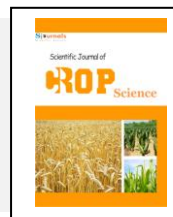


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ROP ScienceJournal homepage: www.Sjournals.com**Original article****Evaluation of physicochemical properties of *Jatropha curcas* seeds from four different agro-climatic areas of Ghana****S.K. Chikpah*, B. Demuyakor**

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ABSTRACT

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Jatropha curcas is a drought resistant multipurpose small shrub/tree with significant economic importance because of its several potential agricultural, industrial and medicinal uses. The study was conducted to provide quantitative data on the physical and chemical properties of seeds and kernels of *Jatropha curcas* grown locally in four different agro-climatic areas of Ghana (1. Nyankpala, Northern Region, 2. Dambai, Volta Region, 3. WA, Upper West Region, 4. Techiman, Brong Ahafo Region), that differ in agro-climatic conditions. The average seed weight was between 0.65 – 0.73 g and average kernel and shell weights ranged between (0.41-0.45 g) and (0.24 – 0.28 g) respectively. The kernel forms larger proportion of the seed and the percentage kernel weight of whole seed was highest in seeds obtained from Nyankpala, sample 1 (62.74 %) and lower in seeds from WA, sample 3 (61.19 %). The seeds have dry matter content of (93.13 – 94.18 %), crude protein (18.21 -19.97 %), lipid (36.52-38.64 %), carbohydrate (14.70 – 16.64 %), crude fibre (14.14 – 19.04 %) and total ash (5.03 – 5.71 %). The kernels of *Jatropha* samples were very rich in lipid (55.51 – 56.83 %) that did not vary significantly ($p > 0.05$) among the four samples. The kernels also contained high crude protein that varied between (23.08 – 25.88 %). Crude fibre was low in the kernels (3.68 – 5.52 %). The seeds and kernels of *Jatropha curcas* samples also contained varying amounts of antinutrients such as crude phorbol ester, phytic acid and tannins. Crude phorbol ester concentrations in the seed samples are sample 1(5.35 mg/g), sample 2 (6.20 mg/g), sample 3(5.30 mg/g) and sample

4(6.82 mg/g). However, the concentration of crude phorbol ester in the kernels (1K, 2K, 3K, and 4K) ranged between (5.0 – 6.45 mg/g). The phytic acid content (% dry matter) in seeds and kernels were between the range (8.71 -10.15 %) and (7.88-9.50 %) respectively. Tannins content in the kernel was low (0.05 – 0.09 % tannic acid equivalent).

