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Antimicrobial Activities and Chemical Compositions Of Chrysophyllum Cainito (Star Apple) Fruit

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The pulp and seed of Star apple, *Chrysophyllum cainito*, was analyzed for its nutrients, anti-nutrients, antimicrobial potentials and microbial profile, using standard methods. The microbial count of the fruits ranged from $1.0x10^9$ to $2.4x10^{10}$ for the total aerobic plate count, $1.0x10^7$ to $2.0x10^7$ for fungal count and $1.0x10^8$ to $1.2x10^9$ for coliform count. The identities of the normal flora on the surfaces of healthy fruits and of spoilage organisms were confirmed to include species of *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Micrococcus*, *Acinetobacter*, *Enterococcus*, and *Pseudomonas* (which are all pathogenic microorganisms). The fungal isolates include species of *Rhizopus*, *Aspergillus*, *Penicillum* and *Saccharomyces*. The high bacterial and fungal counts and their presence, portend a serious health implication. *Aspergillus* and *Penicillium* species produce mycotoxins involved in mycotoxicosis of humans and animals. *Staphylococcus* and *Bacillus* species produce potent toxins implicated in food borne illnesses, while the presence of *Enterococcus* indicates faecal contamination. Vitamins such as vitamin A (0.027mg to 0.089mg) and vitamin C (10.0mg to 43.54mg) were also present. Minerals such as calcium, magnesium, phosphorus, potassium and sodium were present at concentrations of 37.0mg, 5.0mg, 8.0mg, 38.0mg and 21.0mg respectively, for the pulp extract's. *Chrysophyllum cainito* is a good source of minerals, which are needed for electrolyte balance, neurotransmission, development of strong teeth and bones. The proximate composition of star apple consists of protein (1.96 g to 4.63g), moisture (56.04g to 75.90g), fat (0.88g to 15.81g), fibre (2.31g to 4.19g), ash (0.56g to 0.84g) and carbohydrate (18.39g to 78.49g). Varying concentrations of phytochemicals such as spinning, flavonoids, tannin, steroid and cardiac glycoside were detected. The seed and pulp showed varying levels of antibacterial and antifungal activities against some clinical isolates such as *Escherichia coli* and species of *Salmonella*, *Staphylococcus*, *Pseudomonas*, *Aspergillus*, *Candida* and *Penicillium*. *Chrysophyllum cainito* holds great potentials as an antimicrobial agent for chemotherapeutic medicine and it is a rich source of nutrient and phytochemicals. Regular consumption of this fruit should be encouraged for its potential nutrition and health benefits.

Keywords: *Chrysophyllum cainito,* anti-nutritive composition, antimicrobial activity.

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INTRODUCTION

Fruits have been traditionally and nutritionally important food to man for many years (Adepoju and Adeniji, 2012). Fruits are part of plants that produce the seed that are edible and also provide nutrients to man (Adepoju and Adeniji, 2012). The chemical compositions and the antimicrobial sensitivity of some fruits such as *Chrysophyllum albidum* (udara) and *Persea americana* (Avocado pear) have been determined (Arukwe *et al.*, 2012).These fruits have been known to be medicinal and have been applied in curative medicine.

Chrysophyllum cainito also known as star apple is a fleshy fruit with soft endocarp. It is a tropical tree of the family Sapotaceae, and native to the Greater Antilles and the West Indies. It is also sparsely distributed in Nigeria; grows rapidly and reaches 20 metres in height (Luo *et al*., 2002). It has numerous common names including cainito, caimito, star apple, golden leaf tree and also milk fruit (National Research Council, 2008). The tree is hermaphrotic in nature with round; purple-skinned fruit that is often green around the calyx with a star pattern in the pulp. Sometimes there is a greenish – white or yellow variety of the fruit. The skin is rich in latex; though the skin and the rind are not edible. The seed is flat, hard and light brown in colour (Einbond et *al*., 2004). The fruits are delicious as a fresh dessert fruit; it is sweet and best served chilled. The fruit has antioxidant properties (Luo *et al*., 2002; Einbond *et al*., 2004). Infusions of the leaves have been used against diabetes and articular rheumatism (Luo *et al*., 2002). The bark is considered a tonic and stimulant, and a bark decoction is used as an c (Luo *et al*., 2002).

