

**PHYTOCHEMICAL SCREENING OF *Costus spicatus* LEAVES**

**BY**

**OLATUNDE, OLUWATOMISIN. G**

**16010101019**

**A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENT FOR THE DEGREE OF BACHELOR SCIENCE,  
(MICROBIOLOGY) MOUNTAIN TOP UNIVERSITY, OGUN STATE,  
NIGERIA.**

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MOUNTAIN TOP UNIVERSITY, OGUN STATE,  
NIGERIA.

2020.

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HEAD OF DEPARTMENT

## **CERTIFICATION**

I hereby certify that OLATUNDE, OLUWATOMISIN. G carried out this research under my supervision.

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**DR. E.O OYEBANJI**  
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**DATE**

## **DEDICATION**

I dedicate this project to the Almighty God, who has been my strength, provider and my sustainer. Also to my mum (Mrs. Olatunde) and dad (Mr. Olatunde) and my siblings for their unending support.

## **ACKNOWLEDGEMENT**

I give all glory to God Almighty, who has been the solid foundation that has kept me standing through all my days in as a student in Mountain Top University, all adoration be unto his name. I would also want to thank my respected supervisor Dr. E.O. Oyebanji, for her constant supervision, expert guidance, and for pushing me past my limits. Also to all academic staff in the department of Biological sciences.

I deeply appreciate my mother and my father, for their unwavering support. Also, would like to acknowledge the help of my brother, Iyanuoluwa, my sisters, Abigail and Oluwakemi and the guidance of my mentor, Jacque Webster.

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## **ABSTRACT**

Medicinal plants produce compounds that can be used as therapeutics or as precursors to the production of useful drugs. They may be a source of raw materials for the pharmaceutical industry. *Costus spicatus* is a Costaceae plant that is native to Brazil. It has antimicrobial, antifungal, antioxidant, and antidiabetic function, among other pharmacological and therapeutic properties. Because of this, the leaves of *Costus spicatus* were examined for preliminary phytochemical studies to see whether phytoconstituents including alkaloids, flavonoids, steroidal compounds, saponins, tannins, phenols, and cardiac glycosides were present. The aqueous extract was extracted and the presence of phytochemicals was tested in this sample. The findings revealed that the extract contains active bioactive ingredients.

## **CHAPTER ONE**

### **1.0 INTRODUCTION**

#### **1.1 BACKGROUND OF STUDY**

The healing effects of medicinal plants have been used to treat a number of human diseases for hundreds of years. Traditional herbal medicines are used by 60-90 percent of the population in developing countries, who consider them to be a part of primary healthcare (WHO, 2002). Consumer demand for herbal medicine is growing by the day, as these types of healing are seen as safer and more reliable than prescription drugs.

Plants that contain compounds that may be used for therapeutic purposes or are precursors to the synthesis of effective drugs are known as medicinal plants. They may be a source of raw materials for the development of pharmaceuticals. About a quarter of all medicines used around the world today contain natural compounds extracted from plants, whose activities are intensified by chemical manipulation and synthetic chemistry, all of which can be exploited in the field of new drug research and development. Plant-derived drugs have the advantage of being safer than synthetic substitutes while still providing significant clinical advantages and lower treatment costs.

Natural bioactive compounds present in plants are known as phyto-constituents. Phyto-constituents combine with nutrients and fibers to form an integrated part of nutrition that protects against a variety of diseases and stressors. Phytochemicals are compounds found in medicinal plants, leaves, vegetables, and roots that act as a protective mechanism against disease. According to their roles in plant metabolism, phytochemicals are classified into two categories: primary and secondary constituents. Alkaloids, terpenoids, steroids, and flavonoids are among

the primary constituents, while secondary constituents include common carbohydrates, amino acids, proteins, and chlorophyll.

