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Nutritional, Antinitritional and Antimicrobial Activities Of Seed and Pulp of Cola Millenii

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Abstract: *The seed and pulp of Cola millenii were assessed with respect to their nutritional compositions and anti-nutrient factors. Also, the inhibitory activities were equally investigated. The result of the nutritional compositions of seed and pulp were in the range value; protein (4.10 to 5.76 g/100kg), crude fibre (2.22 to 2.83 g/100kg), crude fat (30.03 to 43.00 g/100kg), ash content (2.76 to 3.15 g/100kg), moisture content (6.90 to 9.80g/100g) and carbohydrate (10.46 to 13.79g/100kg). The result for mineral compositions showed that the seed and pulp contained the range value; calcium (168.73 to 394.78mg/100g), magnesium (459.47 to 610.29mg/100g), iron (446.56 to 1901.75mg/100g), zinc (310.26 to 481.53 mg/100g), manganese (100.02 to 555.32 mg/100g), sodium (140.99 to 187.15 mg/100g), potassium (356.47 to 417.72 mg/100g), and copper (7.80 to 9.90 mg/100g). The anti-nutrient factors detected in the seed and pulp was tannin, phytate and oxalate. The level of these anti nutrients were however low and might not pose any health hazard when compared to anti nutrients of 10-60mg/g in diet.The antimicrobial activities indicate that, the seed and pulp inhibits some species of bacteria. The result of the assessment implies that the seed and pulp can be used as a supplement because essential nutrients needed in the body were moderately present. Finally, the nutritional composition compares favourably with some common fruits.*

Keywords: *Cola millenii, nutritional, mineral, tannin, antimicrobial.*

I. Introduction

Wild foods are part of rural people's diets on daily basis. Most dietary studies emphasize the value of calorific intake from staple food, however the amount of world foods consumed, their frequencies of consumption as well as their nutrient content have also been exploded. It is this daily consumption of world foods contributes to over all nutritional well being. The diet o f many rural and urban dwellers is deficient in protein and high in carbohydrates, the implication is high incidence of malnutrition and increase dietary disease, a situation in which children and lactating woman are most vulnerable sadik, (1991). While every measure is being taken by various levels of government to boost food production by conventional agriculture, a lot of interest is been currently focused on the possibility of exploiting the less familiar plants resources of the wild. Anhawange et al, (2004); Abdullahi and Abdulahi, (2004).

The use of wild foods as a component of local response to increasing food insecurity is widely documented. The greatest sources of these phytochemicals are fruits and vegetables Reid et al, (2005). Tropical Africa sub-region is home to many potential valuable fruit species whose potentials have not been fully exploited Umoh, (1998). A good number of this fruit species are not yet domesticated. But tangible economic produce are been harvested from their wild Carnovale et al, (1991). Wild plants which refers to trees, flowers, and grasses, the vines found in the bushes and even the weeds in the lawn, they are what our great grand parents used to doctor the family. Consider the food on the table and also what we need as renewable source of oil, fuel, food, pharmaceuticals and more enhanced the need to discover the potentials present in these wild plants Falade et al, (2003).

Plants have basic nutritional importance by their content of protein, carbohydrates, fats, minerals, vitamins and water responsible for growth and development in man and animals. Report shows that some plants chemicals which have been regarded as anti-nutritional have potentials in reducing the risk of several deadly diseases in man Agte et al, (2000). This phytochemicals reduces the low density lipoprotein, i.e. the cholesterol involves in depositing fat in the arteries, prevent blood clotting which can results in heart attack or stroke Anhwange et al, (2004). They do this either by working alone or in combination with other vitamins Melaku et al, (2005).

