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Comparative Study on Proximate Content of *Cassia occidentalis, Ocimum gratissimum* and *Senna tora* Seeds Obtained from Kafur Local Government Area of Katsina State, Nigeria

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Abstract: *Three different plant seeds samples namely; occimum gratissimum, sennatora and cassia occidentalis seeds were obtained from Kafur Local Government Area of Katsina State, were analysed for the presence of crude protein, crude fat, crude fibre, moisture, Ash, carbohydrate using the method recommended by AOAC 2005. Vitamin C and Cyanide level were also determined. The result of the analysis ranged as follows 6.65 – 13.60% for moisture, 5.25 – 10.4% for Ash, 7.40% - 48.90% for crude, 2.60 – 14.55% for protein and 27.16 – 49.95% for carbohydrate. Vitamin C and cyanide content ranged between 1.70 ±0.06 – 4.10 ± 0.20mg/g and 0.651 ± 0.03 – 0.895± 0.20mg/g. The results of moisture, Ash and protein were above the permissible limit set by RDA and FAO, 2006. However, crude fat, crude fibre and carbohydrate were within the RDA and FAO 2006 limit. The result of statistical analysis indicated no significant difference as p-values were greater than 0.05 (P>0.05). The samples were also tested for cyanide, the result obtained was 0.985 ± 0.2mg/g, 0.749 ± 0.5mg/g and 0.657 ± 0.03mg/g. The vitamin C content of the samples results obtained was found to be; 1.70 ± 0.06mg/g, 2.05 ± 0.20mg/g, and 4.10 ± 0.20mg/g for occimum gratissimum, senna tora and cassia occidentalis seeds respectively. The result obtained were below the permissible limit of RDA and FAO.*

Keywords: *Leaf vegetable, Ocimumgratissimum, Sennatora, Cassia occidentalis seeds, Proximate composition.*

Introduction

Green leafy vegetables are well known for their nutritional importance. The knowledge of the chemical and biochemical composition of foods is important to the health, wellbeing and safety of the consumers (Chauhan, 2019). The word vegetable is derived from Medieval Latin vegetabilis "growing, flourishing" (i.e. of a plant), a semantic change from a Late Latin meaning "to be enlivening, quickening" while the meaning of "vegetable" refers to as a "plant grown for food". (Uhegbu *et al.,* 2011). Vegetables are known as the segments of plants that serve as food to humans and other animals. It is often regarded collectively as edible plant matter, including flowers, fruits, stems, leaves, roots, and seeds. Vegetable gathering from the wild was done by hunter-gatherers. This evolved to the cultivation of vegetables in several parts of the world during the period 10,000 BC to 7000 BC following the development of a new agricultural way of life. Primarily, plants grown locally would have been cultivated. However, with time, various exotic crops from other regions were introduced to the local market (Chauhan, 2019). Presently, the production of most vegetables around the world is highly dependent on the climate and crops may be cultivated as protection for the environments in less appropriate areas. The level of vegetable production varies as a result of the purpose, on the one hand, subsistence farmers supply the needs of their families for food and on the other hand, supply agro-allied businesses (Mirdehyghan, 2009).

Moreso, vegetables are edible when raw or cooked and serve an important role in human nutrition because it has low fat and carbohydrates but high in vitamins, minerals, and dietary fiber. Nutrition experts advised people to consume more fruit and vegetable, also recommended five or more portions a day. Generally, vegetables are rich sources of minerals- especially calcium and iron, and vitamins A and C. while nearly all classes of vegetables are adequate as antioxidants and dietary fiber (Kumar *et al.,* 2020).

Sampling and Samples Preparation

Fresh samples of *senna tora, occimum gratissimum,* and *cassia seeds*, were obtained from Kafur Local Government Area of Katsina State. The plant samples were identified at Department of Plant Science, Bayero University Kano. The samples were thoroughly washed with deionized water to remove some dust, dirt, or possibly parasites. The samples were air-dried at room temperature and then pulvarized with morta and pestle and were packed in airtight containers.