## 1.2 STATEMENT OF PROBLEM

Many medicinal plants have yet to be discovered, grown, processed, and used in the development of new medicines. In relation to numerous research conducted on different species of the Genus, this plant, *Costus spicatus*, has shown great promise in possessing therapeutic values and nutritional significance. With an ever-increasing population and the proliferation of diseases (both new and old), as well as the “mutation” of disease-causing agents in order to respond to certain medicines used to treat them, medicinal plant research is more important than ever to avoid or regulate this phenomenon.

## 1.3 OBJECTIVE OF STUDY

1. To classify medicinally active compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds in *Costus spicatus* leaves plant extract.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 GENUS *Costus*

Spiral Ginger is a species of the *Costus* genus, which belongs to the Costaceae family. There are 100 species in this genus, which includes four genera. *Costus igneus*, *Costus afer*, *Costus spicatus*, *Costus arabicus*, *Costus dubius*, and *Costus speciosus* are only a few of the species on the list. They have a tuberous rootstock and a long fleshy stem. The spirally arranged leaves have silky pubescent underneath them. Both traditional and folk medicine have used *C.speciosus* for therapeutic purposes. Since bioactive compounds such as alkaloids, terpenoids, tannins, glycosides, saponins, and others are present, they have antifungal and antibacterial properties. Ear infections, diarrhoea, colds, catarrhal, fevers, coughs, and dyspepsia have all been treated with the leaves extract, according to studies. *Costus* spp. rhizome is high in macronutrients such as glucose, starch, amylose, protein, lipid, and other lipids, as well as micronutrients such as vitamin A. It's also an alternative source of diosgenin, which is used to treat diabetes and a variety of other ailments. In vitro and in vivo studies have shown that *C.speciosus* alkaloids have anticholinesterase activity. Finally, this family has been successfully used in the treatment and management of a variety of health conditions, as well as for testing and the production of newer drug molecules. As a result, the therapeutic potential should be combined with other pharmaceuticals.

##### 2.1.1 FEATURES OF THE GENUS

The genus has long fleshy stems with tuberous rootstock, according to botany. The leaves are spiraled and silky pubescent underneath. Flowers are closely packed into spikes or terminals. The calyx is a funnel-shaped short tube with ovate teeth. The segments of the corolla tube are wide and sub equal, and the tube is narrow. The connective forms an oblong petaloid process

with the large filament stamens. The ovary is typically three celled, with a filiform style and a semilunar ciliated depression on the stigma. The ovules are numerous and stacked on top of each other. Capsules can be globose or ovoid in shape. Subglobose or obovoid seeds with a short aril

16. The plant blooms between August and October 17th. In terms of the genus, *C. speciosus* is a straight plant with a long leafy stem that grows to a height of 0.6 to 1.8 meters. Tuberous rootstock Sessile, oblong, spirally arranged leaves with silky pubescence on the underside. The calyx is a small tube with ovate teeth, funnel-shaped. The corolla tube is narrow and the bracts are ovate, mucronate, and bright red. White flowers grow in thick spikes. Capsule-shaped, globose trigonus, and red-colored fruit. The aril is white and the seeds are purple. The ovary is usually three-celled, the type filiform, and the stigma has a semilunar ciliated depression. The ovules are numerous and stacked on top of each other. Capsules are globose or ovoid in shape, and they dehisce on one side between the ribs. Subglobose or obovoid seeds with a short aril.

### **2.1.2 PHARMACOLOGICAL VALUE OF COSTUS SPP.**

Saraf (2010) discovered that the rhizome of *C. speciosus* has antibacterial activity in vitro. *Escherchia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* have all been successfully treated with the plants. *Bacillus subtilis*, *Proteus mirabilis*, *E. coli*, and *P. aeruginosa* have all been found to be resistant to the mature leaves of *C. igneus*. The methanol extract had the greatest zone of inhibition against *E. coli*, but the least against *P. mirabilis* (Vasantharaj et al., 2013). *C. speciosus* rhizome extract was found to be effective against *Staphylococcus epidermidis* and *Salmonella typhimurium*, and thus could be used as a bacteriocidal agent (Ariharan et al., 2012). The antibacterial activity of *C. pictus*'s various parts (leaf, flower, stem, and root) was found to be potent against *Shigella flexneri*, *K. pneumoniae*, *B. subtilis*, and *E. coli*. (Majumdar and Parihar, 2012).