Cola millenii is a wild plant of tropical and sub-tropical countries whose place of origin is unknown, but probably from Indonesia. C. millenii belong to the Sterculiaceae family, genus cola and species millenii. Cola millenii is edible fruits of varying characteristics sweetness. It is widely distributed throughout tropical Africa, from Senegal to Cameroon Michel (2004). It was common in the southern Nigeria were they are eaten as edible fruits by the peasant farmers during the peak season. It is a shrub or tree up to 7-10m high somewhat twisted and branchy, with rounded and open crown. The bark is thin, washed pale brown, peeling off in thin

linear scales, with red-brown fibrous slash. The stem is pubescent, grey to brown, lenticellate. The leave is alternate, 2-ranked, 1.5-2.5 cm long 1-5cm across, narrowly elliptic or oblong, odoriferous when crushed. Blade pubescent, are mainly beneath, becoming more or less glabrous, with a pointed or rounded, mucronate apex and a rounded or slightly cordate base. Flowers is solitary or in groups of two or three, in leaf axils, pale yellow, 1.5mm in diameter, with 6 petals curled into blade connected at the base. Fruit composed of several ellipsoid carpels, 5cm long 2.5 cm across, swollen and star-shaped, orange to red when ripe, with several seeds in a whitish pulp Michel (2004).

Previous studies have shown the medicinal application of the root, bark and leave but there is little information about the nutritional, antinutritional and antimicrobial profile of Cola millenii seed and pulp. Therefore this present study is however aimed at assessing the nutritional, antinutritional and antimicrobial profile of Cola millenii seed and pulp.

II. Materials And Method

Materials

Cola millenii used for this study was collected freshly from Obasoto farm, Uso, Ondo State, Nigeria. The pulp was separated from the seed and kept in the clean flask which was stored in the freezer prior to analytical work. The seed were washed, oven dried and milled into flour, both the flour and the pulp were used for analytical work.

Methods

The protein, crude fibre, crude fat, ash content, moisture content were determined according to standard method AOAC, (1990). The protein content was obtained by multiplying the nitrogen content by the factor 6.25. Carbohydrate was obtained by differences that are 100 minus the value of protein, fibre, crude fat, ash content and moisture content. For the mineral composition; samples were digested, each sample, 0.5g was weighed in triplicate into Kjeldali flask and 10ml of concentration trioxonitrate (IV) acid was added and allowed to stand overnight. The samples were then heated carefully until the production of brown trioxonitrate fume had ceased. The flasks were cooled and 2.4ml of 70% perchloric acid was added. Heating was continued until the solution turn colourless. The solutions were transfer into 50ml standard flasks and diluted with distilled water. The mineral content was then analyzed by atomic absorption spectrophotometer.

Reducing sugar was determined from the ethanolic extract by the Ferricyanide method AOAC, (1990). 2ml extract was added to 8ml of Ferricyanide reagent and the absorbance read at 380nm using glucose as the standard. The starch in the original sample was then determined as reducing sugar.

Tannin was determined by the modified Vanillin hydrochloric acid method using 1ml/mg of catechin 1% HCL-MeOH as standard. The colored substituted product was measured at 500nm. Oxalate was determined titrimetrically, being precipitated as calcium oxalate and filtered against standard potassium permanganate. The oxalate was calculated as sodium oxalate equivalent. Anion exchange method was used to determine phytase, using KH_2P0_4 as standard Harland and Oberleas, (1986).

Antimicrobial analysis was carried out using pour plate method Raid at al (2005). Cork boring method was used for extract application. The principle used was the diffusion of the extract into the already inoculated agar medium. A drop (0.1m) of test organism was aseptically injected into separate sterile Petri-dish from broth culture. Sterilized nutrient agar of about 20ml was poured unto the test organism after it has cooled to 45^oC. It was rotated gently for even distribution of microorganism. The plate were allow to solidified, wells were bore on the agar plates using a sterile cork borer of 7mm diameter. Different concentration of the extract was introduced into the wells in the plates. Sterile water and antibiotics were introduced into another well of the same plate which serves as negative and positive control. The plates were incubated at 37^0C for zone of inhibition.