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1. Introduction

Jatropha curcas also known as physic nut or purging nut is a drought-resistant multipurpose shrub or tree belonging to the tribe Joannesieae in the Family Euphorbiaceae, which is cultivated in Central and South America, south-east Asia, India and Africa (Schmook & Seralta-Peraza, 1997; Makkar *et al.*, 2007; Sirisomboon *et al.*, 2007). This plant is well adapted to arid and semi- arid conditions and can grow well on marginal soils with low nutrient content (Heller, 1996; Kumar and Sharma, 2008). *Jatropha* plant can reach a height of 3-5 m and annual seed yield of about 5 t per hectare (Raina and Gaikwad, 1987; Heller, 1996). The plant produces a tri-locular ellipsoidal fruit, the exocarp (hull) remains fresh (green) until the seeds are mature; the seeds are black about 2 cm long and 1 cm thick (Heller, 1996). The number of seeds per fruit may vary between one (1) to four (4) seeds but highest percentage of the fruits of *Jatropha curcas* contain 3 seeds (Makkar *et al.*, 2008). The average seed weight of *Jatropha curcas* varieties from Cape Verde, Nicaragua, Ife-Nigeria, and Non-toxic Mexico ranged between 0.53 – 0.86 g (Makkar *et al.*, 1998) whereas an average seed weight of 0.45- 0.72 g was reported (Martinez-Herrera *et al.*, 2006). The average kernel, and shell weight of *Jatropha curcas* varieties from four provenances of Mexico ranged between 0.31 – 0.49 g and 0.13 – 0.23 g respectively (Martinez-Herrera *et al.*, 2006). The percentage kernel of whole seed of *Jatropha curcas* varieties from Cape Verde, Nicaragua, Ife-Nigeria, and Non-toxic Mexico ranged between (60 – 63.5 %) while the shells were (36.5 – 40 %) of the whole seed weight (Makkar *et al.*, 1998). The seeds of *Jatropha curcas* contained 6.62 % moisture; protein (18.2 %); lipid (38.0 %); carbohydrates (17.3 %); fibre (15.5 %); and 4.5% ash (Gübitz *et al.*, 1999). The kernel is composed mainly of lipid (53.9 – 58.5 %) and proteins (22.2-27.7 %), with very little moisture (< 6 %) and ash. The shell of *Jatropha curcas* seed is composed mainly of fiber (> 83 % NDF) and > 74 % ADF) and lignin (> 45 %) with very little (< 6 %) protein (Makkar *et al.*, 1998). The seeds of *Jatropha curcas* were also reported to be rich in various micro and macro nutrients such as phosphorus, potassium, calcium and magnesium (El-Diwani *et al.*, 2011). *Jatropha curcas* plant has currently received much attention due to its potential applications/ uses in agriculture, medicine /health and industries. The seed oil of the plant serves as a potential renewable energy source for cooking, lighting and substitute for diesel fuel (Ishii *et al.*, 1987; Ouedraogo *et al.*, 1991; Lutz, 1992) as well as raw material for making soap, cosmetics and dye (Gubitz *et al.*, 1999; Openshaw, 2000). *Jatropha curcas* plant and byproducts have potentials for used as bio- insecticide or pesticide since extracts from the plant exhibit molluscicidal properties (Liu *et al.* 1997); insecticidal and rodenticidal properties (Solsoloy A .D., 1995) as well as antimicrobial and fungicidal properties (Ogbebor *et al.*, 2007; Kumar *et al.*, 2006). Although the seed meal, after extraction of oil, is rich in protein, it is toxic to rats, mice and ruminants and therefore cannot be used as an animal feed. Several cases of *Jatropha curcas* nut poisoning in humans after accidental consumption of the seeds have been reported with symptoms of giddiness, vomiting and diarrhoea and in the extreme condition even death has been recorded (Becker & Makkar, 1998). The meal was reported to contain antinutrients like trypsin inhibitor, lectin, phytic acid, saponins and tannins (Makkar *et al.*, 1998). However, phorbol esters have been identified as the main toxic agent responsible for the *Jatropha curcas* toxicity (Adolf *et al.*, 1984; Makkar *et al.*, 1997). The chemical composition in the *Jatropha curcas* seed and other parts of the plant depends on the genotype, soil and climatic conditions (Martinez-Herrera *et al.*, 2006). Several research have been done on physical and chemical characteristics of *Jatropha curcas* varieties from other countries but there is little work down on *Jatropha curcas* grown in locally in Ghana. The plant is under utilized in Ghana, since it is mainly used as fencing plant. For efficient utilization of *Jatropha curcas* seed and its co-products, the physical and chemical characteristics must be known. This study therefore seeks to evaluate the physicochemical properties of *Jatropha curcas* seeds from four different agro-climatic areas of Ghana.

2. Materials and methods

2.1. Collection of *Jatropha curcas* seed samples

Mature seeds of *Jatropha curcas* were obtained from the ripped fruits of locally grown *Jatropha curcas* plant from four different agro-climatic areas of Ghana in the month of December, 2011. The agro-climatic details of the different regions in Ghana, from where the *Jatropha curcas* seeds were collected, are as follows: (1) Nyankpala, Northern Region (Guinea savanna zone, Localization Lat. 09° 25'N, Long. 00° 58' W; Average temperature 28.3°C; Annual rainfall 1043 mm; Average humidity 58 %, SARI, 2005); (2) Wa, Upper west region (Guinea Savanna/Sudan Savanna, Lat. 10° 4' 0.00"N, Long. 2° 30' 0.00" W, Soil type is Lixisols, Annual rainfall 900 mm); (3) Dambai, Volta region (Transitional zone, location: Lat. 7° 40'N and 8° 15'N and Long. 0° 6'E and 0°20'E, Average temperature 27 °C, Annual rainfall 1,120 mm) and (4) Techiman, Brong Ahafo region (Transitional forest, Annual rainfall 1140 – 1270 mm, average temperature 24.5 °C). Soon after the harvesting of the fruits, the seeds were manually removed from the husk and stored in plastic containers at room temperature prior to further use and analysis.

