

ANALYSIS OF BIOACTIVE COMPONENTS
IN ETHANOL EXTRACTS OF
GLYPHAEA BREVIS LEAVES

DIASHI STANLEY CHIDI
MATRIC NO: 12062785

DECEMBER, 2014

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BREVIS* LEAVES**

By

DIASHI STANLEY CHIDI

MATRIC NO: 12/06/2185



**DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY,
ABRAHAM ADESANYA POLYTECHNIC, IJEBU-IGBO,
OGUN STATE, NIGERIA.**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE
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DECEMBER, 2014

DEDICATION

This report is dedicated to my God, the most Merciful, most Gracious,
most Glorious, most Splendors and most Majesty. And to my wonderful
and loving Mr. and Mrs. DLASHI

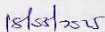
CERTIFICATION

I certify that this research work was carried out by **DIASHI STANLEY CHIDI** with Matriculation number 12/06/1285 under my supervision in the Department of Science Laboratory Technology, Abraham Adesanya Polytechnic, Ijebu Igbo, Ogun State.



Miss O.A JOKOTAGBA

Supervisor



Date

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I give thanks to almighty God for his goodness and kindness upon my life and for enabling me to complete this project.

I humbly appreciate my wonderful parents who brought me into this world MR and MRS DJASHI for giving me this glorious opportunity, a solid foundation for the fulfillment of the divine success upon my life. For all their parental, financial, morally, spiritual support and for their faith in me.

I pray that you will reap the fruit of your labour in good health in Jesus name and you will live to enjoy it. I give a big thanks to my wonderful, supervisor Miss O.A JOKOTAGBA who inculcated in me a sense of responsibility in the course of this project. Her tactical view, observation, suggestion and contribution were the major driving force for the successful completion of this project. I will always remember your impact in my life and my prayer is that God continue bless you and perfect His work in your life.

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ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs, for their history it has been used as a popular folk medicine. *Glyphaea brevis* has medicinal values; ethanolic leaf extract of this plant was analyzed using Gas Chromatography-Mass Spectrometry. Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of 16 compounds. In GC-MS analysis, some of the phytochemicals screened were Phytol, 1,2-Benzenedicarboxylic acid, Oleic Acid and n-Hexadecanoic acid. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Some of the identified compounds have been reported to possess some various medicinal activities such as antioxidant, antimicrobial, anesthetic, antiseptic, antidiabetic, Hypocholesterolemic and e.t.c. The result thus was concluded that *Glyphaea brevis* leaves possess various bioactive compounds and it is recommended as a plant of phytopharmaceutical importances.

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CHAPTER IV

The first part of the chapter discusses the importance of the study of the history of the United States. It is a study of the past which helps us to understand the present and to prepare for the future. The author points out that the history of the United States is a story of growth and development, from a small colony to a great nation. This growth was made possible by the courage and vision of the men who founded the country. The author also discusses the role of the Constitution in the development of the United States. The Constitution is the foundation of our government and it has guided us through many difficult times. The author concludes that the study of the history of the United States is a study of the values and ideals that have made our country what it is today.

The second part of the chapter discusses the role of the individual in the history of the United States. It is the actions of individuals that have shaped the course of our nation. The author points out that many of the great men of our history were men of vision and courage. They were men who were willing to stand up for their beliefs and to fight for the rights of all people. The author also discusses the role of the individual in the development of our government. It was the actions of individuals that led to the creation of the Constitution and the establishment of the three branches of government. The author concludes that the study of the history of the United States is a study of the actions of individuals and the impact that those actions have had on our nation.

The third part of the chapter discusses the role of the individual in the development of our culture. It is the actions of individuals that have shaped our culture and our way of life. The author points out that many of the great works of our literature and art were created by individuals who were willing to stand up for their beliefs and to fight for the rights of all people. The author also discusses the role of the individual in the development of our society. It was the actions of individuals that led to the creation of our laws and the establishment of our institutions. The author concludes that the study of the history of the United States is a study of the actions of individuals and the impact that those actions have had on our culture and our way of life.

