CHAPTER ONE

1.0 INTRODUCTION

1.1Background of study

Medicinal plants have been in use for a long time in traditional medicine. In order to conserve traditional cultures, biodiversity, community health care and drug growth, ethnobotanical data on medicinal plants and their use by indigenous cultures is useful (Ajaib *et al*, 2010). After refinement and addition, the authentic knowledge of the use of medicinal plants went from one generation to another (Haq *et al*, 2011). The traditional recipes are made from either the whole plant or from their different parts, like stem, leaf, root, bark, flower, seed, etc. and also from their secondary product such as gum, resins, and latex. In the human body, through the use of chemical elements, medicinal plants communicate actively or passively with body chemistry. Until the bioactive compounds are absorbed into the blood, by circulating and manipulating the blood vapor, these constituents obtain the requisite benefits (Kolasani *et al*, 2011). Plants provide the human body with nutrients, vitamins and some hormone progenitors, proteins and energy (Antia *et al*, 2006). The study of elements in relation to indigenous medicinal plants has shown that trace elements have an important role in the fight against a variety of human foods and diseases. (Shirin *et al*, 2010).

1.2 Statement of research problem

There has not been any comparative report on the phytoconstituent of *Costus spicatus* flowers and leaves. Thus, necessitating the need to compare the phytoconstituents and highlight their distinctive phytomolecules**.**

1.3 Justification of study

Traditional health practitioners have laid claims that the therapeutic functions of the *Costus spicatus* leaves and flowers are basically one and same. Today, there's not much of a comparative study to clarify the above issue. Therefore, this project report will provide clarification on such claims.

1.4 Aim

The aim of this study is to determine and compare the phytoconstituents in the flower and leaf of *Costus spicatus* via qualitative phytochemical screening, UV spectroscopy and GC-MS analysis.

1.5 Specific objectives of the study

The specific objectives include:

1. To qualitatively determine the secondary metabolites in the aqueous extract of both flowers and leaves via qualitative phytochemical screening;
2. To identify the elemental composition of both flowers and leaves via GC-MS analysis
3. To determine the absorbance of the aqueous mixtures at varying wavelength via UV Spectroscopy analysis
4. To compare the phytoconstituents in the both extracts

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. *Costus spicatus*

2.1.1. Nomenclature

Costaceae is a flowering plant family consisting of 6 species of the genus and 110 tropical and subtropical regions species (Stevens *et al*, 2012). From their main function of the respiratory leaves with a sealed sheath, structured in a single coil up the stem, it is easy to discern species in the Costaceae, even vegetatively. Their inflorescences are usually dense, spicy-capitate, and have big bracts, and in their monosymmetric flowers they have a big labellum and single cotyledons, the pattern flowing between both the two halves of the great anther. With approximately 90 species, the genus Costus is the largest in this family. Popular English names of *C. Spicatus* reflects the habit of growth in which the stems spiral like a corkscrew and the leaves spiral around the main stem themselves.

2.1.2. Cultivation

C. Spicatus grows in the undergrowth of tropical forests and rainy tropical lowland. It is often grown as an ornament in gardens, woods and along sides of the roads, sometimes in warm and humid conditions (Quintans Junior et al., 2010).

C. Spicatus is a medicinal and ornamental herbaceous plant that is currently classified only in Cuba as invasive and in St Lucia as potentially invasive. This plant has dense rhizomes which, once established, are very hard to eradicate. In addition, it is able to grow in full sunlight as well as in heavily dappled light in the undergrowth of the forest area. This situation allows the species to annex both untreated and treated forest resources. (Maas *et al*, 1972).

C. Spicatus is native to the Caribbean region: the islands of Dominica, Guadeloupe, Martinique, the Dominican Republic and Puerto Rico in particular. (Maas *et al*, 1972). It is mainly grown primarily as a decorative and therapeutic herb in tropical and subtropical regions.

There are some differences in the distribution between sources, likely due to a misunderstanding with other Costus species, indicating that the species is indigenous to the Brazilian Atlantic Rainforest. (Tavares *et al*., 2012).

2.1.3 Morphology of leaves

Plants up to 2.5 m tall; folds 1-2 cm in diameter, glabrescent; truncated ligules, 2-10 mm in length; petioles 2-10 mm in length, pubertal to glabrous; blades of leaves broadly elliptic, 7-33, 3.5-8.5 cm or more, shortly acuminated at the tip, rounded at the bottom to cordate, fibrous on both sides(Acevedo-Rodriguez *et al.* 2005).

2.1.4 Morphology of the flowers

Ovoid to cylindrical inflorescence, 5-27 3-4.5 cm; bracts greenish or reddish on the exposed part, reddish on the covered part, broadly ovate, 2-4 cm long and wide, obtuse on the apex, glabrous and coriaceous, fiber-lacerating margin of the covered part; bracts 1,7-3 cm long. 9-16 mm long CALYX. Yellow to pink corolla, 4-5 cm long, glabrous, 1 cm long tube, narrowly obovate lobes, 3-5 cm long. Labellum yellow, 2.5-5 cm long and wide, widely oblong-obovate when spread out, the lateral lobes rolled inward and formed a slender tube, crenulating the margins. Narrowly elliptic, 3-4 cm long stamen; 7-8 mm long anther. Ovary 4-9 mm long, sericeous or rarely glabrous. Capsule ellipsoid, 10-15 mm long; seeds black (Acevedo-Rodriguez *et al.* 2005).

