STUDIES ON THE STEM SAP AND FLOWERS OF TWO VARIETIES OF COCOYAM; *Xanthosoma sagittifolium* AND *Colocasia esculenta.*

BY

AMAECHI PATRICK CHIKAIKE(B. TECH, FUTO)

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A THESIS SUBMITTED TO POSTGRADUATE SCHOOL

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI IMO STATE

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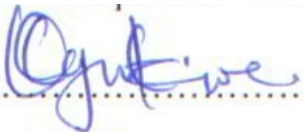
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc.) IN ANALYTICAL CHEMISTRY

DECEMBER, 2016.

CERTIFICATION

This is to certify that this research work on ‘’ Studies on the flowers and the stem sap of two varieties of cocoyam; *Xanthosoma sagittifolium* and *Colocasia esculenta*’’ was carried out by Amaechi Patrick Chikaike with Registration Number 20124763568 in partial fulfillment of the requirements for the award of the degree of Master of Science (M.Sc.) in Analytical Chemistry in the Department of Chemistry, Federal University of Technology Owerri Imo State.



…….. 12/07/2016

Dr. (Mrs.) C. E Ogukwe Date

(Supervisor)

…….. 12/07/2016

Dr.C. K Enenebeaku Date

(Co-Supervisor)

…… 17/08/2016 .

Prof. M. O. C Ogwuegbu Date

(Head of Department)

…….. 18/08/2016 ….

Prof. B.C Anusionwu Date

(Dean School of Physical Science)

…….. 6/12/16 …….

Prof. S.A. Odoemenam Date

(External Examiner)

DEDICATION

This research work is dedicated to Almighty God and to my amiable parents, Chief Sir& Lady G.U Amaechi for their indefatigable encouragement and financial support.

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ABSTRACT

This study is focused on the analysis of the flower extracts and stem extracts (sap) of two varieties of cocoyam: *Xanthosoma sagittifolium* and *Colocasia esculenta.* Phytochemical analysis was carried out on the flowers and stem sap. The proximate analysis was also carried out on the flowers and T-test statistical tool was employed. Gas Chromatography-Mass Spectrophotometric (GC-MS) evaluation was performed using GCMS-QP2010 (SHIMADZU). Standard methods and conditions were employed in this analysis. Phytochemical analysis of *X. sagittifolium* and *C. esculenta* revealed that the flowers are rich in saponins (6.61% and 5.50% respectively), alkaloids (6.20% and 9.80% respectively) and phenolic compounds which were deeply present in both flowers. The stem extracts of *X. sagittifolium* and *C. esculenta* contains more saponins (4.71% and 4.20%, respectively), and alkaloids (4.31% and 5.50%, respectively). The proximate analysis of *X. sagittifolium* and *C. esculenta* revealed that the flowers posses high protein content (37.87% and 22.56%, respectively), high moisture content (16.10% and 16.35%, respectively). *C .esculenta* showed high percentage of total carbohydrate of 25.11%. T-test statistical tool at (P = 0.05) shows that there is no significant difference between the values of the proximate analysis except in crude protein of the two species and total carbohydrates. It also revealed that there is no significant difference between the quantitative phytochemical analysis result values between the two species except in saponins content of the two flowers and tannin contents in the two stems. GC-MS analysis revealed that the flowers of the two varieties contain phenolic compound and alkaloids. The stem extracts (saps) of the two varieties contains high percentage concentration of Permethrin. Studies on the reactive effects of Permethrin as a compound has shown that the itchiness of cocoyam stems could therefore be attributed to the presence of Permethrin in the stem extract.

Keywords: *Xanthosoma esculenta*, *Colocasia esculenta*, GCMS, proximate, phytochemicals, permethrin

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Cocoyam is a herbaceous perennial plant belonging to the family Araceae. It constitutes one of the basic food crops of major economic importance in Nigeria. Cocoyam ranks the third after cassava and yam in terms of total production, land area under crop and consumption (Chukwu and Nwosu, 2008). It is a root crop cultivated mainly for the edible corms (tuber), although the leaves, petioles and the flowers are used in soup preparation. The corms and the cormels can be boiled or baked and consumed in different forms as soup thickeners, pounded cocoyam, can also be roasted in fire as well as prepared as porridge (Ajala and Obiechina, 1990).

Cocoyam is usually classified as edible aroids. According to FAO (2006), it is generally classified as *Colocasia esculenta* commonly known as taro and *Xanthosoma sagittifolium* which is known as tannia. They are the most important specie of edible aroids grown in tropical and subtropical countries. They contribute significantly to the carbohydrate diet, even though ranked less important after yam, cassava and potatoes (Obomeghei, Obafemi and Oyibo 1998). Cocoyam is used mainly for human food and it is commonly grown amongst small scale farmers who operate within the subsistence economy (Enwelu, Asogwa, Nwalieji and Ezeano,2014).

Colocasia esculenta is considered by most botanists to be polymorphic species with several botanical varieties. The two main varieties of *Colocasia esculenta* are; *esculenta var. esculenta* which actually produces a large edible main corm and few lateral cormels and *C. esculenta var. antiquorom* which produces a small or medium size corm and a large number of edible cormels (FAO corporate document 2006). According to Purseglove (1972), *Colocasia esculenta var. esculenta* is characterized by the possession of large cylindrical central corm (tuber) and very few cormels. Agronomically, it is referred to as the dasheen type of cocoyam while the *Colocasia esculenta var. antiquorom* has small globular central corm with several relatively large cormels arising from the corm and agronomically this is referred to as the eddoe type of cocoyam. *Colocasia esculenta* is a genius of flowering plants in the family of Araceae, native of South Eastern Asia and the Indian Sub-continent. Some species are also widely cultivated and naturalized in other tropical and subtropical regions (Purseglove, 1972).

On description, *Colocasia esculenta* is a perennial herb of about 3-7feet tall, leaves are basal from a corm, blades are about 1-2feet, upper surface velvety is green to bluish-black between primary veins, petioles is between green to violet and usually produce a yellow coloured flower. In the south eastern part of Nigeria, it is called ‘’ede ofe’’ or ‘’ede cocoindia’’(Nwanekezi, Onwuamanam, Ihediohanma and Iwouno 2010)

Xanthosoma sagittifolium is commonly known as tannia and generally referred to as new cocoyam. It is a plant species which belongs to the family Araceae and grows exclusively in the tropical areas (Tropical Biodiversity). *Xanthosoma sagittifolium* (tannia) originally came from tropical America and is often confused with taro as the corms have the appearance of rather large tubers. The leaves of tannia are arrow-shaped and 45-90cm long (FAO, 2006). In south eastern part of Nigeria, it is either called ‘’ede ocha’’ or ‘’ede uhie’’ (Nwanekezi *et al*., 2010).

There are common names which are attributed to cocoyam. Purseglove (1972) identified some of these names and they are called vernacular names. Some of these vernacular names include; eddoe, dasheen, mjambe, mjimbi, colcas, alcocas, kalo (Ivancic and Lebot 2000). Some other articles related these vernacular names to the various species. For instance, yautia, tannia and malanga belong to the specie *Xanthosoma sagittifolium* while the eddoe belong to the specie *Colocasia esculenta antiquorom* (Power your diet, 2001). Other reports related these vernacular names as common and these common names are with respect to where this cocoyam is cultivated. For instance, in Azores, cocoyam is known as inhame or inhame-coco, in Bangladesh, it is known as mukhi kochu, in Brazil or other Portuguese speaking countries, the common name is inhame. In China, Honkong and Taiwan, it is called yutou. In east African countries such as Kenya, Uganda and Tanzania, cocoyam is called arrow root, nduma. In Egypt, it is called kolkas, the Indians called it taro or eddoe.

Those from Lebanon call it kilkas while the Japanese call it satoimo and the Philippines call it gabi, south Koreans call it toran (Koichaar, 2006). In West African countries such as Ghana, Nigeria and some Anglophone countries, it is called cocoyam generally. But in francophone countries such as Cameroun, it is called macabo (Power your diet, 2001). In Nigeria, cocoyam has various names; such common names include koko or poso in Yoruba, gwaaza in Hausa, akasi ite or ede aro in Igbo, mkpong or ata mkpong in Benin and then iyokho in Efik (Power your diet, 2001). In south eastern part of Nigeria, it is called ede ocha, ede uhie, ede ofe or ede cocoindia (Nwanekezi *et al*., 2010).

On the uses of cocoyam, reports have shown that the soft white flesh corm or tuber of taro can be eaten when boiled, fried or roasted or are used in making pounded cocoyam. This corm can also be used in preparing soups, beverages and puddings. The leaves and the leaf stalks are used in leafy vegetables and the pot herb for soups and sauces or as relish. These are especially popular in parts of West Africa, north-eastern India and Caribbean regions (Onwueme, 1978). The stem sap is used by Wargo as a treatment for wasp stings (Tuse, Harle and Bore 2009).

Right from the ancient times, man has seriously depended on plants for survival. From historical perspective, it is evident that man’s fascination for plants is as old as mankind itself. The plant kingdom represents rich store of organic compounds many of which have been used for medicinal purposes and some others used for food preservative and seasoning agents. This is because plants

are the richest source of drugs, foods and food supplements, spices and food flavouring agents which in turn, nourishes, vitalizes and maintains vigour (Nakade, Mahesh, Kiran and Vinayak, 2013)

However, during the 19th century and the early 20th century, people of this period usually used dried flower of cocoyam as food additive which either acts as spices. This dried flower of cocoyam was used in preparing local soups and dishes. This was used to improve the quality and the nutritional value of the meal thereby making it palatable (Agboola 1979; Ukpong, Abasiekong and Etuk 2014). Thus this dried flower of cocoyam was used in place of modern day synthetic spices. Also the effects of the stem (petioles) sap of cocoyam on the skin of farmers leads to itchiness of the skin. These have become significant for a subject of research.

1.2PHYTOCHEMICALS

Phytochemicals are chemical compounds that occur naturally in plants. They can either be nutritive or non-nutritive plant chemicals which occur naturally in plants and protective or disease preventive properties. It is a well known fact that plants produce these chemicals to protect them. But research has demonstrated that these phyto-constituents can also protect humans from disease.

There are thousands or more of known phytochemicals. However, some of the well known phytochemicals can be classified as alkaloids, flavonoids, tannins, steroids, saponins, glycosides, phenols and terpenoids.

1.3 NATURE AND PROPERTIES OF SOME PHYTOCHEMICALS

The various classes of some of the known photochemical are as follows;

ALKALOIDS

These are naturally occurring organic base which contain the pyridine ring. They are natural plant compounds that have basic character and contain at least one nitrogen atom in a heterocyclic ring. They have diverse and important physiological effects on humans and other animals. Alkaloids are found primarily in plants and are classified on the basis of their chemical structure. Some of the examples include; cocaine, caffeine, nicotine, morphine and quinine.

FLAVONOIDS

Flavonoids are polyphenolic compounds that are common in nature and are usually categorized according to the chemical structure into flavonols, flavones, isoflavones and chalcones. They are widely distributed in plants performing many functions (Finar, 1975).Over 4000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages. They are the most important plant pigment for flower coloration producing red, yellow, or blue pigmentation in petals designed to attract pollinators.

TANNINS

Tannins are widely distributed in plants. They are colourless non- crystalline substances which form colloidal solutions in water and these solutions have astringent taste, thus forming tannic acid.

There are two groups of tannins; the hydrolysable tannins which are esters of Gallic acid, also glycoside of these esters and the condensed tannins which are polymers derived from various flavonoids. The name tannin is derived from its ability to tan with leather. They occur in many trees and the best source of it is the nut gall and the bark of sumac. Tannins are any phenolic compounds of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups to form effectively strong complexes with protein and other macro-molecule (Finar, 1975).

STEROIDS

The steroids form a large group of structurally related compounds which are widely distributed in animals and plants. Included in the steroids are the sterols, vitamin D, the bile acids, a number of sex hormones, the adrenal cortex hormones, some carcinogenic hydrocarbons, and certain sapogenins. Their structures are always based on the 1, 2-cyclopentenophenanthrene skeleton.

SAPONINS

Saponins are a group of naturally occurring oily glycosides that foam freely when shaken with water. They occur in a wide variety of plants including

acacia, soap wort, soap root, California pigweed and many other plants. Saponins have been and sometimes still are used as cleaning agents and as foam producers notably in fire extinguishers fluids. They have a bitter taste and when ingested orally are practically non-poisonous to warm blooded animals. When injected directed into the bloodstream, they are very dangerous and they quickly dissolve red blood cells (Finar, 1975).

GLYCOSIDES

In chemistry, a glycoside is a molecule in which sugar is bounded to a non-carbohydrate moiety usually a small organic molecule. Glycosides play numerous roles in living organisms like fungi, bacteria, moots etc. Many plant store chemical in form of inactive glycosides. These can be activated by enzyme hydrolysis which causes the sugar parts to be broken off; making the chemical available for use. Many of such plants glycosides are used in medications (Finar, 1975).

PHENOLS

The term phenol is used for any group of related acidic compounds that are hydroxyl derivative of aromatic hydrocarbon such as cresol and resorcinol. Phenols were formally called carbolic acid which is an aromatic organic compound. It is a weak acid and resembles the alcohols in structure.

1.4SPICES AND CONSTITUENTS

Ukpong *et al.*, (2014) reported that cocoyam seldom flowers but *Xanthosoma sagittifolium* species does. This flower is the inflorescence which is the reproductive part of the plant. It produces a sweet smell once the flower matures and this attracts the pollinators. The inflorescence commonly called etrong in Ibibio is edible and is used locally as spice or seasoning to prepare foods like oto mboro, ekpang nkukwo etc. (Ukpong *et al.*, 2014). Agboola (1979) wrote that in Central America, the young inflorescence is cooked and eaten with other vegetables.

A spice is a dried seed, fruit, root bark or vegetable substances primarily used for flavouring, colouring, or preserving food. Sometimes spice is used to hide other flavours (Terrence, 1995). It is also a plant substance from indigenous or exotic origin, aromatic or strong taste used to enhance the taste of food.Harsha, Sridevi, Lakshin, Rani, Divya and Vani (2013) described it as the dry part of plant such as the roots, leaves and seeds which impact to food a certain flavor or pungent stimuli. Then by clubbing spices and condiments into one group, the International Organization for Standardization, illustrated that the term spices or condiments applies to such natural plants or vegetable product or mixtures thereof in whole or ground form as used for imparting flavor, aroma and piquancy to food substances and for also seasoning food (ISO, 1972).Researches and recent studies have shown that spices contain phytochemicals which include alkaloids, phenolics, saponins, flavonoids, tannins, glycosides, steroids, and

terpenoids(Hirasa and Takemasu 1998). Other studies have also shown that certain spices also contain carbohydrates, fats and protein (Zachariah, 1995).

1.5 STATEMENT OF PROBLEM

Cocoyam is a plant that is claimed to have a lot of economic values ranging from medicinal values, nutritional values and flavouring properties. These claims have not been clearly verified. It has also been observed that the stem sap of cocoyam causes serious itchiness on the skin of the farmers. This research work is therefore centered on nutritional evaluation of the flowers of two varieties of cocoyam and the phytochemical constituents. And also to analyse the stem sap of the two varieties of cocoyam to find out the compounds responsible for the itchiness of the sap or fluid.

1.6OBJECTIVES OF THE STUDY

This research work is aimed at analyzing flower extracts and the stem saps of two varieties of cocoyam: *Colocasia esculenta and Xanthosoma sagittifolium* with the following objectives:-

i) To run both qualitative and quantitative phyto-constituents analyses on the flowers and stem saps of the two varieties.

ii) To carry out a proximate analysis on the flowers of the two varieties.

iii) To run a GC-MS analysis on the flowers extract to determine the compounds that are present in the flower.

iv) To run a GCMS analysis on the stem saps of the two varieties to identify the compounds responsible for the itchiness of the sap.

v) To perform comparative phyto-constituents analysis between the two species

1.7 SIGNIFICANCE OF THE STUDY

This research work will be of much importance to the various foods and the spice industries and the potential persons who have interest in going into spice and food production business. This research work also will add value to the economic importance of cocoyam as a plant.

1.8 HYPOTHESIS

The results of this analysis will be expressed as the mean ± standard deviation. The statistical analysis will be done using t-test statistical tool. Difference between the mean will be considered to be significant at 95% confidence level.

The hypothesis is given as thus

1. $H_{0:}$ there is no significant difference between the mean values of *xanthosoma sagittifolium* and *colocasia esculenta*

$H_0 : \mu_1 - \mu_2 = 0$

2. H_a : there is significant difference between the mean values of *xanthosoma sagittifolium* and *colocasia esculenta*

$H_a : \mu_1 - \mu_2 \neq 0$

Decision rule; If $t_{stat.} > t_{crit.}$, reject H_0, and accept H_a

If $t_{stat.} < t_{crit.}$, accept $H_{0,}$ and reject H_a

1.9 SCOPE OF THE STUDY

The scope of this research work centres on the studies of two varieties of cocoyam, *Xanthosoma sagittifolium* and *Colocasia esculenta*. The flowers and the stem saps of the two varieties of cocoyam will be analysed. Proximate analysis will be carried out on the flowers to determine the nutritional value. Qualitative and quantitative phyto constituent analysis will be done on the flowers and the stems. GC-MS analysis will be carried out on both the flowers and the stems sap to identify the compounds present.

CHAPTER TWO

LITERATURE REVIEW

2.1 COMPOSITION OF COCOYAM

In most African countries, cocoyam it is mainly cultivated by small scale farmers. Like many plants of Araceae family, cocoyam grows from fresh corm. The corm supply easily digestible starch and are known to contain substantial amount of protein, vitamin C, thiamine, riboflavin and niacin and significant amount of dietary fiber (Niba, 2003). The flour of the cocoyam can also be used for the preparation of soups, biscuit breads and puddings (Niba, 2003). Depending on the varieties and the local cultural traditions, other part of the plant such as the leaves, flowers and stems are also consumed especially in sauces, purees, stews and soups (Ejoh, Mbiapo and Fokon, 1996).

Apart from the nutritional importance of cocoyam, it also possesses some pharmacological importance. A report has shown that the edible corms and leaves are traditionally used for hepatic ailments. The leaf juice of this plant is applied over scorpion sting or in snake bite (Tuse *et al.,* 2009). The tuber is also used as abortifacient to treat tuberculosis, ulcers, pulmonary congestion, crippled extremities, fungal abscesses in animals and as an anthelmintic. It's foliage is used as a styptic and poultice. The stem sap is used by Wargo as a treatment for wasp stings. Then poi, a fermentated product made from corm shavings is used to improve muscle tone by bathing the sickly in it and allowing the poi to dry on the body (Tuse *et al.*, 2009).

In the analysis of the oxalate content of some Nigeria tubers using titrimetric and UV spectrophotometric method, Popoola, Doherty, Odusami and Durowoju (2014), showed that in titrimetric method, *Colocasia esculenta* had 2.56mg/100gFW while *Xanthosoma sagittifolium* had 1.54mg/100gFW. In UV method, *Colocasia esculenta* had 2.38mg/100gFW while *Xanthosoma sagittifolium* had 1.53mg/100gFW. From the results of Popoola et al (2014), *Colocasia esculenta* showed a higher oxalate content than *Xanthosoma sagittifolium*. Onwuka (2005) reported that it is difficult to ascertain whether the tubers of cocoyam can be relied upon as a good source of minerals because of the presence of anti-nutrition substances such as oxalates which renders the minerals in them unavailable to the consumers. The report further said that the presence of oxalates in the cocoyam results in its bitterness and astringent taste. But Akban and Umoh (2004) said that cooking the cocoyam may lead to further reduction of the low oxalate content to insignificant values in the tubers, thus rendering them more nutritive. Sangketkit, Savage, Martin and Mason (2001) wrote also that the processing of the tubers by either boiling or steaming before consumption leads to the reduction of the oxalate level. FAO (2003) wrote that the presence of oxalates in food impairs the absorption of most metals. The consumption of oxalate rich foods could further aggravate the already existing deficiencies in metals such as calcium and iron.

Olaleye, Owolabi, Adesin, and Isiaka (2013) showed that the leaves of red and white cocoyam are composed of anti-nutrient species such as phytates, oxalates

and tannins. The various values for the leaves of the red and white cocoyam species are as follows: phytates (10.10mg and 9.10mg respectively), oxalates (5.83mg and 8.28mg respectively) and tannins (0.02mg and 0.05mg respectively). Olaleye *et al* (2013) further showed that the leaves of red and white cocoyam contain minerals such as sodium, potassium, calcium, magnesium, manganese, zinc and iron. Also the qualitative and quantitative analysis of the cocoyam leaves showed the presence of saponins, tannins, flavonoids, phenols and alkaloids.

In the phytochemical screening, Ukpong *et al*., (2014) reported that *Xanthosoma sagittifolium* inflorescence contain terpenoids, cardiac glycosides, tannins, flavonoids, alkaloids, saponins and steroids. In the mineral analysis of *Xanthosoma sagittifolium* inflorescence, Ukpong *et al.*, (2014) revealed the high concentration of metals like potassium (52.99mg/100g), iron (39.33mg/100g), zinc (31.33mg/100g), sodium (19.33mg/100g), calcium (17.67mg/100g), manganese (7.66mg/100g) and copper (3.83mg/100g).

According to Bradbury and Nixon (1998), different species of cocoyam especially *Colocasia esculenta* cultivars have sharp pungent taste and should not be eaten raw because they can cause swelling of the lips, mouth and throat. To enable comfortable ingestion, the tubers have to be quickly peeled and cooked over a long period of time (Saikai 1979).Onayemi and Nwigwe (1987), identitified that *Colocasia esculenta* corm is composed of digestible starch, high quality protein, essential amino acids, vitamin C, thiamin, riboflavin and niacin.

Lee (1999) observed that cocoyam is one of the only major staple foods where both the leaves and the underground parts are use and have equal importance for human consumption. The excellent digestibility of the small starch of *Colocasia esculenta* suggests efficient release of nutrients during digestion and absorption of this food (Lee 1999). Ghan, Kao-Jao and Nakayama (1977) reported that the corms of taro (*Xanthosoma sagittifolium*) contain pigment anthocyanins such as cyanidin-3-glucoside, pelargonidin-3-glucoside and cyanidin-3-rhamnoside and anthocyanogens.

2.2 CHEMISTRY OF SPICES

Spices have played a significant role in the civilization and in the history of nations. The delightful flavor and the pungency of spices make them indispensable in the preparation of palatable dishes in addition there are arguments and reports stating that they posses several medicinal and pharmacological properties and hence find position in the preparation of a number of medicines.

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste and are used to enhance the taste of food (Harsha *et al*., 2013). Other reports define spices as the dry part of a plant such as roots, leave, seeds or flowers which impact a certain flavor and pungent stimuli (Hirasa *et al*.,1998). International Standard Organization (ISO, 1972) defines spices as the natural vegetable products or mixtures thereof without any extraneous matter that is used for flavouring, seasoning and impacting aroma to

foods. The USA Food and Drug Administration (FDA, 2015) defines spices as aromatic vegetable substances in the whole, broken or ground form whose significant function in food is seasoning rather than nutrition.

2.3 CHEMICAL COMPOSITION OF SPICES

The chemistry of spices has been a significant study in food industry and technology. Spices give aroma, colour and taste to the food preparations and sometimes mask undesirable odours (Parthasarathy, Chempakam, and Zachariah 2008). According to Menon (2000), the essential (volatile) oils give the aroma and the oleoresins give the taste. Aroma compounds play a significant role in the production of flavourants which are used in the food industry to flavour, improve and increase the appeal of their products. These aroma compounds are classified according to their functional groups. They are alcohols, aldehydes, amines, esters, ethers, ketones, terpenes, thiols and other miscellaneous compounds. In spices, the volatile oils constitute these compounds (Menon, 2000). Another report has also shown that plants used as spices are usually aromatic and pungent and that these properties are as a result of varying amounts of essential oil (Zachariah, 1995).

Analysis of 26 spices by Shan, Cai, Sun and Corke (2005) has revealed that spices have anti-radical capacity and the compounds responsible for this are phenolic compounds. Analysis has also been carried out on commercial spices such as bay, rosemary, parsley, turmeric, basil and oregano. The result revealed that they contain phytochemicals. Quercetin, apigenin, glycosides and

flavonoids were common among bay, rosemary and parsley. Whereas, turmeric is rich in polyphenol curcumin. Rosemary, basil and oregano contains high amount of hydroxycinnamic acids (Alezandro, Lut, Lajolo and Genovese 2011).

A review on the chemical composition of spices has shown that phytochemicals in spices are secondary metabolites which originated for the protection of plants from herbivorous insects, vertebrates fungi, pathogens and parasites. Most probably also, no compound is responsible for flavours; but a blend of different compounds such as alcohols, phenols, esters, terpenes, organic acids, resins, alkaloids and sulphur containing compounds in various proportions produce the flavours (Maney and Shedaksharaswami 1997). Besides these flavouring components, every spice contains the usual components such as proteins, carbohydrates, fiber, minerals, tannins or polyphenols (Etonihu, Obelle and Nweze 2013).