CHAPTER ONE

1.0 INTRODUCTION

Herbal medicine has its root in prehistory making every bit as ancient tradition as farming or cooking. In the Graeco-Roman era, Hippocrates (father of medicine), Theophrastus (father of botany), Galen (originator of pharmaceutical "galenicals") and Dioscorides were all herbalists (Moody, 2007). Also about one-quarter of the prescription drugs dispensed by community pharmacy in the United States contain at least one active ingredient derived from plants (Farnsworth and Morries, 1976).

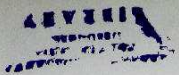
Also in Nigeria, around 205 medicinal plant species are endemic in nature in the Northern, Western, Central and Eastern zones of the country (FEPA, 1992). Beyond the problem of trying to test herbal preparations that may contain active ingredients are the question of whether the research eventually will lead to the isolation of single active ingredient that can be packaged and sold separately. Intense debate surrounds the issue of how to conduct clinical trials of herbal medicine according to western pharmaceutical clinical standards.

Critics say there is an inherent problem with the single active ingredient approach preferred by pharmaceutical companies that are actively involved in herbal medicine research. It is argued that isolating a single compound

may not be the most appropriate approach in situations where a plant's activity decreases on further fractionation (separation of active ingredients by using solvents) or where the plants contain two or three active ingredients that must be taken together to produce the full effect (Chaudhury, 1992).

Beckstrom-Stember and Duke (1994) have documented several cases where synergy has been lost by using the single ingredient approach to developing drugs from plants. Other notable problems associated with herbal medicines include but not limited to how to conduct clinical trials of herbal medicine according to western pharmaceutical clinical standards, issue of dosage specifications, prominent doubts about herbal preparations such as lack of proof of their efficacy, safety, proper packaging problems, appropriateness of their degree or level of hygiene, cost of production and their level of acceptability especially among the elites in the healthcare team who continues to prescribe only orthodox medicines in hospitals and clinics (Pharmanews, 2010).

Nevertheless, the public pay high prices for orthodox medicines because the cost for experimental techniques through research and development (R and D) is enormous. Another common perception is that orthodox medicine which is scientifically based is more reliable, safer and more



Orthodox medicine began over a century ago during the period of Renaissance. As at then the objective thinking of the causative theory of modern science replaced the ecological model which had predominated for over 2000 years (Bhikha, 2004). The new paradigm is often termed the

medicine (Rees and Shuter, 1996).
Recently in Nigeria, the National Agency for Food Drug Administration and Control (NAFDAC) banned the use of Novalgin (a potent analgesic and antipyretic agent) because of its severe side effects that led to the death of children. Although, the history of orthodox medicine traces its root back to Hippocrates, the father of medicine, the practice of orthodox medicine today is not strictly in line with the principles of the fathers of

harmful bacteria (Bradstreet, 1998).
Again, antibiotics which created false hope that modern medical science could eradicate diseases caused by bacteria, ended up killing bacteria that are beneficial to human body thereby reduces the body's resistance to

sickness gave birth to deformed babies.
example when hundreds of women given thalidomide for early morning even fatalities. The thalidomide fiasco of the 1950s and 60s was a tragic are often withdrawn from the market for causing severe side effects and effective. This notion may be wrong because drugs once thought to be safe

Cartesian model being named after the French philosopher, Rene Descartes (1596-1650). This model, it was claimed, invalidated the humoral concepts of the holistic principles of Hippocrates. Galen and Ibn Sina promoted the ideology that man was separate from nature could be viewed objectively through experiment (Boussel *et al.*, 1982).

This heralded the birth of scientific or orthodox medicine. The frontiers of orthodox medicine were further broadened by Rudolph Virchow (1821-1902) who demonstrated that disease begins with changes in living cells and by Louis Pasteur (1822-1895) whose role in the development of the germ theory of infection was of key importance (Rees and Shuter, 1996; Gilbert *et al.*, 1998, Bhikha and Haq, 2000).

