PROXIMATE ANALYSIS OF *Costus spicatus* LEAVES AND FLOWERS

BY

OSOMADE V. CHIDINMA

MATRIC NUMBER: 16010101015

A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE (B.SC), MICROBIOLOGY.

MOUNTAIN TOP UNIVERSITY, OGUN STATE,

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Dr. E.O OYEBANJI	Dr. A.A ADEIGA
SUPERVISOR	HEAD OF DEPARTMENT

DECLARATION

I, hereby solemnly declare that the research work title “Proximate Analysis of *Costus spicatus*” leaves and flowers submitted by the undersigned has been carried out under the supervision of Dr. E.O Oyebanji, Department of Biological Sciences. Mountain Top University, Ogun State. It is further declared that the research work presented here is original work. Any reference to work done by any other person or institution or any material obtained from other sources have been duly cited and referenced.

CERTIFICATION

I hereby certify that OSOMADE CHIDINMA VANESSA carried out this research under my supervision.

______________________ ______________________

Dr. E.O OYEBANJI Date

Supervisor

DEDICATION

I dedicate this project to the God Almighty, who has been my strength, provider and my sustainer. Also, to my family for their unending support.

ACKNOWLEDGMENT

I am much indebted and would like give all glory to God almighty for His unending and sufficient grace that has kept me standing through all my days as a student in Mountain Top University.

I would also like to express my sincere gratitude and esteem to the HOD Dr. A.A. Adeiga, my respected supervisor Dr. E.O. Oyebanji for her constant supervision, expert guidance, enthusiastic encouragement to follow new ideas and constant support throughout the entire period of my research work. Without her supervision and help this dissertation would not have been possible. She offered invaluable assistance, encouragements, and direction to complete my work on time.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The species *Costus spicatus* Jacq. (Spiked spiral flag ginger) is a medicinal plant popularly known as ‘cana-do-brejo’ and a native to the Caribbean regions often grown as an ornamental and medicinal herb principally used as a diuretic, anti-inflammatory, antiseptic, anthelmintic, stimulant and tumor therapy. The aim of this present study was to determine the nutritional values in the aqueous extract of the plant with regards to protein, moisture content, ash content and crude fiber using standard methods. The leaf and flower parts of the plant were found to contain varying levels of nutritional factors. Crude fiber and protein content were highest in the leaves whereas ash and moisture content were highest in the flowers. These indicates that these parts of the specie contain appreciable amount of nutrients which could be included in our diets to supplement our daily nutrients needs.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Natural products are the starting point of all synthetic compounds or products, since something natural seems to have been produced by nature (Brahmachari, 2012). The term natural product applies to any naturally occurring material, but is typically taken to mean a secondary metabolite, a small molecule that is not involved in the cell's main life processes, i.e. primary metabolism, such as growth and development, instead forming a peculiar off-shoot along unique biogenetic pathways (McMurry, 2010).

It has been estimated that well over 300,000 secondary metabolites exists and its thought that their primary function is to increase the likelihood of an organism's survival by repelling or attracting other organisms. Natural products such as plant extract, either as standardized extracts or as pure substances help students, scientists or researchers in discovery of novel products like drugs (for viral, fungi, bacterial, inflammatory and chemotherapeutic agents) and starting or intermediate chemicals (like phenol, benzene, food supplements like vitamins, minerals, fibers etc.) for production of goods for human consumption. 21st century are now going back on the natural products by using herbs to cure their diseases and to keep their body system fit. Nutrient values present in unique natural extracts such as leaves, roots, seeds, stem, etc. of plants are analyzed in a proximate analysis.

1.2 MEDICINAL PLANTS

Rudyard Kipling wrote (1910), "To our ancient ancestors, anything green that grew out of the mould was an excellent herb." Medicinal plants are plants that have been tested or experimentally recognized for medicinal use. They range from plants used in the manufacture of conventional pharmaceutical products to plants that are used in the preparation of herbal medicine. It is possible to find medicinal plants emerging in various settings worldwide. For millennia, the healing properties of different medicinal plants have been used to cure many human diseases (Jeyakumar, 2012). As estimated, traditional herbal medicines are used by about 60-90% of the population of developing countries, finding them to be a natural part of primary health care (WHO, 2002). Day by day, customer demand for herbal medicine has risen as they view these ways of healing as healthy and effective than prescription drugs (Motaleb, 2011). This practice of using complementary and complimentary healthcare has encouraged scientists to examine the various biological activities of medicinal plants (Wendakoon et al. 2011). These plant-derived products contain a broad variety of phytochemicals with various health-related effects such as antibacterial, antidiabetic and carcinogenic properties, such as phenols, flavonoids, tannins and other phyto-constituent (Bidlack et al., 2000). One of the plant that contains such properties is *Costus spicatus.*

1.3 SPIKED SPIRALFLAG GINGER (*Costus spicatus*)

Costus spicatus is a perennial, herbaceous plant up to 7 feet (2.1m) tall that grows mostly from rhizomes. It has tropical-looking foliage with leaves that spiral around the main stem. The leaves are up to1 foot (30 cm) long and up to 4 inches (10 cm) wide. It produces a short red cone, from which red-orange flowers emerge one at a time. It flowers in spring and summer or year-round in tropical conditions. In Brazilian folk medicine, *C. spicatus* tea is used with depurative purposes, astringent and diuretic (Borras, 2003). Nascimento et al. (2016) speculated that the aqueous extract studied *Costus spicatus* has a potential hypoglycemic action which could be related to the synergic action of molecules with antioxidant profile which can enhance the secretion of insulin by the pancreas and the increase in the absorption of tissue glucose level.