2.4 PHARMACOLOGICAL ACTIVITIES OF SPICES

Many reports and literature has shown that spices are not only used as flavouring or seasoning agents, they also have medicinal and pesticidal properties (Pundir, Jain and Sharma 2010; Ahmed, Seth and Banerjee 2000; Christensen and Brandt 2006; Dwivedi, Pandey, Pant and Logani 2002; Guynot, Ramos, Sets, Purroy, Sanchis and Marin 2003). In recent years, there has been an increasing interest of the food industry in incorporating ingredients with health beneficial properties. Among these ingredients are spices. They were not only used because of their flavouring or colouring properties, many of them are

known for their numerous health benefits such as anti-inflammatory, anti-microbial, anti-mutagenic, antioxidant and hypolipidemic activities (Su, 2007). Christensen *et al*., (2006) reported that the effect of polyacetylenes in the celery leaves towards human cancer cells, their human bioavailability and their ability to reduce tumor formation in a mammalian invivo model indicates that they may also provide health benefits. Curry leaves have also been proven to be effective against *Rhizopus stolonifer* and *Gloeosporium psidii* infecting guava (Dwivedi *et al*., 2002). Guynot *et al*.,(2003) also reported that bay leaf has been used as herbal medicine and has pharmaceutical activitywhich includes antibacterial, antifungal, antidiabetes and anti-inflammatory effects. Nutmeg oil posses strong antibacterial, antifungal and insecticidal properties.Myristicin which imparts hallucinogenic properties is also reported to be an effective insecticide while the lignin types of the constituents in the nut are anticarcinogenics (Narasimhan and Dhake 2006).

2.5 PHYTOCHEMICALS AND SPICES

Reports have shown that spices contains phytochemicals and these phytochemicals in spices occur as secondary metabolites which actually originated for the protection of the plants from herbivorous insects, vertebrates, fungi, pathogens and parasites (Etonihu *et al.*,2013; Shan *et al* 2005). These phytochemicals in conjunction with other compounds such as organic acids, resins, esters and sulphur containing compounds produce the flavours in the spices (Maney *et al.*, 1997).Studies has also shown that the pharmacological

activities exhibited by spices such as antioxidants activity, antimicrobial activity, and anti-cancer activities is because of the presence of the phytochemical compounds present in them (Oliver, Mila and Mladen 2006; Christensen *et al*., 2006).

2.6 PHARMARCOLOGICAL ACTIVITIES OF COCOYAM

Studies have revealed that cocoyam possesses certain pharmacological properties. Evidence of this is seen on the corm (tubers), stem and leaves. The leaves of cocoyam have been reported to contain alkaloids, flavonoids, saponins and tannins (Biren, Nayak, Bhatt, Jalapure and Seth 2007). Phytochemical analysis of the leaves of cocoyam indicated that the ethanol extract of the leaves contain sterols, flavonoids, glycosides, tannins, carbohydrates and vitamin A and C. It has been reported that anti-diabetic and anti-hyperglycemic activity of cocoyam leaves may be due to the presence of several bioactive compounds (Kumawat, Chaudhani, Wani, Deshmukh and Patil 2010).

Studies on the antimicrobial activity of the leaves of cocoyam have received concern. According to reports, the antibacterial susceptibility assay indicates that the ethyl acetate extract of cocoyam leaves showed a remarkable activity against pathogenic bacterial strains and thus can be used in the treatment of typhoid, pneumonia, urinary tract infection and diarrhea infection (Nakade *et al.,* 2013). Studies on the phyto-constituents of the leaves of cocoyam by Ferreres, Goncalves, Gil-Izquierdo and Silver (2012) has revealed the presence of phenolic compounds, anthocyanins (cyanidins and pelargonidin derivatives)

and flavones (apigenin and luteolin derivative). The study on the anti-inflammatory activity or property of the leaves of cocoyam has revealed that it possesses wound healing property and thus can be related to its antioxidant activity such as its super-oxide radical scavenging potential and the inhibition of hyaluronidase thus protecting the skin cells from oxidative damage and accelerating the recovery of wounds (Ferreres *et al.*, 2012).

Another report has shown the efficacy of the extract of cocoyam leaf in treating wounds. According to the report, the leaf juice of cocoyam can be applied to scorpion sting or to snake bite as well as it being used in treating food poisoning of plant origin. Its corm is used as an abortifacient to treat tuberculosis, ulcers, pulmonary congestion, crippled extremities, fungal abscess in animals and as an anthelmintic. Its foliage is used as a styptic and poultice. The stem sap is used by the Wargo as a treatment for wasp stings. Poi which is a fermented product made from corm shavings is used to improve muscle tone by bathing the sickly person in it and allowing the poi to dry on the body(Tuse *et a*l., 2009).

2.7 NUTRITIONAL VALUE OF COCOYAM

Proximate analysis of the corm (tuber) of cocoyam has revealed that cocoyam has nutritional values and contains vitamins and minerals. According to Chandra, Saklami and Singh (2012), 100g of edible portion of cocoyam tubercontains 3.4% crude protein, 26.98% carbohydrates, 7.5% crude fiber and 4.80% ash content. The calcium content is 0.83mg/gm, magnesium is

0.94mg/gm, potassium is 1.24% and phosphorus is 0.58%. Another article has stated that cocoyam corms are rich in carbohydrates while the leaves are good source of vitamin A and Vitamin C (ascorbic acid) and contains more proteins than the corms. A report (All Nigerian Recipes, 2014), also stated that the corms and the cormels of cocoyam which are the major economic part contains about 15-39% carbohydrates, 2-3% protein and 70-77% water. The leaves contain about 2% protein and also rich in vitamin C, thiamin, riboflavin, niacin,calcium, phosphorus, and Iron.

A report on the nutrient composition of cocoyam leaves has shown that the leaves contain about 888g/kg of moisture, 204g/kg of fiber level, 307g/kg of crude protein, ash contents is 98g/kg. The principal mineral contents are iron and zinc and moderate amounts of calcium, phosphorus and magnesium (Tuse *et al.*, 2009). Eleazu, Iroaganachi and Eleazu (2013) reported that the tuber of cocoyam contains 3.64% moisture, 10.67% ash, 1.5% crude fiber, 3.42% lipids, 8.44% crude protein, 73.83% carbohydrates. The mineral analysis indicated that it contains 38.41mg/100g magnesium, 113.78mg/100g calcium, 35.38mg/100g potassium, 195.81mg/100g phosphorus, 1.84mg/100g iron and 0.8mg/100g zinc. Tigodoe and Nip (1994) reported that flours made from *Colocasia esculenta* corm are rich in starch and total dietary fibre and low in fat, protein and ash content.

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS USED

CHEMICALS

Anhydrous sodium sulphate, copper sulphate, tetraoxosulphate (vi) acid, boric acid, methyl red, sodium hydroxide, hydrochloric acid, petroleum ether(diethyl ether), lead acetate, acetic acid, ferric chloride, acetic anhydride, ethanol, n-butanol, sodium chloride, ethyl acetate, ammonium hydroxide solution, tannic acid, sodium trioxocarbonate(iv), potassium iodide, silver trioxonitrate (v).

REAGENTS

2% boric acid, 40%, 1.25%, 2.5% NaOH respectively, 1.25%H_2SO_4, Meyer's reagent, 2%HCl, 0.1%$FeCl_2$, 20%ethanol, 5%NaCl, 1%NH_4OH, Folin-Denis reagent, Standard tannic acid, 5%KI, 10%CH_3COOPb(lead acetate). All these reagents were of ANALAR quality.

APPARATUS

Power edge electric blenders, 6530 GenLab Oven , Nalgene dessicator, Mettler analytical balance, Rectangular muffle furnace, kjeldahl flask, micro kjeldahl distillation apparatus,

Soxhlet extractor, steam bath, Genway electronic spectrophotometer, GCMS-QP2010 PLUS SHIMADZU.

3.2 EXPERIMENTAL PROCEDURE

3.2.1 Sample Collection and Preparation

The cocoyam flowers and stem were collected from a farm at Ogwa in Mbaitolu Local Government Imo State. Also from farms in Ihiagwa community and Nekede community both in Owerri West Local Government, Imo State. They were taken to the Department of Crop Science Technology, School of Agriculture and Agricultural Technology, FUTO for identification. The flowers and the stem were air dried at room temperature and under the sun and ground to fine powder using a mechanical blender and then stored in air tight containers for laboratory analysis.

3.2.2 Geography of The Study Area

The samples were collected from Ogwa in Mbaitolu LGA, Ihiagwa and Nekede all in Owerri West LGA all in Imo State. From the location map in Fig. 3.1 below, Ogwa has a coordinates of $5^0\,36^1$ N and $7^0\,03^1$ E, Ihiagwa has coordinates of $5^0\,23^1$ N and $6^0\,57^1$ E while Nekede has a coordinates of $5^0\,24^1$ N and $6^0\,59^1$ E.

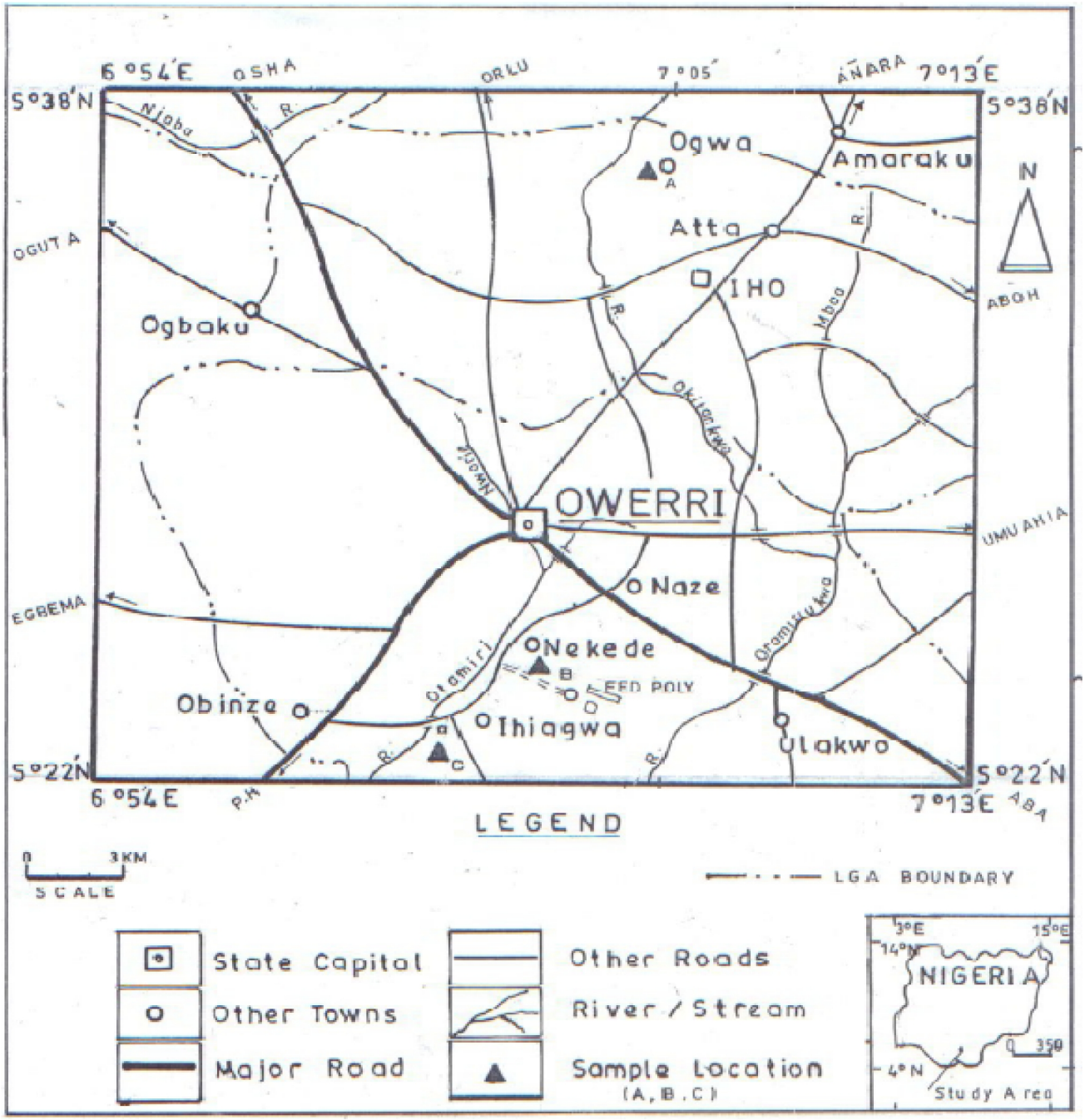

Figure 3.1: Location Map Of The Study Area

3.2.3Extraction of the Plant Samples

The ground flowers and the stem of the cocoyam species were extracted with 95 % ethanol in Soxhlet extractor for a period of 4 hours. The extract was concentrated on a water bath. This ethanol extract was used for GC-MS analysis and phytochemical screening.

3.3 PROXIMATE ANALYSIS

The parameters determined under proximate analysis include moisture content, ash content, crude protein, crude fibre, crude fat and total carbohydrates. The methods used are as described by Association of Official Analytical Chemists (AOAC, 2000).

Moisture content

The method used is the drying method. Two aluminum dishes were washed thoroughly and allowed to dry in the oven. After drying, the dishes were put inside desiccators to cool. After cooling, the aluminum dishes were weighed and their weights recorded.

5 g of the fine grinded cocoyam flowers were taken and transferred to the aluminum dishes. This was put in the oven and allowed to dry at temperature of 105° C for some time. After 4hours, the container and content were taken out and reweighed. It was also taken back to the

oven. It was also reweighed again after 30 minutes. This was now done at 30 minutes intervals until a constant weight was obtained.

The percentage moisture content was thus calculated using:

$$\%\, Moisturecontent = \frac{W_2 - W_3}{W_2 - W_1}\, X\, \frac{100}{1}$$

Where; W_1 =weight of the empty dish,

W_2 =weight of the dish + sample before drying,

W_3 = weight of the dish + sample after drying.

Ash content

The crucibles were washed thoroughly and dried in the oven. After drying, the crucibles were cooled in desiccators and then weighed.

5 g of the sample were transferred into the weighed crucible. The crucible and the content were placed in a muffle furnace. The temperature of the furnace is maintained at 550^0C. This temperature was maintained until the sample became carbonized and charred.

The furnace was switched off, the crucible and the content were cooled in a dessicator and weighed.

The percentage ash content was calculated as thus;

$$\%\ AshContent = \frac{W_3 - W_1}{W_2 - W_1} X \frac{100}{1}$$

Where; W_1 = weight of the crucible

W_2 = weight of crucible + sample before ignition

W_3 = weight of crucible + ash after ignition

Crude fibre

5g of the sample was placed in a hot 200 ml of 1.25 % H_2SO_4 and boiled for 30 minutes. The solution was filtered through Buckner funnel. The residue was washed with hot water until the washings are no longer acidic. This was done by testing with litmus paper. The residue was transferred to another beaker and boiled with 200 ml of 1.25 % NaOH for 30 minutes. This was filtered and also washed with hot water.

The residue was also washed with small quantities of alcohol twice and three times with petroleum ether. The residue was drained and transferred completely to the crucibles and dried in the Gen lab oven to a constant weight.

The dried residue was transferred to a muffle furnace and incinerated at 600°C for 2 hours. The crucible and the content were cooled in the dessicator and weighed. The weight loss on the incineration was the crude fibre and the percentage crude fibre was calculated as;

$$\%\ CrudeFibre = \frac{M_2}{M_1} X \frac{100}{1}$$

Where M_1 = mass of the crucible and content after drying in the oven

M_2 = mass of the crucible and content after incineration

Crude protein

The method used is the micro kjeldahl method. Here 2 g of the ground cocoyam flower sample was weighed and transferred into the kjeldahl flask. 4 g of anhydrous Na_2SO_4 was added to the sample followed by addition of 1 g of $CuSO_4$ with a speck of selenium.

Into the mixture concentrated H_2SO_4 (20 ml) was added. This was now heated in the fume cupboard. The mixture was heated until it became clear. This was now cooled to room

temperature. The clear mixture was transferred to already rinsed 100 ml volumetric flask. This was now made up to 100 ml with distill water. Also the same procedure was carried out now as a blank experiment without the sample.

20 ml of the digest was transferred to a distillation flask. 10 ml of 2 % Boric acid was measured into a receiver (small beaker) and two drops of methyl red indicator added. And the micro kjeldahl distillation apparatus was set up in such a way that the delivery tube extends below the surface of the 2 % boric acid solution in the small receiver beaker. Quickly 35 ml of 40 % NaOH was added to distillation flask. This was heated until 30ml of the distillate was collected.

This same procedure was carried out on blank experiment. The distillate was titrated against 0.1N of HCl. The percentage crude protein was calculated using;

$$\%\ CrudeProtein = \frac{(T-B)XN_{HCl}X\ 6.25\ XVX\ 0.00014}{AXM} X \frac{100}{1}$$

Where : T = titre value of sample,

B = titre value of blank

N_{HCl} = Normality of HCl used,

A = sample volume taken (Aliquot)

V=Volume made up to(100ml)

M = Mass of Sample

Crude fat

The method used is the Soxhlet extraction method. The flask in the extraction chamber is first weighed. 5 g of the sample was also weighed and carefully wrapped and tied with thread. The filter paper and the contents was placed in the Soxhlet extraction column and extracted for about 6 hours. The solvent becomes clear in the column when the fat must have been extracted. The defatted sample was now carefully removed and the solvent recovered. The flask was then oven dried to remove all the solvent, and then cooled in the dessicator.

The flask and its content were now reweighed. The percentage crude fat was now calculated using;

$$\%\ CrudeFat = \frac{M_2 - M_1}{M_3} X \frac{100}{1}$$

Where; M_1 = mass of the flask,M_2 = mass of flask + fat

M_3 = mass of the sample

Total carbohydrate

The percentage carbohydrate content was determined by

% Carbohydrate = 100 – (% crude protein + % ash + % crude fat + % moisture content)

3.4 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Test for Alkaloids

The method used was Meyer's test. Here, 1 ml of extract of the sample is shaken with 5 ml of 2 % HCl on a steam bath and filtered. To 1ml of the filtrate is treated with Potassium Mercuric Iodide solution (Meyer's reagent). A cream precipitate gives a positive test.

Test for Flavonoids

The method used is the Lead Acetate test. To 1 ml of the extract, 1 ml of 10 % Lead acetate is added. The formation of yellow precipitate confirms the presence of flavonoids.

Test for Glycosides

The method used is keller kiliani test. 5 ml of the extract is treated with 2 ml of glacial acetic acid which is followed by the addition of few drops of ferric chloride solution and 1ml of

concentrated H_2SO_4. Formation of brown ring at the interface confirms the presence of glycosides.

Test for Phenols

The method used is Ferric Chloride test.1ml of extract was treated with few drops of ferric chloride. Formation of dark green colour indicates the presence of phenolic compounds

Test for Saponins

The method used is froth test.1ml of the extract is boiled with 5 ml of distilled water for 5minutes and decanted while hot. 1 ml of the filtrate is diluted with 4ml of the distilled water shaken vigorously and observed for standing. After a while, the existence of froth formation confirms the presence of saponins.

Test for steroids

The method used is Libermann-burchard test**.** Here, 2 ml of acetic anhydride was added to 0.5 ml of the extract and 2 ml of concentrated H_2SO_4 slowly along the side of the test tube. Change of colour from violet to bluish green indicates the presence of steroids.

Test for Tannins

The method used was Ferric Chloride test**.** 1ml of the extract was added with 5ml of distilled water and kept for boiling in hot water bath. After boiling sample was cooled .then 0.1% ferric chloride solution was added. Appearance of brownish green coloration confirms the presence of tannins.

3.5 QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Saponin determination

Saponins content was determined according to the method described by Obadoni and Ochuko (2001). 20 g of the dry ground sample was weighed and dispersed in 200 ml of 20 % ethanol. The suspension was heated for 4 hours in a hot water bath maintained at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol. The combined extract was reduced to 10 ml over water bath at temperature of 90°C. The concentrate was transferred into 50 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded and the purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous NaCl. The organic layer was separated

using a separatory funnel and thereafter evapourated to dryness using a water bath. After evaporation, the samples were dried in the oven to constant weight. The percentage saponins can be thus calculated using;

$$\%\, Saponin = \frac{W_2 - W_1}{W_3} X \frac{100}{1}$$

Where, W_1 = weight of flask.

W_2 = weight of flask + dried sample.

W_3 = weight of sample used.

Flavonoids determination

The method used is that described by Harborne (1973). Here, 5 g of ground sample was boiled in the 100 ml of 2M HCl solution for 40 minutes. It was allowed to cool to room temperature before filtering through Whatman filter paper No. 42 to obtain the extract.

In drop wise, concentrated ethyl acetate was added to the extract until in excess. Flavonoids in the extract were precipitated. The flavonoids precipitate was recovered in a weighed filter

paper following filtration. After drying in the oven and cooling in the dessicator, the weight of the flavonoids was obtained using the formular;

$$\%\ Flavonoids = \frac{W_2 - W_1}{W} X \frac{100}{1}$$

Where; W = weight of sample

W_1 = weight of empty filter paper

W_2 = weight of filter paper + flavonoids precipitate

Alkaloids determination

The method used is that of Harborne (1973) which involves alkaline precipitation gravimetric method. Here, 5 g of the sample was dispersed in 100 ml of 10 % acetic acid in ethanol solution. The mixture was shaken vigorously for 4 hours at room temperature with shaking every 30minutes. At the end of this period, the mixture was filtered through Whatman filter paper No. 42. The filtrate was concentrated by evaporation to a quarter of its original volume. The filtrate was treated in drop wise with NH_4OH solution to precipitate the alkaloids. This was continuously added until the ammonia was in excess.

The precipitated alkaloids were filtered using a weighed whatman filter paper No. 42. The precipitate was washed with 1 % NH_4OH solution. The precipitated alkaloids were dried at 60°C and weighed after cooling in dessicator. The percentage alkaloid was calculated as thus;

$$\% \, Alkaloid = \frac{W_2 - W_1}{W} X \frac{100}{1}$$

Where W = weight of the sample

W_1= weight of the filter paper

W_2 = weight of the filter paper + alkaloid precipitate

Tannins determination

The method used is Folin-Denis spectrophotometric method as described by Pearson (1976).Here 5 g of the sample was dispersed in 100ml distill water and agitated. This was left to stand for 30minutes at room temperature being shaken every 5 minutes. At the end of the 30minutes, it was filtered using Whatman filter paper No. 42. 2.5 ml of the extract was mixed with equal volume of Folin-Denis reagent in a 50ml volumetric flask followed by 2.5 ml of saturated Na_2CO_3 solution. The mixture was diluted to mark in the flask and incubated for 90 minutes at room temperature. 2.5 ml of standard tannic acid solution was dispersed in a

separate 50ml volumetric flask and also equal volume of Folin-Denis reagent was also mixed with it followed by 2.5 ml of saturated Na_2CO_3 solution and also incubated for 90 minutes at room temperature. The absorbance of the standard and the sample were measured at 250 nm in a Genway model 6000 electronic spectrophotometer. The tannin content was calculated using the formular

$$\%\, Tannin = \frac{A_n}{A_s} XCX \frac{100}{W} X \frac{V_f}{V_a}$$

Where; A_n = absorbance of test sample

A_s = absorbance of standard solution

C = Concentration of standard solution

W = weight of sample

V_f = total volume of the extract

V_a = volume of extract analyzed

Glycosides determination

The method used is that of AOAC (2000). Here, 1.0 g of the sample is weighed into a 250ml round bottom flask. 200 ml of distilled water is added and allowed to stand for 4hours for autolysis to occur. Distillation is carried out and 150 ml of the distillate was collected in a 250ml conical flask containing 20 ml of 2.5 % NaOH. However before the distillation, an antifoaming agent (tannic acid) was added. To 100ml of the distillate containing the glycoside 8ml of 6N NH_4OH and 2 ml of 5 % KI were added and mixed. This was titrated with 0.02N $AgNO_3$ using a micro burette against a blank. Permanent turbidity indicates end point. The glycoside content of the sample is calculated as thus;

$$\%\ Glycosides = \frac{V_T X\ 1.08\ X V_E}{V_A X W} X\ \frac{100}{1}$$

Where; V_T = Titre Value,

V_A = Volume of Aliquote

V_E = Volume of Extract,

W = Sample Weight

3.6 GC-MS ANALYSIS

GC-MS analysis of the extracts was performed using GCMS-QP2010 (SHIMADZU). The gas chromatograph interfaced to a mass spectrometer was equipped with Elite-I, fused silica capillary. For detection, an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.99%) was used as the carrier gas at column flow rate of 1.58mL/min. Injection volume of 2µL was employed. Injection temperature was 250°C, ion source temperature of 230°C. The oven temperature was programmed from 80°C with an increase of 10°C to 200°C then 5° C/minute to 280°C ending with 5minutes isothermal at 280°C

The relative percent amount of each component was calculated by comparing its average peak area to the total areas. Interpretation of the spectrum was conducted using the data base of National Institute of Standards and Technology (NIST). The various spectra were compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and the structure of the components of the test materials were ascertained.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 PROXIMATE ANALYSIS

The results for the proximate analysis are presented in the Table 4.1 below.