Under the germ theory, disease was associated with specific microorganisms. Since, then technology through research and development (R and D) had played tremendous roles in the propagation of orthodox medicine which is scientifically based and evolve along certain specifications or routes. These routes led to the manifestations of plethora of specialists in disorders of specific organs, tissue and cells such as cardiologists, dermatologists and neurologists among others.

Hence, it has been advocated that patients should be regarded as collections of separate body parts and organ systems (Thomas, 2002).

Generally, the philosophy of orthodox medicine is exclusively based on the physical world and excludes any explanation that goes beyond this (Hammond-Tooke, 1989; Gilbert *et al.*, 1998).

For instance, health and illness are seen as a relationship between the body's components and sub-structure while the mind is considered independent of the body. The causes of disease are therefore, scientific and presented in terms of such concepts as chemical imbalance, virus replication, serum level overload and so on (Bhikha, 2004).

Technology based scientific research in herbal medicine perhaps has made some significant impact in addressing some prominent doubts about herbal preparations such as packaging problems, level of hygiene and dosage regimen. Presently, most herbal medicines sold in Nigeria.

1.1 HERBALISM

Herbalism ("herbology" or "herbal medicine"). Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body.



Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias.

Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.

The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in

Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine.

WHO notes that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems.

For example, ephedra is a herb used in Traditional Chinese Medicine for more than two thousand years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily.

Another example of the use of herbal preparation in modern medicine is the foxglove plant. This herb had been in use since 1775. At present, the powdered leaf of this plant is known as the cardiac stimulant digitalis to the millions of heart patients it keeps alive worldwide.

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1.2 ADVANTAGES OF HERBAL MEDICINE

There are a number of advantages associated with using herbal medicines as opposed to pharmaceutical products. Examples include the following:

1.2.1 Reduced risk of side effects: Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicine, and may be safer to use over time.

1.2.2 Effectives with chronic conditions: Herbal medicines tend to be more effective for long-standing health complaints that don't respond well to traditional medicine. One example is the herbs and alternative remedies used to treat arthritis. Vioxx, a well-known prescription drug used to treat arthritis, was recalled due to increased risk of cardiovascular complications. Alternative treatments for arthritis, on the other hand, have few side effects. Such treatments include dietary changes like adding simple herbs, eliminating vegetables from the nightshade family and reducing white sugar consumption.

1.2.3 Lower cost: Another advantage to herbal medicine is cost. Herbs cost much less than prescription medications. Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs.

1.2.4 Widespread availability: Yet another advantage of herbal medicines are their availability. Herbs are available without a prescription. You can grow some simple herbs, such as peppermint and chamomile, at home. In some remote parts of the world, herbs may be the only treatment available to the majority of people.

1.3 DISADVANTAGES OF HERBAL MEDICINE

Herbs are not without disadvantages, and herbal medicine is not appropriate in all situations. These are a few of the disadvantages to consider:

1.3.1 Inappropriate for many conditions: Modern medicine treats sudden and serious illnesses and accidents much more effectively than herbal or alternative treatments. An herbalist would not be able to treat serious trauma, such as a broken leg, nor would he be able to heal an appendicitis or a heart attack as effectively as a conventional doctor using modern diagnostic tests, surgery, and drugs.

1.3.2 Lack of dosage instructions: Another disadvantage of herbal medicine is the very real risks of doing yourself harm through self-dosing with herbs. While you can argue that the same thing can happen with medications, such as accidentally overdosing on cold remedies, many herbs

do not come with instructions or package inserts. There's a very real risk of overdose.

1.3.3 Poison risk associated with wild herbs: Harvesting herbs in the wild is risky, if not foolhardy, yet some people try to identify and pick wild herbs. They run a very real risk of poisoning themselves if they don't correctly identify the herb, or if they use the wrong part of the plant.