1.4 PROXIMATE ANALYSIS

Proximate analysis is a method used to determine the macro nutrients present in food quantitatively. It is composed of six categories by means of their chemical properties or nutritive values. They are Crude Ash, Crude Fiber, Crude Protein, Moisture content, Carbohydrate (also known as Nitrogen Free Extracts).

1.5 STATEMENT OF PROBLEM

- The usefulness of the herbal medicine plant to treat diseases
- Nutritional qualities held by the leaves and flower of the plant
- The beneficial nature of the plant for human health.

1.6 AIM AND OBJECTIVES OF STUDY

In this study, extract from samples of *Costus spicatus* (aqueous) were collected and investigated

On the basis of this, the objectives of the present study are as follows:

- Extraction of aqueous extract from *Costus spicatus* plant (leaves and flowers)
- To analyze for the following parameters (ash content, crude Fiber, crude protein and moisture content) of the leaves and flowers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Costus spicatus*

2.1.1 History and Use

Costus spicatus (Jacq.) belong to the family Zinziberaceae / Costaceae popularly known in Brazil as "cana de macaco" or "cana do brejo" which stands for "cane of swamp is a native species found in humid south of Mexico, Yucatan, Costa Rica, northern Colombia and Brazil. The "cane of the swamp" name is used to designate two species of *Costus*: *Costus spiralis* Rosc. and *Costus spicatus* Swartz, since both have the same use in popular therapy. The clade has been dated back c.47 million years old. It is most closely related to Zingiberaceae in which it was previously included as a subfamily. Found throughout the Brazilian territory, *Costus spicatus* is a medicinal plant found in wet coastal forests and is often grown as an ornamental and medicinal herb. In traditional medicine, the plant leaves, fruits, seeds and rhizomes are used in diuretic, anti-inflammatory, antiseptic, anthelmintic, stimulant and tumor therapy. (Couly, 2004; Lars, 2007; Duke, 2009; USDA-ARS, 2014). Herbal tea made from the *Costus spicatus* leaves is widely used to treat diabetes (hyperglycaemia) in traditional West Indies medicine (i.e., Dominican Republic). A recent research, however, concluded that this therapy had little success in treating hyperglycaemia caused by obesity (Keller et al, 2009). Heated leaf cataplasms of this genus are used in Brazil for the treatment of kidney and bladder tumours (Couly, 2004; Favro and Brebbia, 2010).

Quintans junior et al. (2010) reports that an infusion of the aerial part of the plant is taken to treat inflammation and pain in northeast Brazil. In the Amazon region many species are used as spies, seasonings, drugs, flavouring agents and a source of certain dyes (Gasparri, 2005). Also an infusion of the aerial part is used for treating colds, sore throat, dysentery, diarrhoea and treatment of diabetes (Cruz, 1965). Flavone glycosides have also been isolated from the leaves and demonstrated an inhibitory activity on nitric oxide production by activated macrophages (Da Silva et al, 2000).

2.1.2 Botany of *Costus spicatus*

Costaceae is a family of flowering plants including 6 genera and 110 species with pantropical distribution (Stevens, 2012). Species in the costaceae family can easily be recognized even vegetatively, from their ligulate leaves with a closed sheath that are arranged in a single spiral up the stem. The genus *Costus* is the largest in this family with approximately 90 species (Acevedo-Rodriguez and Strong, 2005; Stevens, 2012). The English common names of *C. spicatus* (Jacq.) reflect the growth habitat, in which the stems spiral like a corkscrew, and the leaves themselves spiral around the main stem. *Costus spicatus* (Jacq.) will grow in the sun if kept moist. Grows to about 6 to 7 feet tall. Leaves are about a foot long and about 4 inches wide. Plant produces a short cylindrical red cone with red-orange flowers emerging one at a time, long lasting and used as a cut flower. These plants often have close interactions with ants. The plants make a sugary nectar which is attractive to many different kinds of ants. Ants protect the developing seeds which are under the bracts from insect enemies. Some plants have very specialized ants, they form an alliance with one ant specie, which not only get food from the plant but also get a place to rest.

2.1.3 Taxonomic Hierarchy

Rank	*Scientific and common names*
Domain	Eukaryota

Kingdom	Plantae
Subkingdom	Tracheobionta
Infra-kingdom	Streptophyta
Super division	Embryophyta
Division	Magnoliophyta
Subdivision	Spermatophyta
Class	Magnoliopsida
Subclass	Zingiberidae
Superorder	Lilianae
Order	Zingiberales
Family	Costaceae
Genus	*Costus*
Species	*Costus spicatus* (Jacq.) Sw. – Spiked spiral flag.

Table 1.0 Scientific classification of *Costus spicatus* (ITIS, 2020).

2.1.4 General Characteristics

It is described as a plant that grows 1-2.5 m tall; sheaths 1-2 cm in diameter, glabrescent with a truncate ligule 2-10 mm long. The petioles are 2-10mm long, puberulous to glabrous, the leaf blades are narrowly elliptic, 7-33 ×3.5-8.5 cm or more which shortly acuminate at the apex, rounded to cordate at base, glabrescent on both surfaces.

It grows best in warm and very humid areas from sea level to 1000m (USDA-ARS, 2014). In cooler zones, the foliage will die back to the ground in winter, but the rhizomes may survive to allow regeneration. It can grow in shaded conditions. *Costus spicatus* forms a terminal inflorescence comprised of greenish to red non-appendage bracts (green near the apex, reddish on the covered part) broadly ovate, 2.4cm long and broad, obtuse at the apex, glabrous and coriaceous, the margin of the covered parts lacerating into fibers, the bracteoles; 1.7-3 cm long. Calyx 9-16 mm long and tubular flowers with yellow to pink corolla; 4-5 cm long, glabrous, the tube 1cm long, the lobes narrowly obovate lobes, 3.5 cm long and yellow labellum; broadly oblong-obovate when spread out, 2.5-5 cm long and wide, the lateral lobes rolled inward and forming a slender tube, the margins crenulated. The Stamen is narrowly elliptic; 3-4cm long; anther 7-8 mm long. Ovary 4-9mm long, sericeous or rarely glabrous and the capsule ellipsoid; 10-15 mm long; seeds black (Acevedo-Rodriguez and Strong, 2005).