The Ayurvedic system of medicine has described various fruits in the treatment of diseases, which play an important role in modern health care and curing various ailments and diseases. There are several reports on the chemical composition and antimicrobial activities of some fruits and their extracts that inhibits various bacteria. However, studies on the chemical compositions and antimicrobial activities of *Chrysophyllum cainito* are limited. Therefore, scientific evaluation of this fruit (cainito) is important to check its chemical composition as well as its antimicrobial activity in order to support its use as food and alternative medicine in the treatment of some infections, especially enteric diseases.

This study reports on the nutritional and phytochemical properties of the fruit. The antimicrobial potential of the fruit was also determined.

MATERIALS AND METHOD

Sample collection

One hundred fresh and well ripened star apples were harvested from the tree located within the premises of Federal University of Technology, Owerri (F.U.T.O), Imo state, Nigeria between the months of February and March, 2013.

Microbiological analysis of the external surface of the healthy fruit and pulp

The fruit was aseptically washed with 100 ml normal saline into a sterile beaker and serial dilution was made by transferring 1ml portion until 10^{-7} dilution was obtained. Aliquot portion (0.1ml) was inoculated onto bacteriological and mycological media. The inocula were spread evenly to ensure countable colonies, and incubated at 37^0C for 24 hours (Cheesbrough, 2000).

Twenty grams of the pulp were weighed and aseptically introduced into a sterile stomacher blender containing 180ml of normal saline and agitated to mix thoroughly. 1ml was aseptically transferred into 9ml normal saline and further diluted decimally. An aliquot portion (0.1ml) was inoculated into the media, spread evenly and incubated at the appropriate conditions (Cheesbrough, 2000; Sharma, 2003).

Microbiological analysis of deteriorated pulp and outer skin surface of the fruit

This was done using the method applied for healthy fruit, according to Cheesbrough (2000) and Sharma (2003).

Enumeration and identification of microorganisms

Total count of bacterial and fungal isolates were done using Gallemkamp digital colony counter and hand lens respectively (Harrigan and McCance, 1990; Sharma, 2003), and expressed as colony forming units per grams/milliliters (Cfu/g/ml). The identity of the bacterial isolates was done on the basis of their colonial, microscopic and biochemical characteristics (Beishir, 1987; Cheesbrough, 2000 and Pelczar *et al*., 1993; Sharma, 2003). The identity of the fungal isolates was done based on their mycelial arrangement, sporulation, pigmentation on reverse and surface of the media (Domsch *et al*., 1993). The identities of bacteria and fungi isolated were confirmed with reference to standard identification atlas and keys (Buchannan and Gibbon, 2000; Barnnet and Hunters, 1987).

Nutritional evaluation of the pulp

Moisture analysis was determined by gravimetric method (AOAC, 2000). Crude protein was determined by Kjedahl method (Chang, 2003) in which the nitrogen content was determined and multiplied by 6.25 to obtain the protein content. Ash content was determined by furnace incineration gravimetric method (James, 1995). Soxhlet solvent extraction method by James, 1995, was employed in the determination of fat content. Crude fibre was determined by the Weende gravimetric (AOAC, 2000).

Determination of anti-nutritional components

Chemical tests were carried out on the pulp and seed extracts using standard procedures to identify the constituents as described by Denwick (2002), Buhler (2000), George *et al*. (2002) and Heslem (1989). Active constituents analyzed include alkaloid, anthraquinone, steroid, flavonoid, cardiac glycoside, tannin and saponin.

Determination of mineral contents of the pulp

Potassium and sodium were determined using Flame Photometer method (Bonire *et al*., 1990). Phosphorus was determined by Vanado-molybdate colorimetric method (Ologhobo and Fetuga, 1983). Calcium and magnesium were determined by complexiometric method using EDTA (Bonire *et al*., 1990).

Analysis of vitamins

Vitamin C was determined by visual titration method (Ness *et al*., 1996). Vitamin A was determined using B-carotene estimation method (Kirk and Sawyer, 1998).