2.1.5 Ethnobotanical usage

2.1.5.1 Leaves

Costus spicatus is a genus commonly used for the treatment of renal diseases in traditional Brazilian medicine. An ethno - medicinal study of the Dominican group in New York City found the popular need for tea from the insulin plant to relieve hyperglycemia. Costus spicatus was known as Insulina (Keller, 2009). Not much is known for specific usage of the flowers as it mostly used alongside with the leaves or it is left out.

2.1.5.2 Flowers

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-neohesperidosídeo quercetin (Silva *et al*, 2000). In its chemical composition is also recorded the presence of oxalic acid, tannins, saponins, mucilages and pectin(Silva *et al*, 2000).

2.1.6 Pharmacology

2.1.6.1 Leaves

C. spicatus leaf aqueous extract has a significant inhibitory effect on edema and migration of inflammatory cells, mainly at the lowest concentrations tested. Moreover, CSE(*costus spicatus* extract) significantly reduced Bothrops atrox venom(BAV)-induced nociception in the first and second phases(. CSE presumably acts through a central inhibitory mechanism while inhibiting prostaglandin synthesis as well. The research confirmed the conventional application of *C. Spicatus* leaf application, as well as those incurred by snake venomous bites, against inflammatory diseases, emphasizes the value of common awareness in the quest for complementary therapies for snakebites (Picanço, 2016).

2.1.6.2 Flowers

Costus spicatus flowers through phychemical analysis is shown to have compound like alkaloids which interact in cardiovascular sessions of the body systems of Ca^{2+} ions (Qian, 2002). Although the associations of terpenes that could create synergies with pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, pseudomonas and bacterial diseases (including Staphylococcus aureus immune to methicillin) could be handled.

2.2 Phytochemicals

Phytochemicals are non-nutritious plant chemicals which have properties that prevent or defend against disease. Plants produce phytochemicals to protect themselves but recent research demonstrated that they can also protect humans against diseases (Ralfelson *et al.*, 1980).

2.2.1 Alkaloids

Alkaloids are the natural nitrogenous compounds present in plants and are known to be protein-containing substances that disintegrate. Solid vegetable toxins and morphine are also found in the alkaloids. Compounds such as caffeine and theobromine which are closely linked to natural purine (a substance which can be processed into uric acid) are generally known as alkaloid (Hamilton, 1995).

2.2.2 Tannins

Tannins are astringent, sour plant phenolics that either link or degrade proteins and condense them (Harold, 2004). The word 'tannin' relates with the use of animal hides for leather tanning; however, the term is commonly applied to any significant phenolic acids compounds

that contain enough hydroxyl and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3,000 (Harold, 2004). Everywhere throughout the plant kingdom, they are propagated. Both gymnosperms and also angiosperms are usually observed. Tannins are usually found in the plants' vesicles or surface wax. These sites are where tannins do not interact with the life cycle of plants, so it's only after apoptosis that the biochemical influences of tannins are involved. There are tannins in the plant leaves, bud tissues, seed tissues, rhizosphere tissues, and stem tissues. They are also present in coniferous heartwood and can play a part in safeguarding microbial activity, contributing to the natural resilience of the wood. (Harold, 2004).

Tannins may be employed medicinally as antidiarrheal, hemostatic, and antihemorrhoidal agents (Harold, 2004). All signs of gastritis, esophagitis, enteritis, and upsetting bowel disorders are regulated by the anti-inflammatory effect of tannins (Harold, 2004). Not only can tannins treat burns and heal wounds, but they also stop infection. The capacity of tannins to form a layer of protection over the tissue compromised prevents the injury from any further infection. Tannins may also be used to remove toxins from poison oak or bee stings, resulting in immediate relief. Tannins help eliminate all skin irritants because tannin is an astringent that tightens pores and extracts fluids from the skin (Bajaj, 1999).

2.2.3 Anthraquinones

Anthraquinone (9, 10-dioxoanthracene) is an aromatic organic compound. It is a derivative of anthracene which exists as a crystalline powder of yellow or light grey to grey-green solids. It is semi soluble in water but dissolves in alcohol, aniline and nitrobenzene. Chemically, under normal conditions, it is reasonably stable (Macleod, 1943).

In certain plants (e.g. aloe, senna, rhubarb, and Cascara buckthorn), fungi, lichens, and insects, anthraquinones naturally occur where they act as a simple skeleton for their pigments. The laxative effects of natural anthraquinone derivatives appear to be present (Muller- Lissner, 1993).

2.2.4 Saponins

Saponins are a class of chemical compounds, one of several secondary metabolites present in natural sources, with a particular abundance of saponins found in different types of plants. The word sapon means' soap,' referring to the saponins of permanent froth that are combined with water. In the carnation family, the quintessential saponin-producing plant is Soapwort, Saponaria officinalis, also called Bouncing Bet. They are found in different parts of the plant: leaves, stems, roots, bulbs, blossom, and berries. Their bitter taste and their tendency to hemolyze red blood cells define them. (Dharmananda, 2000). To maintain a solid soapy foam, saponins absorb moisture; this is believed to be due to their amphiphilic character. The plant contains mildly poisonous saponins, as reflected from the genus name from the Latin word sapo, meaning soap (Dharmananda, 2000).