Their inflorescence are usually ovoid to cylindrical, 5-27 × 3-45 cm dense, spicate-capitate and have large bracts, and their mono symmetric flowers have a large labellum and a single stamen, the style running between the two halves of the large anther.

2.1.5 Natural Distribution

C. spicatus originates from the Caribbean region: the islands of Dominica, the Dominican Republic, Guadeloupe, Martinique and Puerto Rico in particular (Maas, 1972; USDA-ARS, 2014). It is mostly cultivated mainly in tropical regions of the world as an ornamental and medicinal herb. The distribution below in this overview table is based on all of the available information.

Continent/country/region	Distribution	Origin	Invasive	Reference

NORTH AMERICA			
Cuba	Present	Introduced Invasive	Oviedo Prieto et al. (2012)
Dominica	Present	Native	Broome et al. (2007)
Dominican republic	Present	Native	Goavaerts (2014)
Guadeloupe	Present	Native	Broome et al. (2007)
Haiti	Present	Native	Goavaerts (2014)
Martinique	Present	Native	Broome et al. (2007)
Mexico	present		Cruz and Dirzo (1987)
Panama	Absent, Invalid presence record(s)		Panama Checklist (2014)
Puerto Rico	Present	Native	Acevvedo-rodriguez and strong (2012)
Saint Lucia	Present	Introduced	Graveson (2012)

Trinidad and Tobago	Present	Native and introduced	Lans (2007)
SOUTH AMERICA			
Brazil	Present		CABI (Undated)
Amazonas	Present		IICT (2007)
Para	Present	Introduced	Couly (2004)

Table 2.0 Distribution Table of *Costus spicatus*

2.1.6 Cultivation and Propagation

Costus spicatus are usually grown in fertile, organic, moist, well-drained soils in shade (Whistler, 2000). Tropical climate with high humidity and minimum temperature of 13^{O}C is best for its cultivation. *Costus* or Crepe ginger grows from thick fleshy structures called rhizomes. A single rhizome will produce new shoots and increase to a 3ft wide clump in the second year under ideal growing conditions. *Costus* reproduces vegetatively by rhizomes, division of culms, stem cuttings. It can also be grown through seeds, but the percentage of seed germination was found to be low (Merina Benny, 2004). Therefore, there is a need for development of standard cultivation methods for species of *Costus*. Spiral flag grows in either full sun or partial shade. It needs fertile soil and ample moisture, and is often planted near water. Propagation is by division of the clumps, cuttings, or by separating the offsets or plantlets that form below the flower heads.

Mites and nematodes can be a problem, especially on light, sandy soil. Plant has no diseases that are of major concern.

2.2 GENUS *COSTUS*

2.2.1 Medicinal Properties of Genus *Costus*

In Brazilian folk medicine, *C. spicatus* tea is used with depurative purposes, astringent and diuretic (Borras, 2003). Boorhem et al. (1999) describe the decoction of the vegetative plant parts of the species active in the treatment of vaginal irritation, leucorrhoea and ulcers. The juice of fresh stem dilute is effective in the treatment of gonorrhoea, syphilis, nephritis, insect bites, bladder problems and diabetes (Albuquerque, 1989; Borras, 2003). The sheets can be combined with *Bonamia ferruginea* "vine-Tuira" (choisy) in the form of combinations (potions) in the treatment of malaria, hepatitis and diabetes (Silva, 2000). Their use in traditional medicine includes the use of leaves, stems and rhizomes as a diuretic and tonic (Lorenzi and Matos, 2008). It is also used to make sexual hormones and contraceptives (Warrier et al, 1994; Rastogi and Mehrotra, 1991).

The antioxidant action of species *Costus* is not yet well understood. However, studies show that many species of Zinziberaceae can sequester free radicals (Haraguchi and Inada, 1996).

The oxygen species (ROS) are molecules with an unpaired electron in the final orbital making them highly unstable, extremely reactive and enormous capacity to combine with various member molecules of the cellular structure, such as DNA, proteins, carbohydrates and lipids (Gasparri, 2005). The ability to combine the cellular structures is associated with multiple pathological processes such as cancer, Atherosclerosis, and Alzheimer's disease, and other disorders and normal aging process (Salvador, 2004).

Chemical studies of the aerial parts of *Costus spicatus* allowed the isolation of new flavonoid diglycosides such as tamarixetin 3-O-neohesperidoside the Kaempferol 3-O-neohesperidosidesix flavonoids and other compounds such as 3-O-neohesperidosideo quercetin (Silva, and Bernardo, 2000). In its chemical composition is also recorded the presence of oxalic acid, tannins, saponins, mucilage and pectin (Vieira, 1998) which appears to have significant antioxidant activity, able to reduce the harmful effects caused by free radicals (Gasparri, 2005).

However, it has also been shown to report suggesting that a diet rich in phenolic compounds display pro-oxidant and cytotoxic properties under certain conditions (Sugiyama, Izawa and Inoue, 2000).

Azevedo et al. (2014) found that the *Costus spicatus* displays all classes of metabolites described above, some of which have biological activity already known, making it necessary to carry out quantitative studies and research demonstrating their pharmacological effects, which contribute to the development of new drugs.