Proximate Analysis Procedure

The proximate analysis of all samples were determined. The moisture and ash content were determining using weight difference method. The fiber content was estimated from the loss in weight of crucible and its content on ignition. Carbohydrate was determined when the sum of percentages of moisture, ash, crude protein, ether extracts and crude fiber were subtracted from 100. The nitrogen value which is the precursor for protein of a substance was determined by micro kjeldahl method described by Pearson (1976). Involving digestions, distillation, and finally titration of the sample. The nitrogen was converted to protein by multiplying with a factor of 6.25. Carbohydrate was determined by difference method. All the proximate values were reported in percentage (AOAC 1990; AOAC, 2005).

3.6.1 Moisture Content Determination

Procedure: A clean crucible was dried in an oven at 105C for 20 mins and then transferred to a desiccator to cool. It was then weighed (W_1). 2g of the dried and fresh sample were individually weighed in the crucible, and then placed in an oven and dried at 135^0C until a constant weight was achieved. After drying, the crucible was then transferred to a desiccator to cool then reweighed with its content (W_2) (AOAC, 2005).

Calculation:

$$\%\text{moisture} = \frac{W_1 - W_2}{W_1 - W} \times 100$$

Where

W_1 = weight of sample before drying in (g)

W_2 = weight of sample after drying in (g)

W= weight empty crucible in (g)

Lipid Content Determination

Procedure: A 5g of dried sample was carefully weighed (W_1) in to a folded fat free filter paper. It was weighed again to obtain the weight of the sample together with the filter paper (W_2), and was carefully place in the soxhlet extractor. The whole apparatus was then connected after the addition of about 300ml N-hexane in to the extraction flask. The extraction was then carried out for about 3 hours or until the N-hexane coming out of sample compartment become very clear while ensuring continuous flow of water in the condenser. The sample was then remove, air dried and then placed in an oven at about 75^0C until a constant weight was obtain (W_3) the lipid content was then calculated as the percentage lipid (AOAC, 2005).

Calculation:

$$\%\text{lipid} = \frac{\text{loss of weight on extraction}}{\text{Weight of sample taken}}$$

$$\%\text{lipid} = \frac{W_2 - W_3}{W_1} \times 100\%$$

Where W_1 = weight of sample

W_2 = weight of filter paper + sample

W_3 = weight after extraction

Crude Fiber Content Determination

Procedure: The crude fiber was determining by subjecting 3g (W_3) of the dried sample from moisture analysis and ether extraction to successive treatments with boiling 200cm^3 of 0.255M of H_2SO_4 solution, washed thoroughly with hot water until it was acid free. It was then treated with boiling 200cm^3 of 0.1275M NaOH solution, washed thoroughly with hot water until it was base free. It was then dried to a constant weight in an oven at 100^0C, cooled in a dessicator and

weighed (W_1). The weighed sample was then incinerated in a muffle furnace at 550^0C for 2 hours until a constant weight was obtained (W_2). The crude fiber was calculated as the loss in weight on ashing (AOAC, 2005).

Calculation:

$$\% \text{ crude fiber} = \frac{W_1 - W_2}{W_3} \times 100\%$$

Where

W_1 = weight of residue + filter paper

W_2 = weight of "W_1" after ashing

W_3 = weight of sample used (3g)

Crude Protein Determination

Procedure: 0.2g of dried (moisture free) sample was weighed and the content was transferred in to the kjeldahl digestion flask. 0.8g of catalyst (0.7g sodium sulphate, 0.06g copper sulphate and 0.03g mercury (ii) oxide was added in to the digestion flask; $2cm^3$ of concentrated sulphuric acid was also added and the mixture was heated on the heating mantle for 1 hour until the liquid become clear. The digest was cooled and made alkaline with $15cm^3$ of 40% NaOH. The digest was then transferred to- the steam out apparatus. The ammonia steam condensed in to $10cm^3$ of 2% boric acid solution with 5 drops of methyl red indicator for 15 minute. The distilled ammonia was then titrated with 0.02M HCl (AOAC, 2005).