Antimicrobial assay of the pulp and seed of *Chrysophyllum cainito*

Pure cultures of multiple drug resistant *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans, Aspergillus* and *Penicillium* were obtained from the microbiology unit in the Federal Medical Center, Owerri, Imo State, Nigeria. The identities of the cultures were confirmed using routine laboratory methods (Sharma, 2003), maintained on agar slant at 4^0C prior to use.

The antimicrobial activity was performed by agar well diffusion methods (Dalitha, 2008). The antimicrobial diffusion test was carried out using a cell suspension obtained from McFarland turbidity standard number 0.5. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone around the extract.

Minimum inhibitory concentration (MIC) test

Minimum inhibitory concentrations of the extracts (seed and pulp) were determined by two-fold serial dilution method (Chandrasekaram and Venkatesalu, 2004). The dose levels of 250mg/l, 125mg/l, 62.5mg/l, 31.25mg/l and 15.625mg/ml concentrations each of the extracts were used for MIC determination. After a suitable incubation, the least concentration of the sample extracts with no visible growth was taken as the MIC. This was further confirmed spectrophotometrically at an optical density (OD) of 520nm.

Minimum bactericidal concentration (MBC)

A sample was taken from the tubes with no visible growth in the MIC test and plated out according to the method of Banso and Adeyemo (2007) and incubated at 37^0C for 24 hours. After the incubation, the plates were observed for growth. The lowest concentration of the extracts without any growth was noted as the minimum bactericidal concentration (MBC) in mg/ml.

RESULTS

The mean total microbial count is shown in Table 1. The Total aerobic plate count ranges from 1.0×10^9 to 2.4×10^{10}, the coliform count ranged from 1.0×10^8 to 1.2×10^9 with the fungal count ranging from 1.0×10^7 to $2.0 \times 10^{7.}$

The microorganisms (fungi and bacteria) isolated from both healthy and the deteriorated fruits are shown in Table 2. Species of *Bacillus*, *Staphylococcus*, *Pseudomonas, Enterococcus*, *Micrococcus*, *Corynebacterium*, *Acinetobacter*, *Penicillium*, *Fusarium*, *Saccharomyces* and *Rhizopus* were isolated.

TABLES 1: MEAN TOTAL MICROBIAL COUNT (Cfu/g)

Sample	Total aerobic plate count	Coliform count	Fungal count
A1	1.1×10^{10}	1.0×10^{8}	1.1×10^{8}
B1	1.0×10^{9}	1.0×10^{8}	1.0×10^{8}
C1	1.2×10^{9}	1.0×10^{8}	1.0×10^{7}
D1	1.2×10^{10}	1.0×10^{8}	1.0×10^{7}
E1	1.3×10^{10}	1.1×10^{10}	1.2×10^{8}
A2	1.0×10^{9}	1.0×10^{8}	1.3×10^{8}
B2	1.1×10^{9}	1.0×10^{8}	1.3×10^{8}
C2	2.0×10^{9}	1.0×10^{8}	1.0×10^{7}
D2	1.0×10^{9}	1.0×10^{8}	1.0×10^{7}
E2	$2.4x\ 10^{10}$	1.2×10^{9}	2.0×10^{7}

TABLE 2: MICROBIAL ISOLATES FROM SAMPLES

SAMPLE	MICROBIAL ISOLATES
HEALTHY FRUIT	*Bacillus* spp., *Staphylococcus aureus*, *Micrococcus* spp., *Corynebacterium* spp., *Acinetobacter* spp., *Rhizopus* spp., *Aspergillus* spp., *Penicillum* spp. and *Saccharomyces* spp. and *Enterococcus* spp.
DETERIORATED FRUIT	*Bacillus* spp., *Staphylococcus aureus*, *Micrococcus* spp., *Corynebacterium* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Enterococcus* spp., *Rhizopus* spp., *Fusarium* spp., *Penicillum* spp. and *Saccharomyces* spp.