Plant Part Used	*Phyto-Constituent*	*Activity*
Whole plant	Disogenin	Astringent, aphrodisiac, purgative, anthelmintic, depurative and expectorant

Roots	Diosgenin, sitosterol, dioscin, gracillin, cycloartanol, cycloartenol and cycloalaudenol	Antibacterial, antifungal, tonic, expectorant and stimulant.
Rhizomes	Diosgenin, dioscin, gracillin and Beta-sitosterol	Antispasmodic, antidiabetic, anti-inflammatory, anti-vermin, anti-arthritic, cardiotonic, hydrochloretic, diuretic and CNS depressant
Leaves	Disogenin	Fever, dysentery, diabetes, eye and ear infections, diarrhoea and mental disorders

Table 3.0 Medicinal importance of different parts of genus *Costus*

2.2.2 Pharmacological Activities of Genus *Costus*

Leaves and rhizomes of the *Costus* species are known as antidiabetic in nature and are used in the treatment of diabetes mellitus (Bhat Vishnu et al., 2010). Complications such as coronary heart disease, cerebrovascular disease, renal failure, blindness, neurological complications, and premature death are caused by hyperglycemia according to epidemiological research and clinical trials by (Eliza et al, 2009).

Similarly, Bavara (2008) measured the anti-hyperglycemic, anti-hyperlipemic and antioxidant potency of *Costus speciosus* root ethanol extract in alloxan-induced diabetic male rats. The research showed that *Costus speciosus* can be helpful for the treatment and complications of diabetes. Possible protective effect of *Costus speciosus* rhizome extract on streptozotocin biochemical parameters (STZ)-induced male diabetic Wistar rats was investigated by Daisy et al. (2008).

The extract of *Costus speciosus* rhizome hexane is known to have anti-hyperglycemic and hypolipidemic activity and is able to reduce serum glucose levels and normalize other biochemical parameters of diabetic rats (Eliza et al, 2009). The *Costus* plants have anti-inflammatory, purgative, anti-arthritic and antifungal functions (Bandara et al, Kirtikar and Basu, 2005).

These plants are used internally for infections of the eye and ear that can be attributed to the anticholinesterase activity of the alkaloids of the plant (Mishra et al., 2009). It was confirmed by Bhattacharya et al. (1972) that *C. Speciosus* alkaloids in both in vitro and in vivo methods possess anticholinesterase activity. Singh et al (2008) showed anti-carcinogenic activity of *Costus speciosus* and found that the minimum inhibitory concentration ranged from 0.78 to 10 mg/ml. The pharmacological aspects of different alkaloids and presence of anticholinesterase activity in *Costus speciosus* was noticed by Mandal and Chatterjee. (1985).

There are anti-inflammatory and antipyretic properties to the ethanoic extract of the rhizome of *Costus speciosus*. The value of diosgenin has resulted in comprehensive exploration of its various sources. The Das Gupta and Pandey (1970) study confirming the presence of diosgenin in the rhizomes of *Costus speciosus* increased researchers' interest in studying various *Costus* species as a possible source.

Study work to determine the anti-diabetic effect of the insulin plant has been carried out. In a cross-sectional clinical trial, patients who ingested either one fresh leaf or 1 teaspoon of shade-dried *Costus igneus* powder/day in combination with other treatment modalities produced good glycemic control in diabetics. However, for the analysis of GLUT4 translocation and glucose uptake activity, an in vitro study of ethanoic extract from *Costus pictus* leaf was analyzed, showing no direct peripheral action at a dose of 300μg/ml comparable to insulin and metformin. A research evaluated the capacity of a tea made from Costus spicatus leaves to alter the homeostasis of glucose in C57BLKS/J (KS) db/db mice model of hyperglycemia caused by obesity, with gradual depletion of beta-cells. After the 10-week study duration, intraperitoneal (IP) insulin tolerance testing showed that ingestion of Costus spicatus tea did not alter insulin sensitivity, indicating that at the dose given, tea made from Costus spicatus leaves did not have any efficacy in the treatment of hyperglycemia caused by obesity.

2.3 PROXIMATE ANALYSIS

The total ash, moisture, crude fat, protein and fiber content given as the percentage composition of the sample is calculated by proximate analysis (Self, 2005).

2.3.1 Moisture Content

A sample's moisture content is measured as the difference in mass following dehydration (Self, 2005). The mass after dehydration is the value of dry matter (DM) or overall solids. Oven drying and freeze drying are the most widely used techniques. Moisture or water which is a universal solvent can function in dissolving other substances and carrying nutrients throughout the body system making the organs such as the kidney, liver and stomach to function properly and effectively (McDonald et al., 1998).

2.3.2 Crude Protein (CP)

Proteins which are relevant in the body for the production of hormones, enzymes (globular protein) and blood plasma are also immune boosters which help in the growth and division of cells in the body (Okeke and Elekwe, 2006). The total Nitrogen content of the sample which is determined after the digestion, salt neutralization and titration of the ammonia produced against standard acid (i.e., the Kjeldahl method) is calculated as total nitrogen by adding a conversion factor of 6.25. For calculating the total protein, a conversion factor is added. Most times some functional groups, $-NO_2$ and $-N=N$ do not respond.

2.3.3 Crude Fiber (CF)

The crude fibre material is classified as an indicator of the structural carbohydrate in a sample. Fibers are components of fruits, grains and vegetables that the human digestive system is unable to absorb (Agarwal and Rastogi, 1974). Also, in the human body, dietary fibers slow down the absorption of glucose into the blood, decreasing the risk of hyperglycemia (Bouttwell, 1998).They can also function in reducing levels of plasma cholesterol, colon cancer and cardiovascular diseases. (Davidson et al., 1975). A sample is heated sequentially with dilute acid and then with dilute alkali, and then washed consecutively with ethanol and diethyl ether, subtracting the residue from its ash and identifying the result as crude fiber.