$$\% \text{ N} = \frac{0.014 \left(\frac{\text{MeN}}{100\text{g}}\right) \text{titre value x volume of digest X Normality of acid}}{\text{Weight of sample X volume of aliquot (10ml)}}$$

The percentage of protein content of each sample was calculated as follows: -

Protein (%) = Nitrogen (%) x 6.25 (Conversion factor)

Total Ash Determination

Procedure: A crucible was dried in an oven cooled and weighed (W1). 2g of the dried sample was placed in the crucible and weighted (W2) which was then incinerated in a muffle furnace at 550^0C until a constant weight was obtained. The resulting ash was then covered with a lid and cooled in a desiccator prior to weighing (W3) (AOAC, 2005).

Calculations

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

Where

W_1 = weight of crucible,

W_2 = weight of sample + crucible

W_3 = weight after ashing

Carbohydrate Content Determination

Procedure: The carbohydrate content was determined by difference.

% carbohydrate = 100-(crude fibre+lipid+protein+moisture+ash) (debola *et al*. 2017).

Cyanide Test (HCN)

Procedure: The alkaline picrate colorimeter method describes by Balagopala *et al* (1988) was used. Stripes of filter paper were cut from whatman No. 1 filter paper. An alkaline picrate solution was prepared by dissolving 1g of picrate and 5g of sodium carbonate in a small volume of minimally warm water and volume was made up to 200cm^3 with distill water. The picrate paper was prepared by dipping rectangular pieces of filter paper in picric acid solution and dried. 1g of each test sample was dispensed into 200cm^3 of distilled water in 200cm^3 conical flask. An alkaline picrate paper was suspended in the flask and held in a place with the stopper used to cork the flask. Care was taking to ensure that the picrate paper did not touch the surface of the flask. They were incubated at room temperature for 18 hours (overnight) and then each picrate paper was carefully removed and eluted in 60cm^3 of distilled water. A standard cyanide solution was prepared (0.05M). The absorbance of the sample solution and standard was measured spectrophotometrically at 540nm using the reagent as blank to set the instrument at zero. A cyanide was content determined by calculation as shown below !

HCN mg/kg = $\frac{100}{W}\ X\ \frac{Au}{AS}\ X\ C$

Where:

W = weight of sample

Au = Absorbance of Sample

As= Absorbance of Standard solution

C= Concentration of standard solution

Determination of Vitamin C

Procedure: 1g grams of each samples were macerated with 20ml of 0.4%oxalic acid for 10min. and centrifuged for 5 minute. The supernatant (1ml) was transferred into test tubes to which 9ml of 2.6dichlorophenol indophenols (12mg/l) was mixed thoroughly by shaking. The absorbance of the resulting solution was measured at 520nm at 15sec. and 30sec. with corresponding blank.

Concentration (mg/g) = = $\frac{Absorbance*Dilution*Pathlenth}{\text{Extraction coefficient}}$

Statistical Analysis

The data obtained from the analysis of *Cassia Occidentalis*, *Ocimumgratissimum* and *sennatora* seeds collected from Kafur Local Government in Katsina State were statistically processed and presented as mean ±standard error. Statistical significance of the comparison of data were analysed using one way analysis of variance (Anova) followed by LDS multiple range test using software statistical package for social science (SPSS) and significance level at P –value < 0.05 was considered significant.

Results and Discussion

Results: The results of proximate analyses of the three (3) plant seeds samples analyzed are presented in fig. 1-6.