1.3.4 Medication interactions: Herbal treatments can interact with medications. Nearly all herbs come with some warning, and many, like the herbs used for anxiety such as Valerian and St. John's Wort, can interact with prescription medication like antidepressants. It's important to discuss your medications and herbal supplements with your doctor to avoid dangerous interactions.

1.3.5 Lack of regulation: Because herbal products are not tightly regulated, consumers also run the risk of buying inferior quality herbs. The quality of herbal products may vary among batches, brands or manufacturers. This can make it much more difficult to prescribe the proper dose of an herb.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *GLYPHAEA BREVIS* (SPRENG)

Pharmaceutical industries have come to consider traditional medicines as a source of identification of bioactive agents that can be used in the preparation of synthetic medicines. Extract from medicinal plants are sold in the partially purified or crude form for the treatment and prevention of all kinds of diseases. These herbal products lack scientific backing for the various efficacies claimed. Plants extracts are used for disease conditions such as mental disorders, diabetes, sickle cell anemia, malaria, tuberculosis and a host of other diseases in traditional medicine throughout the world (Odugbemi, 2008).

Glyphaea brevis is a spreading shrub, climber or small tree up to 8m high. It is very common in undergrowth of closed forest, secondary jungle and on river-banks, lowlands to sub-mountain and wide spread in tropical Africa (Burkill, 1985). It is valued there as vegetable (Okafor, 1990) and various therapeutic uses such as treatment of hepatitis and poisoning have been reported (Terasima and Ichikawa, 2003).

Glyphaea brevis (Spreng) which is popularly called Aloanyasi (Ibo) or Atori (Yoruba). It has been reported to have multiple physiological and

pharmacological activities. It is used in the treatment of sleeping sickness and as aphrodisiac, as an antibacterial in the treatment of eye infection and in gum cleaning. It is also reported to be effective in the treatment of impotency (Vasilea, 1969). It has carminative effects and is used as an anticonvulsant, especially in children, where it is either used singly or in combination with other herbs (Ijomah, 1996; Ogbonnia et al, 2003)

Therapeutic activities of various medicinal plants are sometimes related to their antioxidant properties (Agboret *al.*, 2007). Therefore antioxidant activity could be accountable for the medicinal properties of *Glyphea brevis* through a contribution to redox homeostasis.

2.2 BOTANNICAL DESCRIPTION HABITAT

Glyphea brevis is often in flower and fruit which makes identification easy. The stipules fall off very early but otherwise it has a classic suite of sterile characters which indicate that it is the Malvaceae. These characters are; strong stringy bark, two secondary nerves at the base of the leaf, a swollen top to the petiole and Stella hairs.

Occasionally this family can be confused with Euphorbiaceae without flowers and fruit, but the strong bark, the thinner leaves and some sliminess in the slash usually indicate Malvaceae. It is most often a small

tree about 3m high, but it occasionally reaches the size of 25cm in diameter. There are usually the basal veins, on either side of the midrib.

2.3 ECOLOGY AND DISTRIBUTION

Disturbed areas on terra firma and seasonally flooded forest and riverbank.

Distributed in Guinea Bissau to Sudan, Tanzania and Angola.



Figure 2.1 Glyphaea brevis

2.4 COMMON NAMES

English: spreng, Yoruba: ewe Atori, Ibo: Aloanyasi

2.5 TAXONOMY OF Glyphaea brevis

Kingdom: Plantae (unranked); Angiosperms (unranked); Eudicots

(unranked); Rosids Order: Malvales Family: Malvaceae Subfamily:

Grewioideae Genus: Glyphaea

2.6. PHYTOCHEMICAL PROPERTIES

2.6.1 Tannins: A tannin (also known as *vegetable tannin*, *natural organic tannins*, or sometimes *tannoid*, i.e. a type of biomolecule, as opposed to modern synthetic tannin) is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids.