Table 4.1. Proximate analysis result of the two species of cocoyam

Parameter	Values (%)

	Xanthosoma sagittifolium	*Colocasia esculenta*
moisture content	16.10 ± 0.1	16.35 ± 0.02
crude protein	37.87 ± 0.02	22.56 ± 0.015
ash content	6.23 ± 0.02	6.61 ± 0.04
crude fat	13.13 ± 0.045	14.05 ± 0.01
fibre content	12.92 ± 0.026	15.32 ± 0.025
total carbohydrates	13.75 ± 0.03	25.11 ± 0.03

Values expressed as Mean ± Standard deviation

4.2 DISCUSSION OF THE PROXIMATE RESULT

The result of the proximate composition of the flowers of *Xanthosoma sagittifolium* and *Colocasia esculenta* is shown in Table 4.1 above. From the table, *Colocasia esculenta* gave moisture content value of 16.35% while *Xanthosoma sagittifolium* has a value of 16.10%. However, the values obtained from the two species of cocoyam gave a relatively high value when compared to Food and Agriculture Organisation standard for spices from flower which is between 5-11% (FAO, 1997) and also relatively high when compared to United State of

Department of Agriculture standard for moisture content of spices which is 8.46% (USDA, 2015). These values were also significantly higher than the moisture content of spices like garlic (4.55%), ginger (6.37%), and pepper (5.70%) as reported by Otunola, Oloyede, Oladiji and Afolayan (2010).

The percentage crude protein is an indication of the amount of amino acids present in the sample. *Xanthosoma sagittifolium* had a significantly a very higher percentage of crude protein of 37.87% as against the value for *Colocasia esculenta* (22.56%). The two values show that the flowers of the two cocoyams are protein rich. Standal (1983) also reported that *Xanthosoma sagittifolium* contains more amino acids than cassava, yam and sweet potatoes. The percentage crude protein obtained in this study for *Xanthosoma sagittifolium* (37.87%) and *Colocasia esculenta* (22.56%) were significantly higher than the values reported for ginger (8.58%,), garlic (17.35%,), onions (10.45%) and Ashanti pepper (12.50%) by Nwinuka *et al*., (2005). However, these values are higher than the USDA standard value for spices which is 6.09% (USDA, 2015).

Ash content is the residue remaining after all the moisture has been removed as well as organic materials. Ash content is a reflection of mineral content present. High ash content will imply high mineral content. From Table 4.1, *Xanthosoma sagittifolium* has an ash

content of 6.23% which is lower than that of *Colocasia esculenta* which is 6.61%. Thus *Colocasia esculenta* has more mineral content than *Xanthosoma sagittifolium*. This result is in agreement with the result of Njoku and Ohia (2007) in the analysis of the corm of *Colocasia esculenta* and *Xanthosoma sagittifolium* which showed that *Colocasia esculenta* has higher percentage of ash content of 7.78% than *Xanthosoma sagittifolium* which has 4.60%. According to Okonkwo and Ogu (2014), the ash content of four spices such as nutmeg, rosemary, African nutmeg (ehuru) and Ashanti pepper gave 9.84%, 11.78%, 10.49%, and 6.33%, respectively. The value of Ashanti pepper is closed to the values of the cocoyam flowers while others are relatively higher. However the Food and Agriculture Organisation standard for spices from flowers is 4.7-8%(FAO, 1997).

Fat content of samples is an indication of the presence of lipid substances. *Colocasia esculenta* showed greater amount of fat content of 14.05% while *Xanthosoma sagittifolium* had 13.13%. These values are greater than the fat content of the corm of *Xanthosoma sagittifolium* and *Colocasia esculenta* in Tanzania and Uganda which had values 0.43% and 0.44% respectively as reported by Ndabikunze, Talwana, Mongi, Issa-Zacharia, Serem, Palapala and Nandi (2011). Fat content of nutmeg and rosemary spice gave 11.50% and

14.30%, respectively as shown by Okonkwo *et al* (2014). However, the USDA nutrient database standard of fat content for spices is 8.69% (USDA, 2015).

The crude fibre represents the combustible organic residue that is left after other biomolecules have been removed. These can be referred to as the materials that are indigestible in human and animal organism. *Colocasia esculenta* had a greater percentage of crude fibre content of 15.32% than *Xanthosoma sagittifolium* that had 12.92%. These values are relatively high when compared to the values obtained by Odebunmi, Oluwaniyi and Bashim(2010) in the proximate analysis of garlic (0.73%), ginger (2.93%) and pepper (2.61%).

When the values for crude fibre content of the cocoyam flowers in Table 4.1is compared with the results of Olaleye *et al*., (2013) in the crude fibre content of cocoyam leaves, *Xanthosoma sagittifolium* leaves had 17.17% whereas *Colocasia esculenta* leaves had 17.41%. The fibre content values in Table 4.1 are significantly lower than these values.

In comparison with the crude fibre values of other known spices such as nutmeg (12.52%), rosemary spice (14.26%) and African nutmeg (13.66%) as reported by Okonkwo *et al.*, (2014), the values of the crude fibre for the two varieties of cocoyam as shown in Table 4.1

are also higher. However the USDA nutrient standard of crude fibre for spices is 21.6% (USDA, 2015).

The total carbohydrate is the portion or composition remaining after moisture, crude protein, ash, fat and fibre compositions has been removed(AOAC, 2000). It is an indication of energy giving ability of the sample. *Colocasia esculenta* had significantly higher amount of total carbohydrate of 25.11% as against *Xanthosoma sagittifolium* that had 13.75%. These values are significantly smaller when compared with the values of four spices; ginger (72.84%), garlic (73.03%), onions (76.71%), Ashanti pepper (67.59%) as reported by Nwinuka *et al*., (2005). The total carbohydrate of other three spices; nutmeg (41.57%), rosemary (45.84%) and African nutmeg(44.84%) as shown by Okonkwo *et al* (2014) were also relatively higher than the flower extracts.

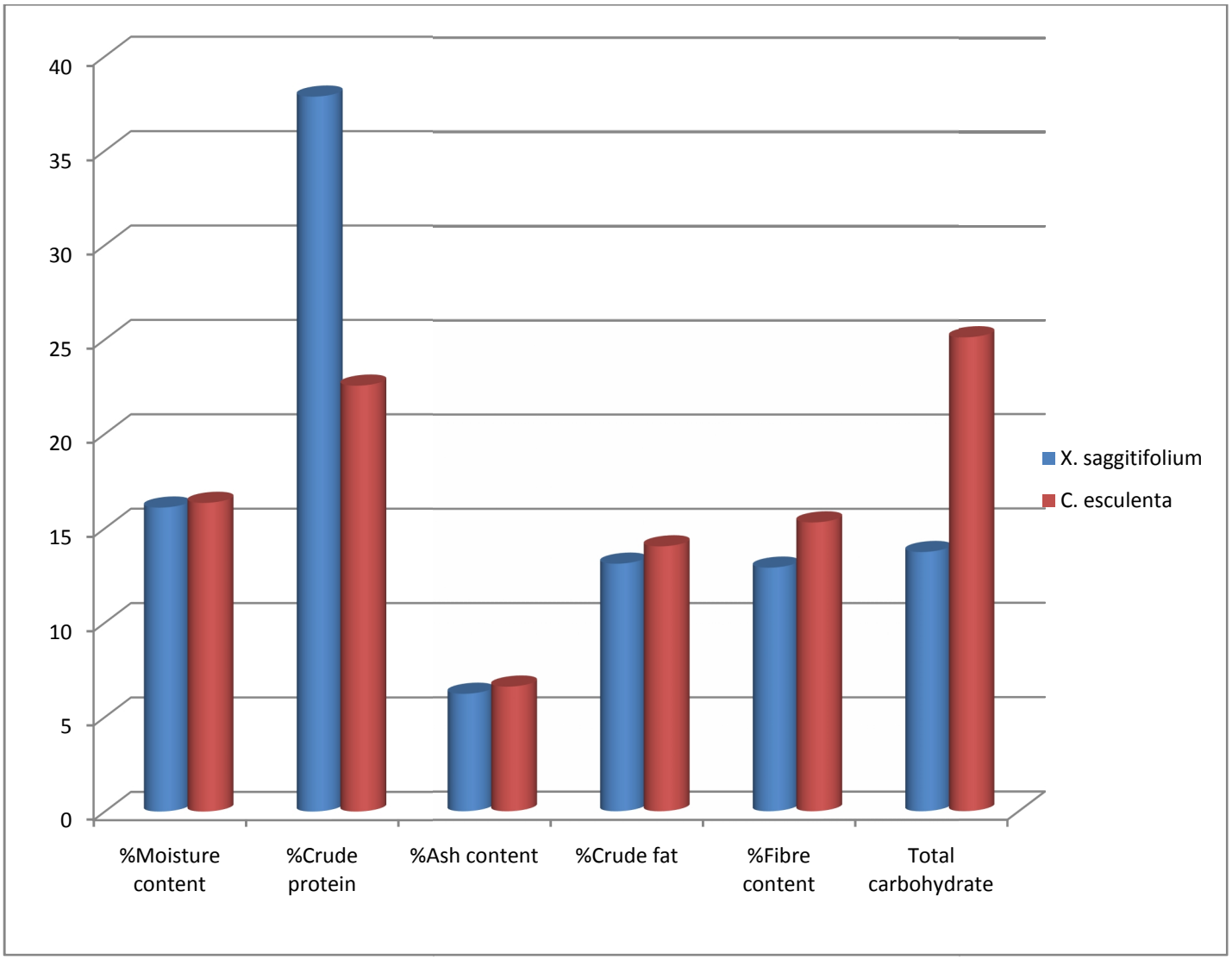

Figure 4.1: Bar chart for the Proximate Analysis of *Xanthosoma sagittifolium* and *Colocasia esculenta* flowers.

Table 4.2 Qualitative phytochemical analysis results for *Xanthosoma Saggitifoleum*

PHYTOCONSTITUENTS	OBSERVATION		INFERENCE	
	Flower	Stem	Flower	stem
Alkaloids	A deep creamy precipitate observed	A slight creamy precipitate observed	+++	++
Flavonoids	A very slight yellow precipitate observed	A very slight yellow precipitate observed	+	+
Glycosides	A very slight brown ring formed at interface	A very slight brown ring formed at interface	+	+
Phenols	A deep dark green colour observed	A slight deep dark green colour	+++	++
Saponins	A deep froth observed	A slight froth observed	+++	++
Steroids	A very slight bluish green colour observed	A very slight bluish green colour observed	+	+
Tannins	A very slight brownish green colouration	Very slightly brownish green colouration	+	+

KEY: (+) =very slightly present, (++) = slightly present, (+++) = deeply present

4.3:DISCUSSION OF QUALITATIVE PHYTOCHEMICAL RESULT FOR *Xanthosoma sagittifolium*

Table 4.2 shows the result of the qualitative phyto-analysis of *Xanthosoma sagittifolium* flower and stem extracts. Phytochemical analysis result shows that alkaloids, flavonoids, glycosides, phenols, saponins, steroid, tannins where all present in the flower extract of *Xanthosoma sagittifolium*. Phenols, alkaloids and saponins were deeply detected, while tannins, flavonoids, steroids and glycosides are very slightly detected.

From Table 4.2 also, the stem extract of *Xanthosoma sagittifolium* showed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroid, and tannins. The analysis reveals that phenols, saponins and alkaloids were slightly detected while, glycosides, steroids, flavonoids, tannins were very slightly detected.

Table 4.3 Qualitative phytochemical analysis results for *Colocasia esculenta*

PHYTOCONSTITUENTS	OBSERVATION		INFERENCE	
	Flower	Stem	flower	stem
Alkaloids	A deep cream precipitate observed	A slight cream precipitate observed	+++	++
Flavonoids	A slight yellow precipitate observed	A very slight yellow precipitate observed	++	+
Glycosides	A very slight brown ring formed at interface	A very slight precipitate brown ring formed at interface	+	+
Phenols	A deep dark green colour observed	A slight deep dark green colour	+++	++
Saponins	A slight froth observed	A slight froth observed	++	++
Steroids	A very slight bluish green colour observed	A very slight bluish green colour observed	+	+
Tannins	A very slight brownish green colouration	A very slight brownish green colouration	+	+

KEY; (+) =very slightly present, (++) = slightly present, (+++) = deeply present

4.4 DISCUSSION OF QUALITATIVE PHYTOCHEMICAL RESULT FOR *Colocasia esculenta*

Table 4.3 shows the qualitative phytochemical analysis of *Colocasia esculenta* flower and stem. The analysis shows that alkaloids, flavonoids, glycosides, phenols, saponins, steroid, tannins where all present. In the flower extract, phenols and alkaloids were deeply detected. Flavonoids and saponins were detected slightly whereas glycosides, tannins and steroids were very slightly detected.

The stem extract of *Colocasia esculenta* showed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroid, and tannins. Saponins, alkaloids and phenols were slightly detected. Glycosides, tannins, flavonoids and steroids were very slightly detected.

Table 4.4: Quantitative phytochemical analysis result of flowers and stem sap of *Xanthosoma Sagittifolium* and *Colocasia esculenta*

Phytochemical	Flower(%)		Stem(%)	
	X. sagittifolium	*C. esculenta*	*X. sagittifolium*	*C. esculenta*
Saponins	6.61 ± 0.015	5.50 ± 0.03	4.71 ± 0.02	4.20 ± 0.02
Flavonoids	1.32 ± 0.02	3.20 ± 0.015	0.51 ± 0.012	1.80 ± 0.012
Alkaloids	6.20 ± 0.02	9.80 ± 0.01	4.31 ± 0.02	5.50 ± 0.02
Tannins	0.50 ± 0.01	1.26 ± 0.01	1.25 ± 0.015	1.16 ± 0.03
Glycosides	0.14 ± 0.3	0.13 ± 0.3	0.05 ± 0.4	0.09 ± 0.25

Values expressed as Mean ± Standard Deviation

4.5: DISCUSSION OF QUANTITATIVE PHYTOCHEMICAL RESULT FOR *Xanthosoma sagittifolium* AND *Colocasia esculenta* FLOWERS AND STEM SAP

Table 4.4 above shows the results of the quantitative analysis of *Xanthosoma sagittifolium* and *Colocasia esculenta* flowers and stems extracts. The flower of *Xanthosoma esculenta* gave 6.61% saponins, 1.32% flavonoids, 6.20% alkaloids, 0.50% tannins and 0.14% of glycosides. The value of the alkaloids are relatively high while the value for flavonoids and tannins are relatively low when compared to the results of Raney and Krishnakumari (2015) in the analysis of nutmeg spice which has alkaloid (3.17%), flavonoids (4.89%) and tannins (2.23%). Analysis of *Colocasia esculenta* flower as shown in Table 4.4 revealed that it contains 5.50% saponins, 3.20%flavonoids, 9.80% alkaloids, 1.26% tannins and 0.13% glycosides. The values of saponins and glycosides in *Colocasia esculenta* were comparatively smaller than the values in *Xanthosoma sagittifolium* whereas the values of flavonoids, alkaloids and tannins were higher in *Colocasia esculenta* than in *Xanthosoma sagittifolium*.

Analysis of the stem sap of *Xanthosoma sagittifolium* revealed that saponins content(4.71%), flavonoids (0.51%), alkaloid (4.31%), tannin(1.25%), glycoside (0.05%) as shown in Table 4.4. These values are relatively high when compared to the result presented

by Olaleye *et al.*, (2013) on the analysis of the leaves of *Xanthosoma sagittifolium* in which the saponins was 1.44%, tannin was 0.47%, alkaloids was 0.02%. Analysis of the stem sap of *Colocasia esculenta* as shown in Table 4.4 shows that the stem contained 4.20% saponins, 1.80% flavonoids, 5.50% alkaloids, 1.16%tannins and 0.09% glycosides. The saponins, tannin and alkaloids contents of the stem of *Colocasia esculenta* are comparatively higher than the value obtained from the leaves as reported by Olaleye *et al*., (2013) in which saponins was 1.50%, tannin was 0.76%, alkaloid was 0.75%.

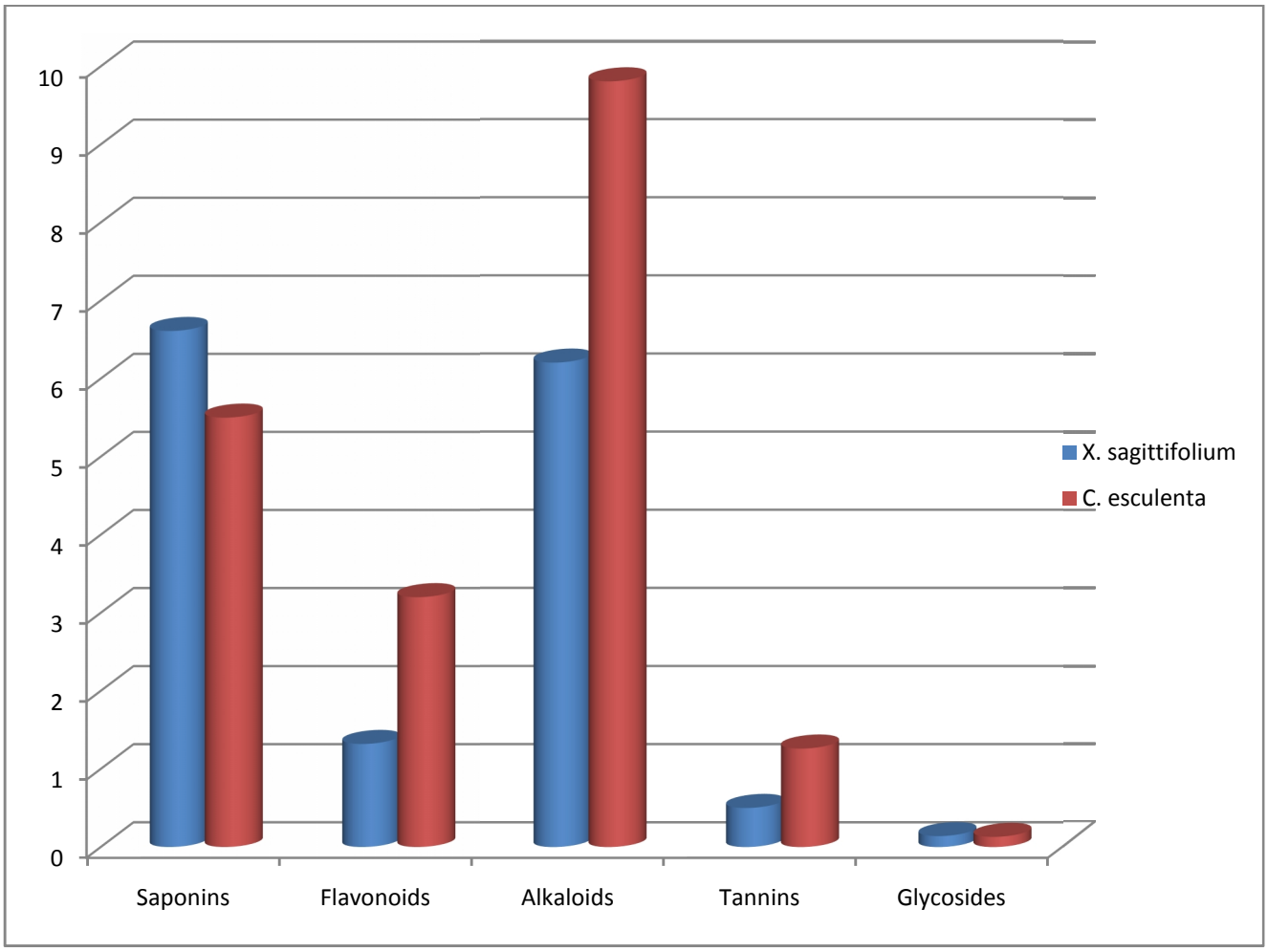

Figure 4.2; Bar chart for the quantitative phyto-analysis of the flowers extract of *Xanthosoma sagittifolium* and *Colocasia esculenta*.

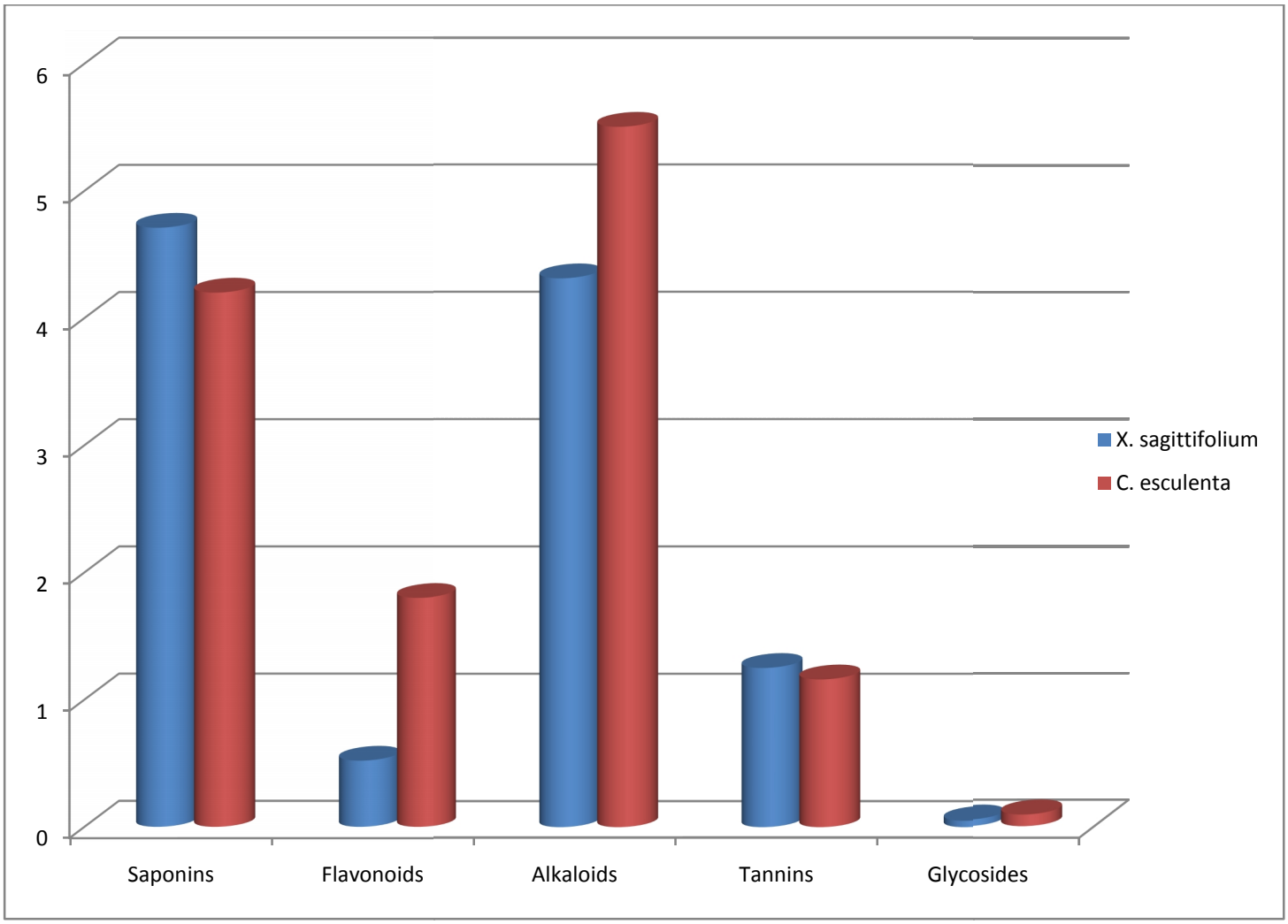

Figure 4.3; Bar chart for the quantitative phytochemical analysis of the stems sap of *Xanthosoma sagittifolium* and *Colocasia esculenta*

4.6 STATISTICAL ANALYSIS

The results of this analysis were expressed as the mean ± standard deviation. The statistical analysis was carried out using t-test. Difference between the means was considered to be significant at 95% confidence limit.

4.6.1 Statistical Analysis of Proximate Analysis Result.

Table 4.5T-test statistical table for proximate analysis

Parameter	Degree of freedom	Calculated value($t_{stat.}$)	Table value ($t_{crit.}$)
%Moisture content	2	-4.25	4.3026
%Crude protein	4	1053.94	2.7765
%Ash content	3	-14.72	3.1824
%Fat content	2	-34.65	4.3026
%Crude fibre	4	-114	2.7765

From the decision rule stated in chapter one, If $t_{stat.}$is greater than $t_{crit.}$(t_{stat}> t_{crit}), reject H_0 and accept H_a. But if $t_{stat.}$is less than $t_{crit.}$(t_{stat}< t_{crit}), accept H_0 and reject H_a.

In the Table 4.5 above, the calculated values (t_{stat}) of the moisture content, ash content, fat content and crude fibre are all less than the table value ($t_{crit.}$) thus the alternative hypothesis (H_a) is rejected while the null hypothesis (H_0) is accepted. But the calculated value in crude protein is greater than the table value, i.e t_{stat}>t_{crit} .Thus the null hypothesis (H_0) is rejected and the alternative hypothesis (H_a) is accepted.

In summary, there is no significant difference in the mean values of moisture content, ash content, fat content and crude fibre of the two cocoyam species. However there is significant difference in their crude protein content.