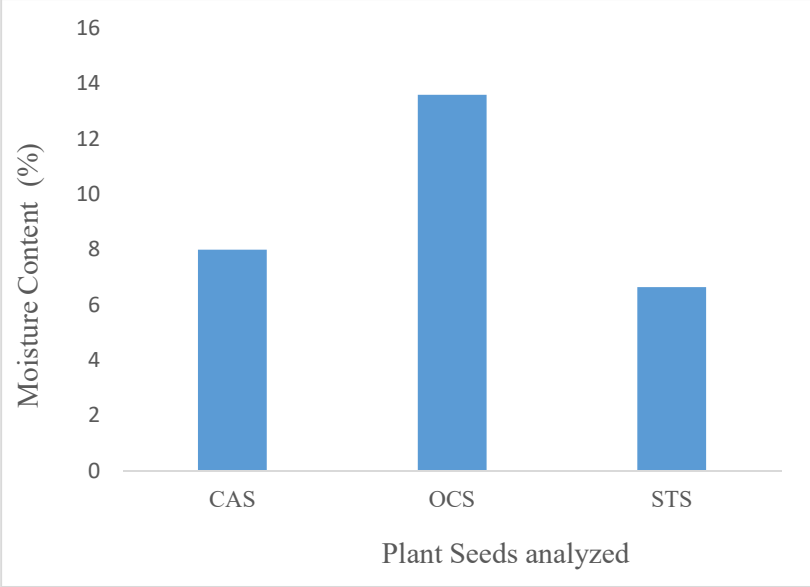

Fig. 1: Moisture content of the plant seeds analyzed (%).

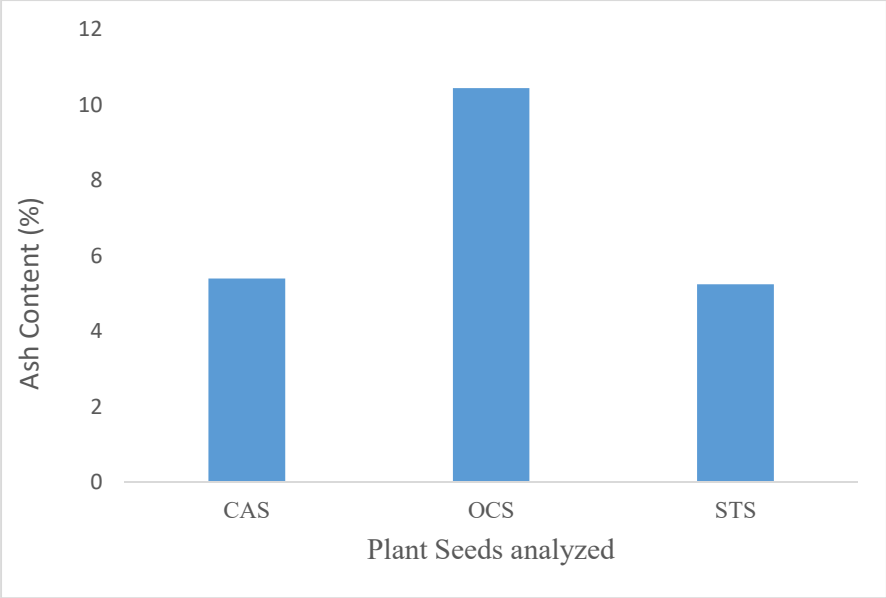

Fig. 2: Ash content of the plant seeds analyzed (%).

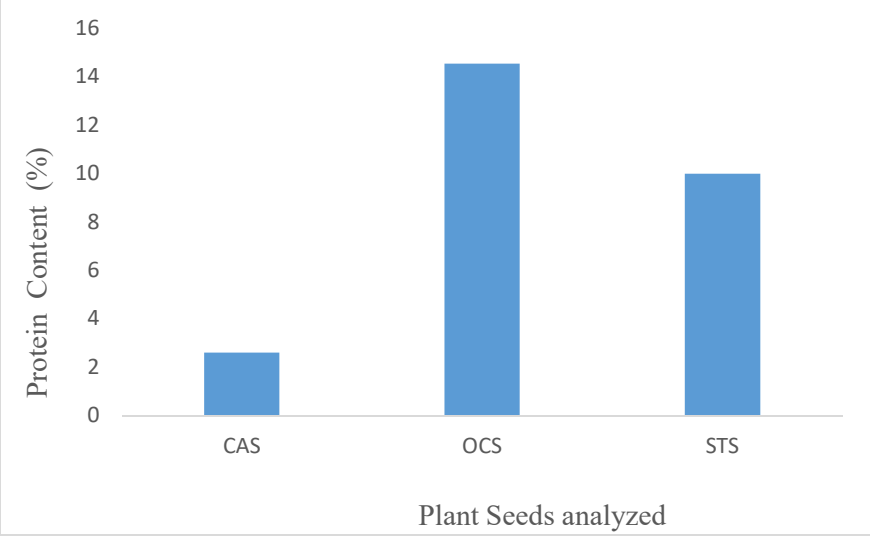

Fig. 3: Protein content of the plant seeds analyzed (%).