Fungal strains including *Penicillium* spp. and *Mucor* spp. were inhibited by a methanol extract of *C. speciosus* leaves. As a result, the plant has antifungal properties. Extracts from various sections of two species of *C. speciosus* and *C. pictus* were found to be successful against the fungus *Pythium aphanidermatum*, but not against the fungi *Colletotrichum capsici* and *Sclerotium rolfsii*. *C. afer* leaves were found to be effective amoebicidal agents. Using a polyxenic culture of *Entamoeba histolytica*, the extract was tested for anti-amoebic activity in vitro (Moundipa et al., 2005). Helminthes are parasitic or intestinal worms that live in the human intestine and feed on their living hosts. Natural and appropriate helminthes treatments can be found in medicinal plants (Kanthal et al 2012). The aerial parts of *C. speciosus* can be used as a promising anthelmintic agent, according to Srivastava et al., 2011. The extracts of the aerial parts were tested on *Pheritima posthuma* as experimental worms, and they were found to have a major paralyzing effect.

Antioxidants have the ability to trap reactive oxygen species and free radicals. These free radicals may cause degenerative disease by oxidizing nucleic acids, proteins, lipids, or DNA. Antioxidants such as phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxy, according to Vishalakshi and Urooj (2010), and thus inhibit the oxidative pathways that lead to degenerative diseases. Using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, Nahak (2009) demonstrated free radical scavenging behavior of an ethanolic extract of *C. speciosus* rhizome. With an IC<sub>50</sub> value of 25g/ml, the highest degree of free radical scavenging activity was found to be 71.61 ± 0.02 percent. The antioxidant function of *C. pictus* methanol and aqueous extracts was determined using the DPPH radical scavenging assay. *C. pictus* methanol extract had the highest antioxidant activity. Majumdar and Parihar (2012) (Majumdar and Parihar, 2012) (Majumdar and Parihar,

*C. speciosus* rhizome extract demonstrated mild anti-cancer activity in adenocarcinomic human alveolar basal epithelial cells- A549, SK-OV-3, human melanoma cell line- SK-MEL-2, and colo-carcinoma- HCT15 cells, according to Chang et al., (2005).

In human leukaemia cells, rhizome extracts induced apoptosis via reactive oxygen species (ROS) and B-cell lymphoma2 (Bcl-2)-dependent mitochondrial permeability transitions, as well as ROS-mediated JNK activation (Nahak, 2009).

*Costus* spp. rhizome extracts are also used to treat inflammation and anaphylactic shocks. The anti-inflammatory and antipyretic properties of the ethanolic extract of *C. speciosus* rhizome have been discovered. Carrageenin-induced edema formation in rats at 800mg/kg was found to have a significant anti-inflammatory effect, as was cotton pellet granuloma formation in rats at 400mg/kg and 800mg/kg (Binny et al., 2010). Hepatoprotective properties of this plant are also well-known. The use of this plant as a hepatoprotective agent was investigated in an experimental setting. The ethanolic extract reduced serum glutamyl oxalacetic acid transaminase (SGOT), serum glutamyl pyruvate transaminase (SGPT), alkaline phosphatase (ALKP), serum bilirubin (SBLN), and liver inflammation, indicating hepatoprotective operation (Verma and Khosa, 2009).

