QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS AND ETHANOL LEAVES AND STEM BARK OF *ANDOGRAPHIS PANICULATA*

BY

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B.Sc, Hons) IN BIOCHEMISTRY

SEPTEMBER 2023

CERTIFICATION

We the undersigned, certify that **EZE-NWAOBASI ONYINYECHI PRINCESS** with matriculation number **LSC1806301** carried out, compiled and reported this project work in partial fulfillment of the requirements for the award of Bachelor of Science degree (B.sc) in Biochemistry.

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DEDICATION

This project is dedicated to God Almighty for all his love, mercy, blessings, and for keeping me alive all through my years in the school. I also dedicate this work to my Parents for their love, financial support and words of encouragement.

ACKNOWLEDGEMENT

Firstly my gratitude goes to GOD ALMIGHTY for seeing me throughout this work. My profound gratitude goes Sto my Parents Mr. and Mrs Felix Nwaobasi and my siblings for their love and support. I also want to appreciate My Project Supervisor Dr O. Osemwenkhae for his guidance and support during and after the project. To my project members in persons of Ms Esther, Jessica, Patience, Victory, Dunsin and Victor for their efforts and cooperation during the work, I am grateful. I wouldn't fail to appreciate My Course Adviser, Dr Cyril Ugbeni for his genuine love and guidance in the course of this research. To my friends; Miracle, Oshomah, Tega, Tosan, Cynthia and Peace for their moral support throughout this undergraduate project.

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ABSTRACT

Andrographis paniculata (Burm. f.) is a widely utilized medicinal plant globally, that holds significant recognition in traditional medicine systems. This plant has been studiedfor its wide range of pharmacological properties including, anti-hepatotoxic, anti-microbial, antioxidant, antibacterial, anti-immunosuppressive properties anti-inflammatory, antimalarial, antipyretic, antiretroviral, antivenom, cardioprotective, and immunomodulatory effects. In this research, the aqueous and ethanol extract of the leaves and stem bark of *Andrographis paniculata* was qualitatively and quantitively analysed, to determine its phytochemical composition. The qualitative analysis carried out between the aqueous and ethanol extract of *Andrographis paniculata* showed positive for saponin, tannin, phenol, flavonoids, cardiac glycosides, proanthocyanidin and terpenoids which are phytochemicals responsible for the plant's ethnomedicinal uses. Although these phytochemicals are present in both extracts, Flavonoids (66 QE/g), Phenol (78 GAE/g), Tannin (54 TAE/g) and Proanthocyanidin (174 mgAAE/g) were found in higher concentration in the aqueous extract of *Andrographis paniculata* than the ethanol extract. This clearly indicates that the aqueous extract of *Andrographis paniculata* is more advantageous for herbal medicine practice. Upon statistical analysis it was concluded that though the level of flavonoids, proanthocyanidin, tannins and phenol were more higher in the aqueous extract than ethanol extract though, there was no significant difference ($p \geq 0.05$) between these extracts, suggesting that the solvent ethanol was just as effective in the extraction of these phytochemicals when compared with the aqueous solvent.

CHAPTER ONE

1.0 INTRODUCTION

1.1 HERBAL MEDICINE

Beneficial plants have devised a method for discovering new medications and have proven to be a primary source of active pharmaceutical ingredients used in the treatment of severe illnesses such as AIDS, Hepatitis, and inflammatory conditions, including antihypertensive drugs (PlantNet., 2008). *Andrographis paniculata* stands out as one of the most widely utilized medicinal plants globally. It goes by the common name "king of bitters" and belongs to the Acanthaceae family. This tropical shrub is extensively cultivated in Europe, Asia, and Africa, and it has a broad range of applications in managing various ailments (Kumar *et al*., 2020; Lalitha *et al*., 2015). *Andrographis paniculata,* with its branched and upright growth pattern, plays a significant role in promoting cardiovascular health and serves as an antioxidant. Additionally, it has proven efficacy in treating snakebites, insect stings, fevers like malaria and dengue, diarrhea, flu, skin conditions, and upper respiratory infections using traditional methods. Its habitat spans forests, roadsides, hills, and villages. The biological effects of andrographolide, a compound found in *Andrographis paniculata*, have been highlighted in a study that demonstrated its ability to reduce acute brain injury in Wistar rats (Tao *et al*., 2018). This annual herb is known for its role in managing various liver disorders and is recognized for its hepatoprotective and hepato-stimulative properties. It belongs to the Acanthaceae family and is extensively used in traditional Indian medicine (Nayak *et al*., 2020). Traditionally, the aqueous extract of this plant's leaves has been employed to treat various liver conditions and jaundice. Furthermore, it is used to address sexual dysfunctions and acts as a contraceptive. All parts of this plant are utilized to extract active phytochemicals, although the