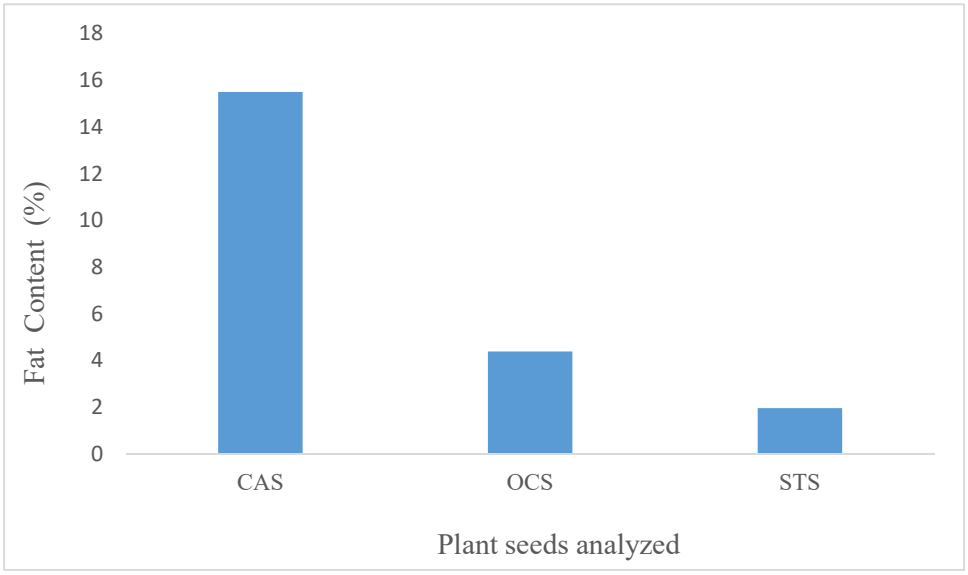

Fig. 4: Fat content of the plant seeds analyzed (%).

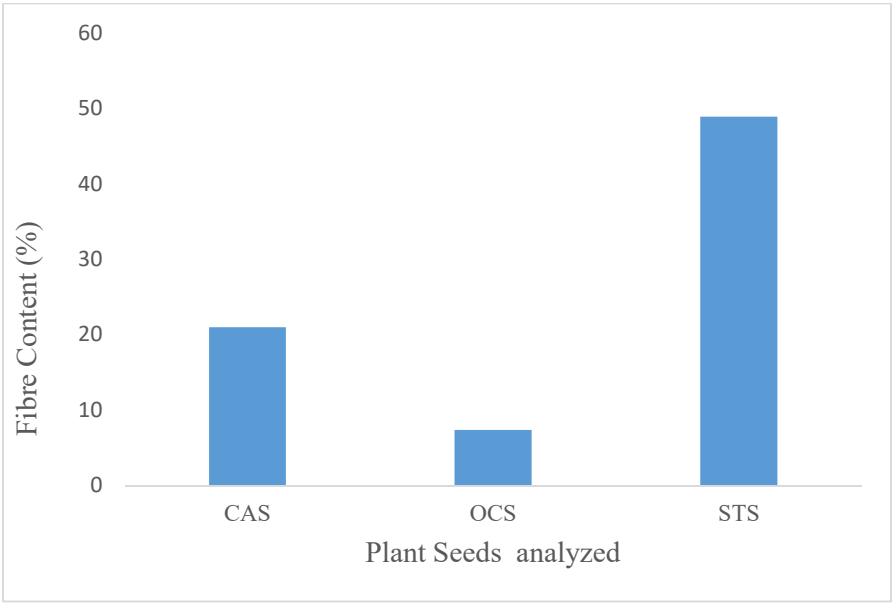

Fig. 5: Crude Fiber content of the plant seeds analyzed (%)