Qualitative phytochemical analysis shows the presence of saponins, tannins, flavonoids, steroids, cardiac glycosides and anthraquinones (Table 3). Table 4 shows the result of the quantitative values of moisture, carbohydrate, fat, protein, fibre and ash, as well as the total energy.

TABLE 3: PHYTOCHEMICAL COMPOSITION OF SEED AND PULP EXTRACTS

Sample	Saponin	Tannin	Alkaloid	Flavonoid	Steroid	Cardiac glycoside	Anthraquinone
PULP	**+++**	**+++**	**-**	**+++**	**+++**	**+++**	**-**
SEED	**-**	**+**	**-**	**+++**	**+**	**-**	**-**

KEY: +++ (HIGHLY CONCENTRATED)

+ (TRACE)

- (ABSENT)

TABLE 4: TOTAL ENERGY AND PROXIMATE COMPOSITION (%) OF THE SEED AND PULP EXTRACT

Sample	**Total energy Kcal/100g**	**Moisture**	**Fat**	**Fibre**	**Protein**	**Ash**	**Carbohydrate**
Seed	474.77±1.09	56.04±1.64	15.81 ±0.53	4.19 ±0.46	4.63± 0.12	0.84±0.25	78.49±0.40
Pulp	89.32±0.15	75.90±4.31	0.88±0.31	2.31±0.21	1.96±0.23	0.56±0.10	18.39±0.33

Table 5 showed the vitamin and mineral compositions of the fruit extract. Vitamins analyzed include; vitamin A and C with the pulp extract containing a high amount of vitamin C than the seed extract. The minerals analyzed included calcium, magnesium, phosphorous, potassium and sodium.

TABLE 5: MINERAL AND VITAMIN COMPOSITION OF THE SEED AND PULP OF *CHRYSOPHYLLUM CAINITO*

Parameter	Pulp	Seed
Vit.C (mg/100g)	43.54±0.57	10.0±0.22
Vit.A (mg/100g)	0.089±0.32	0.027±0.44
Calcium (mg/100g)	37.0±0.25	168.0±0.28
Magnesium (mg/100g)	5.0± 0.24	90.0±0.25
Phosphorous (mg/100g)	8.0± 0.25	18.0±0.31
Potassium (mg/100g)	38.0±0.29	78.0±0.32
Sodium (mg/100g)	21.0±0.32	39.0±0.25

Tables 6 and 7 shows the antimicrobial activity results of the pulp and seed extracts, respectively tested against fifty isolates each. Gentamicin and Ketoconazole were used as control drugs.

Table 6: ANTIMICROBIAL ACTIVITY OF PULP EXTRACTS SHOWING THE MEAN TOTAL OF 50 ISOLATES WITH THEIR ZONES OF INHIBITION

PATHOGENS	**PULP EXTRACT** Zone of inhibition (mm)	**ANTIBIOTIC** Zone of inhibition (mm)	**STANDARD FOR ZONE OF INHIBITION FOR COMMERCIAL ANTIBIOTICS** (mm)
		Gentamicin	**Gentamicin**
Salmonella spp.	1	19	19-25
Staphylococcus spp.	6	18	17-28
Escherichia coli	5	19	18-26
Pseudomonas spp.	10	17	16-21
		Ketoconazole	**Ketoconazole**
Aspergillus spp.	2	16	14-16
Penicillium spp.	1	19	19-21
Candida spp.	3	20	20-22

Source for the Standards: Erhabor *et al*, 2013.

Table 7: ANTIMICROBIAL ACTIVITY OF SEED EXTRACTS SHOWING THE MEAN TOTAL OF 50 ISOLATES WITH THEIR ZONES OF INHIBITION

PATHOGENS	**SEED EXTRACT** Zone of inhibition (mm)	**ANTIBIOTIC** Zone of inhibition (mm)	**STANDARD ZONE OF INHIBITION FOR COMMERCIAL ANTIBIOTICS** (mm)
		Gentamicin	**Gentamicin**
Salmonella spp.	1	19	19-25
Staphylococcus spp.	8	18	17-28
Escherichia coli	10	19	18-26
Pseudomonas spp.	2	17	16-21
		Ketoconazole	**Ketoconazole**
Candida spp.	1	16	14-16
Aspergillus spp.	2	19	19-21
Penicillium spp.	0	20	20-22

Source for the Standards: Erhabor et al., 2013.