Diabetes mellitus is a chronic metabolic disease that affects about 4% of the global population and is projected to rise to 5.4 percent by 2025. (Ignacimuthu 2008). High blood glucose levels are a symptom of the disorder, which is caused by an absolute or relative lack of circulating insulin (Vishalakshi and Urooj 2008). Eliza et al. (2009) extracted costunolide from *Costus speciosus* crude extract and administered it to diabetic male wistar rats induced with streptozotocin (STZ) at various doses, finding that plasma glucose was significantly reduced in a dose-dependent manner as compared to the control. Costunolide was found to inhibit the release

of nitric oxide synthase, assisting in the correction of secretary defects in diabetes (Gupta et al., 2008).

b). *C. speciosus* extracts, both aqueous and methanolic, were extremely successful at lowering blood glucose levels (Rajesh et al., 2009). Daisy et al. (2008) used *C. speciosus* rhizome extracts to examine biochemical parameters in STZ-induced male diabetic wistar rats. STZ treatment (50 mg/kg) resulted in hyperglycemia and physiologic and biochemical changes, while hexane, ethyl acetate, and methanol extracts administered to STZ rats resulted in reduced and normal glycaemic rats. In diabetic albino rats induced with Streptozotocin (STZ), Kalailingam et al. (2011) investigated the antihyperglycemic and hypolipidemic activities of a methanol extract of *C. igneus* rhizome. Fasting blood glucose, serum TC, TG, LDL, and VLDL levels were all significantly lower in diabetic rats, while serum HDL levels were significantly higher. In alloxan-induced diabetic male rats, Bavara et al. (2008) evaluated the antihyperglycemic, antihyperlipemic, and antioxidant potency of an ethanol extract of *C. speciosus* root. *C. speciosus* is believed to have anti-diabetic properties and is used in Indian traditional medicine (Rajesh et al., 2009).

Methanol extract of *C. Pictus* leaves, given as a single dose per day for 21 days to diabetic rats, resulted in substantial blood glucose reductions, indicating that *C. Pictus* leaves appear to be an effective anti-diabetic agent (Jothivel et al., 2007).



## 2.2 *Costus spicatus*

### **2.2.1 BOTANY OF *Costus spicatus***

Costaceae is a flowering plant family of six genera and 110 species that has a pantropical distribution (Stevens, 2012). Species in the costaceae family have ligulate leaves with a closed sheath that are arranged in an extremely single spiral up the stem, making them easy to identify even vegetatively. With approximately 90 species, the genus *Costus* is the most common in this family (Acevedo-Rodriguez and powerful, 2005; Stevens, 2012). The expansion habitat of *C. spicatus* (Jacq.) is reflected in the land common names, in which the stems spiral rather a corkscrew, and then the leaves spiral around the main stem. If kept moist, *Costus spicatus* (Jacq.) can develop in the light. It reaches a height of 6 to 7 feet. The leaves are about a foot long and 4 inches thick. Plant grows a fast-growing cylindrical red cone with one-by-one red-orange flowers that are long-lasting and can be cut. Ants are often seen in close proximity to these plants. The plants produce a sugary nectar that is appealing to a variety of ants. Ants defend the growing seeds underneath the bracts from insect predators. Some plants have very specialized ants: they form an alliance with one ant species, which not only receives food from the plant but also receives a portion of the plant's remainder.

### **2.2.2 HISTORY AND USE OF *Costus spicatus***

*Costus spicatus*, also known as "spiked spiralfang ginger," is a perennial herbaceous plant. It's a vascular plant with ligulate leaves with a closed sheath that spiral up the stem in an extremely single spiral. It belongs to the Costaceae family and the genus *Costus*, which is the largest of the Costaceae. It reaches a height of 6 to 7 feet. *C. spicatus* is commonly grown for ornamental and medicinal purposes. Traditional medicine uses the leaves, fruits, seeds, and rhizomes as a diuretic, anti-inflammatory, antiseptic, anthelmintic, stimulant, and for the treatment of tumors (Couly, 2004; Lars, 2007; Duke, 2009; USDA-ARS, 2014). Tea made from the leaves of *C. spicatus* is commonly used to treat diabetes in traditional archipelago medicine (i.e., Dominican Republic) (hyperglycaemia). (*Costaceae*) is a common medicinal herb used by Dominicans in the Dominican Republic and the United States to treat diabetes, which is a growing epidemic among Hispanics. An ethnobotanical survey of the Dominican community in New York City reported that a tea made from the insulin plant is the preferred treatment for hyperglycemia. *Costus spicatus* was found to be the source of insulin. The plant had antinociceptive and anti-inflammatory properties that were promising. In C57BLKS/J (KS) db/db mice, a model of obesity-induced hyperglycemia with progressive cell depletion, the leaves of *Costus spicatus* were found to alter glucose homeostasis. Two flavonol diglycosides, tamarixetin 3-O-neohesperidoside, kaempferide 3-O-neohesperidoside, and the well-known quercetin 3-O-neohesperidoside, were isolated from the leaves of *Costus spicatus*, along with six other flavonoids. The flavonol diglycosides were tested for their ability to stop activated macrophages from producing gas (Da Silva et al., 2000).