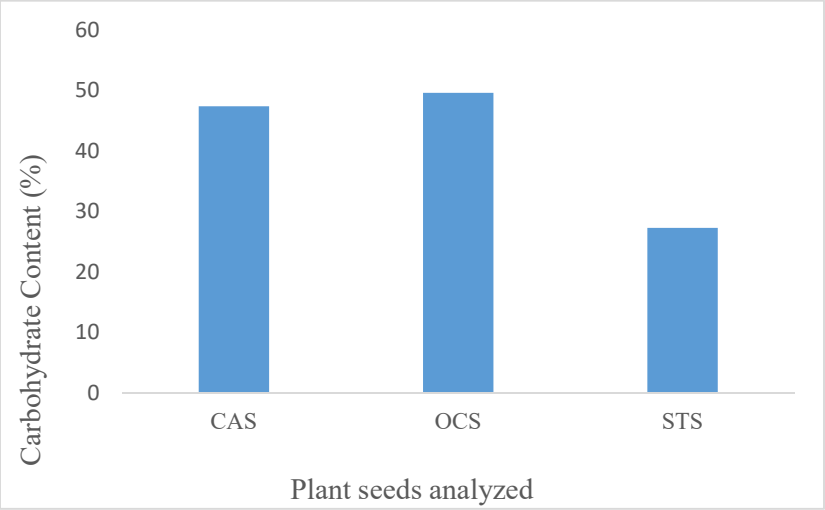

Fig. 6: Carbohydrate content of the plant seeds analyzed (%).

Table 1: Result of Hydrogen Cyanide and Vitamin C contents of the samples analyzed:

S/n	Seeds Extract	HCN mg/g	Vitamic C mg/g
1.	*Occuimum G. Seed*	0.895±0.2	1.70± 0.06
2.	*Senna T. Seed*	0.749±0.50	2.05±0.20
3.	*Cassia O. Seed*	0.65±0.03	4.10± 0.20

Discussion

Proximate Composition of Selected Seeds Sample.

Fig 1. Moisture: Showed moisture content of seeds samples ranged between 6.65% for *Senna tora* to 13.60% for *Occimum gratissimum*. 8.0% moisture was obtained in *cassia* seed. In this study were higher than the RDA standard set aside by Food and Agricultural Organization (FAO) (2006) of the united nation. The moisture content observed in this study was lower than the result obtained 21.96.0±0.30% by Aja *et al* (2017) in the *cassia occidentalis* plants leaves. Similar study by Imran *et al,* (2007) found moisture content in the range of 81.31 and 90.54% in some selected species. The result of statistical analysis (Anova) indicated no significant difference at (P>0.05) between the moisture contents in the analyzed plant sample. The level of moisture in the plant samples analyzed, were above 0.7-3.8g/d (RDA/FAO 2006). This could be because the plants were grown in a humid environment. These results are in agreement with values reported by Bamigboye *et al.,*(2010), that reported 6.4 0.04% moisture in Nigerian sesame seed, Olumide *et al*., (2019), reported 13.60% moisture in *Occimium gratissimum* seed extract and Akpabio *et al*; (2012) reported 8.7% moisture in *cassia hirsute* seed. Moisture is considered as a good sources of water and it is necessary that 20% of the total water consumption must come from food moisture (FNB, 2005).

Fig 2. Ash content: Shows *Occimum gratissim*um has the highest ash content (10.46%) followed by *cassia* seed extract with 5.9% ash and the least was found in *Sennatora* seed extract (5.25%). The result of statistical analysis (Anova) indicated no significant difference at (P>0.05) between the ash contents in the analyzed plant samples. The level of ash content in plant samples analyzed were above 0.8g/d (RDA/FAO 2006). A similar value (10.5%) of ash was obtained by Olumide *et al*., (2019) and Idris *et al* (2011) in the *Ocimum gratissimum*. A slightly higher value of 6.93% was obtained for Ash content in *Senna singueana* leave by Alsiede *et al*. (2015) and a slightly lower value of 4.95% was obtained for Ash content in *cassia Auriculata* seed extract by Bamigboye *et al*; (2010). Rahimullah, (2016) reported higher percentage of 17% for ash in *cassia tora* seed. This might be attributed to the contamination of the plants with ash from either soil or water.