2.2. Determination of Physical Characteristics of *Jatropha curcas* Seeds/kernels

The average seed, kernel, and shell weights of *Jatropha curcas* seed samples were determined according to the method described by Makkar *et al.* (2008). Forty seeds were randomly selected from each group of *Jatropha curcas* seeds for determination of average weight of seed, shell, and kernel. These 40 seeds were weighed separately to calculate the average seed weight. The forty seeds were cracked manually and the shells carefully removed and the weight of each kernel was recorded. The average kernel weight was therefore calculated from the total weight of the 40 kernels obtained. More also the average shell weight was calculated from the total seed weight minus kernel weight of the respective seeds. The weights of shells and kernels were also expressed as percentage of whole seed weight.

2.3. Chemical analysis

2.3.1. Proximate composition

The moisture/dry matter (DM), crude protein, lipid, crude fiber, and ash content of *Jatropha curcas* seeds and kernels were determined in accordance with the standard methods of AOAC (1990). Carbohydrates (Nitrogen free extracts) in samples were determined by difference. The analyses were conducted in triplicate and all reagents were of analytical grade.

2.3.2. Antinutrients analysis

2.3.2.1. Extraction and estimation of crude phorbol ester

Phorbol ester in seeds and kernels of *Jatropha curcas* samples were extracted according to the method described by Hass and Mittelbach (2000). In this study, 5 g of ground *Jatropha curcas* seed/kernel sample was poured into a flask containing 20 ml of methanol. The mixture was shaken thoroughly using a shaker operated at 250 rpm for 5 minutes. The mixture was then filtered using Whatman No. 4 filter paper to obtain the methanol and its extract. The residue on the filter paper and the extract were collected separately. This process was repeated and the residue extracted four additional times. The extract fractions from all five extractions were pooled together and dried under vacuum at 40°C using rotavaporator in order to remove methanol leaving a phorbol ester rich fraction. This dried phorbol ester rich fraction was measured using an analytical scale to obtained amount of crude phorbol esters (mg/g) and not pure phorbol esters.

2.3.2.2. Phytic acid analysis

Phytic acid contents of *Jatropha curcas* samples were determined using the method of Young and Greaves (1940) as adopted by Lucas and Markakes (1975). 0.2 g of finely ground *Jatropha* seed/kernel samples was weighed into different 250 ml conical flasks. Each sample was soaked in 100 ml of 2 % concentrated HCl for 3 hours. The samples were then filtered. 50 ml of each filtrate was dispersed in 250 ml beaker and 100 ml of distilled water added to each sample. 10 ml of 0.3 % ammonium thiocyanate solution was added as indicator and titrated with standard Iron (III) Chloride solution which contained 0.00195 g iron per ml.

The percentage phytic acid in the sample was calculated using the formula:

$$\text{Phytic acid (\%)} = \frac{\text{Titre Value} \times 0.00195 \times 1.19}{2} \times 100$$

2.3.2.3. Determination of tannins

Tannins concentration in *Jatropha curcas* seed and kernel samples were analyzed according to the titrimetric method of International Pharmacopoeia (2003) as described by Atanassova and Christova-Bagdassarian (2009) with some modifications. In this study, 5 g of finely ground *Jatropha* seed/kernel sample was dispersed in 50 ml of distilled deionized water (dd H₂O). The mixture was shaken using mechanical shaker at 150 rpm for 15 minutes. The mixture was then allowed to stand for 4 hours at room temperature and then filtered through Whatman No. 42 filter paper. 25 ml of the extract was dispensed into 1 litre conical flask, then 25 ml of Indigo solution and 750 ml distilled deionized water (dd H₂O) are added. 0.1 N aqueous solution of KMnO₄ was used for titration until the blue coloured solution changes to green colour. Then few drops are added at time until solution became golden yellow. The same procedure was used for the blank test titration but this time no extract was added (25 ml Indigo solution mixed with 750 ml of dd H₂O was titrated with 0.1 N KMnO₄). The standard solution of Indigo carmine was prepared as follows; 6 g of Indigo carmine was dissolved in 500 ml of distilled deionized water by heating. After cooling 50 ml of 95 – 97 % H₂SO₄ was added and the solution was diluted to 1 L and then filtered. All samples were analyzed in triplicates.