The zones of inhibitions (ZOI) of the different concentrations of the pulp extract of *Chrysophyllum cainito* measured in millimeter (mm) are represented in Table 8. Antimicrobial activity is high against *Staphylococcus*, *Pseudomonas* and *Salmonella* at concentrations 250mg/ml, 250mg/ml and 31.25mg/ml respectively.

Table 8: ZONES OF INHIBITION OF VARIOUS CONCENTRATIONSOF THE PULP EXTRACT OF *CHRYSOPHYLLUMAINITO*

Pathogens	Conc. of pulp extract (mg/ml)	Z0I (mm)
Staphylococcus spp.	250	6
Staphylococcus spp.	125	4
Staphylococcus spp.	62.5	1
Staphylococcus spp.	31.25	1
Staphylococcus spp.	15.62	1
Salmonella spp.	250	1
Salmonella spp.	125	1
Salmonella spp.	62.5	3
Salmonella spp.	31.25	9
Salmonella spp.	15.62	1
E.coli	250	5
E.coli	125	1
E.coli	62.5	1
E.coli	31.25	1
E.coli	15.62	5
Pseudomonas spp.	250	10
Pseudomonas spp.	125	0
Pseudomonas spp.	62.5	1
Pseudomonas spp.	31.25	1
Pseudomonas spp.	15.62	1
Candida spp.	250	3
Candida spp.	125	1
Candida spp.	62.5	1
Candida spp.	31.25	1
Candida spp.	15.62	1
Aspergillus spp.	250	2
Aspergillus spp.	125	1
Aspergillus spp.	62.5	1
Aspergillus spp.	31.25	1
Aspergillus spp.	15.62	4
Penicillium spp.	250	1
Penicillium spp.	125	1
Penicillium spp.	62.5	1
Penicillium spp.	31.25	1
Penicillium spp.	15.62	2

KEY: Z0I = Zone of Inhibition
mm = millimeter

The zone of inhibition measured in millimeter (mm), of the seed extract of *C. cainito* is shown in Table 9. Antimicrobial activity is prominent at the higher concentrations (250mg/ml and 125mg/ml). The seed and pulp extracts exhibited higher antimicrobial activity against *E.coli* and S*taphylococcus aureus* than other test organisms including the fungi.

Table 9: ZONES OF INHIBITION OF VARIOUS CONCENTRATIONS OF SEED EXTRACT OF *CHRYSOPHYLLUMCAINITO*

Pathogens	Conc. of pulp extract (mg/ml)	Z0I (mm)
Staphylococcus spp.	250	8
Staphylococcus spp.	125	6
Staphylococcus spp.	62.5	4
Staphylococcus spp.	31.25	2
Staphylococcus spp.	15.62	0
Salmonella spp.	250	1
Salmonella spp.	125	10
Salmonella spp.	62.5	1
Salmonella spp.	31.25	1
Salmonella spp.	15.65	0
E.coli	250	5
E.coli	125	6
E.coli	62.5	3
E.coli	31.25	2
E.coli	15.65	2
Pseudomonas spp.	250	2
Pseudomonas spp.	125	2
Pseudomonas spp.	62.5	1
Pseudomonas spp.	31.25	1
Pseudomonas spp.	15.65	1
Candida spp.	250	3
Candida spp.	125	1
Candida spp.	62.5	1
Candida spp.	31.25	1
Candida spp.	15.65	1
Aspergillus spp.	250	1
Aspergillus spp.	125	1
Aspergillus spp.	62.5	0
Aspergillus spp.	31.25	2
Aspergillus spp.	15.65	4
Penicillium spp.	250	0
Penicillium spp.	125	1
Penicillium spp.	62.5	3
Penicillium spp.	31.25	1
Penicillium spp.	15.65	1

KEY: Z0I = Zone of Inhibition
mm = millimeter

The minimum inhibitory concentration of various concentrations of pulp and seed extract taken at optical density (OD) at 520nm is shown in Tables 10 and 11 respectively. Optical density (OD) was measured with an ultra violet Spectrophotometer.