Saponins are medically used in the treatment of prolonged salivation, epilepsy, chlorosis, and migraines as an expectorant, emetic, and contraceptive. Saponins inhibit some kinds of cancer cell tumor growth in animals, particularly lung and blood cancers, without killing normal cells (Dharmananda, 2000). Due to the aphrodisiac property of saponin, it serves as precursors for sexual hormones like testosterone, corticosterone, aldosterone, progesterone, estrogens and androgens (Abo-Doma *et al.*, 1991). Steroidal saponins therefore boost the level of sex hormones in the body as well as trigger their actions, hence, its use as aphrodisiacs. The aphrodisiac nature

of steroidal saponins makes it useful for the treatment of impotency and it is also used for muscle building by wrestlers and weight lifters (Kintia *et al.,* 1996).

2.2.5 Phenolics

A series of chemical compounds comprising of a hydroxyl group (-OH) bound to an aromatic hydrocarbon group are phenols, also referred to as phenolics. The simplest of the class is phenol (C6H50H). Although alcohols are similar, Phenols have distinctive characteristics and are not known as alcohols (since the hydroxyl group is not linked to a saturated carbon atom). Because of the tight coupling of the aromatic ring with oxygen and a comparatively loose bond between oxygen and hydrogen, they have relatively higher acidity. The acidity between carboxylic acids and aliphatic alcohols in the hydroxyl group in phenols is typically intermediate (their pKa is usually comprised between 10 and 12). The displacement of a positive hydrogen ion (H+) from hydroxyl group of a phenol is formed by a negative phenolate ion. (Carmen, 2006).

2.2.6 Flavonoids

Flavonoids are compounds with strong antioxidant properties that are plant-based. Flavonoids are widely dispersed in plants that perform many functions, including the cultivation of flowers with yellow or red/blue pigmentation and defense from microbial and insect attacks. The rich diversity of flavonoids, their abundance and their comparatively low toxicity imply that many species, including humans, consume significant quantities in their diets compared to most other active plant products (for example, alkaloids).

They are synthesized by a phenylpropanoid metabolic pathway in which 4-coumaroyl-CoA is produced using the amino acid phenylalanine. This can be combined with malonyl-CoA,

a group of compounds called chalcones that contain two phenyl rings, to yield the true backbone of flavonoids. The familiar shape of flavonoids, the three-ringed structure of a flavone, results in the conjugate ring-closure of chalcones.

The metabolic pathway for the development of lavabo dihydroflavonols, anthocyanins, continues via a series of enzymatic modifications. Many products can be developed along this path, including flavonols, flavan-3-ols, proanthocyanidins (tannins) and a host of other polyphenolics (Lotito, 2006).

Due to clear scientific evidence of their intrinsic ability to alter the body's reaction to allergens, pathogens, and carcinogens, flavonoids have been referred to as "nature's biological response modifiers" They illustrate anti-allergic, anti-inflammatory, anti-microbial and anti-cancer behaviors (Filippos *et al.,* 2007). Most commonly, flavonoids are known for their antioxidant function. The human body absorbs flavonoids poorly (less than 5 percent), and much of what is ingested is rapidly metabolized and eliminated from the body (Lotito, 2006).

2.2.7 Terpenoids

Terpenes are a broad and diverse class of organic compounds that are usually obtained via a wide range of plants and consisting of six isoprene units with a molecular formula of $C_{30}H_{48}$. Terpenes are a large and diverse group of hydrocarbons, produced primarily by a wide range of plant species, especially conifers. They are the key components of resin and resin-produced turpentine. Terpenes are important biosynthetic building blocks within almost every living species, in respite to their functions as end products in many organisms.

The key component of shark liver oil, the linear terpene squalene, is obtained from the reduced coupling of two farnesyl pyrophosphate molecules. Squalene is then biologically

processed to create either lanosterol or cycloartenol, the progenitors of all steroids structurally (Carmen, 2006).

Terpenes and terpenoids are the principal components of the natural products of all types of plants and flowers. Sterol variants and currently a plant sterol are a variety of animal hormones; brassinolide has indeed been found to impact plant growth (Carmen, 2006). Terpenes frequently exhibit bioactivity as antifungals, antivirals, and antibacterials.

2.2.8 Steroids

Steroids are secondary plant metabolites consisting of twenty carbon atoms bound together to form four fused rings: three rings of cyclohexane (rings A, B and C in the figure to the right) and one ring of cyclopentane (the D ring). The steroids differ according to the functional groups attached to this four-ring center and the rings' oxidation state. (moss, 1989). Steroidal compounds are of importance and interest in pharmacy because they serve as precausor for sex hormones (Okwu, 2001).

2.3 UV-Spectrosopy

This method of absorption spectroscopy is premised on the reality that when radiation passes through a material, the material will absorb a part of the radiation. This absorption of various radiation energy levels (wavelengths) depends on the different levels of electrical energy displayed by the molecules contained in the material (Eric S, 2008).

Principle of UV-Spectrosopy

It is guided by 2 major laws.

1. Beer's Law, which states that the absorbance is proportional to the absorbing material concentration.
2. The Law of Lambert implies that the level of light absorbed is proportional to the density of the absorber (length) (Cuvette).