Tannin content (T, %) in sample was calculated using the formula:

$$T (\%) = \frac{(V - V_0) \times 0.004157 \times 50}{g \times 25} \times 100$$

Where V = volume of 0.1 N aqueous solution of KMnO₄ for titration of sample, ml;

V₀ = volume of 0.1 N aqueous solution of KMnO₄ for titration of blank sample, ml;

0.004257 = tannins equivalent in 0.1 N aqueous solution of KMnO₄

g = quantity of the sample extracted for the analysis, g

50 = volume of the distilled water used for the extraction, ml

2.4. Statistical analysis

The data obtained from the study was analyzed using the General Linear Model (GLM) of the Analysis of Variance (ANOVA) of Minitab Statistical Package, Version 15 (Minitab, 2007). Where significant differences were found, the means were separated using Tukey Pair Wise comparison, at 5 % level of significance.

3. Results and discussion

3.1. Physical properties of *Jatropha* samples

The Physical characteristics of *Jatropha curcas* seeds from four different agro-climatic areas of Ghana are shown in Table 1. The average whole seed weight differed significantly ($p < 0.05$) between all the four seed samples. The average whole seed weight ranged between 0.65 - 0.73 g. Sample 4 (0.73 g) had the highest average seed weight followed by sample 2 (0.71 g) while sample 1 (0.65 g) and sample 3 (0.67 g) had the lowest seed weight. The average kernel weights of samples 4 (0.45 g) and 2 (0.44 g) did not vary significantly ($p > 0.05$) but were higher than the kernel weights of sample 1 and 2 which had the same kernel weight of 0.41 g. However, the percentage kernel of whole seed weight was highest in sample 1 (62.74 %) and lower in sample 3 (61.19 %). Further, the average shell weight was significantly higher ($p < 0.05$) in samples 2 (0.27 g); 3 (0.26 g), and 4 (0.28 g) and lower in sample 1 (0.24 g). The variations observed among the physical characteristics of the *Jatropha curcas* seed samples (1, 2, 3, and 4) may be as a result of differences in agro-climatic conditions. This observation confirmed that of Martinez-Herrera *et al.* (2006) where *Jatropha curcas* seeds from different provinces of Mexico with different agro-climatic conditions had different seed, kernel and shell weights. The values of average whole seed weight of all the samples (1, 2, 3, and 4) were very similar to those reported by Aderibigbe *et al.* (1997); Makkar *et al.* (1997), (1998); Martinez-Herrera *et al.* (2006). However, the average shell weights of all *Jatropha* seed samples (1, 2, 3, & 4) in the present study are higher than those described earlier by Martinez-Herrera *et al.* (2006). The kernel forms larger proportion of the seed as indicated in Table 1. The kernel to shell ratio of all *Jatropha* seed sample (1, 2, 3, and 4) were within the range reported (Makkar *et al.*, 1998).

Table 1Physical characteristics of *Jatropha curcas* seeds collected from four different agro-climatic areas of Ghana.

J. seed samples	average seed weight (g)	average kernel wt(g)	average shell wt (g)	% kernel wt of whole seed wt	% shell wt of whole of seed wt
1	0.65 ^d ± 0.010	0.41 ^b ± 0.006	0.24 ^b ± 0.004	62.74	37.26
2	0.71 ^b ± 0.022	0.44 ^a ± 0.014	0.27 ^a ± 0.009	61.97	38.03
3	0.67 ^c ± 0.035	0.41 ^b ± 0.022	0.26 ^a ± 0.014	61.19	38.81
4	0.73 ^a ± 0.026	0.45 ^a ± 0.016	0.28 ^a ± 0.010	61.64	38.36

Samples 1, 2, 3, and 4 are *Jatropha curcas* seeds collected from Nyankpala, Dambai, WA, and Techiman respectively. Average weight = Mean ± SD. Means with different superscripts within a column are significantly different ($p < 0.05$). SD = Standard deviation.