4.6.2: Statistical Analysis of Quantitative Phyto-Analysis Result

Table 4.6:T-test statistical table for quantitative phyto-analysis of *Xanthosoma sagittifolium* flower and *Colocasia esculenta* flower.

Parameter	Degree of freedom	Calculated value($t_{stat.}$)	Table value ($t_{crit.}$)
Saponins	3	56.29	3.1824
Flavonoids	4	67.97	2.7764
Alkaloids	3	130.04	3.1824
Tannins	4	-93	2.7764
Glycosides	4	-232	2.7764

From the Table 4.6 above, all the calculated values (t_{stat}) of the tannins and glycosides are less than the table values ($t_{crit.}$). Thus H_0 is accepted and H_a is rejected. But in saponins, flavonoids, alkaloids, (t_{stat}) > ($t_{crit.}$), Thus H_0 is rejected and H_a is accepted.

This implies that there is no significant difference in the, tannins and glycosides contents between *Xanthosoma sagittifolium* and *Colocasia esculenta* flowers. However there is significant difference in the saponins, flavonoids and alkaloids content of their flowers.

Table 4.7: T-test statistical table for quantitative phyto-analysis of *Xanthosoma sagittifolium* stem and *Colocasia esculenta* stem.

Parameter	Degree of freedom	Calculated value($t_{stat.}$)	Table value ($t_{crit.}$)
Saponins	4	-4.23	2.7764
Flavonoids	4	137.53	2.7764
Alkaloids	4	72.87	2.7764
Tannins	3	-31.23	3.1824
Glycosides	4	-2.45	2.7764

From the Table 4.7 above, all the calculated values (t_{stat}) of the saponins, tannins, and glycosides are less than the table values ($t_{crit.}$). Thus H_0 is accepted and H_a is rejected. But in flavonoids, alkaloids, (t_{stat}) > ($t_{crit.}$), Thus H_0 is rejected and H_a is accepted.

This implies that there is no significant difference in the saponins, tannins and glycosides contents between *Xanthosoma sagittifolium* and *Colocasia esculenta* stem. However there is significant difference in the flavonoids, alkaloids content of their stems.

4.7 GC- MS RESULT PRESENTATION

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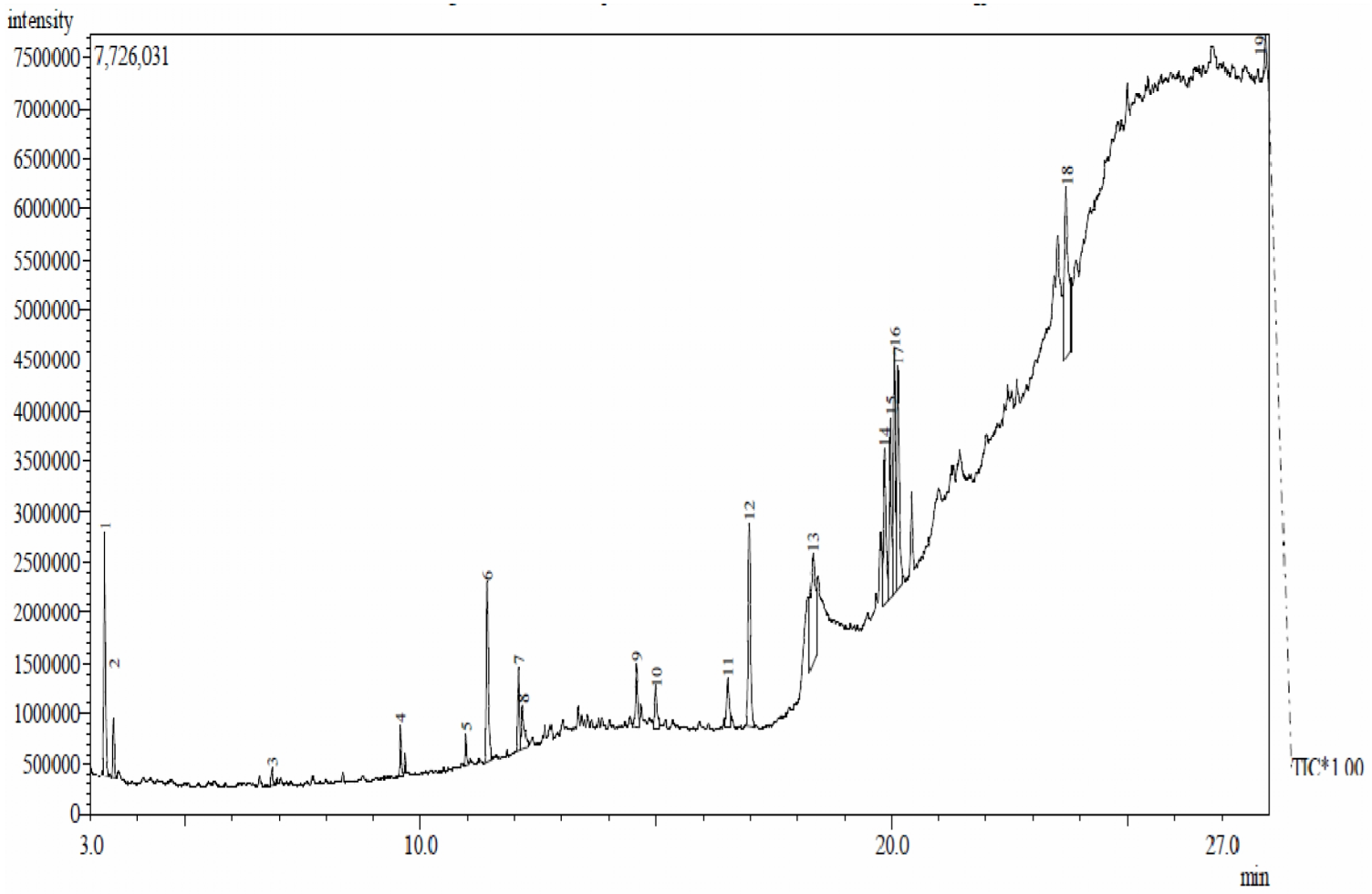

FIGURE 4.4; GC-MS Chromatogram of*Xanthosomasagittifolium*crude ethanol flower extract.

Table 4.8 ; Peak Report of Chromatogram of *Xanthosoma sagittifolium*crude ethanol flower extract

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.288	3.250	3.383	6458317	8.32	2417559	11.14	2.67	V	
2	3.489	3.383	3.567	1518299	1.96	599251	2.76	2.53	V	
3	6.843	6.800	6.900	401589	0.52	185976	0.86	2.16		
4	9.582	9.533	9.650	1244702	1.60	523247	2.41	2.38		
5	10.961	10.917	11.033	724731	0.93	302309	1.39	2.40		
6	11.412	11.358	11.525	5668559	7.31	1783938	8.22	3.18		
7	12.087	12.033	12.133	1925882	2.48	831129	3.83	2.32		
8	12.173	12.133	12.275	1664244	2.14	430172	1.98	3.87	V	
9	14.593	14.500	14.650	2116192	2.73	634086	2.92	3.34	V	
10	14.990	14.933	15.058	1596697	2.06	439644	2.03	3.63	V	
11	16.522	16.467	16.592	2044663	2.64	488603	2.25	4.18	V	
12	16.975	16.900	17.092	7954757	10.25	2031393	9.36	3.92		
13	18.329	18.258	18.392	6934068	8.94	1076930	4.96	6.44	V	
14	19.842	19.800	19.917	5455404	7.03	1533425	7.06	3.56	V	
15	19.967	19.917	20.008	5392603	6.95	1779659	8.20	3.03	V	
16	20.055	20.008	20.100	7919901	10.21	2427976	11.19	3.26	V	
17	20.136	20.100	20.250	7815528	10.07	2201631	10.14	3.55	V	
18	23.703	23.642	23.792	9784644	12.61	1691754	7.79	5.78	V	
19	27.923	27.858	27.958	970886	1.25	327901	1.51	2.96		
				77591666	100.00	21706583	100.00			

Table 4.9: Compounds identified in *Xanthosoma sagittifolium* flower extract.

Number	Compound Name	Retention Time (min)	Molar Mass	Molar Formular	Concentration (%)
1	3-Methylbutan-2-one	3.29	86	$C_5H_{10}O$	11.4
2	3-Methylpentan-2-one	3.49	100	$C_6H_{12}O$	2.76
3	2-Tridecene	6.84	182	$C_{13}H_{26}$	0.86
4	3-Tetradecene	9.58	196	$C_{14}H_{28}$	2.41
5	Tridecane	10.96	184	$C_{13}H_{30}$	1.39
6	2,6-Di-tert-butylphenol	11.41	206	$C_{14}H_{22}O$	8.22
7	Neodene 16	12.09	224	$C_{16}H_{32}$	3.83
8	2,3,5,8-tetramethyldecane	12.17	198	$C_{14}H_{30}$	1.98
9	1-Octadecene	14.59	252	$C_{18}H_{36}$	2.92
10	Dodecanal	14.99	184	$C_{12}H_{24}O$	2.03
11	2-Tetradecanone	16.52	212	$C_{14}H_{28}O$	2.25
12	Methyl Caprate	16.98	186	$C_{11}H_{22}O_2$	9.36
13	3-Hexadecene	18.33	224	$C_{16}H_{32}$	4.96
14	4-Cyclohexyl-2-butanone	19.84	154	$C_{10}H_{18}O$	7.06
15	Methyl-9,12-hexadecadienoate	19.97	266	$C_{17}H_{30}O_2$	8.2
16	Methyl-13,16-Octadecadienoate	20.06	294	$C_{19}H_{34}O_2$	11.19
17	11-Dodecen-2-one	20.14	182	$C_{12}H_{22}O$	10.14
18	Tridecane-2,4-dione	23.7	212	$C_{13}H_{24}O_2$	7.79
19	Squalene	27.92	410	$C_{30}H_{50}$	1.51

4.8DISCUSSION OF GC- MS RESULT FOR *Xanthosoma sagittifolium* Flower Extract

Figure 4.4 shows the GC-MS chromatogram ethanol extract of *Xanthosomasagittifolium* flower. The figure showed 19 peaks which indicate the presence of 19 compounds. The phytochemical constituents in comparison with the mass spectra of the constituents with the NIST library, the compounds were characterized and identified as shown in Table 4.9. From the chromatogram, the most prominent peaks are numbered 1, 6, 12, 14, 15, 16, 17 and 18. These peaks have significant percentage concentration. The chromatogram shows the relative concentration of the various compounds getting eluted as a function of time. The peak heights and the area under the peak indicates the relative concentration of the components present the sample. The mass spectrometer analyses the components eluted at different times to identify the compounds as shown in the Appendix 1to Appendix19.

These compounds can be characterized mainly as alkanones (ketones) which include; 3 methylbutane-2-one (PEAK 1) with percentage concentration of 11.4%. From the mass spectrum in Appendix 1, the molecular peak ion is at m/z =86 which corresponds to $[C_5H_{10}O]^+$ ion, the base peak(largest relative abundance) is found at m/z = 43 which is characteristics of alkanones and is due to the loss of $CH_3CH^+CH_3$ and the fragmentation m/z =71 is due to the loss of Methyl ion ($^+CH_3$). 4-Cyclohexyl-2-butanone (PEAK 14) with

percentage concentration of 7.06% shows a molecular peak ion of m/z = 154 which corresponds to $[C_{10}H_{18}O]^+$, base peak of m/z = 43 is due to the formation of $CH_3{}^+CO$. Other fragmentation patterns are m/z =58 $(C_3H_5O)^+$, m/z =71$(C_4H_7O)^+$, as shown in appendix 14.11-dodecen-2-one (PEAK 17) with percentage concentration of 10.14% showed a minor molecular ion peak at m/z = 182 which corresponds to $[C_{12}H_{22}O]^+$, a base peak fragmentation at m/z =43 which is characteristics of alkanones. And other fragmentation patterns at m/z =58 $(C_3H_6O)^+$, m/z =71$(C_4H_7O)^+$ as shown in appendix 17. Tridecene-2,4-dione (PEAK 18) with percentage concentration of 7.79% showed a minor molecular ion peak at m/z = 212 which corresponds to $[C_{13}H_{24}O_2]^+$ withbase peak fragmentation due to the formation of CH_3CO^+ at m/z =43. Other major fragmentation patterns at m/z =85 $(C_4H_5O_2)^+$, m/z = 100 $(C_5H_8O_2)^+$ as shown in appendix 18. Ketones compounds or alkanones have economic importance or industrial applications as they are widely used as solvents and polymer precursors.

Other compounds identified are classified as phenolic compounds such as 2,6-Di-tertbutylphenol (PEAK 6) with percentage concentration of 8.22%From the mass spectrum in Appendix 6, the molecular peak ion at m/z =206 which corresponds to $[C_{14}H_{22}O]^+$ ion, had base peak fragmentation at m/z =191 due to loss of Methyl ion ($^+CH_3$).This is an

alkylated phenol compound used as synthetic intermediate in the production of higher molecular weight phenolic antioxidants. It is also used as oxidation inhibitor and stabilizers for fuel, oil and gasoline. Also incorporated in plastic and rubber production.

The remaining of the compounds is characterized as carboxylic acid and esters. They are; Methyl Caprate (PEAK 12) and percentage concentration of 9.36% which has molecular ion fragmentation pattern at m/z =186 which corresponds to $[C_{11}H_{22}O_2]^+$ ion, base peak ion fragmentation at m/z =74 due to formation of ($^+CH_2COOCH_3$) ion, and other major fragmentation patterns at m/z =87($^+CH_2CH_2COOCH_3$), m/z = 43($^+CH_2CH_2CH_3$), m/z =41($^+CH_2CH_2CH_2$) as shown in appendix 12. Methyl-9,12-hexadecadienoate (PEAK 15) with percentage concentration of 8.2% which has molecular peak ion fragmentation pattern at m/z =266 which corresponds to $[C_{17}H_{30}O_2]^+$ ion, base peak ion fragmentation at m/z =67 $(C_5H_7)^+$ ion, and other major fragmentation patterns at m/z = 55 $(C_4H_7)^+$ ion, and m/z =41$(C_3H_5)^+$ ion which is characteristics of alkenes as shown in appendix 15.Methyl-13,16-Octadecadienoate (PEAK 16) with percentage concentration of 11.19% which has molecular ion fragmentation pattern at m/z =294, base peak ion fragmentation as m/z =55 which is due to formation of ($^+CH_2CHCHCH_3$), and other major fragmentation patterns at m/z =41 from ($^+CHCHCH_3$), m/z = 74 from ($^+CH_2COOCH_3$), m/z =87 from ($^+CH_2CH_2COOCH_3$) as shown

in appendix 16. Industrial application of carboxylic acids and esters cannot be over emphasized. Carboxylic acids and esters are used in the production of polymers, pharmaceutical solvents and food additives.

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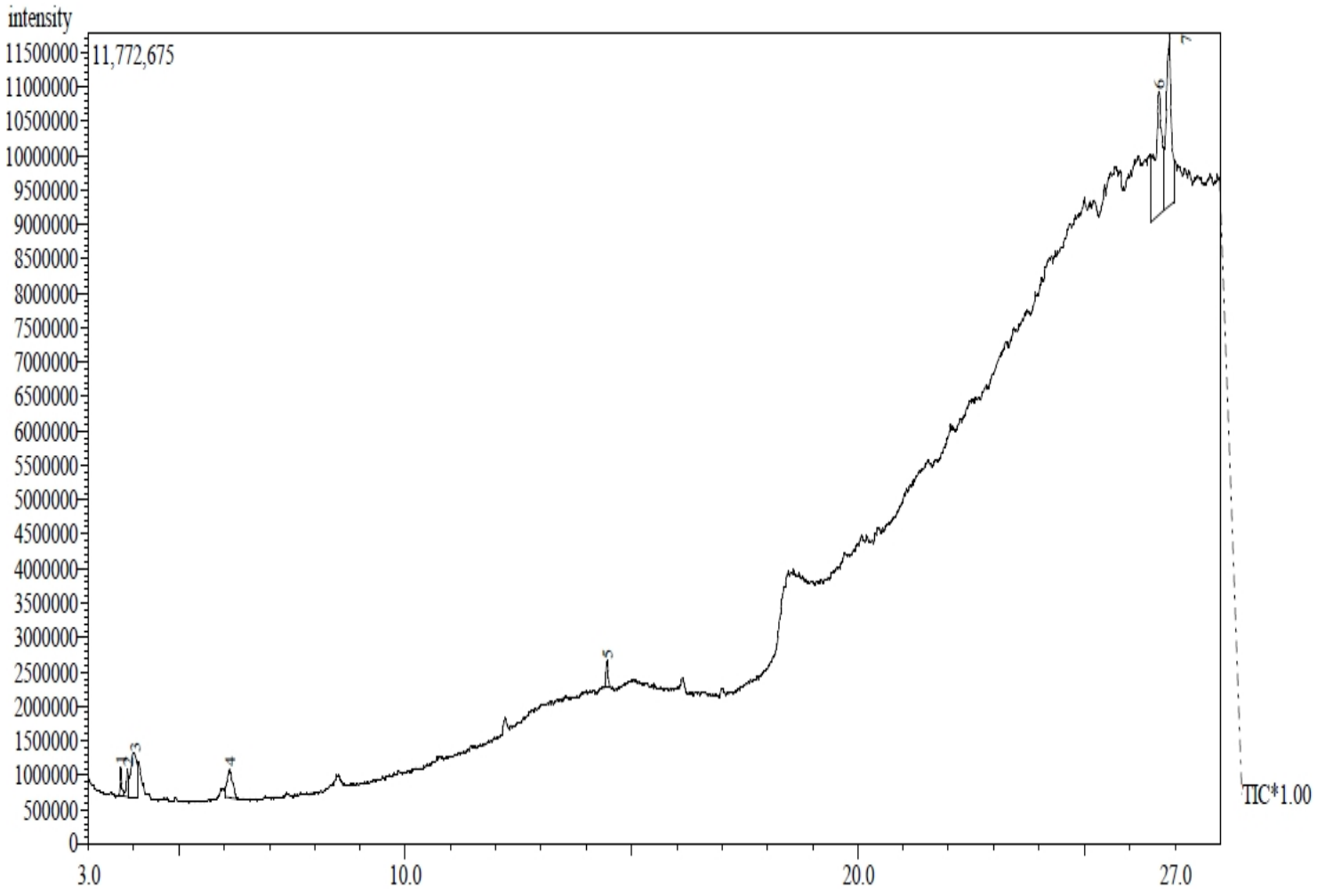

FIGURE 4.5: GC-MS Chromatogram of *Xanthosoma sagittifolium* stem sap extract

TABLE 4.10: Peak Report of Chromatogram of *Xanthosoma sagittifolium*crude ethanol stem extract

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.720	3.683	3.783	1026248	1.99	420019	6.41	2.44		
2	3.868	3.783	3.892	1200674	2.33	395603	6.03	3.04	V	
3	4.019	3.892	4.092	6004609	11.66	639334	9.75	9.39	V	
4	6.125	6.033	6.292	3344535	6.49	415717	6.34	8.05	V	
5	14.463	14.400	14.517	1287871	2.50	387949	5.92	3.32		
6	26.645	26.475	26.758	19507980	37.87	1792269	27.34	10.88	V	
7	26.876	26.758	26.975	19140345	37.16	2504575	38.21	7.64	V	
				51512262	100.00	6555466	100.00			

TABLE 4.11 Compounds identified in *Xanthosoma sagittifolium* stem extract

Number	Compound Name	Retention Time (min)	Molar Mass	Molar Formular	Concentration (%)
1	2-methylcyclopentanone	3.72	98	$C_6H_{1O}O$	6.41
2	2,4,5-Trimethyl-1-3-dioxolane	3.87	116	$C_6H_{12}O_2$	6.03
3	Octamethyl tetrasiloxane	4.02	296	$C_8H_{24}O_4Si_4$	9.75
4	2,3-dimethoxy-2-methylbutane	6.13	132	$C_7H_{16}O_2$	6.34
5	Phthalic acid,3,5-dimethylphenyl-4-methylphenyl ester	14.46	376	$C_{23}H_{20}O_5$	5.92
6	Permethrin	26.65	390	$C_{21}H_{20}Cl_2O_3$	27.34
7	Cis-permethrin	26.88	390	$C_{21}H_{20}Cl_2O_3$	38.21

4.9DISCUSSION OF GC- MS RESULT FOR *Xanthosoma sagittifolium* STEM EXTRACT

Figure 4.5 shows the GC-MS chromatogram of *Xanthosoma sagittifolium* stem extract. The figure showed 7 peaks which indicate the presence of seven compounds. The phytochemical constituents in comparison of the mass spectra of the constituents with the NIST library, the phytochemicals were characterized and identified as shown in Table 4.11.From the chromatogram, the most prominent peaks are numbered 6 and 7. These peaks have significant percentage concentrations. The peak heights and the area under the peak indicates the relative concentration of the components present the sample. The most prominent being Permethrin [Cyclopropane carboxylic acid, 3(2,2-dichlorovinyl)-2,2-dimethyl-(3-phenoxyphenyl) methyl ester] at PEAK 6 and PEAK 7 with total percentage concentration of 65.55%. From the mass spectrum in Appendix 25 and 26, the base peak is found at m/z = 183 which isdue to the loss of $(C_8H_9Cl_2O_3)^+$. The fragment at m/z = 77 is due to the formation of phenyl cation ($C_6H_5^+$), m/z =91 is due to formation of Tropylium ion, m/z = 163 is due to $^+C_7H_9Cl_2$.

According to National Pesticide Information Centre report (NPIC, 2009), permethrin is a common synthetic chemical widely used as an insecticide, acaricide and insect repellant. It belongs to the family of synthetic chemicals called pyrethroids. They can also be extracted

naturally from chrysanthemum flower. Health effect of permethrin depends on how someone is exposed to it. Mammals like dog and cat that have permethrin on their skin may act strangely, twitch their skin or ears or roll on the ground. Animals that have licked the skin treated with permethrin may drool a lot or smack their skin. When human beings get permethrin on their skin, they may have irritation, burning or itching at that spot. If permethrin gets into the eyes, it can cause redness, pain or burning. And if people eat permethrin, it could cause sore throat, abdominal pain, nausea and vomiting. Medically it is used in the treatment of scabies.

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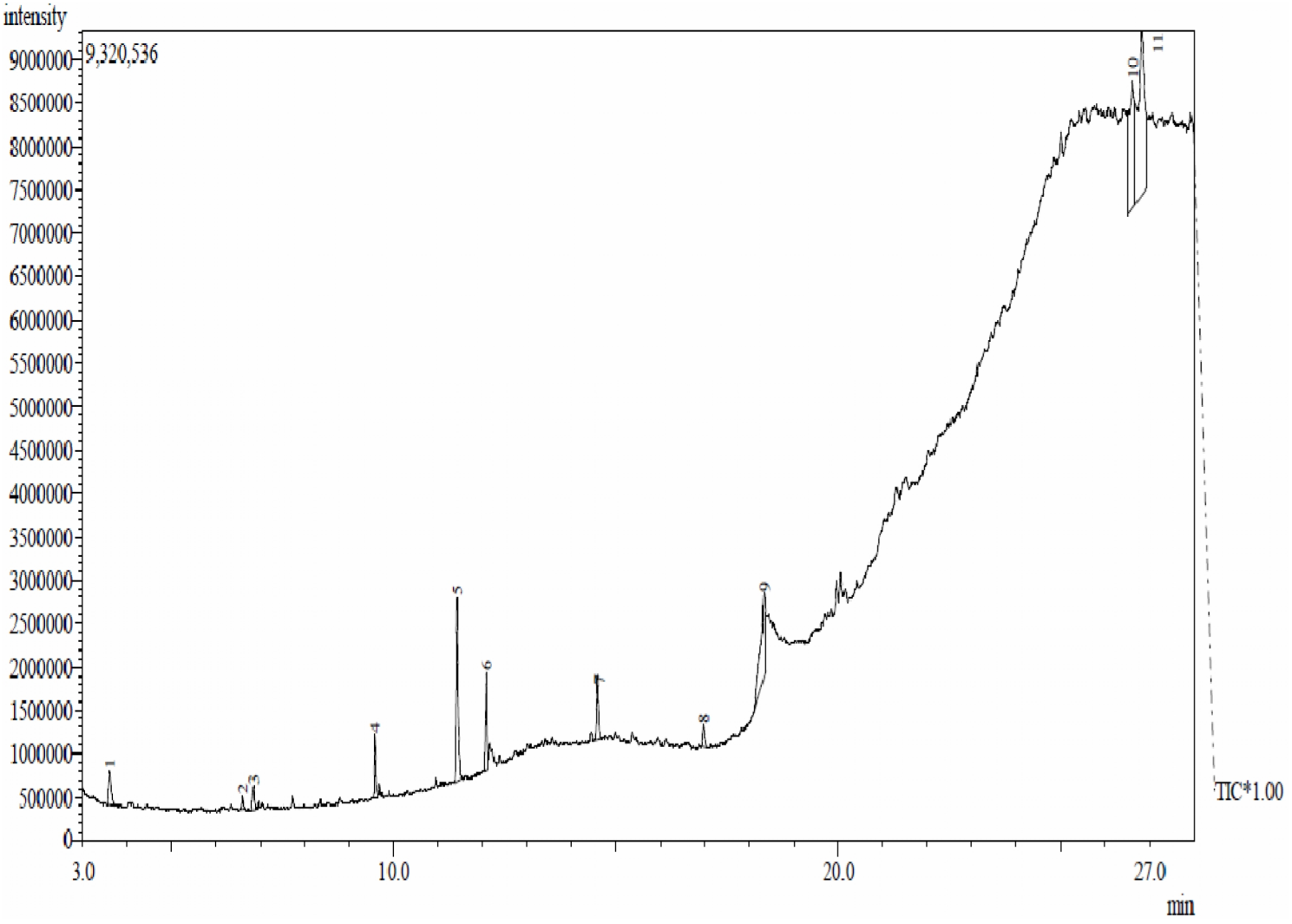

Figure 4.6.GC-MS Chromatogram of*Colocasia esculenta*crude ethanol flower extract.