Fig 3. Protein: Revealed protein level of selected seeds samples. *Ocimum* among other plants was found to obtain the highest amount of protein (14.55%) followed by *Senna* seed extract with 9.79% and the least amount was found in cassia extract (2.6%). Anova result indicated no significant differences at (P>0.05) between protein content in the analyzed plant samples. The level of protein in the analyzed plant samples were above 3.5g/d set by RDA/FAO (2006). This might be because the plants were grown in a nutrient rich soil. Almost similar values were previously reported such as 15.08 ± 0.29% for protein in *Occimumgratissimum* seed extract by (Militant *et al,* 2004) and 2.3% for protein in *cassia Occidentallis* by (Daniyan *et al*., 2011). However, 17.36 ± 1.10% for protein in *tetrapleuratetraptera* seed and 83.56% for protein in *citrullus vulgaris* seed were above the findings of the present research. Protein were reported to provide amino acids for the body to build new tissues, and normal growth of infants. Protein is used to maintain body tissue and replacement of damaged worn out tissue in both adults and children, it served as a source of energy in the absence of carbohydrate, help in the formation of hormones, enzymes and antibodies. (Ojofietimi, 2007).

Fig 4. Crude Fat: Shows crude fat of selected seed samples. *Cassia seeds* extract has 15.5% as the highest percentage of crude fat, followed by *Ocimumgratissimum* seed extract with 4.4% and the least percentage of 1.97% was found in *Sentora seed* extract. The result of statistical analysis (Anova) indicated no significant difference at (P>0.05) between the Fat contents in the analyzed plant samples. The level of Fat content in plant samples analyzed were within 1.5 – 31.0 g/d (RDA/FAO 2006). Fagbohun *et al*., (2012) reported 7.75% crude fat in *Ocimumgratissum*. Almost similar value of 1.6 ±1.0% was reported for crude fat in *Sennaoccidentallis* (Aja *et al*., 2017). Likewise higher values above the findings of the present work were reported such as 31.84 ± 0.02% crude fat in *citrullusalata* seed (Mamman *et al*., 2022) and 70.0 ± 14.1% crude fat in *Ocimum gratissimum* (Aluko *et al.,* 2012). Fat is used to supply energy, help the body to transport fats soluble vitamins (A, D, E, and K), provide the essential fatty acids which help in preventing excessive loss of water, help in protecting the internal organ of the body serving as cushion pad, for example the kidney. Fats give taste and feeling of satisfaction in the meal. (Ojofeitimi, 2007).

Fig. 5. Crude fiber: Showed that *Senna tora* extract has the highest percentage of crude fiber (48.88%) and *Ocimum gratissimum* seed extract the lowest ((7.4 %.) *Cassia* seed extract was found to have 21.0% crude fiber. The result of statistical analysis (Anova) indicated no significant difference at (P>0.05) between the Fiber contents in the analyzed plant samples. The level of fibre content in plant samples analyzed were within 19 - 38 g/d (RDA/FAO 2006). 7.54 ± 1.08% crude

fibre in cassia leaves reported by Muhammed *et al*., (2018) and 7.6% crude fibre in *Occimum gratissimum* leaf extract reported by Olumide *et al*., (2019) agreed with the findings of the present work. However, (Aja *et al*., 2017) reported 19.63% crude fibre in *Senna occidentallis* which is lower than the value obtained in the present study. Fiber is use to lower blood cholesterol, delay the glucose absorption (Gibney *et al.,* 2009).