### 2.2.3 SCIENTIFIC CLASSIFICATION

### 2.2.4 TAXONOMIC HIERARCHY

<b>RANK</b>	<b>SCIENTIFIC NAME AND COMMON NAME</b>
Domain	Eukaryota
Kingdom	Plantae – Plants, planta, vegetal, plantes
Subkingdom	Tracheobionta -Vascular plants
Infrakingdom	Streptophyta –land plants
Superdivision	Embryophyta
Division	Magnoliophyta - Flowering plants
Subdivision	Spermatophyta - seed plants, phanerogames
Class	Magnoliopsida
Subclass	Zingiberidae
Superorder	Lilianaes - monocots, monocotyledons
Order	Zingiberales
Family	Costaceae - Costus family
Genus	<i>Costus</i> L.- Costus
Species	<i>Costus spicatus</i> (Jacq.) Sw. – Spiked spiralflag.

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

### **2.2.5 GENERAL CHARACTERISTICS OF *Costus spicatus***

This perennial herbaceous plant grows up to 6-7 feet tall in Brazil and is known as "cana de macaco" or "cana do brejo," which means "cane of swamp." It's described as a plant with sheaths 1-2 cm in diameter, glabrescent, and a truncate ligule 2-10 mm long that grows to 1-2.5 m tall. The leaf blades are broadly elliptic, 7-33 3.5-8.5 cm or more, shortly acuminate at the apex, rounded to cordate at base, glabrescent on both surfaces, and the petioles are 2-10mm long, puberulous to glabrous. The leaves are about 4 inches wide and a few feet long. Plant produces a short cylindrical red cone with one-by-one red-orange flowers that last for a long time and can be cut. It thrives in wet, humid environments from sea level to 1000 meters above sea level (USDA-ARS, 2014). The foliage in cooler zones may die down to the ground in the winter, but the rhizomes will survive to allow regeneration. It can thrive in shady areas. *Costus spicatus* produces a terminal inflorescence with greenish to red non-appendaged bracts (green near the apex, reddish on the covered part), narrowly ovate, 2.4cm long and thick, obtuse at the apex, glabrous and coriaceous, the covered parts' margins lacerating into fibers, the bracteoles; 1.7-3 cm long. 4-5 cm long, glabrous, the tube 1 cm long, the lobes narrowly obovate lobes, 3.5 cm long and yellow labellum; roughly oblong-obovate when unfolded, 2.5-5 cm long and thick, the lateral lobes rolled inward and forming a slender tube, the margins crenulated. The stamen is narrowly elliptic, measuring 3-4cm in length with an anther measuring 7-8 mm. The ovary is 4-9 mm long, sericeous or glabrous, and the capsule is ellipsoid and 10-15 mm long, with black seeds (Acevedo-Rodriguez and robust, 2005).