Ultraviolet-visible (UV-vis) spectroscopy, primarily for assessing concentrations of identified solutes, is a method most widely used for quantitative analysis of solutions. However, useful insights can also be provided by its implementation to solid-state samples. Its relative ease of it's use, cost-effectiveness, and sequencing speed are among the main benefits of UV-vis spectroscopy. Hence, for solid evidence work or to direct early exploration, it can be a good starting point.

The mechanical concepts that underlie this technique are basic, rendering the equipment easy and powerful. Light of known wavelength and intensity is directed at the sample and its final intensity, after passing through, is measured by a detector. By comparing the incident radiation (I0) and the transmitted radiation (I), the amount of light absorbed by the sample at that particular wavelength can be easily calculated.

2.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

GC-MS is an analytical technique that combines the separation properties of gas-liquid chromatography with the detection characteristics of mass spectrometry to identify different substances within a test sample (Chauhan *et al*., 2014). GC can distinguish with great resolution volatile and semi-volatile compounds, but it can not recognize them while mass spectrometry can provide extensive structural details on most compounds so that they can be accurately classified, but it can not easily separate them (Hussain and Maqbool, 2014). It is used in the determination

of drugs and metabolites in the pharmaceutical area, and molecular weights and elemental composition in complex mixtures. It is used to evaluate the combination of volatile and semi-volatile organic molecules. (Sneddon *et al*., 2007).

Principle of GC-MS

GC/MS combines of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS) to analyze difficult organic and biochemical mixtures. The GC-MS equipment has two main components. Based on their volatility, By circulating an inert gas (mobile phase) that transports the substance through a stationary phase established in the column, the gas chromatography portion distinguishes different compounds in the sample into surges of pure chemicals. Wavelengths of compounds are gathered by the mass spectrometer as they leave a chromatographic column. Inside the mass spectrometer, phase ion separation is accomplished using electrical and/or electromagnetic waves to separate ions. As per their mass-to-charge ratio (m/z), the spectrometer defines and takes into account the chemicals. Such wavelengths could then be monitored and analysed on the computer (Hussain and Maqbool, 2014). In a chromatogram, a plot of this signal as a function of time produces a series of symmetrical peaks, providing some information on the composition of the sample. The retention time of the peaks can help to differentiate the particles by contrasting them with the retention time of a certain norm, whereas the heights of the peaks or the area under the peaks can provide relative evaluation of the total of component (Hussain and Maqbool, 2014).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1 Collection of Plant Material

Fresh flowers of *Costus spicatus* plant were collected at the chapel, library and hostel area of Mountain Top University. The plant was identified at the Botany Department of the University of Lagos, where a voucher specimen (Number 8571) was prepared and deposited.

3.2. METHODOLOGY

3.2.1. Preperation of Aqueous Extract

The identified sample were toughly rinsed under running water to remove contaminants, oven dried at 50 degree Celsius when it attains a constant weight it was pulverized using an electric blender . The Pulverized flower (350g) was weighed and 2600ml of distilled water, kept in a container with a lid and placed in a cupboard for 48hours. It is then sieved with a cheesecloth the extract was poured in a container, watman's filter paper and funnels where then used to further separate concentrate from the residue .The residue was collected in beakers and placed in the oven to concentrate the extract which yielded 47.57g (15.85% yield). The concentrates was then stored in a refrigerator at −4°C.

3.2.2 Qualitative Phytochemical analysis

The aqueous flower extract was tested for the presence of bioactive compounds using standard methods as described by Trease and Evans (1989) with slight modification.

Test for alkaloids (Mayer's test)

0.5 g of methanol leaf extract of *V. amygdalina* (crude extract) was dissolved in 5mls of distilled water. 2 ml of 1% hydrochloride (HCl) was added and heated gently. 3 ml of Mayer's reagent was then added to the mixture. Turbidity of the precipitate shows the presence of alkaloids.

Test for carbohydrates (Molisch's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2mls of Molisch reagent was added and the mixture was shaken properly. 2 ml of conc. sulphuric acid (H_2SO_4) was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase shows the presence of carbohydrate.

Test for saponin (Froth test)

0.5 g of crude extract was diluted with distilled water to 20 ml and was shaken in a graduated cylinder for 5 mins. Formation of foam indicated the presence of saponin.

Test for terpenoids (Salkowski's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2 ml of chloroform was added and 3 ml of conc. H_2SO_4 was meticulously added to which forms a layer. The reddish brown color which appears at the interphase indicates the presence of terpernoids.

Test for phenol (Ferric chloride test)

0.5 g of crude extract was dissolved in 5 ml of distilled water and 4 drops of ferric chloride ($FeCl_3$) solution was added. The formation of bluish black color represents the presence of phenol.

Test for tannin

0.5 g of crude extract was dissolved in 5 ml of distilled water and 2ml of 2% $FeCl_3$ solution was added. The blue-green coloration formed represents the presence of tannin.

Test for protein (Ninhydrin test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2 ml of 0.2% Ninhydrin reagent was added and the mixture was boiled for 5 mins. The formation of violet/blue color indicated the presence of amino acids.

