	37.9
	Daboase (ME-D)	11.0	4.3	59.7	35.9
	Samreboi (ME-S)	10.5	4.1	55.3	32.2
	Sefwi Bodi (ME-SB)	10.6	4.0	61.1	22.9
Wet evergreen forest zone (W)	Asonti (W-AS)	10.9	4.6	51.1	35.4
	Banso (W-BA)	10.9	4.3	51.4	39.5
	Kwansima (W-KS)	11.1	4.8	54.5	38.4
	Nzema Akropong (W-NA)	11.2	4.1	41.9	33.9
Minimum		7.8	3.7	35.6	22.9
Maximum		11.4	4.8	61.1	44.8
Mean		**10.4**	**4.3**	**53.3**	**35.7**
Standard Deviation		1.10	0.30	6.75	5.16

Oilseed cakes can be assessed as a protein feed or source depending on the percentage of protein for animal feeding. Different animals have different protein needs: for instance, broiler chicken feed is formulated to contain 22.0% protein for starter feed and 19.0% for the finisher feed [37], and the leghorn-type (egg-laying) chickens' diets for the first 18 weeks (before first egg-laying) contain 16.0% protein [38]. Considering *Allanblackia parviflora,* the mean kernel cake protein content (10.4%) was low and may not be suitable as a protein source for poultry. Meanwhile, the mean protein content of *Allanblackia parviflora* kernel was com-

parable to palm kernel cake (PKC) protein. Based on this similarity, the kernel cake of *Allanblackia parviflora* can therefore be considered a protein and energy source for ruminants.

According to Makkar *et al.* [39], high levels of shells in seed cakes can prevent their use in animal diets. Seed cakes (undehulled seed cakes) containing high fibre content are always detrimental to animals, especially non-ruminants, due to their poor digestibility [35]. The presence of shells/hulls in *Allanblackia parviflora* seed cakes and the relatively lower mean protein content (4.3%) may undermine its usability as an animal feed. The *Allanblackia parviflora* industry can therefore produce more useful cakes by removing the husk to gain higher protein content as well as increasing the oil yields. Further investigation needs to be conducted on both kernel and seed cakes to determine their fibre composition.

Differences between kernel and seed cakes protein samples were subject to T-test comparisons, and significant differences were recorded. Three samples (SD-AF, SD-F, and SD-NE) recorded conspicuously lower kernel and seed cake proteins, and all represented communities located in the SD ecological zones. Sample SD-F had the least kernel protein (7.8%) while sample ME-B recorded the highest (11.4%) kernel protein content. Regarding seed protein content, sample SD-NE had the least seed protein (3.7%), with sample W-KS having the highest (4.8%). Kernel and seed cakes proteins showed no apparent relationship with ecological zones.

4.2. Nutrition of Oilseed Cake of *Allanblackia parviflora* for Livestock Rearing

The oilseed cake of *Allanblackia parviflora*, a by-product obtained after oil extraction, has potential as an unconventional feed resource for livestock in West Africa. The seeds are rich in oil (68%), with low crude protein (4%) and minerals such as potassium and phosphorus. After oil removal, the cake contains a higher proportion of protein, fibre, and minerals, while residual fat levels depend on extraction efficiency. The oil is dominated by stearic (52% - 63%) and oleic acids (35% - 45%), meaning the residual fat fraction in the cake is largely saturated and stable. This makes the cake potentially useful in ruminant diets, where stearic acid is less disruptive to rumen fermentation compared with unsaturated fats.

Nutritionally, Allanblackia cake could serve as a supplementary protein and energy source, though its crude protein content is expected to be lower than conventional cakes like groundnut or soybean. It is more comparable to shea nut cake, which contains moderate protein and residual fat. However, caution is required due to possible anti-nutritional factors such as phenolics, tannins, and phytic acid, which may limit digestibility and nutrient availability. Heat treatment, fermentation, or enzyme supplementation can help reduce these compounds.

In livestock rearing, the cake may be best suited for ruminants at low to moderate dietary inclusion levels, with recommended fat ceilings (6% - 7% of diet dry matter) respected. For poultry and pigs, inclusion should be more restricted due to sensitivity to residual stearic-rich fat and anti-nutrients. Allanblackia oilseed

cake offers a promising local feed ingredient, but its use should be preceded by proximate analysis, anti-nutrient screening, and feeding trials.