TABLE 10: MINIMUM INHIBITORY CONCENTRATION (MIC) OF VARIOUS CONCENTRATIONS OF SEED EXTRACT, OD MEASURED BY U.V. SPECTROPHOTOMETER (OD: 520nm)

Conc. of Seed Extract **spp** (Mg/l)	Seed extracts against ***E.coli***	Seed extracts against ***Salmonella* spp**	Seed extracts against ***Staph.*spp**	Seed extracts against ***Ps .*spp**	Seed extracts against ***Candida* spp**	Seed extracts against ***Aspergillus* spp**	Seed extracts against ***Penicillium***
250	3.000±0.36	3.000±0.36	3.000±0.36	3.000±0.36	3.000±0.39	2.052±0.01	2.210±0.02
125	2.305 ±0.13	2.523±0.18	2.561±0.19	2.612±0.002	2.646±0.03	1.374±0.01	1.480±0.02
62.5	1.863±0.12	2.015±0.14	1.848±0.07	1.778±0.004	1.444±0.03	0.739±0.01	0.845±0.02
31.25	**1.509±0.07**	**1.576±0.03**	1.680±0.06	**1.602±0.003**	1.618±0.03	**0.144±0.004**	0.657±0.03
15.62	1.584±0.03	1.658±0.02	**1.664± 0.16**	1.611±0.002	**1.364±0.01**	0.150±0.004	**0.614±0.01**

TABLE 11: MINIMUM INHIBITORY CONCENTRATION (MIC) OF VARIOUS CONCENTRATIONS OF PULP EXTRACT, OD. MEASURED BY U.V. SPECTROPHOTOMETER (OD: 520nm)

Conc. of Seed Extract **spp** (Mg/l)	Seed extracts against ***E.coli***	Seed extracts against ***Staph.* spp**	Seed extracts against ***Salm.* spp**	Seed extracts against ***Aspergillus* spp**	Seed extracts against ***Ps .***spp	Seed extracts against ***Candida* spp**	Seed extracts against ***Penicillium* spp**
250	**0.359±0.03**	1.890±0.07	1.821±0.05	1.568±0.01	1.839±0.003	1.575±0.02	1.911±0.01
125	0.511±0.08	1.181±0.02	0.766±0.13	0.818±0.01	0.946±0.003	0.621±0.04	1.005±0.01
62.5	0.416±0.05	0.537±0.06	0.780±0.09	0.336±0.004	0.611±0.005	0.351±0.02	**0.652±0.01**
31.25	1.652±0.02	**0.356±0.03**	0.665±0.04	**0.092±0.01**	0.514±0.003	0.096±0.02	0.881±0.02
15.62	0.648±0.02	0.517±0.06	**0.627±0.01**	0.142±0.004	**0.449±0.004**	**0.090±0.02**	0.669±0.01

The Minimum Bactericidal Concentration (MBC) of the various MIC concentrations of both the seed and pulp extract showed scanty growths and in some cases, no growth at all. These are shown in Table 12.

TABLE 12: MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF SEEDAND PULP EXTRACTS OF *CHRYSOPHYLLUM CAINITO*

Organisms	AT ALL CONCENTRATIONS (mg/ml)	
	SEED	**PULP**
Escherichia coli	Scanty growth	Very Scanty growth
Staphylococcus spp.	Scanty growth	No Growth
Salmonella spp.	Scanty growth	No Growth
Candida spp.	No Growth	No Growth
Pseudomonas spp.	Scanty growth	Very Scanty growth
Aspergillus spp.	No Growth	Scanty growth
Penicillium spp.	Very Scanty	No Growth

Table 13 shows the nutritional content and mineral compositions of the pulp and seed extracts of the fruit (*C.cainito*) as compared with standard values of the Reference Nutrient Intake (RNI) from international agency. The gross energy of the seed is higher than that of the pulp, whereas the pulp shows higher vitamin C (ascorbic acid) than the seed.