## **2.2.6 MORPHOLOGY**

### **2.2.7 MACROSCOPIC STRUCTURE OF *Costus spicatus***

It is described as a plant with sheaths 1-2 cm in diameter, glabrescent, and a truncate ligule 2-10 mm long that grows to 1-2.5 m tall. The leaf blades are broadly elliptic, 7-33 3.5-8.5 cm or more, shortly acuminate at the apex, rounded to cordate at base, glabrescent on both surfaces, and the petioles are 2-10mm long, puberulous to glabrous. It thrives in wet, humid environments from sea level to 1000 meters above sea level (USDA-ARS, 2014). The foliage in cooler zones may die down to the ground in the winter, but the rhizomes will survive to allow regeneration. It can thrive in shady areas. *Costus spicatus* produces a terminal inflorescence with greenish to red non-appendaged bracts (green near the apex, reddish on the covered part), narrowly ovate, 2.4cm long and thick, obtuse at the apex, glabrous and coriaceous, the covered parts' margins lacerating into fibers, the bracteoles; 1.7-3 cm long. 4-5 cm long, glabrous, the tube 1 cm long, the lobes narrowly obovate lobes, 3.5 cm long and yellow labellum; generally oblong-obovate when opened up, 2.5-5 cm long and thick, the lateral lobes rolled inward and forming a slender tube, the margins crenulated The stamen is narrowly elliptic, measuring 3-4cm in length with an anther measuring 7-8 mm. Ovary 4-9mm long, sericeous or occasionally glabrous, resulting in an ellipsoid capsule; 10-15 mm long; seeds black (Acevedo-Rodriguez and powerful, 2005).

### **2.2.8 THERAPEUTIC VALUES OF *Costus spicatus***

The use of *Costus spicatus* in the ancient medicinal method has a long and illustrious history. In the treatment of gonorrhoea, syphilis, nephritis, insect bites, bladder problems, and diabetes, fresh stem juice is useful (Albuquerque, 1989; Borrás, 2003). In the treatment of malaria, hepatitis, and diabetes, the sheets can be mixed with *Bonamia ferruginea* “vine-Tuira” (choisy) hallier in the form of combinations (potions) (Silva, 2000). The leaves, roots, and rhizomes have been used as a diuretic and tonic in traditional medicine (Lorenzi and Matos, 2008). *C. spicatus* tea is said to be used for depurative (body system cleansing), astringent, and diuretic purposes (Borrás, 2003). The decoction of the vegetative plant parts of the species active in the treatment of vaginal irritation, leucorrhoea, and ulcers was reported by Boorhem et al. (1999). Chemical analysis of *Costus spicatus* aerial sections resulted in the isolation of new flavonoid diglycosides such as tamarixetin 3-O-neohesperidoside, Kaempferol 3-O-neohesperidoside, and other flavonoids such as 3-O-neohesperidosideo quercetin (Silva, and Bernardo, 2000). Certain compounds are confirmed in its chemical composition, like acid, tannins, saponins, mucilage, and pectin (Vieira, 1998), which appears to have strong antioxidant function, reducing the harmful effects induced by free radicals (Gasparri, 2005). Azevedo et al. (2014) discovered that the *Costus spicatus* produces all of the metabolites mentioned above, some of which have already been identified as having biological activity, necessitating the conduct of quantitative studies and analysis to demonstrate their pharmacological effects, which aid in the development of new drugs.

## 2.3 PHYTOCHEMICAL SCREENING

Constituents can be therapeutically active or inactive based on their chemical makeup. Several phytochemical surveys are being performed in order to detect different classes of phytochemicals that are currently present. The phytochemical research method is thought to be useful in determining the bioactive profile of therapeutically important plants. The extraction, screening, and detection of medicinally active substances present in plants is referred to as phytochemical screening. Flavonoids, alkaloids, carotenoids, tannin, antioxidants, and phenolic compounds are examples of bioactive substances obtained from plants.

### 2.3.1 FLAVONOIDS

Flavonoids are common in plants, where they serve a variety of roles. They're necessary pigments for the creation of colors that attract pollinating insects. Flavonoids are required in higher-order plants for UV filtration, biological processes, cell cycle inhibition, and chemical messengers. The symbiotic relationship between rhizobia and certain vegetables like peas, clover, and beans is supported by flavonoids secreted by a plant's roots. In response to the presence of flavonoids in the soil, rhizobia develop Nod factors. The plant recognizes these Nod factors, which triggers responses such as ion fluxes and root nodule formation. Some flavonoids also suppress certain spores, which protects plants from certain diseases. Flavonoids are the most common form of polyphenolic compound found in the human diet and are abundant in plants.