3.2.3 GC-MS ANALYSIS

The GC-MS analysis was performed using a Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and a 250 °C MS transfer line temperature injector of the Hewlett Packard 7683 series. The GC was equipped with a capillary fused silica column- HP-5MS (30 x 0.25 mm), 1.0 µm film thickness. The oven temperature was maintained at 50 ° C for 5 min of holding time and increased at a rate of 2 ° C / min from 50 to 250 ° C, using helium gas (99.999 percent) as a carrier gas at a steady flow rate of 22 cm / s. 1.0 microns of extract was injected (1 mg dissolved in 1 ml of absolute alcohol) at a 1:30 split ratio. Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled with Hewlett Packard Gas Chromatograph (Model 6890 series) with NIST08 Library software database was analyzed by MS. Mass spectra were taken at 70 eV/200 °C, 1 scan/s scanning rate. Compound recognition was carried out using the NIST08 Library database. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Library (Ajayi *et al.*, 2011).

3.2.4 UV-visible spectrophotometry

The absorbance and wavelength of the peaks were determined for the aqueous flower extract by a wavelength scan between 200 and 227 nm (Rice-Evans and Miller, 1996). The UV-visible spectra were recorded on a (Lamotte SMART SPECTRO 2, China) UV-Vis spectrophotometer.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Analysis

The result of the phytochemical analysis carried out on the aqueous extract of *C. spicatus* flower and leaves revealed the presence of some important secondary metabolites as shown in Table 1 and 2.

Table 1: Qualitative analysis of aqueous extract of *Costus spicatus* leaves

SECONDARY METABOLITES	LEAVES
Carbohydrate	++

Tannins	- -
Alkaloids	++
Phenols	- -
Flavonoids	++
Proteins	++
Terpenoids	- -
Anthraquinone	- -

Present ++; Absent --

Table 2: Qualitative analysis of aqueous extract of *Costus spicatus* flower

SECONDARY METABOLITES	FLOWER
Carbohydrate	++
Tannins	--
Alkaloids	++
Saponins	++
Terpenoids	++
Phenols	++
Protein	++

Present ++; Absent --

4.2 UV-spectroscopy analysis of aqueous extract of *C. spicatus* flower

The Ultra violet -Visible spectroscopy of aqueous extract *Costus spicatus* flower revealed the varying absorbance of the flower extract at different wavelengths as shown in Figure 2 and 3

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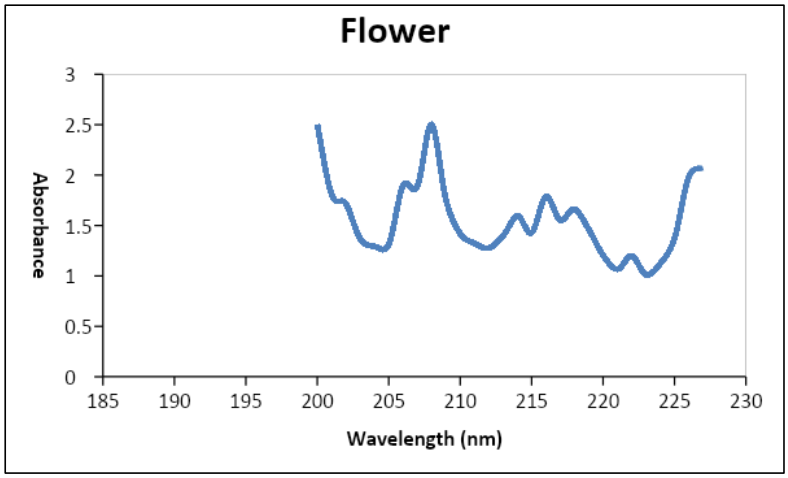

Figure 1: Ultra violet -Visible spectroscopy of *Costus spicatus* flower

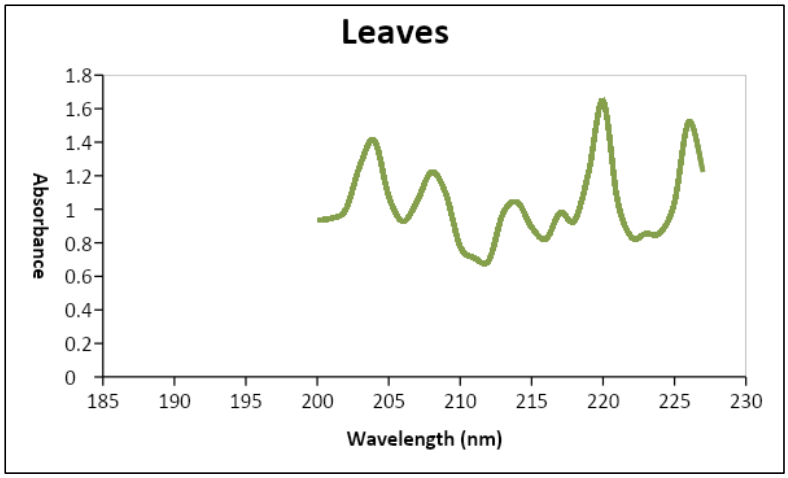

Figure 2: Ultra violet -Visible spectroscopy of *Costus spicatus* leaves

4.3 Gas chromatography-Mass spectrometry (GC-MS) analysis

4.3.1 Chromatogram of aqueous extract of *C. spicatus* flower and leaves

Figure 2 shows the GC-MS chromatogram of aqueous extract of *C. spicatus* flower. Peak 1 with the retention time of 15.279 was identified as Hexadecanoic acid, methyl ester and as the major phyto-component of *C. spicatus* flower while the other peaks were of other phyto-components present in the plant. The peak of value of leaves is 5.55 amd was identified as Bicyclo[4.1.0]heptane, 3-methyl-(C_8H_{14}) Figure 3 and 4.