TABLE 13: NUTRIENT COMPOSITION OF *C. CAINITO* (SEED AND PULP) COMPARED WITH THE REFERENCE NUTRIENT INTAKE (RNI)

NUTRIENT	NUTRIENT CONTENT OF PULP (100g)	NUTRIENT CONTENT OF SEED EXTRACT (100g)	**RNI**
Gross Energy (kcal)	89.32	474.77	**2300**
Protein (g)	1.96	4.63	**50**
Fibre (g)	2.31	4.19	**20**
Potassium (mg)	38.0	78.0	**3500**
Calcium (mg)	37.0	168.0	**400**
Magnesium (mg)	5.0	90.0	**270**
Phosphorous (mg)	8.0	18.0	**550**
Vitamin C (mg)	43.54	10.0	**60**
Vitamin A (µg)	89.0	27.0	**625**
Sodium (mg)	21.0	39.0	**1600**

RNI Source: (Www. nap.edu)

DISCUSSION

The mean total microbial count of the samples was analyzed and the result shown in Table 1. Most of the bacteria and fungi isolated from the sample are widely distributed in nature, particularly in the soil and may contaminate the samples during harvest and handling. The microbial isolates found on the surfaces of the healthy and deteriorated fruit is shown in Table 2. These isolates which includes species of *Bacillus*, *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Acinetobacter*, *Enterococcus*, *Pseudomonas*, *Rhizopus*, *Aspergillus*, *Penicillum*, *Fusarium* and *Saccharomyces* are pathogenic microorganisms whose natural habitats aqueous solution, haemolytic activity, cholesterol - binding properties, etc. (Sodipo and Akiniyi, 2000). Saponins also confer antimicrobial effects. Possible antimicrobial mechanism of saponins is as a result of reduced glucose utilization efficiency in microorganism, thus affecting their growth and proliferation; reducing the activity of key enzymes in physiological metabolism and suppressing the synthesis of relevant proteins and finally executing the antimicrobial effect (Yu *et al*., 2013). Tannins noted for astringency and bitter taste, hasten the healing of wounds and inflamed mucus membrane (Duke, 1992). The result in Table 3 showed that the pulp extract contains a high concentration of tannin, while the seed contains just a trace amount of tannin. Tannic acid was found to be inhibitory to the growth of intestinal bacteria such as *Bacteroides fragilis*, *Clostridium perfringens*, *Escherichia coli* and *Enterobacter cloacae* amongst others. Tannic acid may also work like a siderophore to chelate iron from the medium and make iron unavailable to microorganisms, especially those growing under aerobic conditions that need iron for variety of functions like reduction of ribonucleotide precursor of DNA, formation of haem and other essential purposes (Chung *et al*., 1998). The different mechanisms proposed so far to explain tannin antimicrobial activity includes inhibition of extracellular microbial enzymes, toxic action on the membrane of the microorganisms, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation. A further mechanism involving iron deprivation had been proposed (Chung *et al*., 1998). Tannin is noted to have no antifungal activity due to their (fungi) morphological structure. Fungi have thicker cell walls and contain a higher percentage of chitin (Madigan and Martinko, 2006). Flavonoids are potent water- soluble super antioxidants and free radical scavengers. They prevent oxidative cell damage, have strong anti – cancer activity and protect against all includes the human intestinal tracts, respiratory tracts and or droplets, skin, soil and water. Ingestion of these pathogens, followed by their growth and tissue invasion and or release of toxins may cause food borne infection (Hoge *et al*., 1991). Therefore, it is important that fruits be properly handled and washed before consumption.