Table 4.12: Peak Report of Chromatogram of *Colocasia esculenta* crude ethanol flower extract

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.617	3.558	3.750	1517918	2.68	388479	3.85	3.91		
2	6.603	6.567	6.658	391270	0.69	154980	1.54	2.52		
3	6.845	6.800	6.892	630434	1.11	271258	2.69	2.32		
4	9.585	9.542	9.650	1717340	3.03	732979	7.27	2.34		
5	11.429	11.375	11.550	6835315	12.08	2109325	20.91	3.24		
6	12.090	12.042	12.133	2468231	4.36	1128911	11.19	2.19		
7	14.597	14.492	14.675	2269316	4.01	738714	7.32	3.07	V	
8	16.977	16.925	17.075	906446	1.60	242018	2.40	3.75		
9	18.336	18.108	18.375	7587321	13.41	965665	9.57	7.86		
10	26.625	26.508	26.683	13094502	23.14	1466638	14.54	8.93	V	
11	26.823	26.683	26.933	19180247	33.89	1886706	18.71	10.17	V	
				56598340	100.00	10085673	100.00			

Table4.13 Compounds identified in *Colocasia esculenta* flower extract

Number	Compound Name	Retention Time (min)	Molar Mass	Molar Formular	Concentration (%)
1	Glycerol acetonide	3.62	132	$C_6H_{12}O_3$	3.85
2	3-hexene-2,5-diol	6.6	116	$C_6H_{12}O_2$	1.54
3	2-Tridecene	6.85	182	$C_{13}H_{26}$	2.69
4	3-Tetradecene	9.59	196	$C_{14}H_{28}$	7.27
5	2,6-Di-tert-butylphenol	11.43	206	$C_{14}H_{22}O$	20.91
6	1-tetradecene	12.09	196	$C_{14}H_{28}$	11.19
7	1-pentadecene	14.6	210	$C_{15}H_{30}$	7.32
8	Palmitic acid, methyl ester	16.98	270	$C_{17}H_{34}O_2$	2.4
9	10-Tetradecen-1-ol acetate	18.34	254	$C_{16}H_{30}O_2$	9.57
10	1-(chloromethyl)-4-phenoxybenzene	26.63	218	$C_{13}H_{11}ClO$	14.54
11	Permethrin	26.82	390	$C_{21}H_{20}Cl_2O_3$	18.71

4.10DISCUSSION OF GC- MS RESULT FOR *Colocasia esculenta* FLOWER EXTRACT

Figure 4.6 shows the GC-MS chromatogram of *Colocasia esculenta* ethanol flower extract. The figure showed 11 peaks which indicate the presence of eleven compounds. The phytochemical constituents on comparison of the mass spectra of the constituents with the NIST library, the phytochemicals were characterized and identified as shown in Table 4.13 above. The peak heights and the area under the peak indicates the relative concentration of the components present the sample. Thus from the chromatogram, the most prominent peaks are numbered 5, 6, 9, 10 and 11.These compounds can be characterized mainly as phenolic compounds, carboxylic acids, esters and olefins.

The phenolic compound is 2, 6–Di-tert-butylphenol (PEAK 5) and percentage concentration of 20.91%. The spectrum is shown in appendix 31. The molecular peak ion is at m/z =206 which corresponds to $[C_{14}H_{22}O]^+$ ion, base peak fragmentation at m/z =191 due to loss of Methyl ion ($^+CH_3$). Another major fragmentation is m/z = 57 which is formation of $CH_3{}^+C(CH_3)CH_3$.This is an alkylated phenol compound used as synthetic intermediate in the production of higher molecular weight phenolic antioxidants. It is also used as oxidation

inhibitor and stabilizers for fuel, oil and gasoline. Also incorporated in plastic and rubber production.

1-chloromethyl-4-phenoxybenzene (PEAK 10) showed a percentage concentration of 14.54%. The molecular ion peak fragmentation pattern shown in appendix 36 is m/z = 218 which corresponds to $[C_{13}H_{11}ClO]^+$, base peak fragmentation is m/z = 183 which is due to loss of chlorine atom and another major fragmentation is at m/z = 77 which corresponds to formation of phenyl cation ($C_6H_5^+$)as shown in appendix 36. This could be described as a chloro-aromatic compound mostly used in organic chemicals such as insecticides and pesticides.

The alkanoate such as 10-Tetradecen-1-ol acetate (PEAK 9) with percentage concentration of 9.57%. Base peak fragmentation is m/z = 43 due toCH_3C^+O and other fragmentations are m/z =41($C_3H_5^+$), m/z = 55 ($C_4H_7^+$), m/z 69 ($C_5H_9^+$), as shown in appendix 35. Permethrin (PEAK 11) with percentage concentration of 18.71% has also industrial applications.

The only olefin with high concentration is 1-tetradecene (PEAK 6) with percentage concentration of 11.19%. The molecular ion peak fragmentation pattern shown in appendix 32 is m/z = 196 which corresponds to $[C_{14}H_{28}]^+$ion, base peak fragmentation is m/z = 43

which is due to the formation of ($C_3H_7^+$). Other fragmentation patterns are m/z = 55 ($C_4H_7^+$), m/z = 83 ($C_6H_{11}^+$), m/z = 97($C_7H_{13}^+$), as shown in appendix 32. It can be used in the production of amine and amine oxides, oxo alchohols, alkylated aromatics, alpha olefin sulphonates, epoxides, tanning oils and synthetic fatty acids.

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GCMSANALYSIS

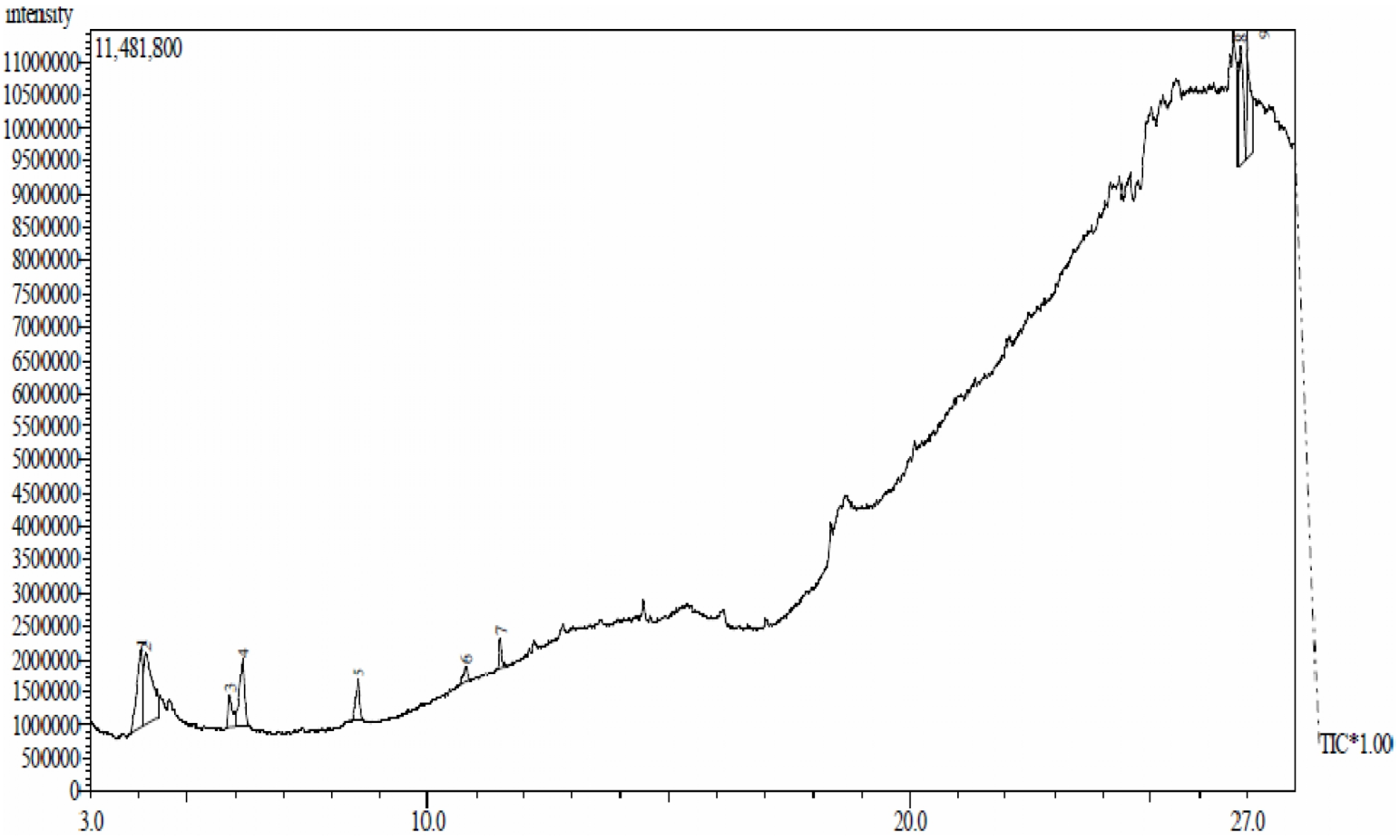

Figure 4.7.GC-MS Chromatogram of *Colocasia esculenta* ethanol crude stem extract.

Table 4.14 : Peak Report of Chromatogram of *Colocasia esculenta* ethanol crude stem extract

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	4.057	3.825	4.100	10244084	16.54	1194160	13.41	8.58		
2	4.153	4.100	4.417	13375448	21.59	1085646	12.19	12.32	V	
3	5.895	5.833	6.000	2824279	4.56	490325	5.51	5.76		
4	6.157	6.000	6.300	8034361	12.97	1038592	11.66	7.74	V	
5	8.550	8.425	8.650	3485635	5.63	625829	7.03	5.57		
6	10.794	10.667	10.842	1177497	1.90	246166	2.76	4.78		
7	11.510	11.458	11.608	1585101	2.56	461472	5.18	3.43		
8	26.855	26.808	26.950	10330749	16.68	1804857	20.27	5.72	V	
9	26.986	26.950	27.108	10886794	17.58	1956595	21.98	5.56	V	
				61943948	100.00	8903642	100.00			

Table4.15: Compounds identified in *Colocasia esculenta* stem extract

Number	Compound Name	Retention Time (min)	Molar Mass	Molar Formular	Concentration (%)
1	Octamethyl tetrasiloxane	4.06	296	$C_8H_{24}O_4Si_4$	13.41
2	Lactic acid	4.15	90	$C_3H_6O_3$	12.19
3	2-Furanmethanol	5.9	98	$C_5H_6O_2$	5.51
4	Decamethyl cyclopentasiloxane	6.16	370	$C_{10}H_{30}O_5Si_5$	11.66
5	Dodecamethyl cyclohexasiloxane	8.55	444	$C_{12}H_{36}O_6Si_6$	7.03
6	3-ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethy(siloxy)tetrasiloxane	10.79	562	$C_{17}H_{50}O_7Si_7$	2.76
7	3,5-di-tertbutylphenol	11.51	206	$C_{14}H_{22}O$	5.18
8	Permethrin	26.86	390	$C_{21}H_{20}Cl_2O_3$	20.27
9	Cis-Permethrin	26.97	390	$C_{13}H_{20}Cl_2O_3$	21.98

4.11DISCUSSION OF GC- MS RESULT FOR *Colocasia esculenta* STEM EXTRACT

Figure 4.7 shows the GC-MS chromatogram of *Colocasia esculenta* stem extract. The figure showed 9 peaks which indicate the presence of nine compounds. The phytochemical constituents in comparison of the mass spectra of the constituents with the NIST library, the phytochemicals were characterized and identified as shown in Table 4.15. The GCMS analysis of *Colocasia esculenta* stem sap revealed the presence of nine phytochemical compounds. The peak heights and the area under the peak indicates the relative concentration of the components present the sample. Thus from the chromatogram, there are five prominent peaks are numbered 1, 2, 4, 8 and 9. These compounds can be characterized mainly as organo-silicon compounds, carboxylic acids and ester.

The organo-silicon compounds are; octamethyl tetrasiloxane(PEAK 1) and percentage concentration of 13.42% usually called ‘’D_4 compounds’’. The most pronounced fragmentation is m/z = 281, which is actually the base peak fragmentation as shown in appendix 38. This is due to the loss of methyl ion ($^{+}CH_3$).Another compound is decamethyl cyclopentasiloxane (PEAK4) and percentage concentration of 11.66% usually called ‘’D_5 compounds’’. The spectra in appendix 41 shows that the base peak fragmentation is m/z = 73. Other major fragmentation is m/z = 267 and m/z = 355. These compounds are used in

the manufacture of silicone polymers and copolymers. Used also in the production health care products or cosmetics such as deodorants, hair sprays and antiperspirants.

The carboxylic acid present is lactic acid(PEAK 2) and percentage concentration of 12.19% The spectra in appendix 39 shows that the base peak fragmentation is m/z = 45 due to the loss of carboxylic group (-COOH) and the molecular peak ion at m/z = 90 which corresponds to $[C_3H_6O_3]^+$. Industrially used in tanning leather and dyeing wool. Also used as a flavouring agent preservative in food industries. Lactic acid can also be used as a raw material or catalyst in numerous chemical processes. The rest being Permethrin (PEAK 8 & 9) having a total percentage concentration of42.25%.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In the proximate analysis, the two flowers showed high moisture content and protein content which is relatively higher than the FAO and USDA nutrient standards for spices. The high moisture content will influence the physical properties of the cocoyam flowers which will affect the nutritional value. Thus this will make it unsuitable to be packaged as spice. The crude fat of the two varieties of cocoyam is higher than the USDA nutrient standard for crude fat. And the total carbohydrate is lower than the carbohydrates of many of the known spices like. These factors have made the flowers of *Xanthosoma sagittifolium* and *Colocasia esculenta* unsuitable to be packaged and marketed as a standard consumable spice. Also the presence of permethrin and chloro-aromatic compound to a significant percentage in the flower of *Colocasia esculenta* makes it toxic.

The study revealed that the flower and stem of *Xanthosoma sagittifolium* are rich in phenolic compounds, alkaloids and saponins. The flowers of *Colocasia esculenta* are rich in

phenolic compounds and alkaloids while the stems contain more of saponins, alkaloids and phenolic compounds.

The GC-MS analysis showed that the flowers of *Xanthosoma sagittifolium* and *Colocasia esculenta* both contain phenolic compounds which are common among spices. The flower of *Xanthosoma sagittifolium* contains about three prominent ketones compounds which can be grouped as flavones or flavonoids. But the flower of *Colocasia esculenta* contains high levels of permethrin and chloro-aromatic compounds. As a result of the presence of Permethrin and chloro-aromatic compounds, the flower of *colocasia esculenta* may be more toxic and therefore pose more health hazard than the flower of *Xanthosoma sagittifolium* if consumed.

Again the analysis revealed the presence of permethrin in the stem of the two varieties of cocoyam to a very significant percentage. Comparatively, *Xanthosoma sagittifolium* contain more percentage concentration of permethrin than *Colocasia esculenta.* From the information provided by National Pesticide Information Centre (2009), it can be said that the itchiness of cocoyam stem sap on the skin could be attributed to the presence of Permethrin in the stem sap of cocoyam.

5.2 RECOMMENDATION

The people living in the rural areas who still consume the flowers of cocoyam, or use them as spices(especially the flower of *Colocasia esculenta*) should be cautious of possible health hazard because of the presence of toxic compounds such as permethrin, chloro-aromatic compounds and other Pyrethroids compounds in them. Again farmers who are based on cocoyam cultivation should not expose their skin or wounded skin to the sap coming out from cocoyam stem as this may lead to permethrin poisoning.

5.3 CONTRIBUTION TO KNOWLEDDGE

This research work has shown that the stem sap of the two varieties of cocoyam contains a high percentage concentration of Permethrin compound which belongs to the family of pesticides called Pyrethroids. Since Permethrin is known to cause itching when in contact with the skin of animals, the itchiness and irritation of cocoyam stem sap may be attributed to permethrin.

Also, the consumption of cocoyam flower is not safe as the flowers contain toxic compounds such as permethrin and chloro-aromatic compound. Thus it is not advisable to use these flowers as a spices or food additives.

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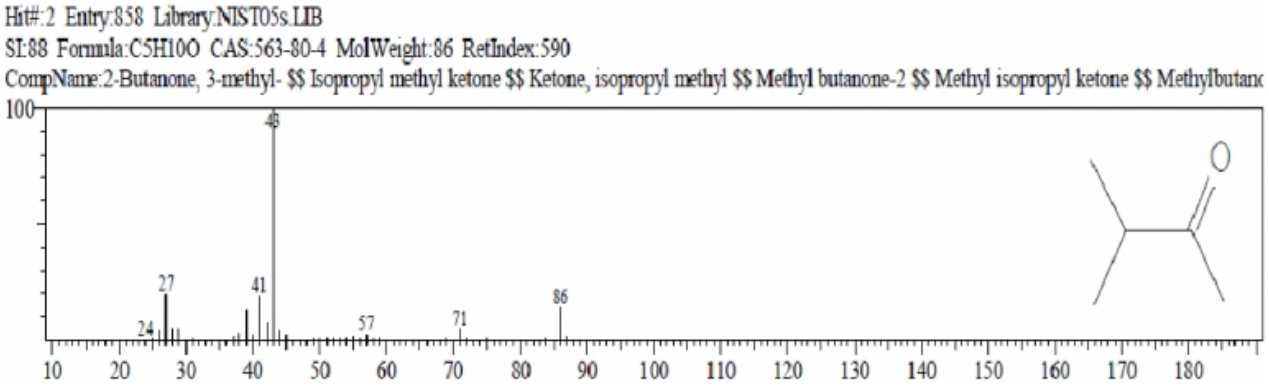
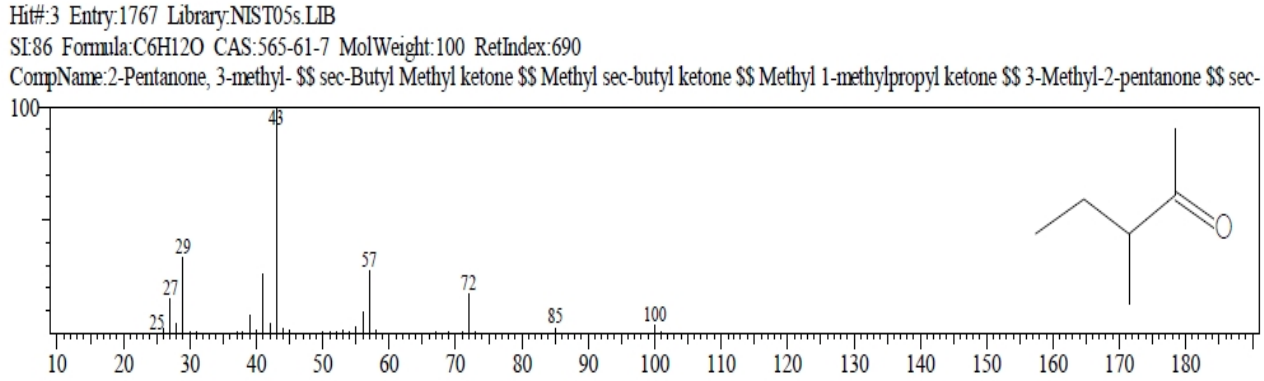
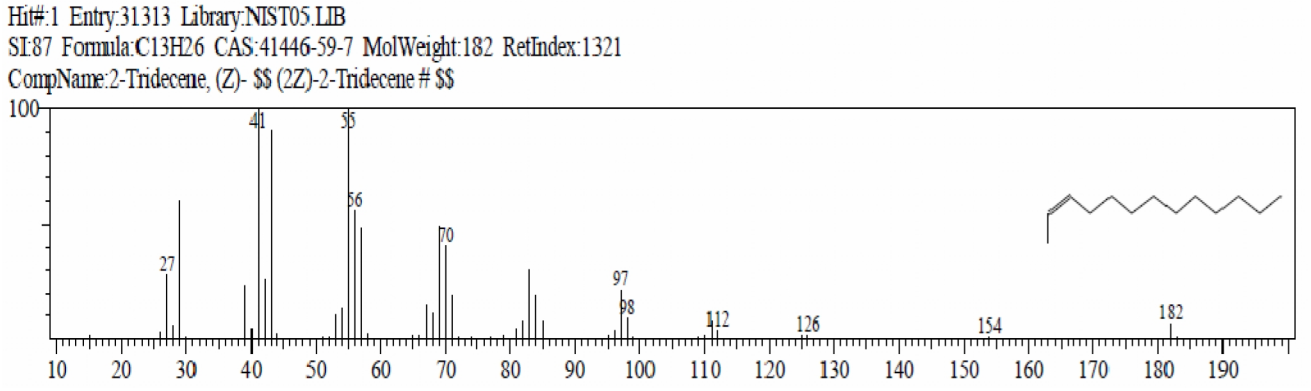
APPENDIX



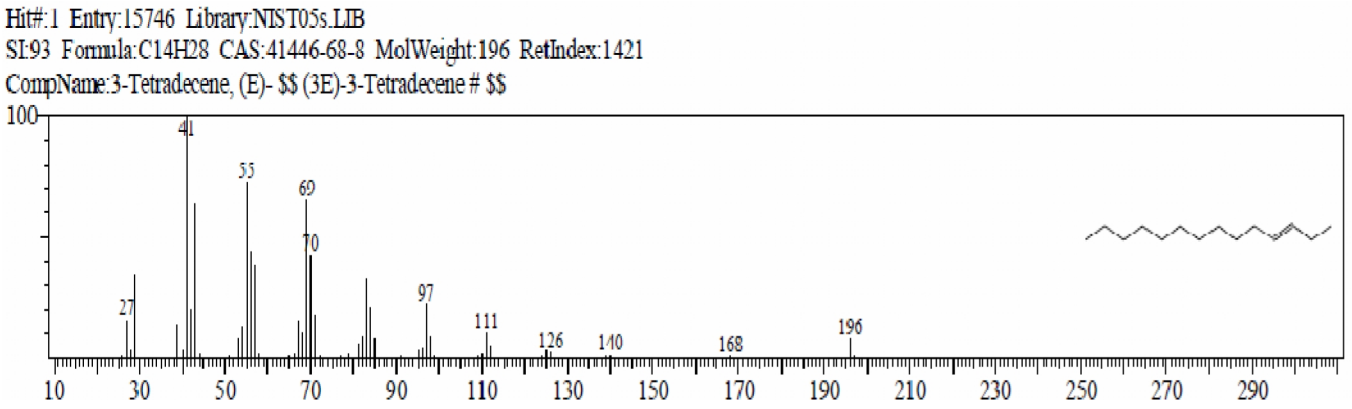
Appendix 1: 3-Methyl butan-2-one (PEAK 1 of *X. sagittifolium* flowerextract)



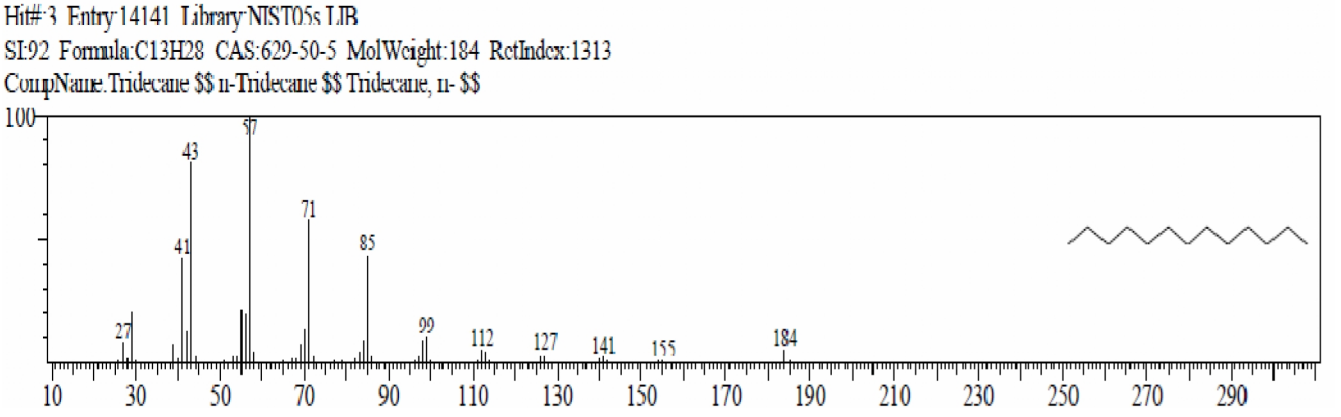
Appendix 2: 3-Methyl Pentan-2-one (PEAK 2 of *X. sagittifolium* flowerextract)