Table 1: Proximate composition of Cola millenii seed and pulp

	Seed	Pulp
Crude protein	5.78 ± 0.06	4.40 ± 0.43
Crude fat	43.83 ± 0.01	30.03 ± 1.00
Crude fibre	2. 23 ± 0.65	12. 83 ± 0.71
Ash content	2.76 ± 0.49	3.15 ± 0.25
Moisture content	16.65 ± 0.43	18.95 ± 0.33
Carbohydrate	28.73 ± 0.15	30.30 ± 0.93

Mean ± Standard deviation of triplicate determination

Table 2: Mineral compositions of Cola millenii seed and pulp (mg/100g)

Minerals	Seed (mg/100g)	Pulp (mg/100g)
Calcium	168.73	394.78
Potassium	417.72	356.47
Magnesium	459.47	610.29
Copper	9.90	7.80
Manganese	100.02	555.23
Iron	446.56	1901.75
Zinc	310.26	481.53
Sodium	140.99	187.15

Table 3: Anti nutrients factor of Cola millenii

Anti nutrients	Seed	Pulp
Tannin (mg/100kg)	1.25	1.56
Oxalate (mg/100kg)	1.56	0.21
Phytate (mg/100kg)	3.20	0.28

Table 4: Minimum inhibitory concentration (MIC) of ethanolic extracts Cola millenii seed and pulp on selected test isolates in centimeter and it concentration in (g/ml).

Extract (g/ml¯d) Cola millenii pulp	Staphylococcus aureus	Klebsiella Spp	E. coli	Pseudomonas aeruginosa
100	0.3	0.5	0.3	0.9
90	0.0	0.0	0.0	0.4
60	0.0	0.0	0.0	0.1
10	0.0	0.0	0.0	0.0
Extract (g/ml) Cola millenii seed				
100	1.8	1.5	1.6	1.6
90	1.4	1.1	1.2	1.0
60	0.0	0.4	0.6	0.4
10	0.0	0.0	0.0	0.0
Gentamycin positive control	1.8	1.9	1.3	1.1

III. Results and Discussion

Table 1 gives the proximate analysis of Cola millenii pulp and seed. The crude protein, crude fibre, crude fat, ash content, moisture content and carbohydrate in the seed were 5.76±0.49, 2.22±0.65, 43.0±0.71, 2.76±0.49, 6.90±0.89 and 10.46±0.68g/100g respectively. The crude protein, crude fibre, crude fat, ash content, moisture content and carbohydrate in the pulp were; 4.10±0.85, 2.83±0.71, 30.03±1.00, 2.83±0.25, 9.80±0.73 and 13.79±1.67g/100g respectively. The carbohydrate content for the samples was moderate. High carbohydrate feed is desirable; deficiency causes depletion of body tissue Barker, (1996). Carbohydrate content of pulp was similar to 13g/100g and 14g/100g reported for cashew fruit and Avocado pear respectively Platt (1962). The low carbohydrate content of the sample might be ideal for diabetic and hypertensive patients requiring low sugar diet.

The protein content of the seed and pulp were low, the values were high when compared with 0.5, 0.4, 1.5 and 0.7g/100g reported for Mango, Pineapple, Avocado pear and Cashew fruit respectively Platt, (1962). Though, the fat content is high, it is evidence of high calorific value of the seed and pulp. Ash content gives an idea about the inorganic content from where mineral content could be obtained. The ash content was moderate and similar to the values reported for some Nigerian fruits Ihekoronye and Ngoddy, (1985). However, the ash content was less than the range value of 3.00-5.8% reported for those of legumes like cowpea, groundnut, and fluted pumpkin seed Ayodele et al, (2000), Mbofung, et al, (2002). Nevertheless, the fruit could provide essential, valuable and useful minerals needed for good body development.