Phytochemicals are important chemicals found virtually in plants at different concentrations. (Duke, 1992). Table 3 shows the result of phytochemicals analyzed. It shows that the pulp extract contained a high concentration of saponin while it is absent in the seed extract. General characteristics of saponins include formation of foams in

stages of carcinogenesis (Salah *et al.,* 1995). Also, flavonoids in intestinal tract lower the risk of heart disease, inflammation and represent the most common and widely distributed groups of plant phenolic compounds. Both the pulp and seed extracts contains high levels of flavonoid and this could be responsible for the anti-inflammatory, anti-cancer and anti-hypertensive properties of the plant and its parts. Catechins (flavonoids in fruits) have been shown to inactivate cholera toxin in *Vibrio cholerae* and inhibit isolated bacterial glucosyltransferases in *Streptococcus mutans,* probably due to complexing activities (Borris, 1996). Also, the correlation between antibacterial activity and membrane interference supports the theory that flavonoids may demonstrate antibacterial activity by reducing membrane fluidity of bacterial cells (Nakahara and Kawabata, 1993). Flavonoids (myricetin, datiscetin, kaempferol and quercetin) exhibit an inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Myricetin was also found to inhibit the growth of multidrug resistant *Burkholderia cepacia*, Vancomycin- resistant Enterococci (VRE) and other medically important organisms such as *Klebsiella pneumonia* and *Staphylococcus epidermidis* (Xu and Lee, 2001). Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope, transport proteins, etc. (Cowan, 1999).

Steroids were found to be present in the pulp and seed extracts, but with a higher concentration in the pulp extract. Steroidal compounds are of importance and so interest as grown in pharmacy due to their association with such compounds as sex hormones (Okwu, 2007). Since the pulp of *C. cainito* contains a high concentration of steroids, its consumption should be encouraged to serve as potent starting materials in the synthesis of sex hormones and also to boost existing sex hormones. Table 3 also shows that the seed extract

contains no cardiac glycoside, while the pulp contains a high concentration of cardiac glycoside. Cardiac glycosides help the heart to beat more efficiently by making the blood get more oxygen and gives nutrients to the body cells. Cardiac glycosides also help in the treatment of congestive heart failure and arrhythmia (Sodipo and Akiniyi, 2000). Alkaloid and anthraquinone were not found in the extracts of both seed and pulp.

Results obtained in Table 4 shows that the sample contains protein (1.96g for pulp and 4.63g for seed) which indicates high nutritional quality. Crude fibre (4.19g for seed and 2.31g for pulp) and ash contents are high and could aid bowel movement and also increase mineral contents respectively in the body (WHO/FAO, 1970). The gross energy values (474.77 for seed and 89.32 for pulp) are high and this could facilitate protein utilization and possibly avert protein-energy malnutrition, which is very common in underdeveloped countries evident by high cost of proteins in the diet (WHO/FAO, 1970).

Moisture contents have a great impact on the preservation of food materials. Moisture content is one of the most important and most widely used parameter in food processing. Moisture contents of the star apple pulp and seed are 75.90 and 54.04 respectively. Fat contents of pulp and seed extracts are 0.88g and 15.81g respectively as shown in Table 4.

Chrysophyllum cainito can serve as a good food supplement, especially for the obese because of its low fat content. Its low carbohydrate content underscore its low value of simple sugar, hence, it can be consumed by diabetic patients. Its decent amount of ascorbic acid (43.54mg) as shown in Table 5 makes it tolerable for people with peptic ulcer. *C*. *cainito* is also believed to be a good source of antioxidants (β-carotene and ascorbic acid) needed by the body to prevent and combat the activities of free radicals. *C*. *cainito* is a good source of minerals such as potassium, calcium and phosphorus (Table 5), which are needed for electrolyte balance, neurotransmission, and development of strong teeth and bones (Roth and Townsend, 2003).

This study shows that *C*. *cainito* has great potentials as antimicrobial agents against selected pathogens and may be used as an alternative medicine in the treatment or control of enteric bacterial infections. The results also showed that the test organisms were potentially susceptible to the seed and pulp extracts based on their zones of inhibition which ranged from 1mm to 10mm (Tables 8 and 9). The results of the antimicrobial susceptibility assay, the MIC assays and the MBC assay, showed promising evidence for the antimicrobial activity of *Chrysophyllum cainito* pulp and seed extracts against enteric pathogens.

Few fruits have the wide range of bioactivity exhibited by *Chrysophyllum cainito*. It appears to have excellent health and medicinal benefits which deserve to be further explored. Compared with other fruits, *Chrysophyllum cainito* could be classified as one of the new super fruits.

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