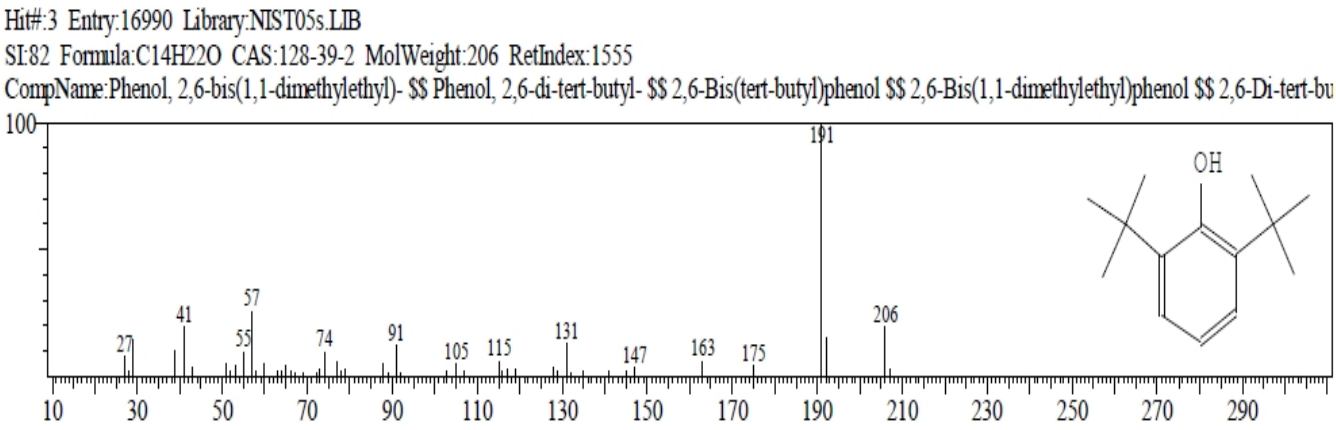
Appendix 3: 2-Tridecene (PEAK 3 of *X. sagittifolium* flowerextract)



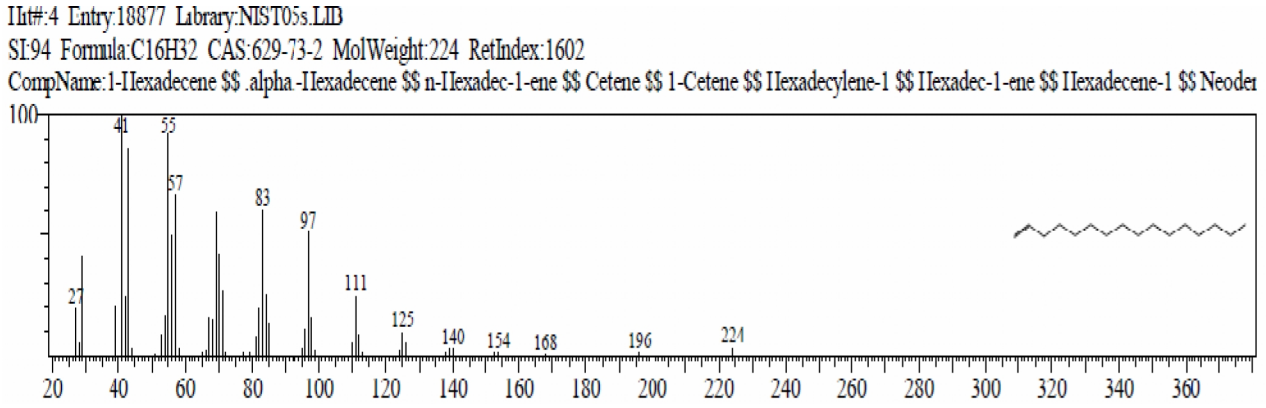
Appendix 4: 3-Tetradecene (PEAK 4 of *X. sagittifolium* flowerextract)



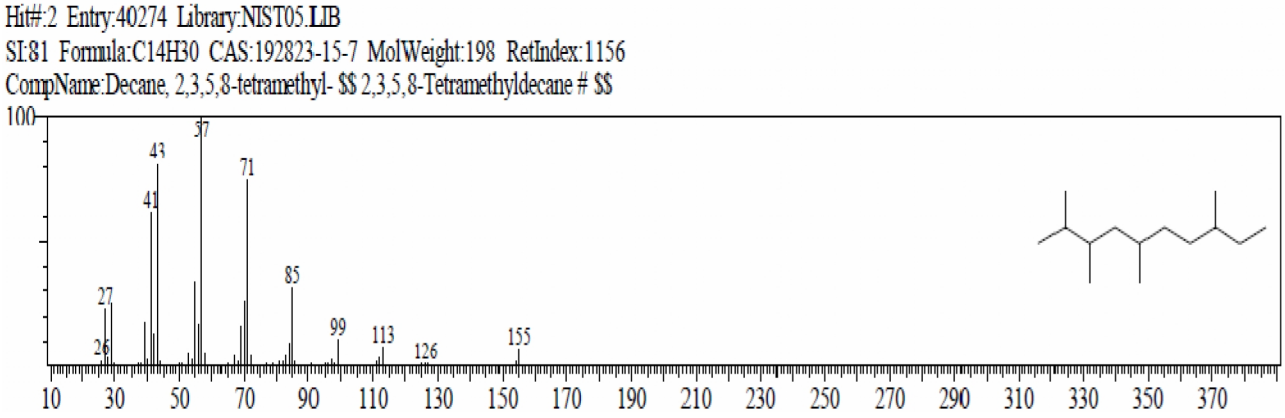
Appendix 5: Tridecane (PEAK 5 of *X. sagittifolium* flowerextract)



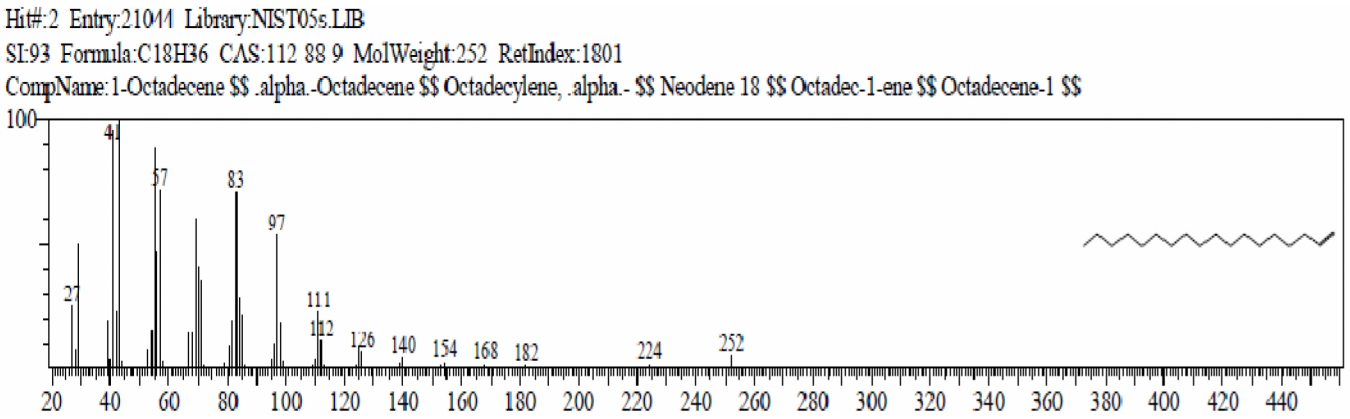
Appendix 6: 2, 6 –Di-tertbutylphenol (PEAK 6 of *X. sagittifolium* flowerextract)



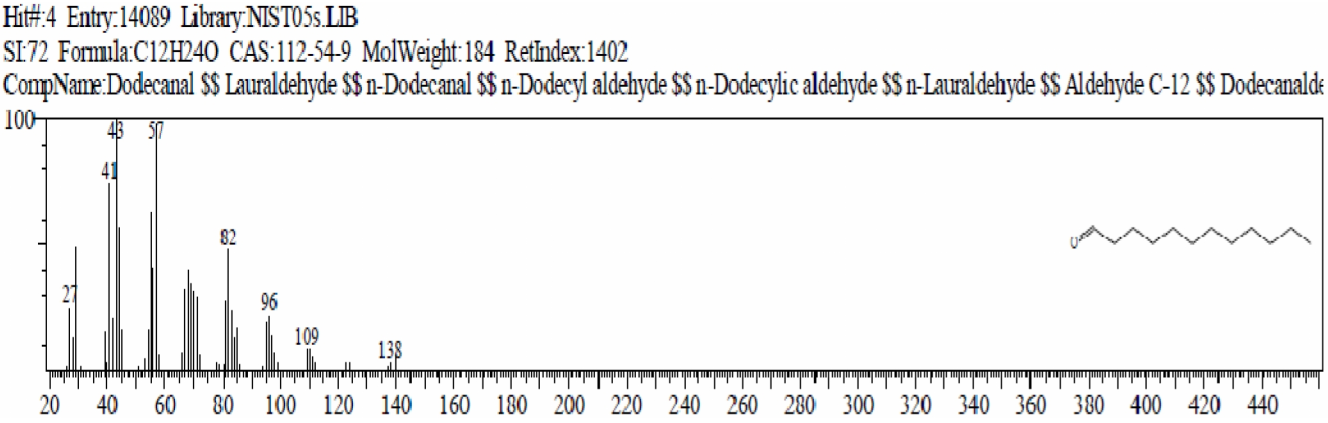
Appendix 7: 1-Cetene (PEAK 7 of *X. sagittifolium* flowerextract)



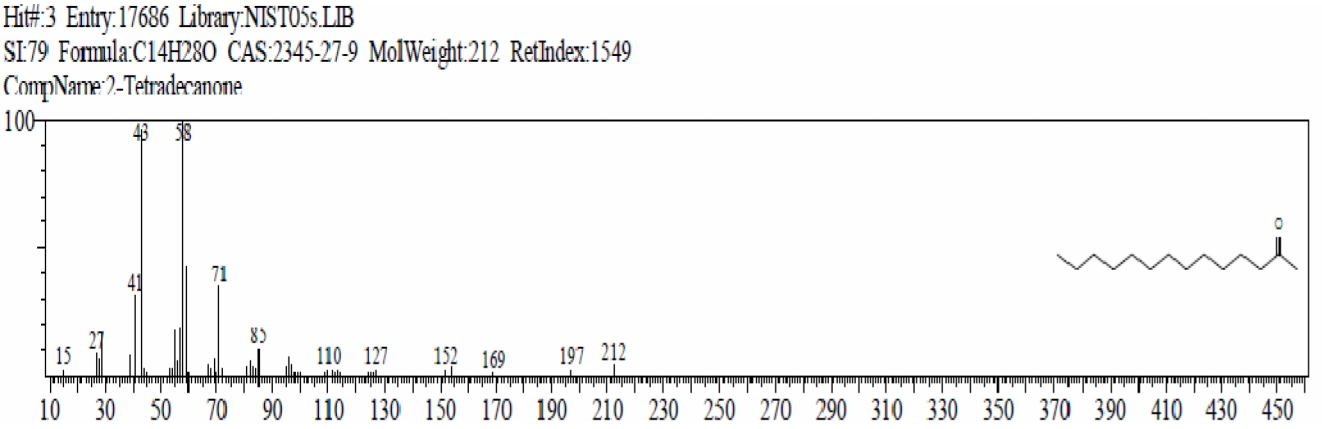
Appendix 8: 2,3,5,8-Tetramethyldecane (PEAK 8 of *X. sagittifolium* flowerextract)



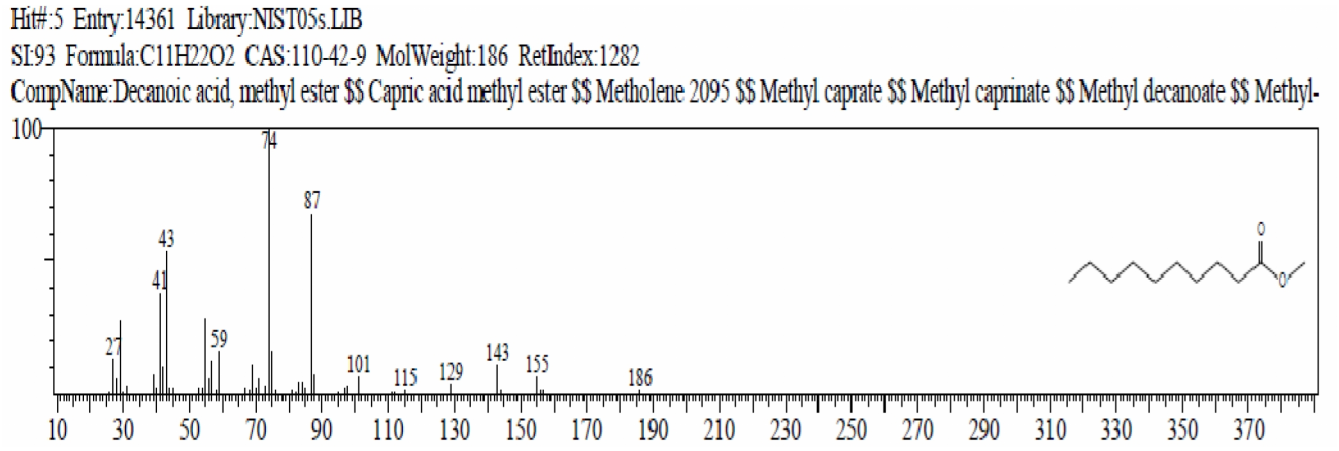
Appendix 9: 1-Octadecene (PEAK 9)



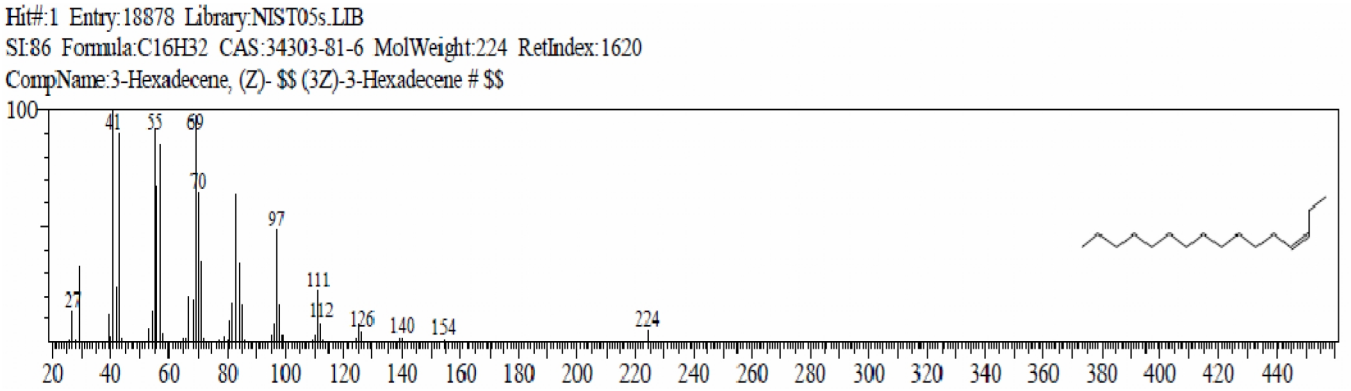
Appendix 10: Dodecanal (PEAK 10 of *X. sagittifolium* flowerextract)



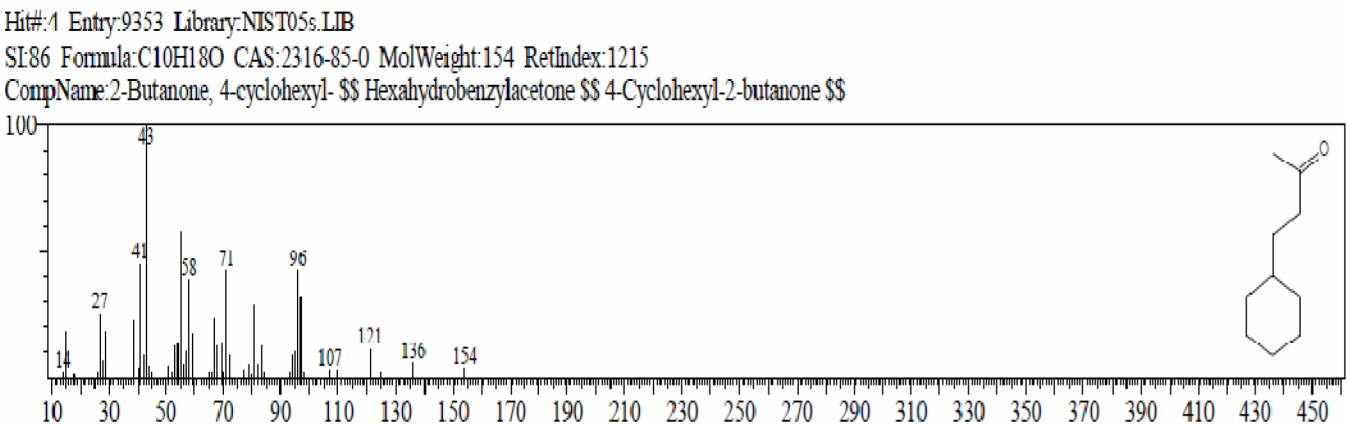
Appendix 11: 2-Tetradecanone (PEAK 11 of *X. sagittifolium* flowerextract)



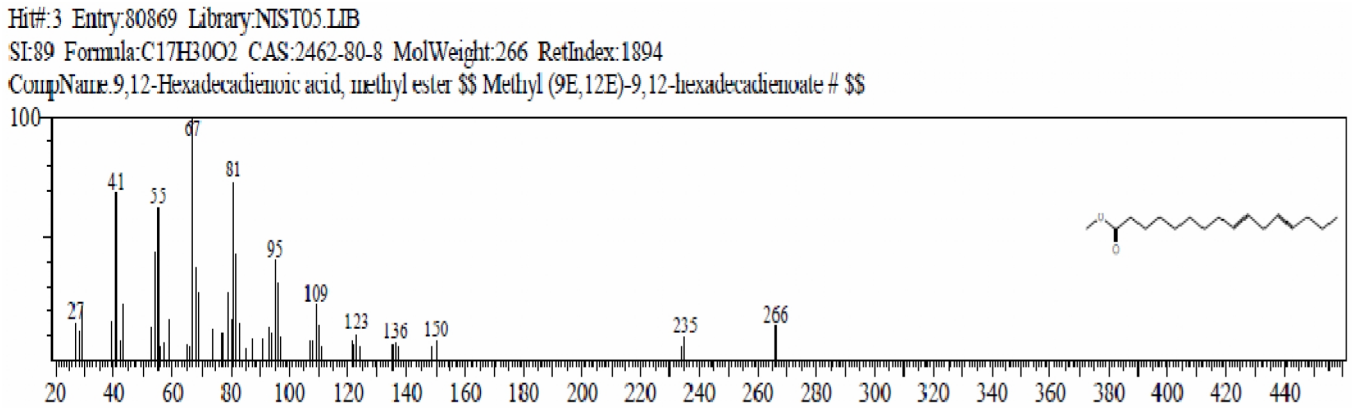
Appendix 12: Methyl Caprate (PEAK 12 of *X. sagittifolium* flowerextract)



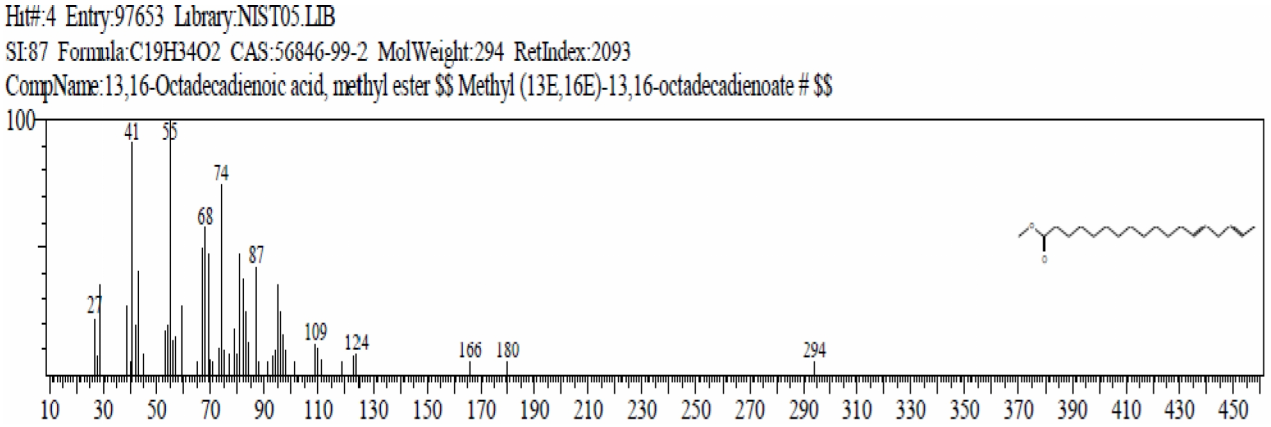
Appendix 13: 3-Hexadecene (PEAK 13 of *X. sagittifolium* flowerextract)



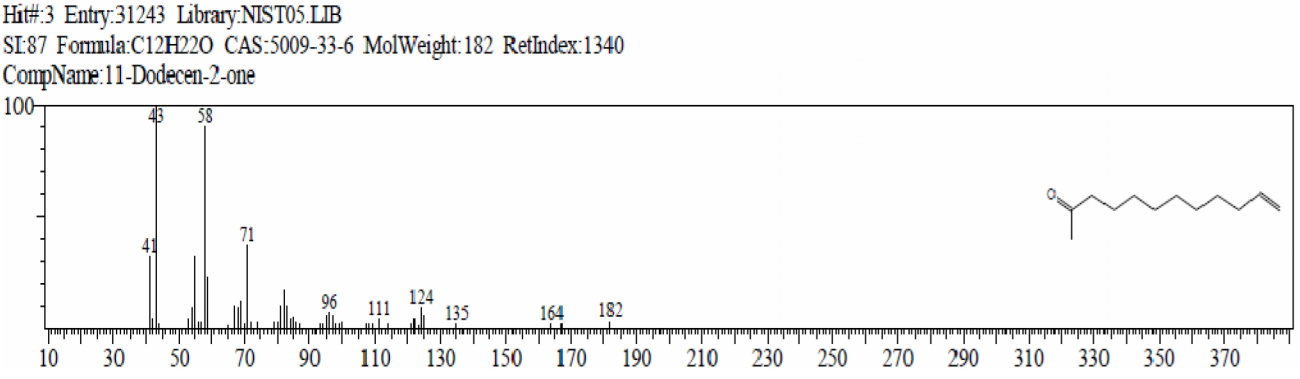
Appendix 14: 4-Cyclohexyl-2-butanone (PEAK 14 of *X. sagittifolium* flowerextract)



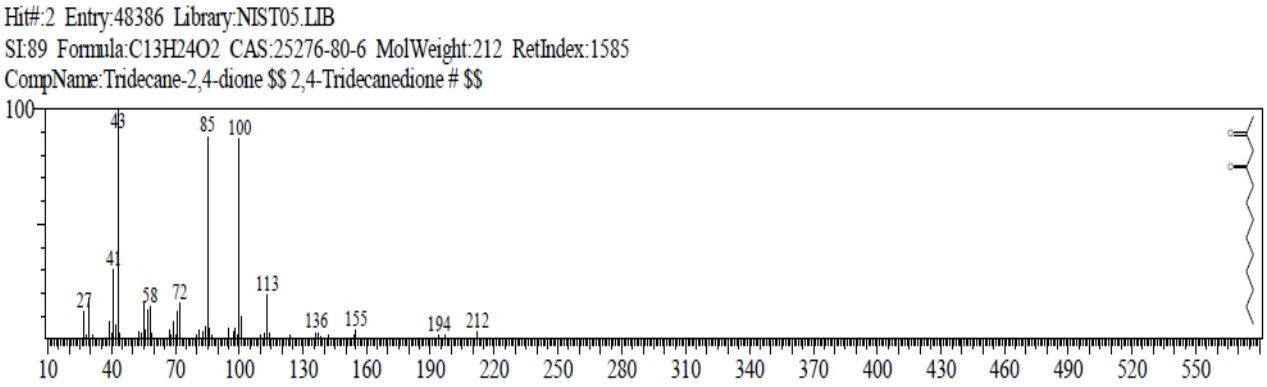
Appendix 15: Methyl-9,12-hexadecadienoate (PEAK 15 of *X. sagittifolium* flowerextract)