composition of these constituents varies widely from one part to another. *Andrographis paniculata* typically reaches a height of 1-2 meters, with quadrangular stems, dark green opposite leaves, and green capsule fruits containing 5-8 seeds. These seeds, in green, yellow, and brown, are small and oval-shaped, remaining dormant for 5-6 months. They serve as the plant's origin and are widespread in its native. The roots exhibit a cylindrical and curved shape, measuring 5-20 cm in length and 1.5-5 cm in diameter. They have a grayish-brown exterior and contain starchy white flesh inside. Both the leaves and stems of the plant are utilized to extract active phytochemicals, with a particular focus on the leaves and roots for medicinal purposes. Traditionally, these leaves and roots have been employed as remedies for a wide range of ailments, including diabetes, hypertension, fever, digestive issues, and as tonics. The plant's extracts are rich in diterpenes, flavonoids, and stigmasterol. This plant has a long history of traditional use in treating common colds, diarrhea, fevers caused by various infections, and jaundice. It is also valued as a health tonic for liver support.

JUSTIFICATION OF THE STUDY

To investigate the bioactive compounds in *Andrographis paniculata* used in drug discovery

AIM OF THE STUDY

To determine the phytochemicals present using both aqueous and ethanol extract of *Andrographis paniculata*

CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1 Taxonomy

Taxonomic hierarchy is as follows:

Domain: Eukaryota,

Kingdom: Plantae,

Subkingdom: Tracheobionta,

Superdivision: Spermatophyta,

Division: Angiosperma,

Class: Dicotyledonae,

Subclass: Gamopetalae,

Series: Bicarpellatae,

Order: Personales,

Family: Acanthaceae,

Subfamily: Acanthoideae,

Tribe: Justiciae,

Subtribe: Andrographideae,

Genus: Andrographis,

Species: *Andrographis paniculata* (Burm. f.) Nees

2.1.2 Vernacular names

Arabic: Quasabhuva

Gujarati: Kariyatu

Tamil: Nilavembu

Kannada: Nelaberu

Telugu: Nelavembu

Marathi: Oil-kiryata

Hindi: Kirayat

Sanskrit: Kalamegha (Kumar *et al*., 2012)

2.1.3 Demographic and Geographical Distribution

Andrographis paniculata originates from the Indian subcontinent, including India, Pakistan, and Bangladesh, as well as Sri Lanka. Over time, it has been introduced to other regions, including the Caribbean islands, the West Indian Ocean, various provinces in China (such as Guangdong, Guangxi, Fujian, Yunnan, Sichuan, Jiangsu, and Jiangxi), Indonesia, Laos, Malaysia, Myanmar, Thailand, Vietnam, and Mesoamerica. This plant thrives in Southern and Southeastern Asia, encompassing countries like India, Java, Sri Lanka, Pakistan, and Indonesia. It is both naturally found and cultivated in regions like India, China, Thailand, Brunei, Indonesia, and various tropical areas in the Americas, including locations like Jamaica, Barbados, the Bahamas, Hong Kong, and southwestern Nigeria. In India, its natural habitat

spans five of the country's 15 specified agro-climatic zones, including regions like the "Middle Gangetic Plains Region" in Bihar and Uttar Pradesh, the "Upper Gangetic Plains Region" in Uttar Pradesh and Delhi, and the "Eastern Plateau and Hill Region" in Bihar, Orissa, and Tamil Nadu.