3.2. Chemical composition

The chemical composition of seeds and kernels of *Jatropha curcas* from four different agro-climatic areas of Ghana are shown in Table 2 and 3 respectively. The dry matter contents of *Jatropha* seed samples (1, 2, 3 & 4) ranged between 93.13 % in sample 4 to 94.18 % in sample 3. There were some variations in crude protein (CP) content and lipid (ether extract) of *Jatropha* seed samples. The CP value was statistical higher in sample 2 (18.95 %) and sample 4 (18.97 %) than the CP content of sample 1 (18.50 %) and sample 3 (18.21 %). The lipid content of *Jatropha* seeds of samples 1(38.63 %), 2(38.64 %), and 4(38.58 %) were significantly the same ($p > 0.05$) but higher than the lipid value of sample 3 (36.52 %). Further, the crude fibre (CF) content of *Jatropha* seeds ranged between (14.14 -19.04 %). The CF content was significantly higher in the seeds of sample 3 (19.04) than samples 1(15.17 %), 2 (14.14 %) and 4(14.42 %). The difference between crude fibre content of sample 2 and sample 4 was insignificant ($p > 0.05$).

Table 2Proximate composition of *Jatropha curcas* seeds obtained from four different agro-climatic locations of Ghana.

Component	Jatropha seed samples				P-Value
	1	2	3	4	
Moisture	5.89 ^c	6.37 ^b	5.82 ^d	6.87 ^a	0.003
Dry matter	94.11 ^b	93.63 ^c	94.18 ^a	93.13 ^d	0.003
Crude protein	18.50 ^b	18.95 ^a	18.21 ^b	18.97 ^a	0.002
ether extract	38.63 ^a	38.64 ^a	36.52 ^b	38.58 ^a	0.001
carbohydrate	16.48 ^a	16.64 ^a	14.70 ^b	16.17 ^a	0.003
crude fibre	15.17 ^b	14.14 ^c	19.04 ^a	14.42 ^c	0.001
Ash	5.33	5.26	5.71	5.03	0.111

Samples 1, 2, 3, and 4 are *Jatropha curcas* seeds collected from Nyankpala, Dambai, WA, and Techiman respectively. Means with different subscripts within a row are significantly different ($p < 0.05$).

Table 3Proximate composition of *Jatropha curcas* kernel obtained from *Jatropha* seeds from four different agro-climatic areas of Ghana.

Component	Jatropha kernel samples				P-Value
	1	2	3	4	
Dry matter	95.54 ^a	95.05 ^b	95.50 ^a	94.71 ^c	0.001
Crude protein	23.78 ^b	25.53 ^a	23.08 ^c	25.88 ^a	0.001
ether extract	56.83	56.02	55.51	56.22	0.293
carbohydrate	6.31 ^a	4.40 ^b	7.47 ^a	5.08 ^b	0.046
crude fibre	4.74 ^a	4.16 ^b	5.52 ^a	3.68 ^c	0.001
Ash	3.88	4.94	3.92	3.84	0.405

Samples 1, 2, 3, and 4 are *Jatropha curcas* seeds collected from Nyankpala, Dambai, WA, and Techiman respectively. Means with different subscripts within a row are significantly different ($p < 0.05$).

Carbohydrates and total mineral (ash) content of seed samples were in the ranges of (14.70 – 16.64) and (5.03 – 5.71 %) respectively. On the other hand the *Jatropha curcas* kernels contained high amount of Lipid (55.51 - 56.83 %) that did not varied significantly ($p > 0.05$) among the four samples. The crude protein (CP) content of *Jatropha* kernels ranged between (23.08 – 25.88 %). The percentage CP in kernels of sample 2 (25.53 %) and 4 (25.88 %) were alike but significantly higher than the CP values measured in the kernels of sample 1(23.78 %) and 2(23.08 %). The crude fibre content was lower in the kernels of *Jatropha* (3.68 – 5.52 %). The dry matter content of *Jatropha curcas* seed and kernel were within the range reported (Gübitz *et al.*, 1999 and Makkar *et al.*, 1997 respectively). The kernels of *Jatropha curcas* contained higher percentage of lipid and crude protein than the whole seed. Therefore to obtain maximum oil production from *Jatropha curcas* seeds, extraction should be done using the kernels and not the whole seed. The values of crude protein and lipid in all *Jatropha curcas* seed samples agreed with that described in earlier publications (Makkar *et al.*, 1998; Makkar *et al.*, 2008; Raja *et al.*, 2011). The crude fibre and ash content of kernels of all the four samples were very similar to other *Jatropha* kernels collected from Cape Verde, Nicaragua, Ife-Nigeria, and Non-toxic Mexico reported by Makkar *et al.* (1997). The higher crude fibre content of the *Jatropha* seed as compared to that of the kernel can be attributed to the presence of shells around the seed, since the shell of *Jatropha curcas* is composed mainly of fibre with very little protein (Makkar *et al.*, 1998).