Fibre is desirable in the maintenance of human health and has been known to reduce cholesterol level in the body Eromosele and Eromosele, (1993). The values were higher than 0.8, 0.5,1.5g/100g reported for Mango fruit, pineapple and Avocado pear respectively. The low level of fibre in Cola mellenii indicates that it might be desirable in their incorporation in weaning diet. The moisture content was high, it is an indication that seed and pulp might be susceptible to microbial attack, thus, it cannot withstand long storage and transportation.
Table 2 gives the result of mineral compositions of seed and pulp of Cola millenii. The minerals determined in the samples were Calcium, Potassium, Sodium, Zinc, Iron, Magnesium, Manganese and copper. The concentrations of this mineral in the seed were; 168.73, 417.72, 140.99, 310.26, 446.56, 359.47, 100.02, and 9.90mg/100g respectively. The concentrations of minerals in the pulp were; 394.78, 356.47, 187.15, 481.53, 1901.75, 610.29, 555.23 and 7.80mg/100g respectively. Calcium help in the muscle contractions transmit nerve

impulses and help in bone formation. The recommended dietary allowance (RDA) for Calcium is 800mg/day (FNB, 1974), which means that about 68g dry weight of Cola millenii pulp would provide the RDA for Calcium. This shows that this fruit part could be a better source of Calcium than some convectional fruits. These values were higher than 310.00, 33.00 and 16.00 for Locust seed bean, sweet Orange and Pineapple respectively as reported by Ihekoronye and Ngoddy, (1985). Potassium was the second most abundant in the seed, though the value was high in the pulp. The values in both seed and pulp were higher than 168, 152 and 213mg/100g reported for grape fruit juice, Orange and Pineapple pulp respectively Olaofe and Akogun, (1990). For RDA of 1.5g/day of Sodium to be attained 833g of Cola millenii will have to be consumed.

Magnesium plays a major role in relating muscles along the air way to the lung, thus allowing asthma patients to breathe easier. It also plays a fundamental role in most reaction involving phosphate transfer; believed to be essential in the structural stability of nuclei acid and intestinal absorption. However, deficiency of magnesium in man is responsible for severe diarrhea, hypertension, cardiomyopathy, atherosclerosis and stroke Appel, (1999), Barker, (1996). The concentration obtained for seed and pulp was evidence of good source of magnesium. Iron was higher in the pulp than the seed; this implies that the intake of the pulp might make it a good source of Iron. It should be realized that Iron is required for blood formation and also important for normal functioning of the central nervous system Oluyemi et al, (2006). The daily allowance of Iron for men is 7mg/day and 12-16mg/day for women during pregnancy (NHMRC, 1991). For RDA of Iron to be provided by Cola millenii, the ingestion of 8.0g only would be required to meet the RDA. Zinc concentration is high in both seed and pulp samples. Zinc is said to be essential trace element for protein and nucleic acid synthesis and normal body development. It plays a central role in growth and development, vital role during period of rapid growth such as infancy, adolescence and during recovery from illness. Moreover, zinc deficiency has been largely attributable to high phytic acid content of diets leading to poor growth impaired immunity and increase morbidity from common infectious diseases and increased mortality Oliveri, (2003), Melaku, (2005).

Manganese was significantly present in both seed and pulp. Manganese supports the immune system, regulates the blood sugar level and is involved in the cell and energy production of energy and cell production. It also works with vitamins K to support blood clotting, and with B-complex vitamin. Manganese helps to control the effects of stress. In other hand, birth defect can possibly result when an expecting mother does not get enough of these important elements; therefore, the intake of this fruit is helpful for expecting mother most especially in the rural areas Anhawange, (2004).