4.3. Total Phenolic Content of *Allanblackia parviflora* Kernel and Seed Cakes

Like for many other parameters in this study, the TPC was lower in the seed compared to the kernel. This trend for TPC is supported by the literature for other seeds. The results obtained for the total phenolic content (TPC) of the kernel and seed cakes expressed as mg/g GAE of cake are shown in **Table 1**. Kernel cake's total phenolics content (KCTPC) ranged from 35.6% - 61.1% while seed cakes' total phenolic content SCTPC) ranged between 22.9% - 44.8%. The means observed for both KCTPC and SCTPC were 53.3 mg/g GAE and 35.7 mg/g GAE, respectively. The mean TPC obtained for the *Allanblackia parviflora* (53.3 mg/g GAE) kernel cakes was comparable to the value (54.4 mg/g FAE) determined for the kernel cake of *Allanblackia floribunda* by Boudjeko [20].

Generally, KCTPC were significantly higher than SCTPC values ($t = 7.765$, $p = 0.000$). Due to the limited literature on *Allanblackia parviflora* or other species, our results were compared to other plant seeds. Our study recorded higher TPC figures for kernels than seeds, in agreement with the work of Abu Bakar *et al.* [40] on bambangan (*Mangifera pajang*), where the phenolic content of kernel (103.30 mg GAE/g) was greater than seed (22.93 mg GAE/g), which was greater than flesh (5.96 mg GAE/g). In contrast, the variations in the distribution of phenolic compounds in various seed parts of peanuts were highest in the seed coats.

Also, Chandrasekara & Shahidi [41] reported that outer layers of seeds (peels, shells, hulls, or skin) of plant materials contain higher phenolic content. According to their report, analysis of raw cashew nuts revealed the following total phenolic content in the various parts of the seed: whole seed (7.01 mg GAE/g), kernel (1.14 mg GAE/g), and testa or hull (269.05 mg GAE/g). This is an indication that the levels of phenolic compounds are distributed in the various parts of plants, depending on the type of plant. The higher phenolic content in the outer layers of most plant seeds may provide a defensive mechanism against pathogens, parasites, and predators, as well as contribute to the colour of the plant.

The sample with the least kernel TPC was 35.6 mg/g GAE from the SD-F community. The highest kernel TPC was observed for sample ME-SB (61.1 mg GAE/g). The minimum SCTPC was observed for sample ME-SB (22.9 mg GAE/g), and the maximum SCTPC was for sample SD-AA (44.8 mg GAE/g) from the SD-AA community. There was also a significant difference ($p = 0.025$) between the moist evergreen (ME) and wet evergreen (W) forest zones' seed total phenolic contents (SCTPC). The differences between the SCTPC of the ME and W ecological zones could be attributed to the variations in their rainfall regimes. Even though the literature on the effect of location on the total phenolic content of *Allanblackia* kernel and seed cakes is limited, previous work by Bilgin & Sahin [42] on the total phenolic yield of olive tree leaves revealed that climatic differences

could make significant differences. Their studies reported that the TPC of olive tree leaves decreased in windy and high percentage humidity locations. The variations in the SCTPC across the moist evergreen (ME) and wet evergreen (W) zones could also be attributed to the variations in growing conditions of these two locations. Comparing the total phenolic content of the *Allanblackia parviflora* kernels to other tree kernels, *Allanblackia parviflora* can therefore be considered a good source of phenolics suitable for the development of healthy food formulations.

4.4. Relationship between Kernel Protein, Seed Protein, and Total Phenolic Content

Phenolic compounds have been identified to interact with proteins, leading to changes in the structural, functional, and nutritional properties of both compounds [43]. This affects the secondary and tertiary structures of the proteins and decreases the solubility of the protein. Additionally, the protein-phenolic compounds interactions might reduce the amount of some amino acids and protein digestibility [43]. According to Ferrer-Gallego *et al.* [44], the interaction of phenolic compounds with proteins affects the level of astringency. The level of astringency of a food material also affects its palatability to animals. The relationships between the proteins and total phenolic compounds of the kernel and seeds are shown in Table 2. No significant relationship was seen between kernel protein and kernel TPC.