Table 4

Crude phorbol ester, tannins, and phytic acid concentrations of *Jatropha curcas* seeds and kernels obtained from four different agro-climatic areas of Ghana.

Jatropha seed samples	crude pe (mg/g)	tannins (% tannic acid equivalent)	phytic acid (% dm)
1	5.35 ^c	0.77 ^c	8.71 ^c
2	6.20 ^b	0.86 ^b	10.05 ^a
3	5.30 ^c	0.75 ^c	8.89 ^b
4	6.82 ^a	0.97 ^a	10.15 ^a
LSD	0.297	0.038	0.154
Sig.	***	***	***
Jatropha Kernels			
1K	5.05 ^c	0.06 ^b	7.88 ^b
2K	5.70 ^b	0.09 ^a	9.25 ^a
3K	5.00 ^c	0.05 ^b	8.16 ^b
4K	6.45 ^a	0.09 ^a	9.50 ^a
LSD	0.240	0.026	0.450
Sig.	***	*	***

Samples 1, 2, 3, 4 are *Jatropha curcas* seeds collected from Nyankpala, Dambai, WA, and Techiman respectively. K = Kernel. Means with different subscripts within each column are significantly different. LSD = Least significant difference.

Sig = significance; * = ($P < 0.05$); ** = ($P < 0.01$); *** = ($P < 0.001$).

3.3. Antinutritional factors

The concentrations of crude phorbol ester (CPE), tannins (tannic acid), and phytate (phytic acid) in *Jatropha curcas* seed and kernel samples are shown in Table 4. The crude phorbol ester (CPE) and not pure, tannins and Phytic acid varied significantly ($p < 0.05$) between the seed samples. The CPE concentration was within the range (5.30 – 6.82 mg/g). Sample 4 (6.82 mg/g) had the highest CPE content followed by sample 2(6.20 mg/g) and sample 1 (5.35 mg/g) and sample 3 (5.30 mg/g) recorded the lowest CPE content in the *Jatropha* seed. Tannins content of *Jatropha* seed samples (1, 2, 3, & 4) ranged between 0.75-0.97 (% tannic acid equivalent). The phytic acid value (% dry matter) was statistically higher in sample 2 (10.05 %) and sample 4 (10.15 %) than sample 1(8.71%) and sample 3 (8.89 %). On the other hand *Jatropha curcas* kernel samples (1K, 2K, 3K and 4K) contained crude phorbol ester (5.0 - 6.45 mg/g), phytic acid (7.88 – 9.50 % dry matter) and negligible amount of tannins (0.05 - 0.09 % tannic acid equivalent). The differences in the antinutritional factors among the four *Jatropha* samples could be attributed to variations in agro-climatic conditions (Martinez-Herrera *et al.*, 2006). Generally the

antinutritional factors were higher in seeds and kernels of samples (2&4) than samples (1& 3). The crude phorbol ester determined in seeds and kernels of *Jatropha* samples were higher than those reported in other *Jatropha* samples (Makkar *et al.*, 1997). The difference may be as a result of differences in the method of analysis. It should be noted that the phorbol ester concentration determined in this research was crude and not expressed as phorbol 12-myristate 13- acetate. The phytic acid of all the *Jatropha* seed and kernel samples were within the range reported in similar studies by Makkar *et al.* (1997); Martinez-Herrera *et al.* (2006). It was also observed in the present study that the antinutritional factors occur in higher concentrations *Jatropha* seed samples (1, 2, 3, 4) than the kernel samples (1K, 2K, 3K,4K).

4. Conclusion

The kernel formed larger proportion of the *Jatropha curcas* seed. The seeds and kernels have high dry matter content with low moisture. The *Jatropha* seed samples contained considerable amounts of lipid, crude protein and crude fibre. The kernels have higher lipid and crude protein content with low fibre than the seed samples. More also, the seeds and kernels contained antinutrients such as phorbol ester, phytic acid and tannins. These antinutrients were high in samples collected from Dambai, Volta region (sample 2) and Techiman, Brong Ahafo region (sample 4) than samples obtained from Nyankpala, Northern region (sample 1) and WA, Upper west region (sample 3).

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