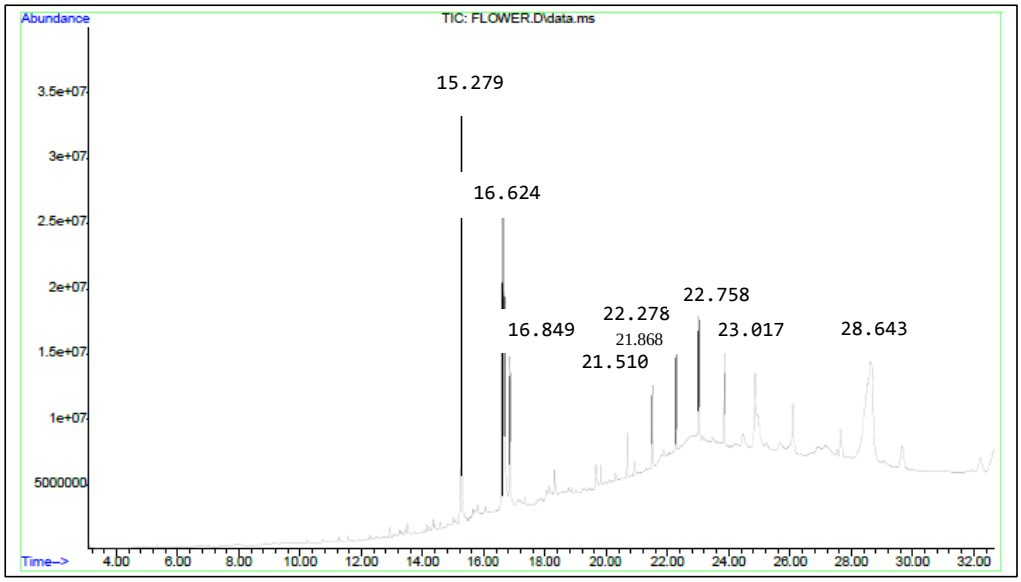

Figure 3: GC-MS chromatogram of aqueous extract of *C. spicatus* flower

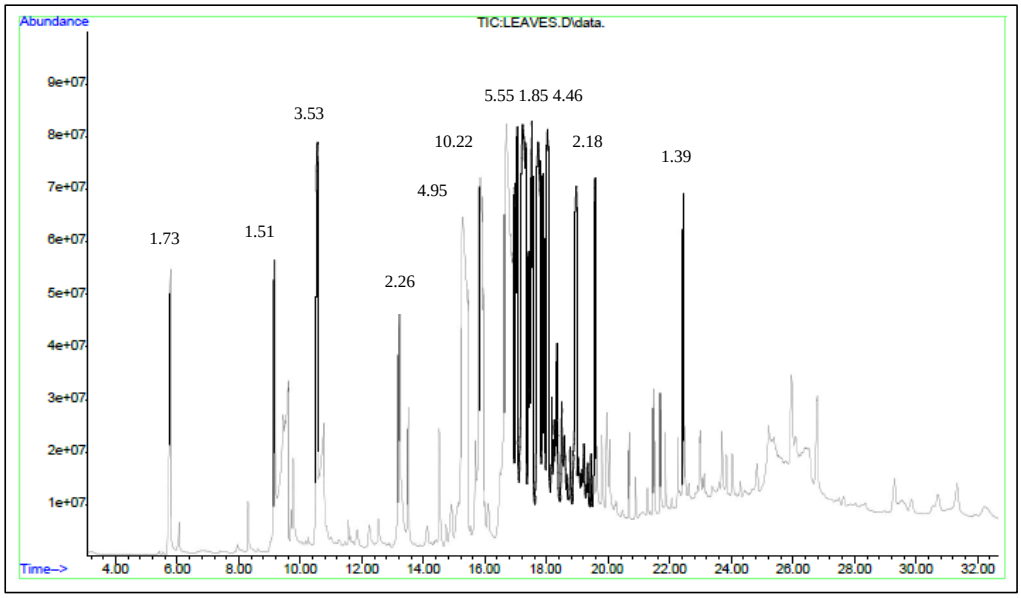

Figure 4: GC-MS chromatogram of aqueous extract of *C. spicatus* leaves

4.3.2 Phyto-component of aqueous extract of *C. spicatus* flower and leaves

Other phytochemical were recorded in the flower and they include Hexadecanoic acid, 9,12-Octadecadienoic acid, Cyclononasiloxane, octadecamethyl, Carbonic acid, but-3-en-1-yl penta decyl ester, [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester and Cyclohexane, 1,1'-(2-methyl-1,3 propanediyl)bis-.