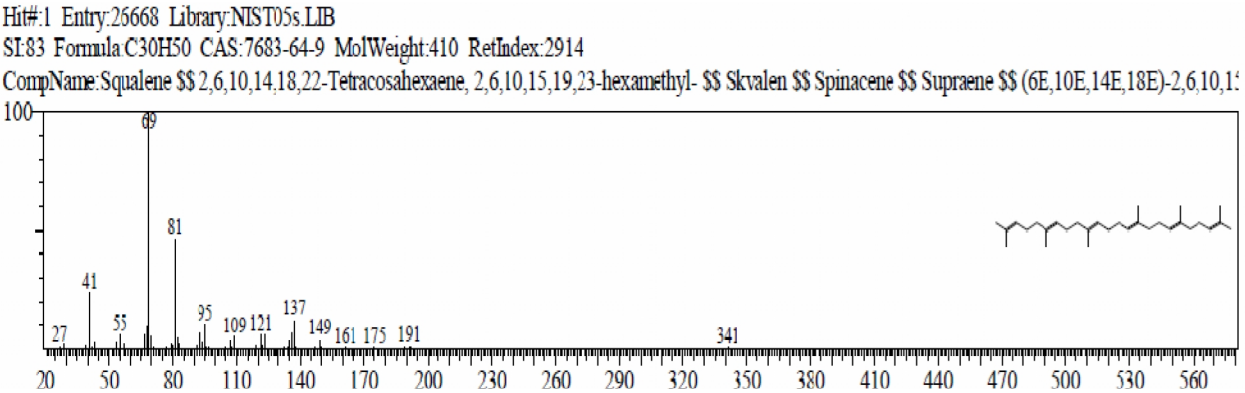
Appendix 16: Methyl-13,16-octadecadienoate (PEAK 16 of *X. sagittifolium* flowerextract)



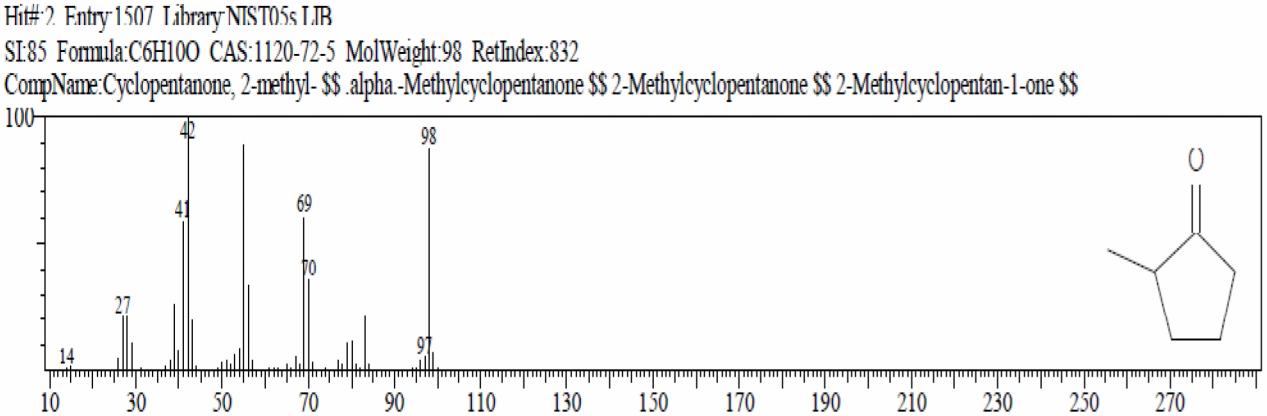
Appendix 17: 11-Dodecen-2-one (PEAK 17 of *X. sagittifolium* flowerextract)



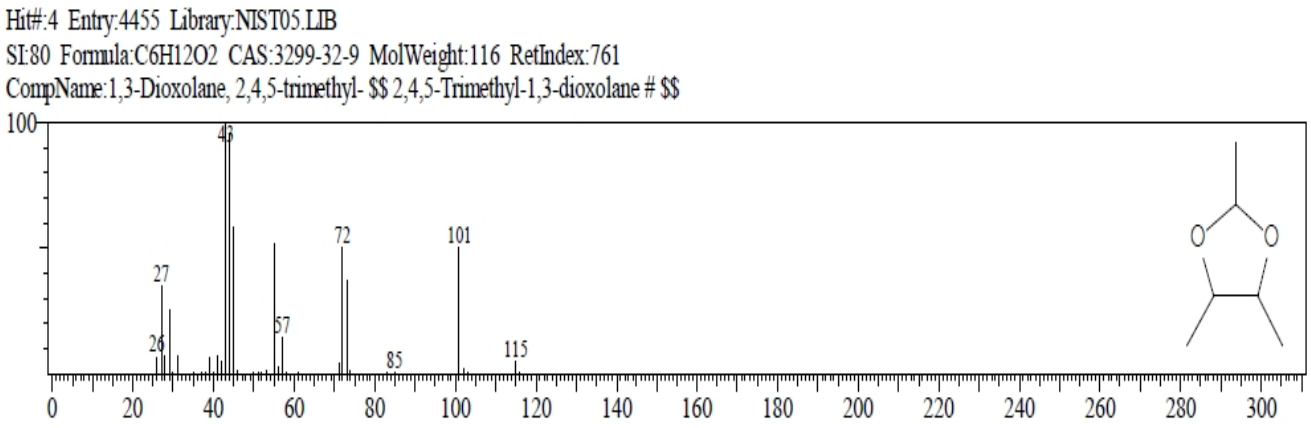
Appendix 18: Tridecane-2,4-dione (PEAK 18 of *X. sagittifolium* flowerextract)



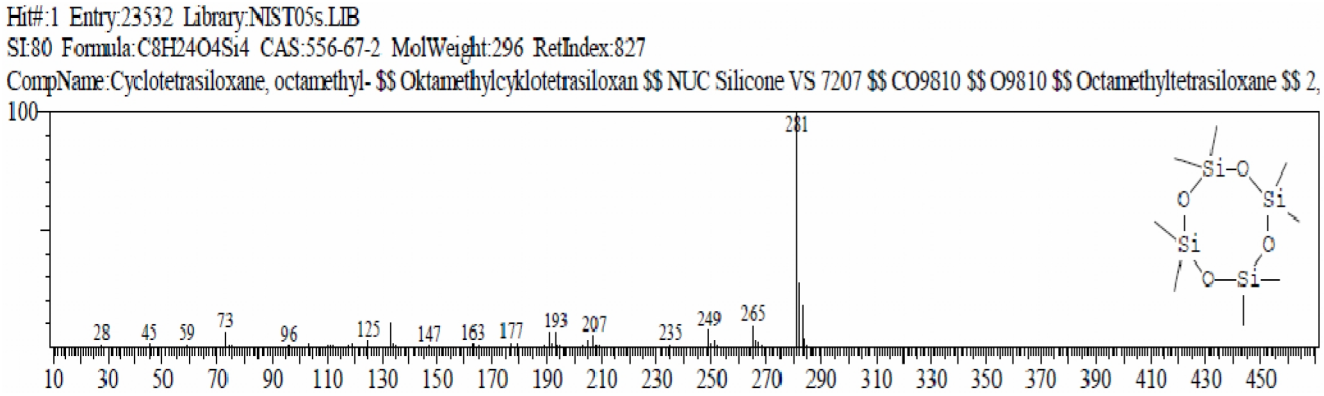
Appendix 19: Squalene (PEAK 19 of *X. sagittifolium* flowerextract)



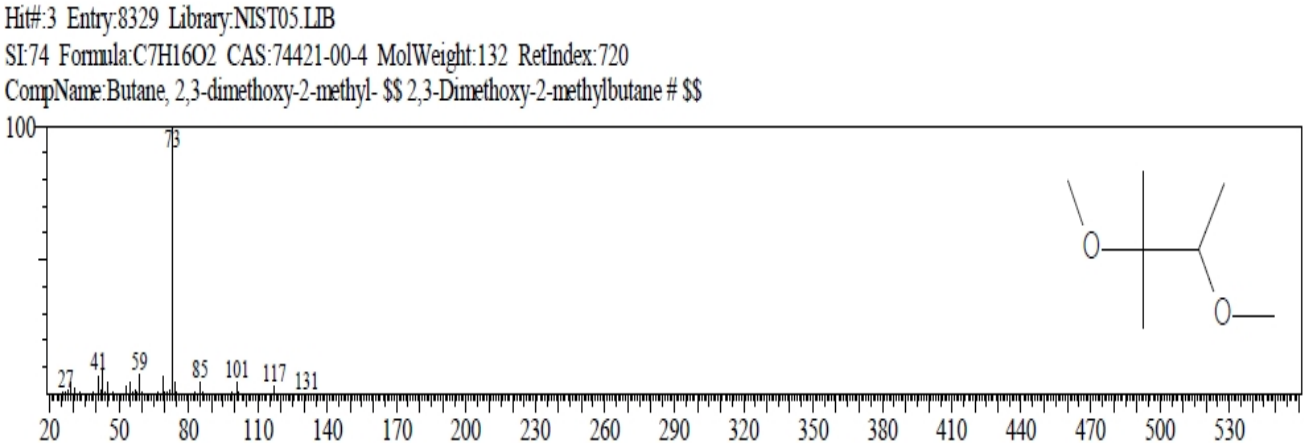
Appendix 20: 2-Methylcyclopentanone (PEAK 1 of *X. sagittifolium* stem extract)



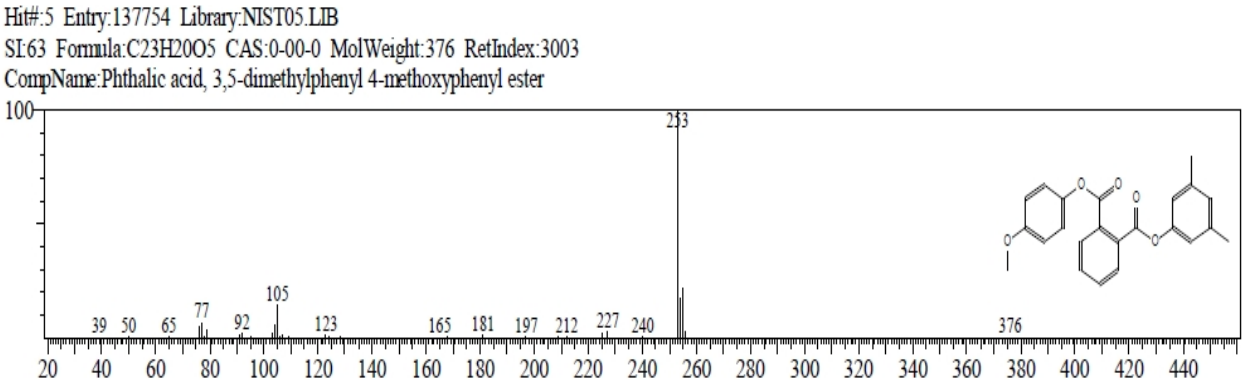
Appendix 21: 2,4,5- Trimethyl-1,3-dioxolane (PEAK 2 of *X. sagittifolium* stem extract)



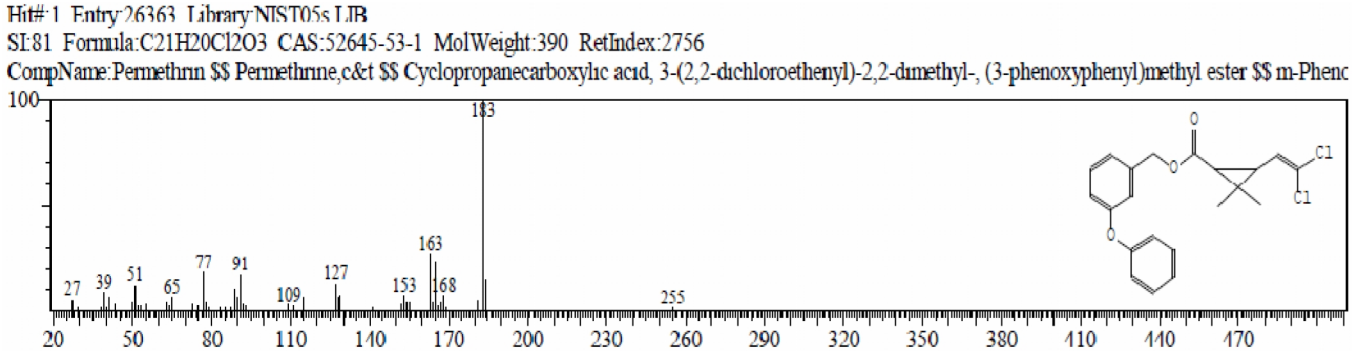
Appendix 22: Octamethyl Siloxane (PEAK 3 of *X. sagittifolium* stem extract)



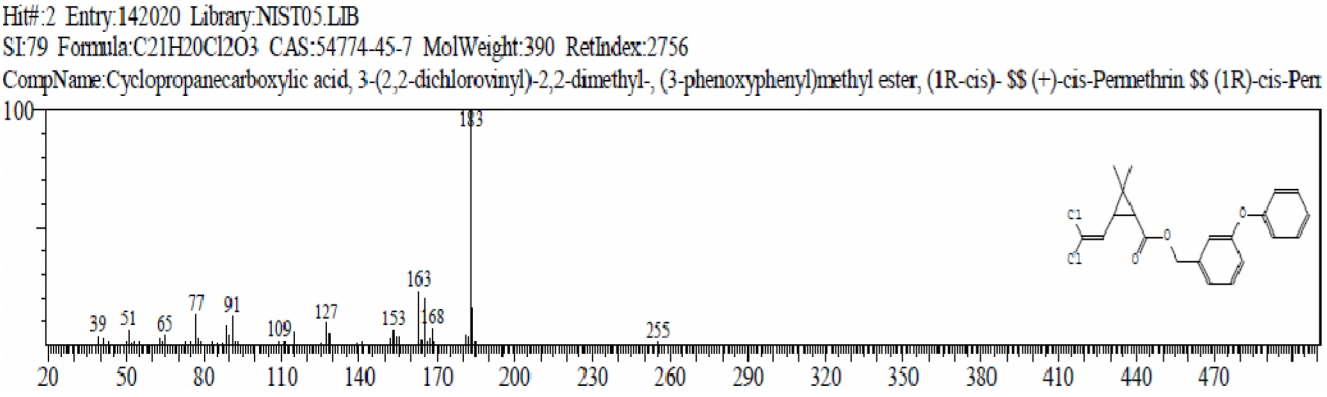
Appendix 23: 2,3-Dimethoxy-2-methylbutane (PEAK 4 of *X. sagittifolium* stem extract)



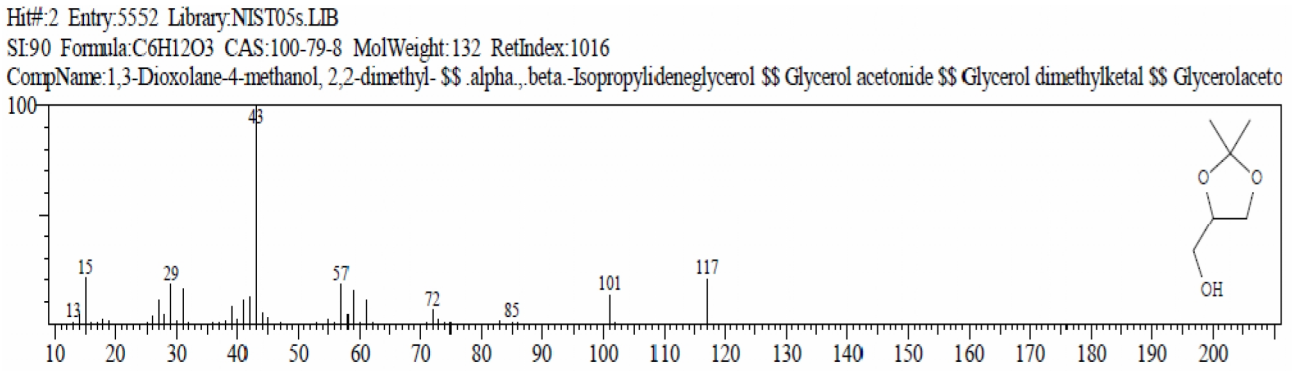
Appendix 24: Pthalic acid,3,5-dimethylphenyl-4-methoxyphenyl ester(PEAK 5 of *X. sagittifolium* stem extract)



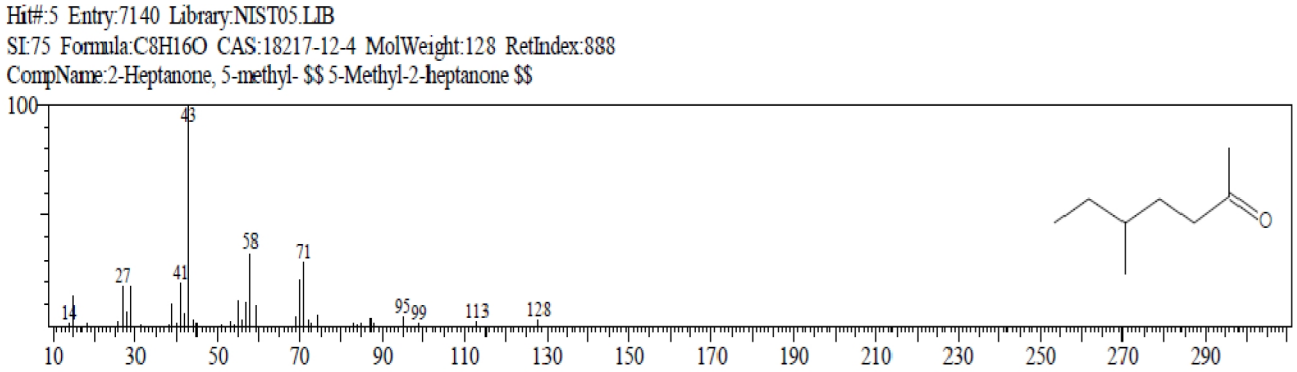
Appendix 25: Permethrin (PEAK 6of *X. sagittifolium* stem extract)



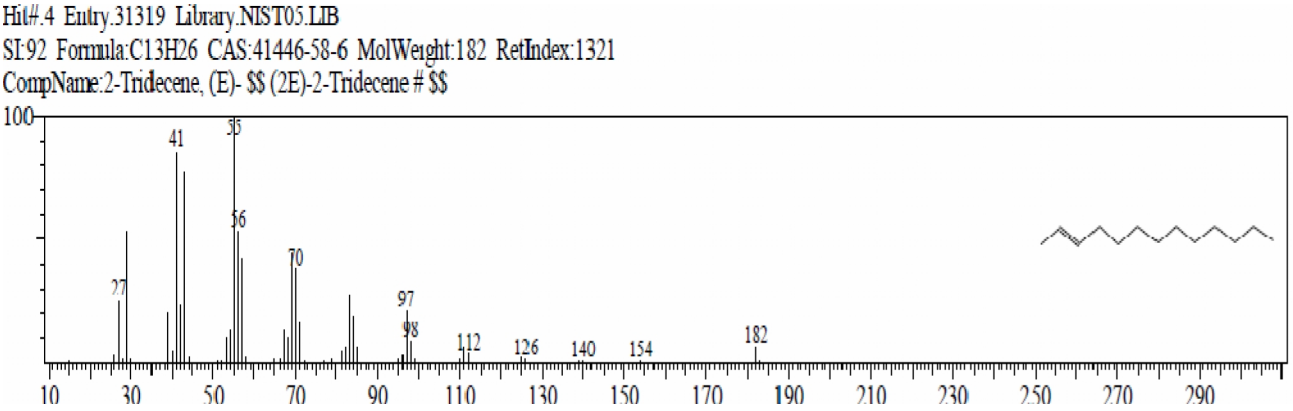
Appendix 26: Cis-permethrin (PEAK 7of *X. sagittifolium* stem extract)



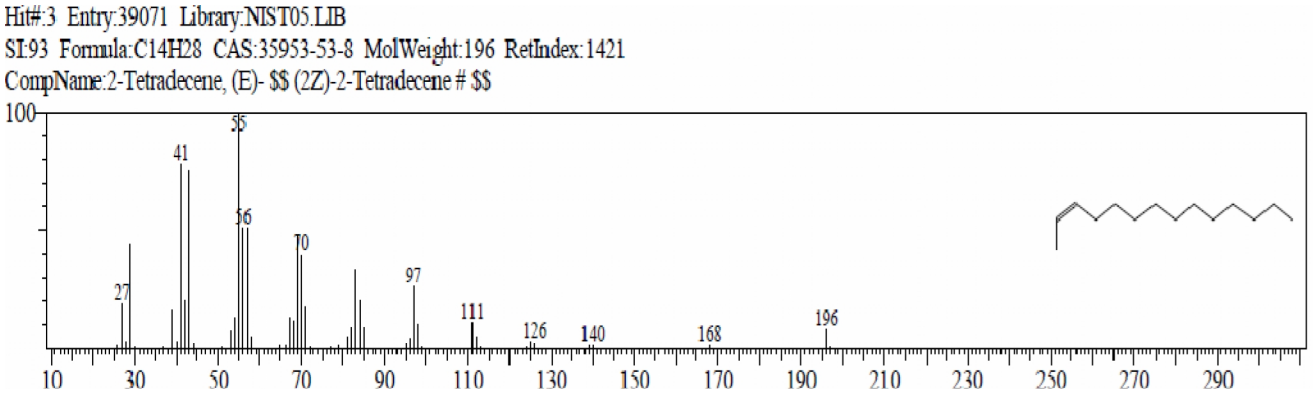
Appendix 27: Glycerol acetonide (PEAK 1of *C. esculenta* flower extract)



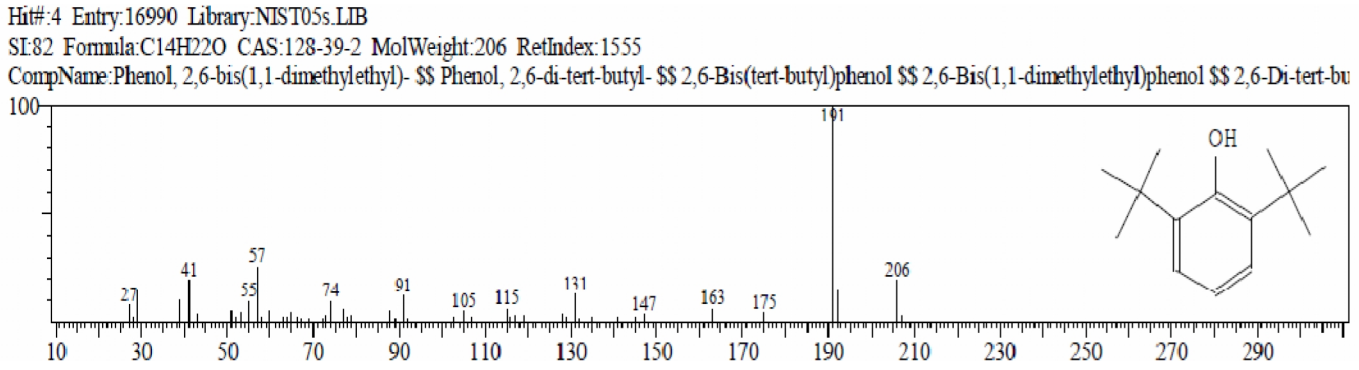
Appendix 28: 5-Methyl, 2-heptanone (PEAK 2of *C. esculenta* flower extract)



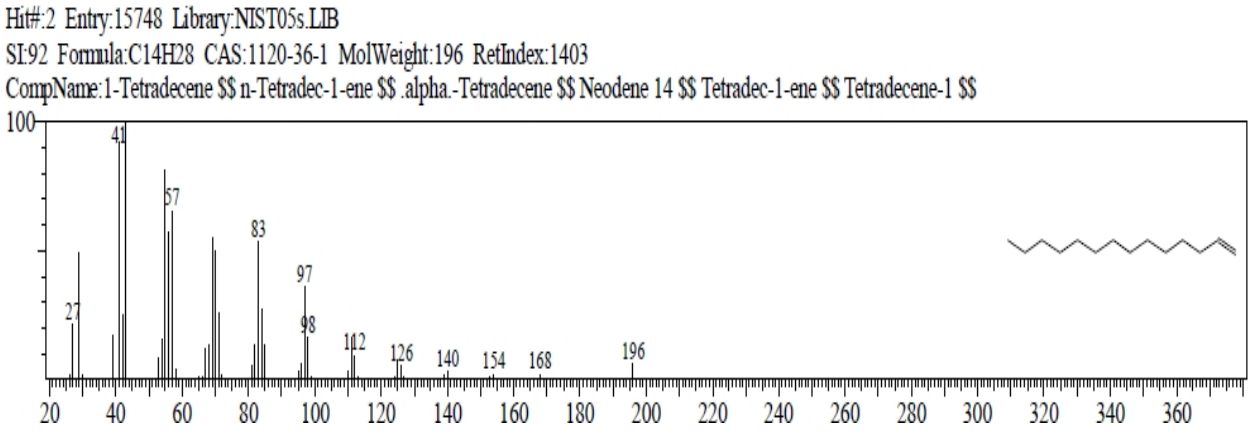
Appendix 29: 2-Tridecene (PEAK 3of *C. esculenta* flower extract)