#### **Health benefits to humans**

Flavonoids are strong antioxidants that have a variety of health benefits. Apart from their antioxidant properties, these molecules have the following advantages:

- Anti-viral
- Anti-cancer

- Anti-inflammatory
- Anti-allergic

### **2.3.2 ALKALOIDS**

Alkaloids are a diverse category of cyclic compounds containing nitrogen. Approximately 2,000 different alkaloids have been discovered, some of which are pharmacologically useful. Morphine, strychnine, atropine, colchicine, ephedrine, quinine, and nicotine are all important alkaloids. They are most commonly found in herbaceous plants, but they can also be found in woody plants, especially tropical species. They mainly serve as defensive compounds in plants against insect pests and herbivorous animals. There are few food plants that contain substantial amounts of alkaloids, apart from the recognized medicinal plant species.

### **2.3.3 SAPONINS**

Saponins are glycosides of steroid and triterpenoid steroids and triterpenoids with a wide range of biological activities. The widespread presence of saponin in plants has led to saponin extraction and identification in a variety of species, owing to the potential for pharmaceutical applications. They were looked into because they contain maesasaponins, some of which have been stated to have anti-cancer properties, but none of which have haemolytic activity, which is normally associated with many saponins.

### **2.3.4 TANNINS**

Tannins are present in tree bark, wood, leaves, buds, stems, fruits, seeds, roots, and plant galls, among other areas. Tannins play a role in protecting individual plant species in both of these systems. Tannins in tree bark protect the tree from bacteria and fungi infection. In this case, the tannins precipitate out the enzymes and other protein exudates produced by bacteria and fungi, preventing infection of the tree.



## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Collection and Identification**

In October 2019, the leaves of *Costus spicatus* Jacq. were collected on the campus of “Mountain Top University” in Ogun State. Taxonomist Dr. Nodza G.I. described and authenticated a voucher specimen of the genus at the Herbarium Unit of the Department of Botany, Federal University of Lagos (UNILAG), Nigeria, and the plant was assigned the voucher specimen number 8559.

#### **3.1.1 Preparation and Storage of Plant Materials**

The fresh plant leaves were washed thoroughly and carefully with distilled water before being dried in a 50°C oven with circulating air for 48 hours. They were then pulverized to powder in the laboratory and stored in an airtight container until required for further extraction and various processes.

#### **3.1.2 Aqueous Extract**

In a volumetric flask, 50 grams of powdered plant leaves were weighed and mixed with 600 ml of distilled water. This was left in a sterile atmosphere for two days before being screened with a Whatman Filter Paper no.40. The filtrate was dried in a water bath at 80-90 degrees Celsius.

#### **3.1.3 Storage of Extract**

The resulting extracts (dry and sticky) were allowed to cool before being collected in 25ml McCartney bottles with a spatula, tightly sealed, and stored at 4°C.

### 3.2 PHYTOCHEMICAL SCREENING OF THE EXTRACTS

Tannins, alkaloids, flavonoids, saponin, protein, hormones, terpenoids, cardiac glycosides, and reducing compounds were all detected in the extract oils using standard tests.

#### 3.3.1 Test for Alkaloids

5 mL of oil extract was weighed and stirred with 5 mL of 1 percent aqueous hydrochloric acid on a steam bath; 1 mL of the filtrate was treated with a few drops of Mayer's reagent; 1 mL of the filtrate was treated similarly with freshly prepared dragendoff's reagent and Wagner's reagent. The existence of alkaloids may be determined by the turbidity of each of these reagents. (Evans 1989).

#### 3.3.2 Tannins

3-4 drops of 10% ferric chloride solution were applied to a portion of the oil extract. The presence of tannins was indicated by the presence of a blue green color. (Talukdar and Choudhary 2010).