Fig. 6. Carbohydrate: Showed that, *Ocimum gratissimum* among other has the highest percentage of carbohydrate 49.95%, followed by *cassia tora* seed with 47.4% and 27.16% for *Senna seed* extract. The result of statistical analysis (Anova) indicated no significant difference at (P>0.05) between the carbohydrate contents in the analyzed plant samples. The level of Carbohydrate content in plant samples analyzed were below 60-120g/d (RDA/FAO 2006). This might be attributed to the age of the plants (matured).49.75% carbohydrate reported in *Occmum gratissimum* by Olumide *et al*, (2019) agreed with the present work. 45.5% carbohydrate in *senna occidentallis* was higher compared to 27.16% in the present study. While 56.72% carbohydrate in *cassia sabrienna* seed reported by Olapade *et al*., (2014) and 58.8% carbohydrate in *Ocimum gratissimum* reported by Adewale, (2014) were higher compared to the values in the present study. Carbohydrate supplies energy which serves as body fuel to both internal and external activities, spares protein from being used as a source of energy, One gram of carbohydrate will supply 4kcal to the body, supplies glucose to the body especially the brain, help the body to burn body part. It help in the formation of genetic materials Deoxyribonuecleic acid (DNA) and Ribonucleic acid (RNA) (Ojofeitimi, 2007).

Cyanide and Vitamin C

The samples had been tested for cyanide, result obtained (0.895 $\pm$ 0.20mg/ml) for *occimum gratissimum* seed having the highest value of cyanide content among the samples. 0.749$\pm$ 0.50mg/ml was obtained from *Senna Tora* seed and the lowest cyanide value 0.657mg/ml ± 0.03 was obtained from *cassia Occidentalis* seed. 3. 75 $\pm$ 0.10mg/ml cyanide content were reported by Frodin *et al;* (2014) on *ocimum G* seed. The sampled results obtained showed mild concentration of cyanide. Acute cyanide poisoning occurs at the concentration of 0.5-3.5mg/kg of body weight according to Borinwa *et al,* (2013). Even though, person would have consume between 0.4 and 4kg of flax seeds to reach acute poisoning, the long term effect of small dose intake are not well documented but should be of concern (Borinwa *et al.* 2013). The potential toxicity of food produced from cyanogenic plants depends on the likelihood that consumption could result in concentrations of hydrogen cyanide (HCN) that are toxic to exposed humans or animals. The lethal dose of orally ingested hydrogen cyanide for a 60kg adult man ranges from 30- 210 mg equivalent HCN (Frodin *et al,* 2014)).

The result of vitamin C obtained revealed 1.70$\pm$ 0.06mg/g for *occimum gratissimum,* the result is in ranged the reported work by Rahul (2018) (1.284 – 1.828mg/g) of vitamin C level in the leaves samples. Vitamin C level of *Sennatora* seed was found to be 2.05 $\pm$ 0.20mg/g, the result obtained in *sennatora* seed is lower than the finding obtained by Rahul *et al*, (2018). 2.940mg/g. Cassia occidental seed vitamin C was found to be 4. 10 $\pm$ 0.20mg/g. The value obtained is higher than 1.061 $\pm$ 0.81 reported by Innocent *et al,* (2017). And lower than the reported work of 8.09 $\pm$ 0.27mg/ml by Batari, (2012). 17.63 $\pm$ 0.02mg/ml were reported for a row seed (Arekemase *et*

al 2019). Vitamin C (ascorbic acid) is water soluble, antioxidant essential for human health. It has been necessary for health response, wound healing, reducing in allergic response and developments connective tissue components such as collagen and for the prevention of diseases (Innocent *et al*; 2022).

Conclusion

The proximate values results obtained in the seed samples shown that, the seeds is worthy of being exploited outside its immediate locality. The seeds having a protein content that is comparable with good source of plant protein by RDA/FAO. The seeds also contain fiber which is essential to lower blood cholesterol. The fat content of the seeds were comparably within the range of values giving by RDA/FAO 2006, which provide essential amino acid to the body and transport soluble vitamin. Ash content of the seeds are in appreciable amount compared to the permissible limit. Proper maintenance and utilization of the seeds should be encourage for the nutritional support giving in human diets. However, further studies should be carried out to elucidate the anti-microbial activities of these plant seeds to affirm the claims by traditional healers of the effectiveness of these seeds in treating diseases.

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