The term tannin (from *tanna*, an Old High German word for oak or fir tree, as in Tannenbaum) refers to the use of wood tannins from oak in tanning animal hides into leather; hence the words "tan" and "tanning" for the treatment of leather. However, the term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with various macromolecules.

Tannins are found in leaf, bud, seed, root, and stem tissues. An example of the location of the tannins in stem tissue is that they are often found in the growth areas of trees, such as the secondary phloem and xylem and the layer between the cortex and epidermis. Tannins may help regulate the growth of these tissues.

2.6.2: Flavonoids: Flavonoids (or bioflavonoids) (from the Latin word *flavus* meaning yellow, their color in nature) are a class of plant secondary

metabolites. Flavonoids were referred to as Vitamin P (probably because of the effect they had on the permeability of vascular capillaries) from the mid-1930s to early 50s, but the term has since fallen out of use.

Chemically, they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). This carbon structure can be abbreviated C6-C3-C6. According to the IUPAC nomenclature.

Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors.

Flavonoids secreted by the root of their host plant help *Rhizobia* in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy. *Rhizobia* living in soil are able to sense the flavonoids and this triggers the secretion of Nod factors, which in turn are recognized by the host plant and can lead to root hair deformation and

several cellular responses such as ion fluxes and the formation of a root nodule.

In addition, some flavonoids have inhibitory activity against organisms that cause plant diseases, e.g. *Fusarium oxysporum*. Flavonoids have been shown to have a wide range of biological and pharmacological activities. Examples include anti-allergic, anti-inflammatory, antioxidant, anti-microbial (antibacterial, antifungal, and antiviral), anti-cancer, and anti-diarrheal activities.

2.6.3 Alkaloids: Alkaloids are a group of naturally occurring chemical compounds (natural products) that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also termed alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and more rarely other elements such as chlorine, bromine, and phosphorus.

Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals. They can be purified from crude extracts of these organisms by acid-base extraction. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g. quinine), antiasthma (e.g. ephedrine), anticancer (e.g. homoharringtonine), cholinomimetic (e.g.

galantamine), vasodilatory (e.g. vincamine), antiarrhythmic (e.g. quinidine), analgesic (e.g. morphine), antibacterial (e.g. chelerythrine), and antihyperglycemic activities (e.g. piperine).

Many have found use in traditional or modern medicine, or as starting points for drug discovery. Other alkaloids possess psychotropic (e.g. psilocin) and stimulant activities (e.g. cocaine, caffeine, nicotine), and have been used in entheogenic rituals or as recreational drugs. Alkaloids can be toxic too (e.g. atropine, tubocurarine). Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.

The boundary between alkaloids and other nitrogen-containing natural compounds is not clear-cut. Compounds like amino acid peptides, proteins, nucleotides, nucleic acid, amines, and antibiotics are usually not called alkaloids. Natural compounds containing nitrogen in the exocyclic position (mescaline, serotonin, dopamine, etc.) are usually attributed to amines rather than alkaloids.

2.6.4 Terpenoids: The **terpenoids** sometimes called **isoprenoids**, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one

another not only in functional groups but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products.

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and ginger, the yellow color in sunflowers, and the red color in tomatoes. Well-known terpenoids include citral, menthol, camphor, salvinorin A in the plant *Salvia divinorum*, the cannabinoids found in cannabis, ginkgolide and bilobalide found in *Ginkgo biloba*, and the curcuminoids found in turmeric and mustard seed.

2.7 AIM AND OBJECTIVES

The aim and objective of this project work are:

- 1) To extract active ingredients in the *Glyphaea brevis* leaf sample.
- 2) To carry out the phytochemical analysis of the extract.
- 3) To identify various medicinal component in the extract using Gas Chromatography-Mass Spectrometer.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 PLANT MATERIAL:

The leaf of *Glyphaea Brevis* was obtained from owu-ikija arca in ogun state, Nigeria in November 2014.