While for the leaves the phyto-components include1-Decyne,Heptanoic acid, ethyl ester,Hepta-4,6-dienoic acid, ethyl este-1-Octyne,Diethyl azelate,Hexadecanoic acid, methyl ester,Hexadecanoic acid, methyl ester,10,13-Octadecadienoic acid, methyl ester, Bicyclo[4.1.0]heptane, 3-methyl-, E,E,Z-1,3,12-Nonadecatriene-5,14-diol and 1-Methyl-2-methylenecyclohexane

Table 3: Phytochemical components identified by GC-MS in aqueous extract of *C. spicatus* flower showing the peak, retention time, library ID, % of total and chemical formula

Peak	Retention time	Library ID	Area %	Chemical formula
1	15.279	Hexadecanoic acid, methyl Ester	11.34	$C_{17}H_{34}O_2$
2	16.624	9,12-Octadecadienoic acid, methyl ester, (E,E)-	13.21	$C_{19}H_{34}O_2$
3	21.510	Cyclononasiloxane, octadecamethyl	1.49	$C_{18}H_{54}O_9Si_9$
4	21.868	Carbonic acid, but-3-en-1-yl penta decyl ester	1.62	$C_{15}H_{28}O_3$
5	22.278	Cyclononasiloxane, octadecamethyl	2.11	$C_{18}H_{54}O_9Si_9$
6	22.758	[1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl	2.47	

		ester		
7	23.017	Cyclononasiloxane, octadecamethyl-	3.28	$C_{18}H_{54}O_9Si_9$
8	28.643	Cyclohexane, 1,1'-(2-methyl-1,3 propanediyl)bis-	18.66	$C_{16}H_{30}$

Table 4: Phytochemical components identified by GC-MS in aqueous extract of *C. spicatus* leaves showing the peak, retention time, library ID, % of total and chemical formula

Peak	Retention time	Library ID	Area %	Chemical formula
1	5.790	1-Decyne	1.73	$C_{10}H_{18}$
2	9.134	Heptanoic acid, ethyl ester	1.51	$C_9H_{18}O_2$
3	10.554	Hepta-4,6-dienoic acid, ethyl este-1-Octyne	3.53	$C_9H_{14}O_2$
4	13.217	Diethyl azelate	2.26	$C_{11}H_{20}O_4$
5	15.267	Hexadecanoic acid, methyl ester	4.95	$C_{18}H_{36}O_2$
6	15.400	Hexadecanoic acid, methyl ester	1.37	$C_{18}H_{36}O_2$
7	16.676	10,13-Octadecadienoic acid, methylester	10.22	$C_{19}H_{34}O_2$
8	17.046	Bicyclo[4.1.0]heptane, 3-methyl-	2.75	C_8H_{14}
9	17.219	E,E,Z-1,3,12-Nonadecatriene-5,14-d Iol	5.55	$C_{19}H_{34}O_2$
10	17.727	1-Methyl-2-methylenecyclohexane	4.46	C_8H_{14}

CHAPTER FIVE

5.0 Discussion, Conclusion and Recommendation

5.1 Discussion

Costus spicatus has many medicinal and Therapeutic action and scientifically validated documents. The phytochemical analysis shows that not all phytochemicals are present in both the leaves and flowers which leads to some distinctions in their pharmacological activities. Due to more presence of flavonoids they have more of an anti-diabetic roles in the system because of its antioxidants and hypoglycemic properties as reported in an article by Nascimento.

The flower, due to more presence of saponins, terpenoids and alkaloids exhibits more of anti-inflammatory, antifungal, antibacterial properties.

GC-MS Analysis of the flower shows that the retention time of 15.279 was identified as Hexadecanoic acid, methyl ester and as the major phyto-component of *C. spicatus* while the other peaks were of other phyto-components present in the plant. The peak of value of leaves is 5.55 and identified as Bicyclo[4.1.0]heptane, 3-methyl-(C8H14).

Absorbance of the flower, results showed that the highest absorbance was recorded at 2.5 and lowest to 1.0 while the leaves exhibit highest absorbance of 1.6 at wavelength of 220nm and lowest absorbance was recorded as 0.7 at 212nm.

Comment [1]: Rewrite this and expanciate on the wavelengths. Support your claims with literature as well

5.2 Conclusion

The comparative study on chemical constituents identified aqueous extract of the plant of *Costus spicatus* via qualitative phytochemical screening, UV-Spectroscopy and Gas Chromatogram-Mass spectrometry (GCMS) analysis reveals the unique phytomolecules and compounds in the flower and leaves which has potential biological importance. Therefore, further studies of the flower and leaves of this plant's phytochemicals via *in silico*, *in vitro* and *in vivo* methods will prove its medicinal importance. This can lead to the discovery of more cheaper and effective drugs that has higher biomass availability.

5.3 Recommendation

Further research should be carried out on the comparative studies on the mineral elements and amino acid composition in the flowers and leaf of *Costus spicatus*..