Fig 1; Image of *Andrographis paniculata*

Source : *Journal of Pharmacy and Biological sciences,* (Lalitha *et al.,* 2015).

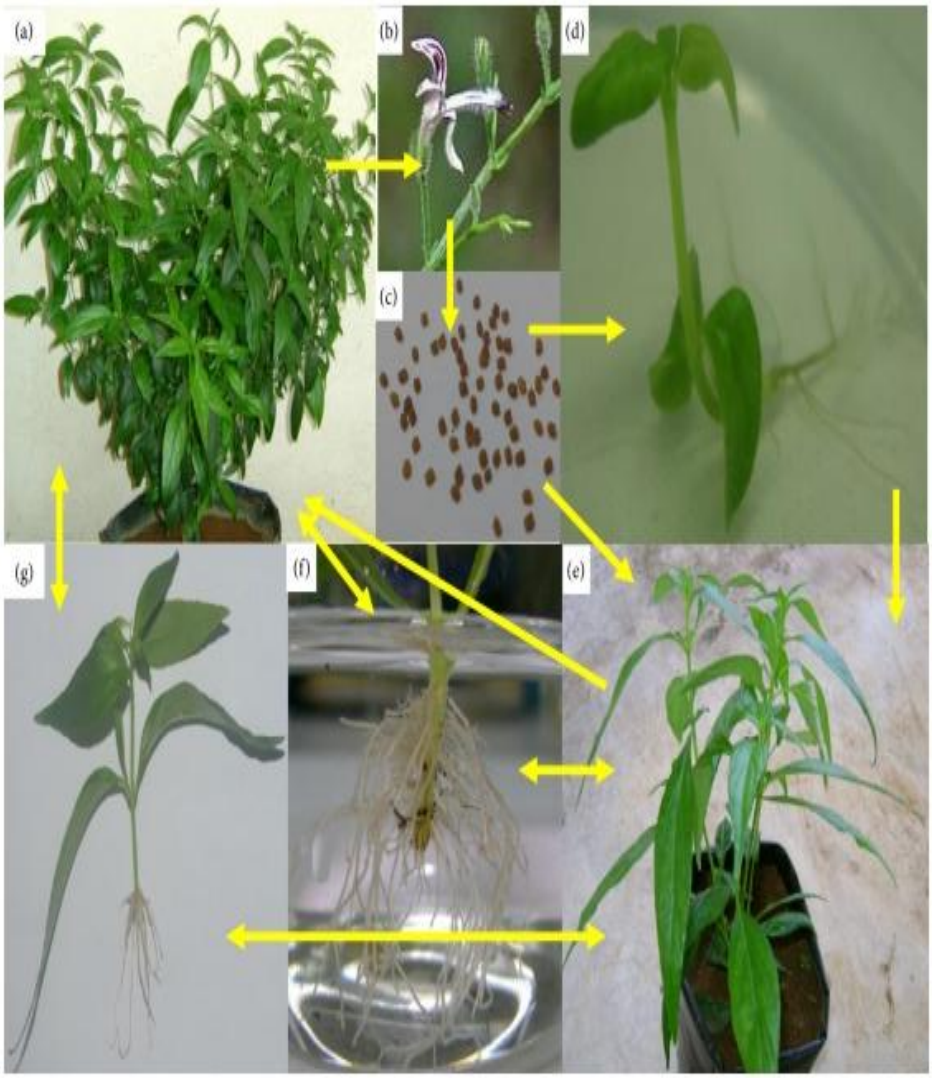


Fig 2: *Andrographis paniculata* and its different parts. (a) Aerial part, (b) flower, (c) pod stage with panicles: mature capsule, (d) fruit, (e) opened capsule, (f) roots, (g) leaves: opposite arrangement,