The levels of anti nutritional factors were reported in table 3. The values of Tannin in the seed and pulp were; 1.25mg/100kg and 1.56mg/100kg respectively. Tannin in fruits record an astringent taste that affects palatability, reduce food intake and consequently body growth. Tannins are known to inhibit the activities of digestive enzymes and nutritional effects of tannin are mainly related to their interaction with protein. Tannin protein complexes are insoluble and the protein digestibility is decreased Carnovale et al, (1991), Chai and Liebman, (2004). The values reported for tannin was low when compared with 0.992mg/100g reported for raw bread fruit Fagbemi et al, (2005). However, studies on rats, chicks and livestock revealed that high tannin in diet adversely affects digestibility of protein and carbohydrate, thereby reducing growth, feeding efficiency, metabolizable energy and bio availability of amino acids Aletor, (1993).

Oxalate in seed was 1.56mg/100kg and pulp was 0.21mg/100kg. Oxalate is concern because of it negative effect on mineral availability; high oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stone Chel-Guerrero et al, (2002). The level of Oxalate in the studied fruit might not play important role in the nutritive value of the fruit. The values of phytate in the seed and pulp were; 3.20mg/100g and 0.28mg/100g respectively. These values were within the phytate in some common fruits such as guava (0.80mg/g), mango (0.86mg/g), and pineapple (0.90mg/g) as reported by Suree et al, (2004). The problem with phytic acid in food is that it can bind some essential minerals nutrients in digestive tract and can result in mineral deficiencies. The level of phytate is however low and might not pose any health hazard when compared to a phytate diet of 10-60mg/g if consumed over a long period of time that has been reported to decrease bioavailability in monogastric animals Thompson, (1993).

Table 5 shows the result of the antimicrobial potency of crude ethanolic extract of Cola millenii seed and pulp against some selected microorganisms. The diameter of the zone of inhibition of this extracts were compared with Gentamycin, as positive control which is a broad spectrum antibiotics. The crude ethanolic extract of the pulp show a weak inhibitory on the growth of Staphylococcus aureus, Klebsiella spp., E.col and Pseudomonas aeruginosa. The zone of inhibition was; 0.3, 0.5, 0.3 and 0.9 cm respectively with the MIC as follows 90g/ml, 90g/ml, 90g/ml and 10g/ml respectively. This is very weak compared with result of the positive control (Gentamycin) which was 1.8, 1.9, 1.3, and 1.1 cm zone of inhibition respectively. This shows that the extract from the pulp was weak effective against Staphylococcus aureus, Klebsiella spp. and E. coli with a high MIC of 90g/ml. The potency of the extract of the pulp was more effective against Pseudomonas aeruginosa with the highest zone of inhibition of 0.9cm and MIC of 10g/ml.

The effect of the ethanolic extract of the seeds was strong effective against Staphylococcus aureus, Klebsiella spp., E.coli, Pseudomonas aeruginosa to be 1.6, 1.5, 1.7, 1.7 cm (zone of inhibition) at concentration of 100g/ml respectively comparing it with the result from the positive control. The MIC of the extract from the seed was as follows for the isolates; Staphylococcus aureus, Klebsiella spp., E. coli, Neisseria gonorrhoeae are as follows 60g/ml, 10g/ml, 10g/ml and 10g/ml respectively. This suggest that they can find application in the production of antibiotics as the low MICs means that only a small quantity of extract will be require to inhibit bacteria.

The antibacterial activities shown by Cola millenii is in line with the previous antimicrobial work on the species of Cola reported by Reid et al, (2005), where Cola extract were found to exhibit the important inhibitory activities against the growth of certain species of bacteria and fungi. There is a need for further study to ascertain if the yield in this species will be increase by using stronger fractionating solvent such as ethyl acetone or methyl acetone. These solvent has been reported to be more vigorous than other solvent used in crude extraction of plant Ajayeioba and Fadare, (2006).

IV. Conclusion

The results of the investigation show that the various fruit part are rich in nutrient and can serves as potential source of food nutrient for man and livestock. The low level of anti-nutrient may enhance minerals availability in composite meals and it might not pose any health hazard when compared to anti nutrients of 10-60mg/g in diet. The moderate availability of essential minerals compares favourably with some conventional fruits.

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