#### 3.3.3 Flavonoids

Sulphuric acid was applied to the filtrate of the extract after five millilitres (5 ml) of diluted ammonia solution was added. The existence of flavonoids is shown by a yellow coloration (Harborne, 1973 and Sofowora, 1993).

### **3.3.4 Saponins**

Every oil extract was put into a test tube with five millilitres (5 ml) and shaken with water before being filtered. The existence of saponins can be determined by frothing that persists after being heated. Two test tubes each received 2 mL of normal saline solution. 2 ml sterile water was applied to each of the test tubes. Each tube contains an isotonic concentration of sodium chloride in comparison to blood serum. Five drops of oil were applied to each test tube, which were then gently combined to ensure that the contents were evenly distributed. The presence of red blood cell haemolysis in the test tube containing the oil extract was taken as proof of saponin presence (Farnsworth, 1966).

### **3.3.5 Cardiac glycosides**

The existence of cardiac glycosides was determined using the method defined by (Sofowora, 1993). A few drops of 2 percent sodium nitroprusside and a few drops of sodium hydroxide were added to 0.5 g of the oil extract dissolved in pyridine.

### **3.3.6 Anthraquinones glycoside**

The filtrate was extracted with chloroform after a quantity of the oil extract (0.1 g) was dissolved in 10 ml of hot water and steamed for 5 minutes. The chloroform layer was stripped and shaken with 5 ml of ammonia solution after being washed with 5 ml of distilled water. Van-Buren and Robinson (Van-Buren and Robinson, 1981).

### **3.3.7 Reducing compounds**

To assess the existence of reducing compounds, Ochuko's methods (2001) were used. The presence of reducing compounds was tested by boiling up to 0.5g of the oil extract in 1 percent aqueous hydrochloric acid.

**Table 2.0: Phytochemical screening of the extracted leaves of *Costus spicatus***

<b>Analyzed phytochemical factor</b>	<b>Test</b>	<b>Water</b>
Tannin	Tannin test	-
Alkaloid	Wagner's Test	+
Flavonoid	Alkaline Test	+
Saponins	Normal saline test	+
Protein	Nihydration Test	+
Steroid	Anthraquinone	-
Terpenoid	Salkowski's Test	-
Cardiac glycosides	Sodium nitroprusside	+
Anthraquinones	Hot water test	-

Reducing compounds	1% hydrochloric acid test	+
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## **CHAPTER FOUR**

### **4.0 RESULTS AND DISCUSSION**

As shown in (Table 2), the extracted leaves used in this study contained tannins, alkaloids, flavonoids, saponin, protein, hormones, terpenoids, cardiac glycosides, and reducing compounds.

The results of the phytochemical screening conducted in this study revealed that the leaves contain alkaloids, tannins, saponins, cardiac glycosides, and reducing compounds. Alkaloids have been commonly documented to have a wide range of pharmacological functions, and as a result, they have been used to make a variety of drugs. According to Onadapo and Owonubi (1993), tannins have antimicrobial properties and can inhibit the growth of microorganisms at low concentrations while acting as an antifungal agent at higher concentrations by coagulating the microorganism's protoplasm. Saponins are said to have a variety of health benefits. Saponin has been shown to have beneficial effects on blood cholesterol levels, bone health, and immune system activation in studies (Rao and Sung, 1995). Plock et al. (2001) have discovered that saponin-containing plant extracts had anti-disease properties. The extract has potentially active compounds, according to the results of the phytochemical screening performed in this report. Azevedo et al. (2014) discovered that the *Costus spicatus* produces all of the metabolites

mentioned above, some of which have already been identified as having biological activity, necessitating the conduct of quantitative studies and research to demonstrate their pharmacological effects, which may aid or contribute to drug development.

## **CHAPTER FIVE**

### **5.0 CONCLUSION**

The study's results revealed the existence of potentially active compounds, suggesting that each part of the plant is beneficial to mankind and can be used as a drug precursor in the pharmaceutical industry's drug synthesis. Clinical trials are required to assess the LD50 and the amount at which the extract is toxic to humans.

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