REFERENCES

1. Ajaib, M., Khan, Z., Khan, N. A. S. R. U. L. L. A. H., & Wahab, M. (2010). Ethnobotanical studies on useful shrubs of district Kotli, Azad Jammu & Kashmir, Pakistan. *Pak. J. Bot*, *42*(3), 1407-1415.
2. Haq, F., Ahmad, H., & Alam, M. (2011). Traditional uses of medicinal plants of Nandiar Khuwarr catchment (District Battagram), Pakistan. *Journal of Medicinal Plants Research*, *5*(1), 39-48.
3. Kolasani, A., Xu, H., & Millikan, M. (2011). Evaluation of mineral content of Chinese medicinal herbs used to improve kidney function with chemometrics. *Food Chemistry*, *127*(4), 1465-1471.
4. Antia, B. S., Okokon, J. E., Nwidu, L. L., & Jackson, C. L. (2006). Effect of subchronic administration of ethanolic stembark extract of Mammea africana Sabine on haematological and biochemical parameters of rats. *African Journal of Biomedical Research*, *9*(2)..
5. Shirin, K., Imad, S., Shafiq, S., & Fatima, K. (2010). Determination of major and trace elements in the indigenous medicinal plant Withania somnifera and their possible correlation with therapeutic activity. *Journal of Saudi Chemical Society*, *14*(1), 97-100..
6. Specht, C. D., Yockteng, R., Almeida, A. M., Kirchoff, B. K., & Kress, W. J. (2012). Homoplasy, pollination, and emerging complexity during the evolution of floral development in the tropical gingers (Zingiberales). *The Botanical Review*, *78*(4), 440-462..

7. Quintans Junior, L. J., Santana, M. T., Melo, M. S., de Sousa, D. P., Santos, I. S., Siqueira, R. S., ... & Santos, M. R. (2010). Antinociceptive and anti-inflammatory effects of Costus spicatus in experimental animals. *Pharmaceutical biology*, *48*(10), 1097-1102.
8. Valderrama, E., Sass, C., Pinilla-Vargas, M., Skinner, D., Maas, P. J., Maas-van de Kamer, H., ... & Specht, C. D. (2020). Unraveling the spiraling radiation: A phylogenomic analysis of Neotropical Costus L. *Frontiers in plant science*, *11*, 1195.
9. Tavares, W. D. S., Salgado-Neto, G., Legaspi, J. C., Ramalho, F. D. S., Serrão, J. E., & Zanuncio, J. C. (2012). Biological and ecological consequences of Diolcogaster sp.(Hymenoptera: Braconidae) parasitizing Agaraea minuta (Lepidoptera: Arctiidae) and the effects on two Costus (Costaceae) plant species in Brazil. *Florida Entomologist*, *95*(4), 966-970.
10. Hallahan, D. L., West, J. M., Wallsgrove, R. M., Smiley, D. W., Dawson, G. W., Pickett, J. A., & Hamilton, J. G. (1995). Purification and characterization of an acyclic monoterpene primary alcohol: NADP+ oxidoreductase from catmint (Nepeta racemosa). *Archives of Biochemistry and Biophysics*, *318*(1), 105-112.
11. Harold, E. R., Means, W. S., & Udemadu, K. (2004). *XML in a Nutshell* (Vol. 3). Sebastopol, CA: O'reilly.
12. Bajaj, L., Takai, M., Ahuja, R., Tang, K., Bagrodia, R., & Gerla, M. (1999). Glomosim: A scalable network simulation environment. *UCLA computer science department technical report*, *990027*(1999), 213.
13. MacLeod, J. (1943). The role of oxygen in the metabolism and motility of human spermatozoa. *American Journal of Physiology-Legacy Content*, *138*(3), 512-518.

14. Müller-Lissner, S. A. (1993). Adverse effects of laxatives: fact and fiction. *Pharmacology*, *47*(Suppl. 1), 138-145.

15. Dharmananda, S. (2000). *Platycodon and other Chinese herbs with triterpene glycosides*. ITM.

16. Doma, M. A. (1991). Effect of onion and garlic oils as well as some of their sulphur constituents on lipid metabolism in albino rats. *J. Drug Res. Egypt*, *20*, 1-11.

17. Kintia, P. K. (1996). Chemistry and biological activity of steroid saponins from Moldovian plants. In *Saponins used in traditional and modern medicine* (pp. 309-334). Springer, Boston, MA.

18. Okwu, D. E. (2001). Evaluation of chemical composition of indeginous species and flavouring agents. *Global Journal of Pure and Applied Sciences*, *7*(3), 455-460.

19. Chauhan, A., Goyal, M. K., & Chauhan, P. (2014). GC-MS technique and its analytical applications in science and technology. *J. Anal. Bioanal. Tech*, *5*(6), 222.

20. Keller, A. C., Vandebroek, I., Liu, Y., Balick, M. J., Kronenberg, F., Kennelly, E. J., & Brillantes, A. M. B. (2009). Costus spicatus tea failed to improve diabetic progression in C57BLKS/J db/db mice, a model of type 2 diabetes mellitus. *Journal of ethnopharmacology*, *121*(2), 248-254.

21. Picanço, L. C. D. S., Bittencourt, J. A. H. M., Henriques, S. V. C., da Silva, J. S., Oliveira, J. M. D. S., Ribeiro, J. R., ... & Silva, J. O. D. (2016). Pharmacological activity of Costus spicatus in experimental Bothrops atrox envenomation. *Pharmaceutical biology*, *54*(10), 2103-2110.

22. Nascimento, C. C. H. C., Vasconcelos, S. D., Camacho, A. C. L. F., Nascimento, S. F., Oliveira, J. F. F., Nogueira, R. I., ... & Diré, G. F. (2016). A literature review on the medicinal properties and toxicological profile of Costus spicatus plant.

23. Jia-Qing, Q. I. A. N. (2002). Cardiovascular pharmacological effects of bisbenzylisoquinoline alkaloid derivatives. Acta Pharmacol Sin, 23, 1086-92.