Table 2. Pearson correlations among *Allanblackia parviflora* kernel, seed protein, and total phenolic content (n = 16; p-values are in bold parentheses).

	Kernel protein (%)	Seed protein (%)	Kernel total phenolic content (mg GAE/g)	Seed total phenolic content (mg GAE/g)
Kernel protein	1			
Seed protein (%)	0.688 **(0.003)***	1		
Kernel total phenolic content (mg GAE/g)	0.394 **(0.131)**	0.293 **(0.271)**	1	
Seed total phenolic content (mg GAE/g)	0.061 **(0.824)**	0.275 **(0.303)**	0.147 **(0.584)**	1

*Correlation is significant at the 0.05 level (2-tailed).

4.5. Determination of Individual Phenolic Content in Kernel and Seed Cakes by HPLC-UV and LC-MS

Seed and kernel cake extracts were analysed by HPLC-UV. A comparison of retention times indicated that the seedcake extracts did not contain detectable amounts of the commonly occurring phenolic acids such as gallic acid, protocatechuic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, sinapic acid, ferulic acid, m-coumaric acid, rutin trihydrate, cinnamic acid and quercetin. However, two peaks were consistently detected in all the extracts from the

16 sampled communities, for both seeds and kernels. These compounds eluted late in the chromatogram, suggesting that they are quite non-polar and/or high in molecular weight. They absorbed strongly at 280 nm, which is consistent with phenolic compounds [45].

While there is no published literature on the phenolic compounds present in *Allanblackia parviflora*, Azebaze *et al.* [46] reported the presence of xanthones (1, 7-dihydroxyxanthone, macluraxanthone) and flavones (morelloflavone and volkensiflavone) in *Allanblackia floribunda*. Therefore, LC-MS was used to determine if the two key compounds contributing to the phenolic content in *Allanblackia parviflora* were xanthones and/or flavones.

A bulked kernel extract was then analysed by high-resolution liquid chromatography-mass spectrometry to identify the key analytes. Using LC-MS, two key peaks eluted after quercetin and were therefore consistent with the LC-UV separation. These were attributed to ions having a mass/charge ratio of 539.0994 (earlier eluting peak) and 555.0932 (later eluting peak). A search against the Thermo Scientific online library failed to identify the compounds; however, the fragment (125.0234), present in both compounds, is consistent with pyrogallol, indicating that the compounds are indeed phenolic and contributors to the TPC. The high-resolution mass spectrometer in negative mode assigned a mass/charge ratio of 539.0994 (earlier eluting peak) and 555.0932 (later eluting peak). Azebaze *et al.* [46] reported the presence of volkensiflavone and morelloflavone in *Allanblackia parviflora*. The corresponding monoisotopic masses for the neutral forms of these compounds are 540.106 and 556.101, respectively. The high-resolution mass spectrometer recorded the same masses for the two peaks X and Y and with less than 2 ppm error.

5. Conclusions

The analysis of the *Allanblackia parviflora* cakes revealed that kernel cakes contain more protein and total phenolics than seed cakes. Kernel and seed cakes from the SD-AF, SD-F, and SD-NE communities had lower protein content compared to those in the other communities, whose protein contents were high. Samples SD-AF and SD-F had low seed cake phenolic content, with SD-AF being the lowest. There is no strong evidence suggesting that the crude protein of *Allanblackia parviflora* kernels differs markedly between the ecological zones. However, the results from our studies suggest that conditions in the SD ecological zone may be responsible for the lower protein and total phenolic contents reported.

HPLC analysis of kernel and seed cakes indicated that the sample did not contain any of our common individual phenolic compounds (gallic acid, protocatechuic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric, sinapic acid, ferulic acid, m-coumaric, o-coumaric, ellagic acid, cinnamic acid, and quercetin). However, further analysis using LC/MS revealed the presence of two compounds (morelloflavone and volkensiflavone) in both kernel and seed cakes.

Therefore, kernel cakes can be considered a potential source of protein in ruminant feed. The presence of morelloflavone and volkensiflavone are useful properties of cakes in the pharmaceutical industry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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