3.2 PREPARATION OF PLANT EXTRACTS

Fresh leaves of *Glyphaea Brevis* were cut and washed with water to remove all contaminants; they were air dried under room temperature and grounded to powder. The powdered leaves were extracted with ethanol using "soxhlet extractor".

3.3 PHYTOCHEMICAL SCREENING

Phytochemical compositions of the leaves were determined using the methods Variously described by Trease and Evans (2002).

3.3.1 Test for tannins: In the test for tannins, 0.5 g of dried powdered sample was boiled in 20 mL of water in a test tube and filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black coloration as indication of tannins.

3.3.2 Test for saponins: Approximately 2 g of powdered material was boiled in 20 mL of distilled water in a water bath and filtered. Next, 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as indication of saponin.

3.3.3 Test for flavonoids: A portion of the powdered material was heated with 10 mL ethyl acetate over a steam bath for 3 mins. The mixture was filtered and 4 mL of the filtrate was shaken with 1mL of dilute ammonia solution. Development of yellow coloration is an indication of the presences of flavonoids.

3.3.4 Test for steroids: In this test, 2 mL of acetic anhydride was added to 0.5 g of extract with 2mL concentrated H_2SO_4 . The color change from violet to blue or green is indication of steroids.

3.3.5 Test for alkaloid: A measured weight of sample was dispersed in 10 % acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 hrs at $28^{\circ}C$. It was later filtered via what man No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise

addition of concentrated aqueous NH_4OH until the alkaloid was precipitated.

The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution dried in an oven at 80°C . Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed. Development of brownish yellow precipitate which turns intense yellow with picric acid is an indication of the presences of alkaloids.

3.3.6 Test for glycoside: 10 mL of extract was pipette into 250 mL conical flask. 50 mL chloroform was added and shaken on a vortex mixer for 1hr the mixture was filtered into 100 mL conical flask and 10 mL pyridine, 2 mL of 2% sodium nitroprusside were added, shaken thoroughly for 10minutes. 3 mL of 20% NaOH was later added to develop a brownish yellow color.

Glycoside standard of concentrations which range from 0-5 mg/ml were prepared from 100 mg/ml stock Glycoside standard. The series of standard 0-5 mg/ml were treated similarly like sample above. The absorbance of sample as well as standard were read on a spectronic 211D Digital spectrophotometer at a wavelength of 510nm%. Trease and Evans (2002).

3.3.7 Cardiac glycoside

One gram of powdered sample was extracted with 10 mL of ethanol for five minutes on a steam bath and filtered. To the filtrate was added and 2-3 drops of lead acetate solution was added shaken gently and then filtered. To the filtrate 2 ml of chloroform was added and then 1ml concentrated H_2SO_4 was carefully added to form a lower layer. A reddish-brown colour at interface was observed for cardiac glycoside.

3.4 GAS CHROMATOGRAPHY MASS SPECTROMETER (GC-MS) ANALYSIS

The plant powder was extracted with ethanol and analyzed using GC-MS analyzer. The data were obtained on an Elite-1 (100% Dimethyl polysiloxane) column (300,25mm Iumdf), Helium (99.999%) was used as the carrier gas with flow rate of 1ml/min in split mode (10:1). An aliquot of 2ul of ethanol solution of sample was injected into the column with the injector temperature at 250°C.

GC oven temperature stated at 110°C and holding for 2mins and it was raised to 200°C at the rate of 10° C/min, without holding. Holding was allowed at 280°C for 9 mins with program rate of 5° C/min. The injector and detector was temperature was set at 250°C and 280°C respectively. Ion source temperature was maintained at 200°C.