2.3.3 Ash Content

A plant sample's ash content is the feature of the mineral elements that are present. Dietary ash has commonly been used to create and maintain an acid-alkaline blood system balance

(Barborka, 1970; Hawkins, 1979) as well as to monitor conditions of hyperglycemia (Gokani et al., 1992). The ash content is the mineral or inorganic residue left after high temperature combustion (Self, 2005) and is used as a crude indicator of mineral content. The Association of Analytical Communities (AOAC, 1995) supports the use of the muffle furnace for sample incineration in the process and is commonly used for proximate analysis.

The ash content is important for determining the quality of certain foods, including their texture, appearance, quality and stability, depending on the concentration and form of minerals they contain.

The nutrients and mineral content present in certain plant extracts, however, does more than just stop diseases of deficiency. Certain vitamins or vitamin precursors such as vitamin C, B, carotene, polyphenols which acts as powerful anti-oxidants are produced by most of these plants.

Anti-oxidants avoid oxidation-related molecular damage and protect against muscle degeneration and cardiovascular diseases (Islam et al., 2002).

It is important to provide empirical data on the nutritional values of some under-used multi-purpose plants such as *Costus spicatus* and it will go a long way to improve their consumption.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION AND IDENTIFICATION

The leaves and flowers of *Costus spicatus* Jacq. were collected within “Mountain Top University” campus Ogun State in October 2019. A voucher specimen of the genus was identified and authenticated at the Herbarium Unit of the Department of Botany, Federal University of Lagos (UNILAG), Nigeria by Taxonomist Dr. Nodza G.I. and the Voucher specimen number 8559 was given to the plant.

3.2 PREPARATION AND STORAGE OF PLANT MATERIALS

The fresh plant leaves and flowers were washed thoroughly and carefully with distilled water and dried in an oven with circulating air at 50^0C for 48 hours, it was pulverized to powder using a grinder in the laboratory and in an air-tight container until needed for further extraction and various processes.

3.3 AQUEOUS EXTRACT

50 grams each of the powdered plant leaves and flowers was weighed and mixed with 600 ml of distilled water in a volumetric flask. This was left for 2 days in a sterile environment and then filtered using a Whatman Filter Paper no.40. The filtrate was placed in water bath at 80-90^{O}C till it was dried out.

3.4 STORAGE OF EXTRACT

The resulting extracts (dry and sticky) was allowed to cool and collected in 25ml McCartney bottles using a spatula, tightly stoppered and stored in a refrigerator at 4^{o}C

3.5 PROXIMATE ANALYSIS

The powdered samples of *C. spicatus* were analyzed for moisture content, crude protein, ash content and crude fiber by standard analytical methods (AOAC, 1990; Kirk and Sawyer, 1980; James, 1995).

3.5.1 Determination of Moisture Content

Moisture was determined by oven drying (Gravimetric method), where 2.0 g of the ground crude sample was accurately weighed in a clean and dry crucible. Then it was placed in an oven at 105^{o}C for 4hours, this procedure was done repeatedly until a constant weight was achieved. The sample was placed in a desiccator to cool for some time. The weight of moisture lost was calculated with the equation (a) below and expressed as percentage of weight of sample analyzed.

$$\text{Moisture Content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = Weight of empty crucible

W_2 = Weight of empty crucible + Wet Sample

W_3 = Weight of empty crucible + Sample dried to constant weight.

Apparatus

- Crucible
- Desiccator
- Drying oven

3.5.2 Determination of Crude Protein

The Protein in the aqueous extract of the plant sample was determined using kjeldahl method described by Chang (2003). The total nitrogen was determined and multiplied by a factor of 6.25 to obtain the protein content. 2.0 g of the sample was taken into a digestion flask containing 25.0 cm^3 of concentrated H_2SO_4 and mixed, a tablet of catalyst (copper). The flask was transferred to kjeldahl digestion apparatus. The mixture was digested until a clear green color was obtained. It was allowed to cool and any black particles showing at the mouth and neck were washed down with distilled water. The digest was transferred into a 250.0 cm3 flask. A solution of 40% NaOH was measured (10.0cm3), slowly added to a conical flask which contained a mixture of 10.0 cm^3 2% boric acid and 3 drops of mixed indicator was used to trap the ammonia being liberated. The conical flask and the Kjeldahl flask were then placed on Kjeldahl distillation apparatus with the tubes inserted into the conical flask, heat was applied to distill out the NH3.

The distillate was collected into the boric acid solution. The distillate was then titrated into the receiving flask using 0.1 M HCL. A reagent blank was also digested, distilled and titrated. The nitrogen/protein content was calculated using the equation (b) below;

1ml of 1N H_2SO_4 = 14mg

Protein (%) = N2 (%) x CF

$$N2\ (\%) = \frac{(Vs - Vb) \times C \times 1.4007}{W}$$

Where:

W = Weight of Sample (2.0g)

Vs = Volume of standard acid used to titrate

Vb = Blank b endpoint

C = Molar concentration of HCL

CF= Conversion factor (6.25)

Apparatus

- Kjeldahl digestion apparatus
- Volumetric flask
- Fume cupboard
- Digestion flask

3.5.3 Determination of Crude Fibre

This was determined by the method of James (1995). 2.0 g of the processed plant sample was boiled in 150 ml of 1.25% H_2SO_4 solution for 30minutes under reflux. The boiled sample was washed in several portions of hot water using a two-fold cloth to trap the particles. It was returned to the flask and boiled again in 150ml of 1.25% NaOH for another 30min under the same condition. After washing in several portions of hot water the sample was allowed to drain dry before being transferred to a weighing crucible where it was dried in the oven at $105^{O}C$ to a constant weight. It was thereafter taken to a muffle furnace where it was burnt, only ash left. The weight of the fiber was determined by difference and calculated using the equation (c) below;

$$\text{Crude Fiber (\%)} = \frac{W_2 - W_3}{\text{Weight of Sample}} \times 100$$