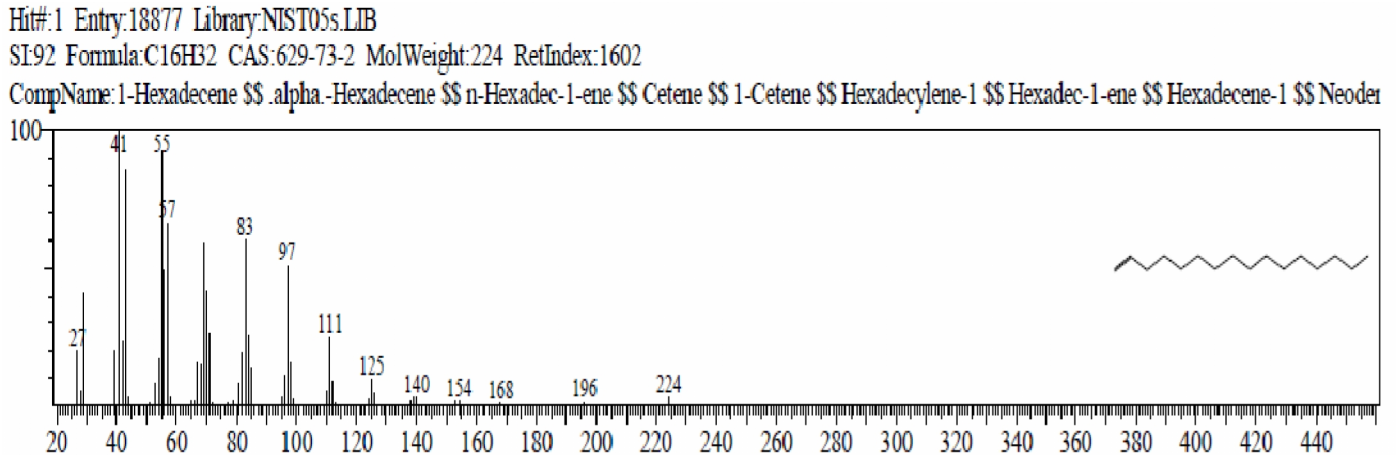
Appendix 30: 2-Tetradecene (PEAK 4of *C. esculenta* flower extract)



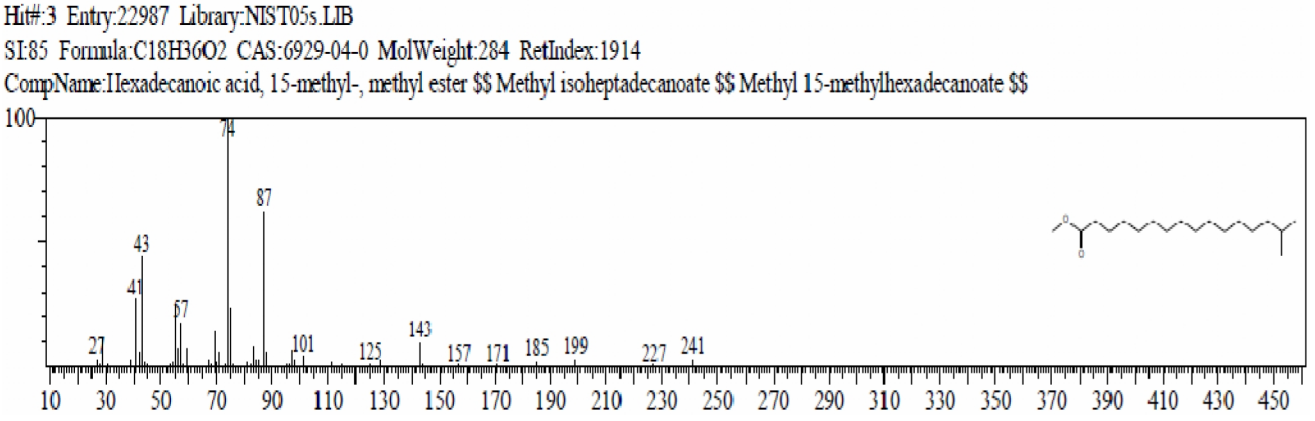
Appendix 31: 2,6-Di-tert-butylphenol (PEAK 5of *C. esculenta* flower extract)



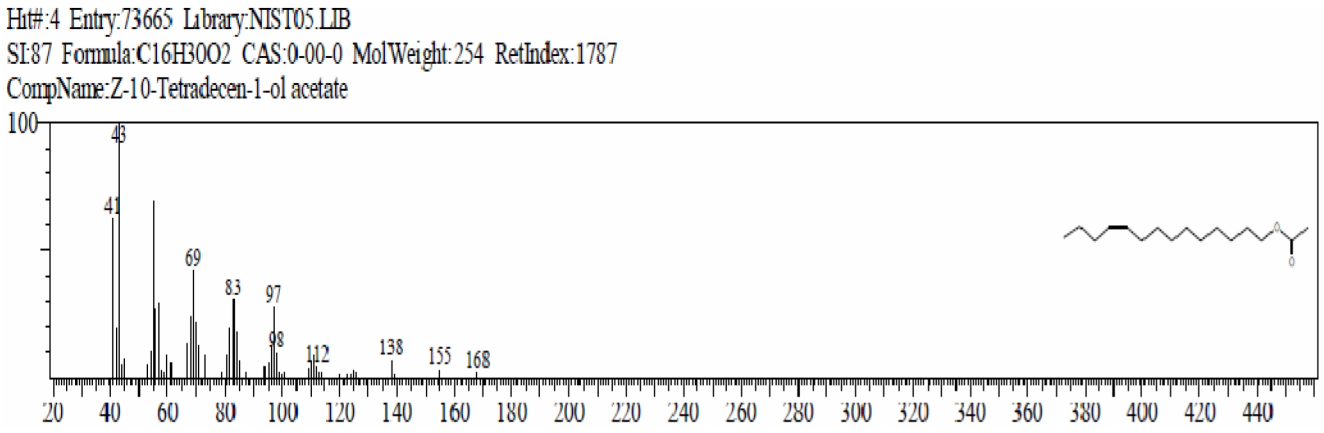
Appendix 32: 1-Tetradecene (PEAK 6of *C. esculenta* flower extract)



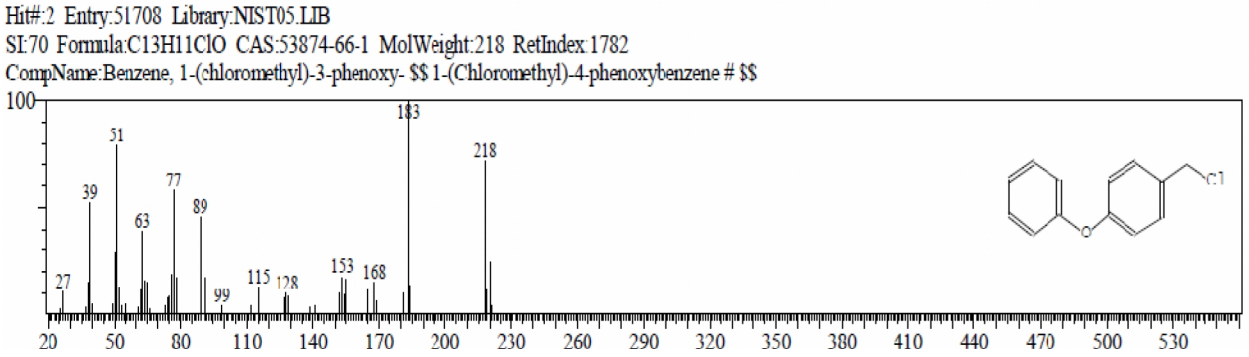
Appendix 33: 1-Cetene (PEAK 7of *C. esculenta* flower extract)



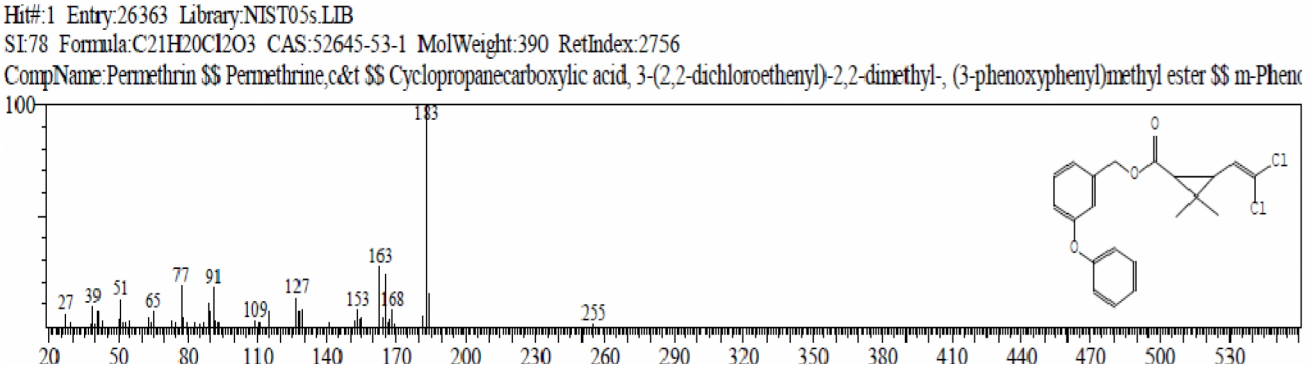
Appendix 34: Methyl Isoheptadecanoate (PEAK 8of *C. esculenta* flower extract)



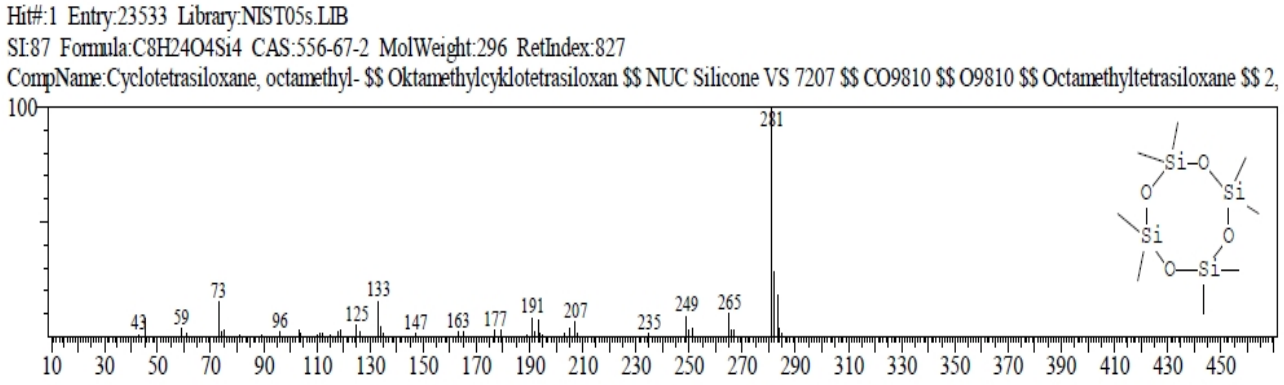
Appendix 35: 10-Tetradecen-1-ol acetate (PEAK 9of *C. esculenta* flower extract)



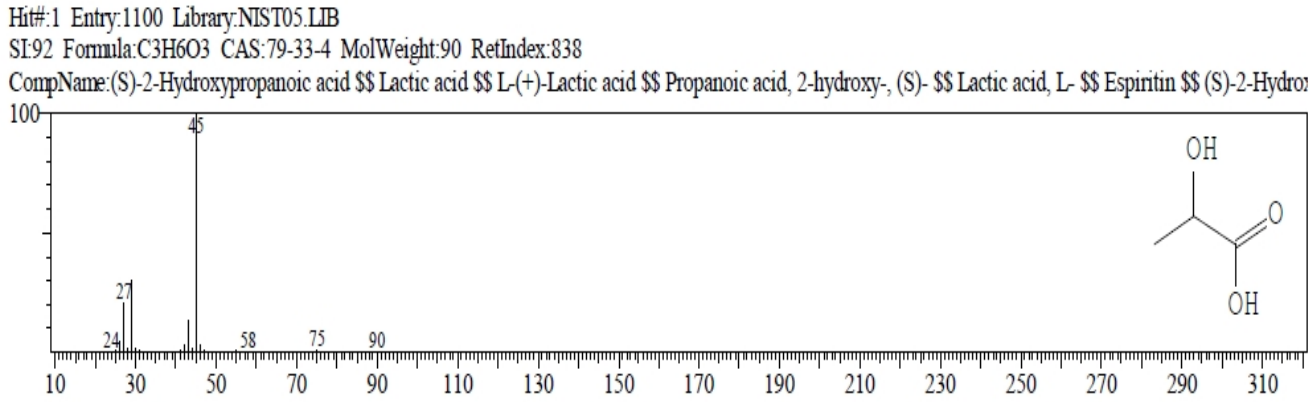
Appendix 36: 1-(Chloromethyl)-4-phenoxybenzene (PEAK 10of *C. esculenta* flower extract)



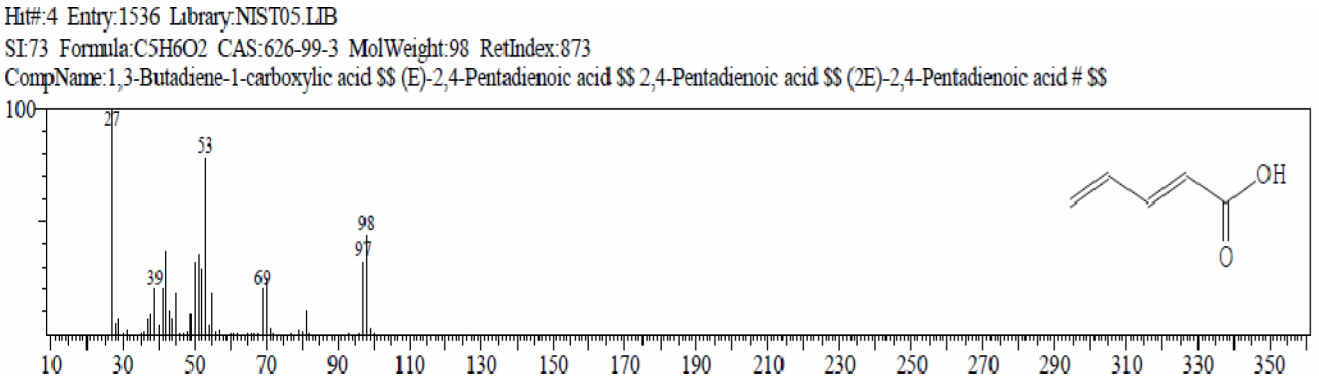
Appendix 37: Permethrin (PEAK 11of *C. esculenta* flower extract)



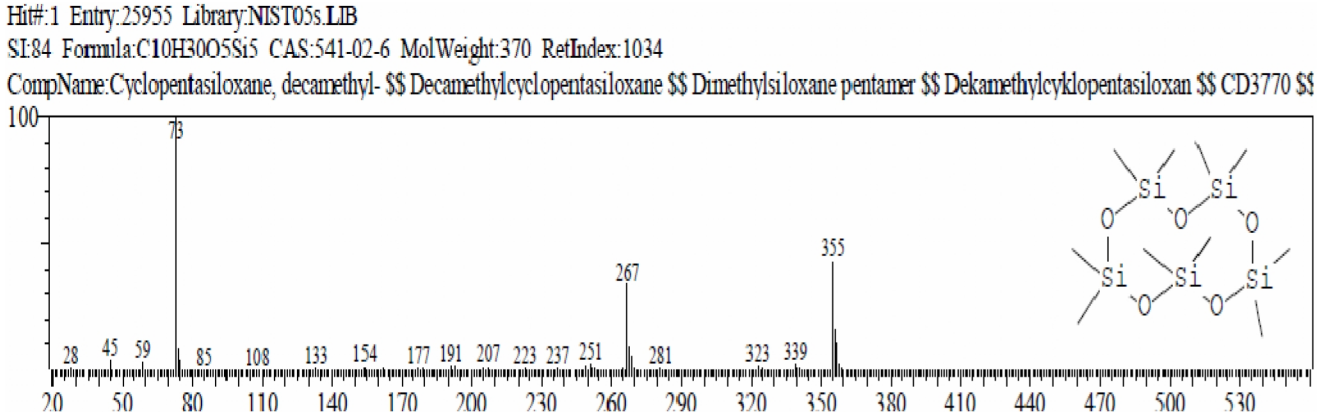
Appendix 38: Octamethyl tetrasiloxane (PEAK 1of *C. esculenta* stem extract)



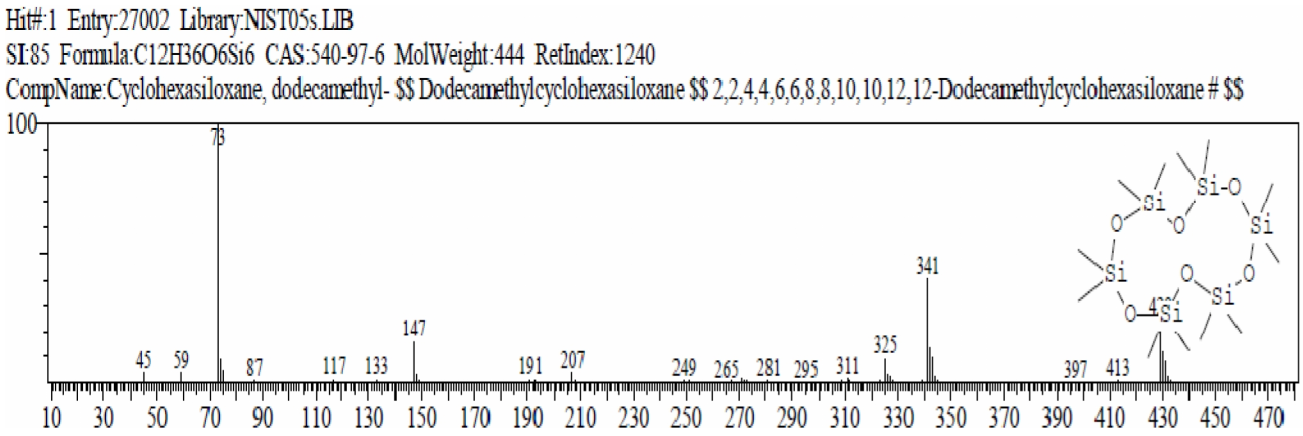
Appendix 39: Lactic acid (PEAK 2of *C. esculenta* stem extract)



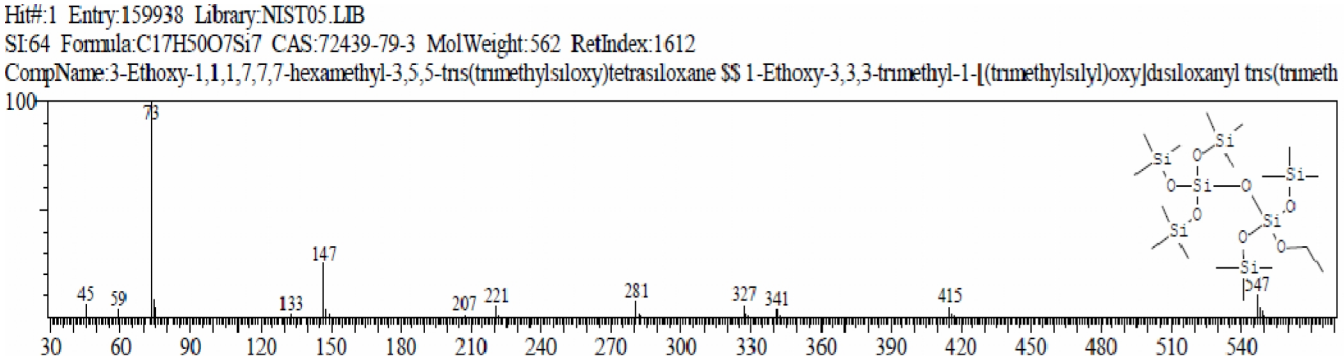
Appendix 40: 2,4-Pentadienoic acid (PEAK 3of *C. esculenta* stem extract)



Appendix 41: Decamethyl cyclopentasiloxane (PEAK 4of *C. esculenta* stem extract)

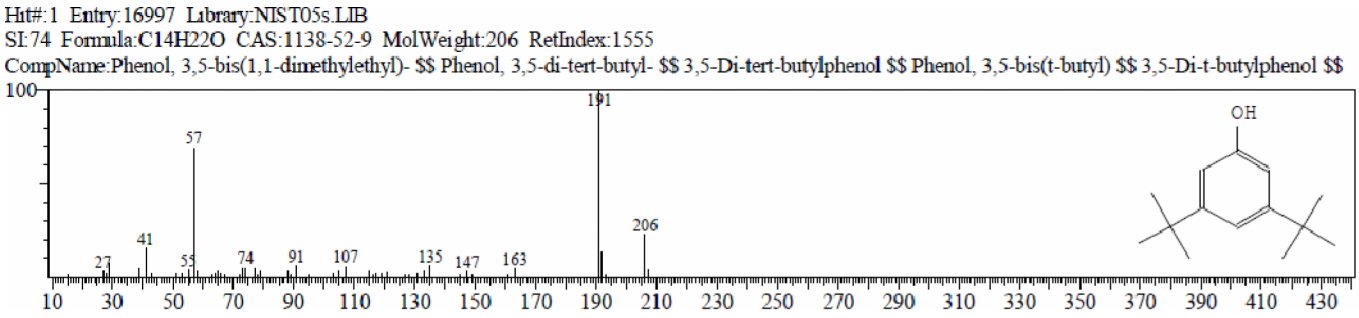


Appendix 42: Dodecamethyl cyclohexasiloxane (PEAK 5of *C. esculenta* stem extract)

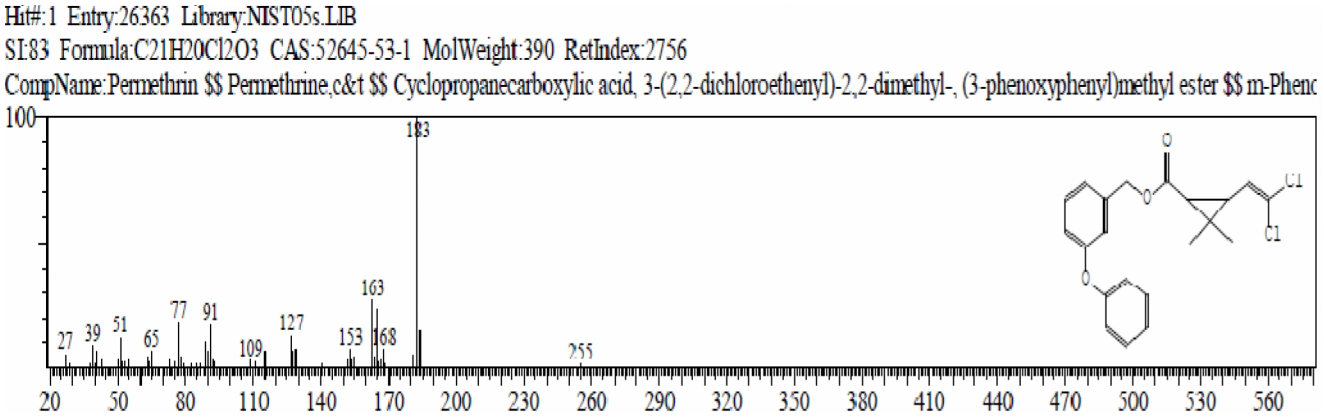


Appendix 43:3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-

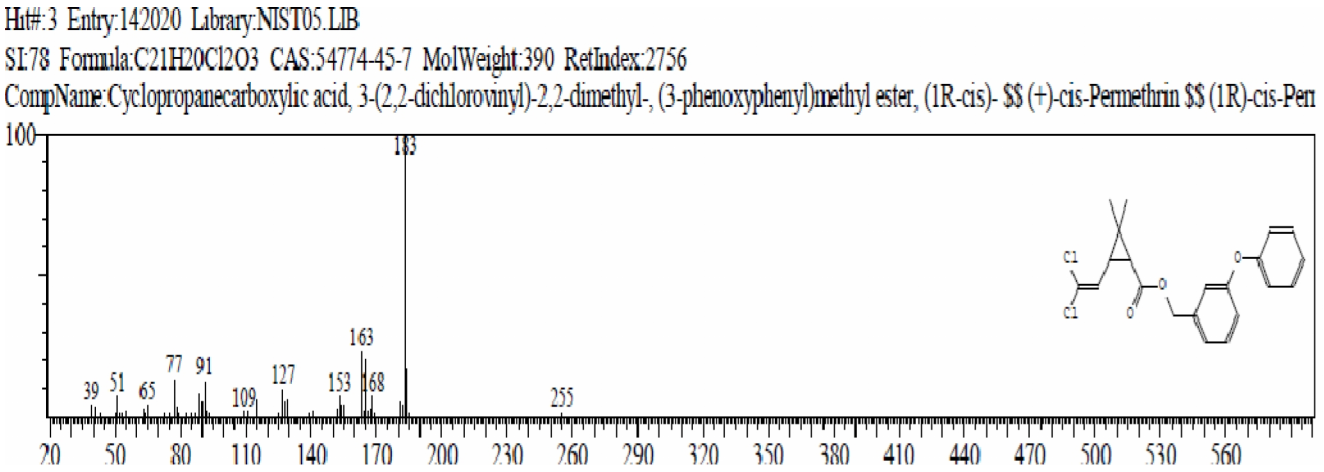
tris(trimethyl(siloxy)tetrasiloxane(PEAK 6of *C. esculenta* stem extract)



Appendix 44: 3,5-Di-tert-butylphenol (PEAK 7of *C. esculenta* stem extract)



Appendix 45: Permethrin (PEAK 8of *C. esculenta* stem extract)



Appendix 46: –Cis- Permethrin (PEAK 9of *C. esculenta* stem extract)