The mass spectrum of compounds in sample was obtained by electron ionization at 70Ev and the detector was operate in scan mode from 45-459amu(atomic mass units). A scan interval of 0.5 seconds and fragment from 45 to 450 Da was maintained. The total running time was 27 minutes.(sermakkani et al;2012).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

Table 4.1 Phytochemicals identified in the ethanolic leaf Extract of *Glyphaea brevis*

<u>Phytochemical component</u>	<u>Result</u>
Tannins	Present
Saponins	Absent
Flavonoid	Present
Alkaloids	Present
Steroids	Absent
Glycosides	Absent
Cardiac glycoside	Absent
Terpenoids	Present

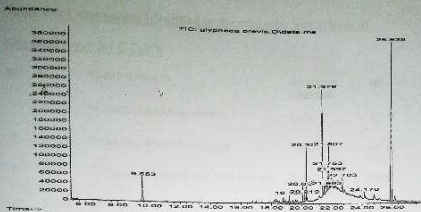


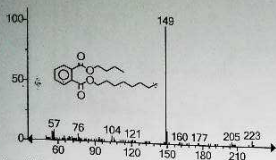
Figure 4.1: GC-MS Spectra of ethanolic extract *Glyphaea brevis*.

Table 4.2: Components Identified in the ethanolic leaf extract of

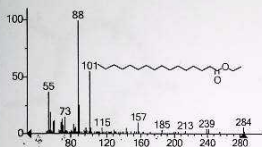
***Glyphaea brevis* by GC-MS**

NO	RI	NAME OF COMPOUND	MOLECULAR FORMULAR	MW
1	9.556	Nonane,2,2,4,4,6,8,8-heptamethyl	C ₁₆ H ₃₄	226.44
2	19.186	2-(Hexyloxy carbonyl)benzoic acid	C ₇ H ₆ O ₂	122.12
3	20.78	1,2-Benzenedicarboxylic acid, butyl octyl ester	C ₂₀ H ₃₀ O ₄	334.5
4	20.216	8,8,10,10 Nonamethylcyclopentasiloxane	C ₁₀ H ₃₀ O ₅ Si ₃	370.77
5	20.330	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.0
6	21.377	Phytol	C ₂₉ H ₆₀ O	296.0
7	21.503	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-		
8	21.500	Trichloroacetic acid, undec-2-enyl ester	C ₇ HCL ₃ O ₂	163.39
9	21.600	Oleic Acid	C ₁₈ H ₃₄ O ₂	282.46
10	21.692	Pentanoic acid, 10-undecenyl ester		
11	21.755	9,12-Octadecadienoic acid, ethyl ester	C ₂₀ H ₃₆ O ₂	280.0
12	21.806	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	278.0
13	21.995	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	284.0
14	22.705	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris(trimethylsiloxy) tetra siloxane	C ₁₄ H ₃₂ O ₇ Si ₂	577.19
15	24.170	4-Cyclohexene-1,2dicarboxylic acid, 4- chloro-, bis(trimethylsilyl) ester		
16	25.938	Bis(2ethylhexyl) phthalate	C ₂₈ H ₅₄ O ₄	390.56

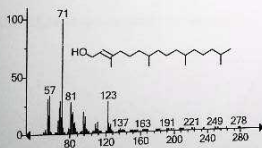
STRUCTURE OF IDENTIFIED COMPOUNDS



(replib) 1,2-Benzenedicarboxylic acid, butyl octyl ester

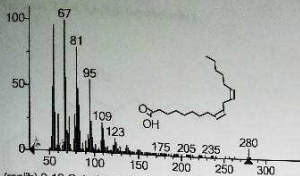


(replib) Hexadecanoic acid, ethyl ester

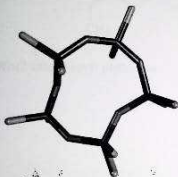


(mainlib) Phytol

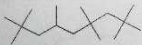
H3C CH3



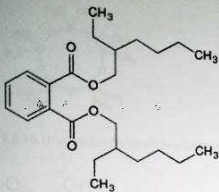
(replib) 9,12-Octadecadienoic acid (Z,Z)-