Where:

W_2 = Weight of crucible + sample after washing, boiling and drying

W_3 = Weight of crucible + Sample of ash

Apparatus

- Crucible
- Muffle furnace

3.5.4 Determination of Total Ash Content

The ash content of the sample was determined by the furnaces incineration gravimetric method which was described by James (1995) and AOAC (1984). 2.0 g of the processed sample was measured into a previously weighed crucible. The sample was burnt was to ashes in a muffle furnace at 550Oc. After it was completely ashed, it was cooled in a desiccator and weighed. The weight of the ash obtained was determined by the difference and calculated using the equation (d) below;

$$\text{Ash (\%)} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

Where:

W_1= Weight of empty crucible

W_2 = Weight of crucible + Ash

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.2 Proximate Analysis

The proximate composition of the aqueous extract *Costus spicatus* plant samples (Leaves and Flowers) was determined by using the official and accepted method of analysis by the Association of Official Analytical Chemists (AOAC) to ascertain the following parameters: Moisture content, Crude protein, Crude fiber and Ash content.

The moisture content in this study was found to be 34.0% and 37.5% in the leaves and flower respectively. More than 10 percent of which is not a reasonable long-term storage cap, Adoms, Sorrells and Liuu (2005). The moisture content of foods can be affected by the type, variety, and condition of storage.

The crude protein content of the leaf and flower of *Costus spicatus* shows 14.57% and 5.38% respectively. This can be due to the geographical position of the fertility of the soil (nitrogen levels of the soil) as protein levels can be affected by soils with low nitrogen levels.

All proximate values were expressed in percentages as shown in Table 4.0a and 4.0b below:

Parameters	***Costus spicatus* Aqueous extract (%)**
	Leaf
Moisture	34.0
Protein	14.57
Ash	0.24
Fiber	12.5

Table 4.0a Percent proximate composition in *Costus spicatus* leaf

Parameters	***Costus spicatus* Aqueous extract (%)**
	Flower
Moisture	37.50
Protein	5.38
Ash	1.6
Fiber	10.0

Table 4.0b Percent Proximate Composition in *Costus spicatus* Flower

The Crude fiber content found were (12.5%) in the leaf which is lower than (10.0%) in the flower sample of *Costus spicatus*. Crude fiber helps in the prevention of heart diseases, colon cancer, diabetes, etc. The high content of fiber makes it a favorable forage since fiber content help in digestion.

The ash content of the leaf and flower of *Costus spicatus* exhibited 0.24% and 1.6% respectively. Ash content is an indication of the mineral content in the plant. Ash contents may vary due to climatic conditions, genetic variations and soil characteristics.

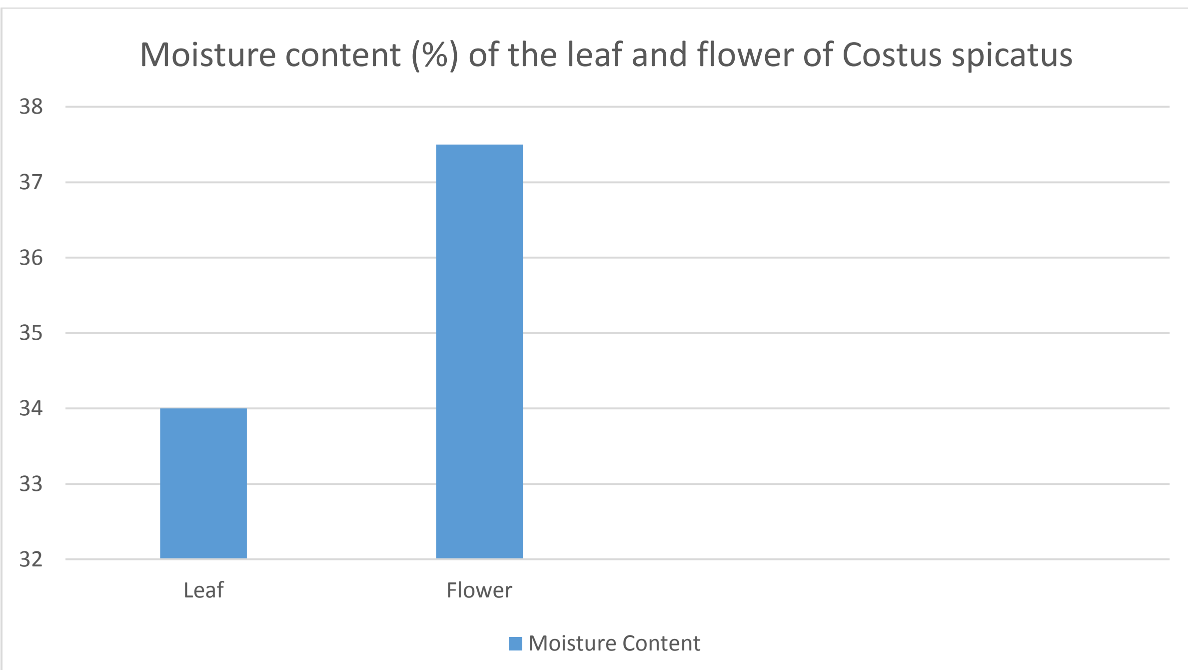

Fig 1.0: Graphical Representation of the Moisture Content

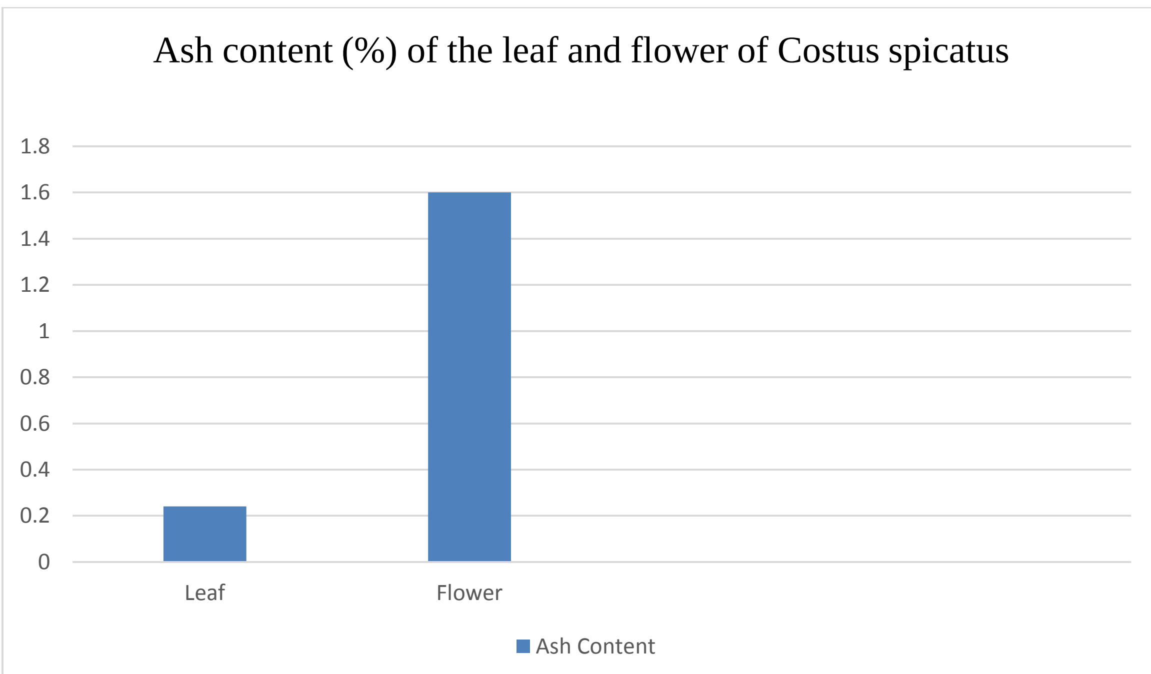

Fig 1.1: Graphical Representation of the Ash Content

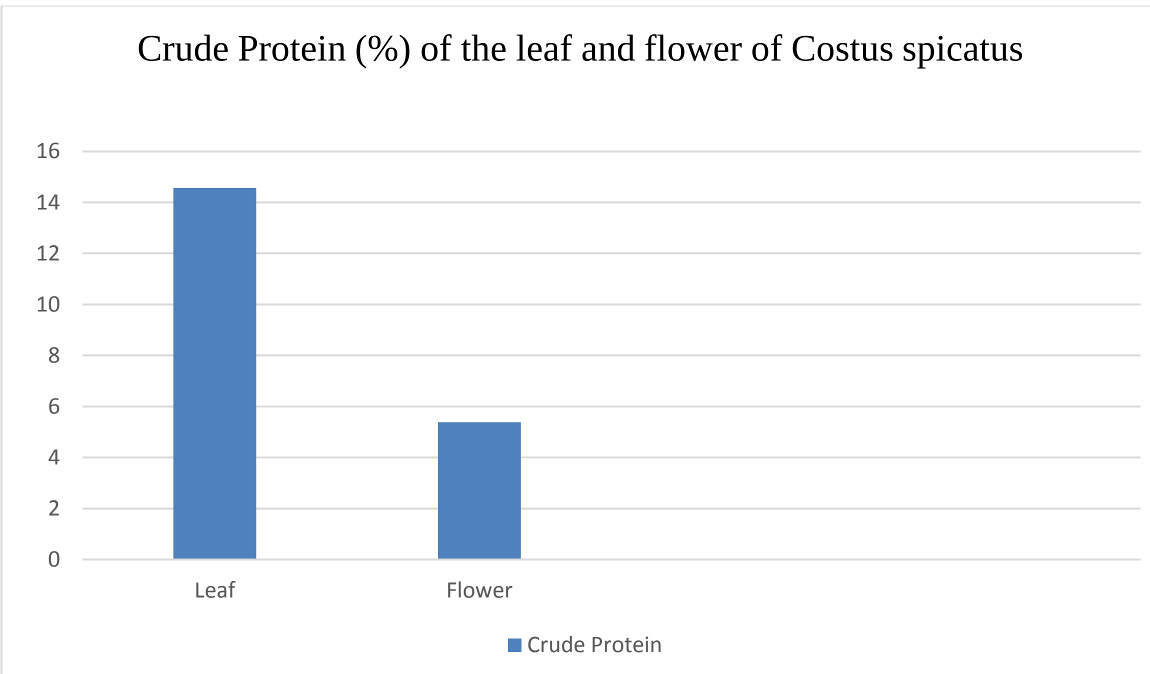

Fig 1.2: Graphical Representation of the Crude Protein Content

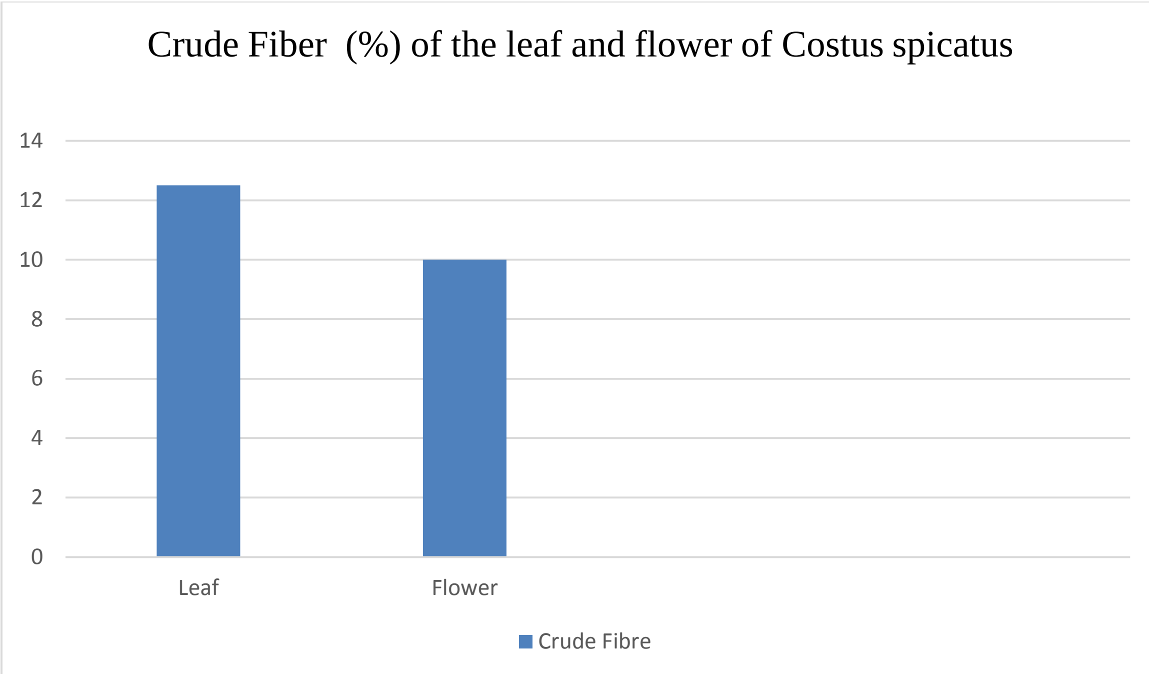

Fig 1.3: Graphical Representation of the Crude Fiber Content

4.2 DISCUSSION

To demonstrate the influential phenomena of coexistence, nature is often a golden sign. The treatment of human diseases is focused on natural products made from plants, animals and minerals (Firenzuoli and Gori, 2007). At present, medicinal plants are in demand and their acceptance is progressively increasing. Undoubtedly, by providing vital services to ecosystems, plants play an important role. Without plants, people and other living things will not exist in a way that they should be able to live. However, herbs, especially medicinal herbs, have constantly been an overall indicator of ecosystem health (Singh, 2002). Since ancient times, medicinal plants have doubtless been considered by human beings. It can be said that before the history and since the early humans recognized and exploited the plants around them for use as fuel, clothing, shelter and food, they became aware of their properties more or less. It can be said that they were more or less conscious of their resources before history and because early humans knew and used the plants around them for use as fuel, clothing, shelter and food.

In nations such as China, Greece, Egypt and India, medicinal plants have been turned into one of the oldest sciences. Plants were widely used as a medicine, disinfectant and aromatic agent in ancient Persia (Hamilton, 2004). Indeed, the use of medicinal plants for the treatment of diseases dates back to the history of human civilization, that is to say, because human beings have found a means of recovering from disease in their world, the use of plants was their only treatment option (Halberstein, 2005). In pharmaceutical and cosmetic products, more than a tenth of the plant species (over 50,000 species) are used.

Undoubtedly, worldwide demand for plant-derived products has increased. More than 85% of the population in the Middle East, Latin America, Africa and Asia rely primarily on traditional medicine, especially herbal medicine, for their health care needs. Approximately 100 million people still use conventional, complementary or herbal medicines in the European Union and in some countries, up to 90 percent of the population. Herbal medicine has a huge market that is growing. The overall sales of Chinese herbal medicines reached more than