CH₃8,8,10,10-nonamethylcyclopentasiloxane



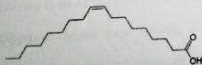
Nonane, 2,2,4,4,6,8,8-heptomethyl



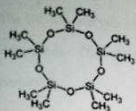
Bis(2 ethylhexyl) phthalate



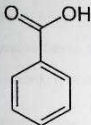
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl



Oleic Acid



8,8,10,10-nonamethylcyclopentasiloxane



2-(Hexyloxy)benzoic acid

4.2 DISCUSSION

Medicinal plants are used in traditional treatment to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Thus, for the above mentioned reason and bearing in mind its medicinal importance, the plant species

Glyphaea brevis were selected to analyze by GC-MS technique and explore the major and minor phyto-constituent present in the respective

plant species. The preliminary phytochemical screening shows the presence of tannins, flavonoid, alkaloids and terpenoids.

The GC-MS analysis of *Glyphaca brevis* revealed the presences of some compounds. The identified compounds possess so many biological properties.

Phytol is one among the seventeen compounds of the present study. Similarly Maria Jancy Rani *et al.* (2011) observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* (2011) in *Mimosa pudica* leaves. Similar result was also observed in the leaves of *Lantana camara* (Sathish kumar and Manimegalai, 2008).

Phytol was observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al.*, 2005).

Moreover, Hexadecanoic acid, ethyl ester determined at (R/T 20.330), also serve as antioxidant, Flavour, [Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Haemolytic, 5-Alpha reductase inhibitor as discovered by sermakanni M. *et al.* (2012).

Furthermore, 9,12 Octadecadienoic acid determined at (R/T 21.600) serves as anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic, as determined by sermakanni M. et al; (2012).

Nevertheless, 9,12,15-Octadecatrienoic acids methyl ester determined at (R/T 21.806) serves as Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, as described by senthamarai Selvi. V et al; (2012). While pentadecanoic acid is used as an Antioxidant. And 1, 2-Benzenedicarboxylic acid, butyl octyl ester is an plasticizer compound which act as a Antimicrobial, antifouling Substances.

However Trichloroacetic acid. It is used in biochemistry for the precipitation of macromolecules, such as proteins, DNA, and RNA. Solutions containing trichloroacetic acid as an ingredient are used for cosmetic treatments, such as chemical peels, tattoo removal, and the treatment of warts, including genital warts. It can kill normal cells as well. It is considered safe for use for this purpose during pregnancy.

Bis(2 ethylhexyl) Phthalate is widely used as a plasticizer in manufacturing of articles made of PVC. It is also used as a hydraulic fluid

and as a dielectric fluid in capacitors. Bis (2 ethylhexyl) Phthalate also finds use as a solvent in glow sticks.

Finally, Benzoic acid which is an aromatic compound serves as a biological uses for Allergenic, anesthetic, antibacterial, anticancer, antimutagenic, antipeptic, antiseptic, antispasmodic, antitumor, candidicide, flavour, insecticide, nematocide, pesticide, sedative, termiticide, tyrosinase inhibitor.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The source of many plants (herbs and spices) can often be identified from the peak pattern of the chromatograms obtained directly from headspace analysis. GC-MS method is a direct and fast analytical approach for identification of terpenoids and steroids and only few grams of plant material is required.

The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in *Glyphaea brevis* suggests that the contribution of these compounds from the extracts will be valuable for treatment of ailments by traditional practitioners.

Therefore, *Glyphaea brevis* leaves can be used as antimicrobial, antiparasitic insecticide, nematocide, pesticide, antiandrogenic, hypocholesterolemic, antioxidant, cancer preventive, anticoronary, antiarthritic, hepatoprotective, neuroactive, analgesic and anesthetic, flavour and allergenic.

5.2 RECOMMENDATION.

The phytoconstituents of *Glyphaea brevis* possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance. Therefore, further studies on isolation and identification of individual constituent are very much needed. It is also timely to explore its pharmacological values at the molecular level with the help of various biotechnological techniques in future.

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