US$83 billion in 2012, which was 20 percent higher than the 2011 market. It has been declared that by 2020, the entire market for all herbal supplements will hit more than US$ 115 billion, which is the fastest growing market in the Asia-Pacific region and the highest growing market in Europe. By the focus on questions about the adverse effects of synthetic drugs, these demands are primarily motivated by female topics. Investment in human resource training may also be a primary source of research growth in order to move from manufacturing to crop production. More than ever, the value of research in the medicinal plant area is felt. The origins of adjuvant treatment in health systems worldwide are some medicinal plants which do not only treat diseases but also to prevent them and maintain health. Despite considerable experience in the use of medicinal plants in traditional medicine, scientific research and the detection and effects of active plant compounds will lead to the discovery in the future of new therapeutic benefits and the development of products based on nature. Medicinal plant extracts provide significant potential for the production of new agents that are effective against infections that are currently difficult to treat (wendakoonn et al, 2011). *Costus spicatus* is one of those plants that is well known for its use in diabetes care, inflammatory pains, tumor therapy, colds, sore throat, dysentery, diarrhoea, etc.

Azevedo et al. (2014) found that all groups of metabolites mentioned above are displayed by the *Costus spicatus*, some of which already have known biological activity, making it important to carry out quantitative studies and research showing their pharmacological effects that lead to the production of new drugs. The plant sample chosen was collected and aqueous extraction was performed.

The results of the proximate analysis of *Costus spicatus* shows that those nutrients (such as the protein, fiber, moisture and ash content) were present in the leaves and flower sample of the plant but seen in varying levels. Moisture and ash contents were highest in the flower when compared to the leaves which had a significant increase in crude fiber and protein content.

CHAPTER FIVE

5.0 CONCLUSION

The result of the study revealed high contents of Moisture, Crude Fiber and the presence of Protein and ash though at a low concentration which suggest that each part of the plant are nutritious and can contribute significantly to human health requirements and animal feeds. Clinical studies is recommended to determine at what level the nutrients and method of plant extraction become toxic to humans and farm animals and also ascertain the side effects.