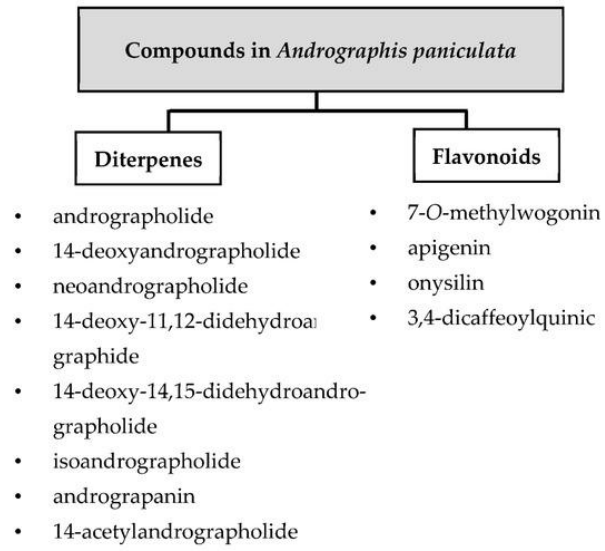
Source: *Journal of Ethnopharmacology, (*Kumar *et al*., 2021)

2.1.4 Biological Importance

Andrographis paniculata holds significant biological importance due to its diverse range of activities. Its primary biological function is as an antidiabetic agent, but it has also demonstrated anti-angiogenic, antibacterial, anticancer, anti-inflammatory, antimalarial, antioxidant, and hepatoprotective properties. These beneficial biological effects are attributed to its bioactive compounds, with the main class being diterpene lactones. Notable members of this group include andrographolide, dehydroandrographolide, neoandrographolide, and deoxyandrographolide (Chithra *et al*., 2016). In traditional Indonesian medicine (jamu), *Andrographis paniculata* is commonly used as an antidiabetic remedy. The therapeutic effects of this plant are closely linked to the concentration of its chemical compounds. Additionally, the choice of solvents used for extraction can impact the extraction of these compounds. Therefore, there is a need for a quality control method to ensure consistent quantification of these compounds in *Andrographis paniculata*, enhancing its therapeutic applications. To address this, a combination of high-performance liquid chromatography fingerprint analysis and chemometrics has been employed to evaluate extracts obtained through various solvent extraction methods (Kadirvelmurugan *et al*., 2014)

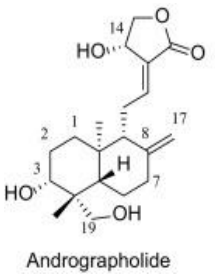
2.1.5 Chemical composition

Andrographis paniculata contains the following chemical compounds:

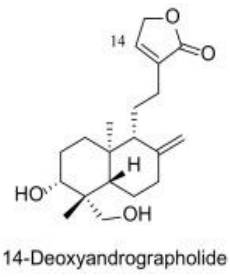


Andrographolide :

It is an extremely bitter diterpenoid that has been isolated from the stem and leaves of *Andrographis paniculata*



Andrographolide



14-Deoxyandrographolide

14-deoxyandrographolide :

This lactone diterpenoid has anticancer, hepatoprotective, antioxidative and antidiabetic properties.

Neoandrographolide : It is a colorless isolated column crystal used for treating acute bacterial dysentery. acute gastroenteritis and upper respiratory tract infection.

14_deoxy-11,12-didehydroandrographide : It acts as a bioactive phytonutrient for the treatment of many diseases

Andrograpanin : Is a hydrosylate hat exhibits anti-inflammatory properties.

2.1.6 Traditional Uses

Traditionally, *Andrographis paniculata* has found use in treating a wide array of health issues. These include atonic dyspepsia (which involves indigestion with weakened stomach muscle tone), digestive problems in children, diabetes, diarrhea, dysentery, flatulence, gastroenteritis, general weakness, loss of appetite, liver issues (including toxic liver damage and infections), recovery from fevers, and various respiratory and skin conditions. In Ayurvedic medicine, it has been employed for centuries to manage intermittent fevers, jaundice, and as an ingredient in more than half of India's multi-ingredient herbal formulations for liver-related conditions. Both the leaves and roots have been integral to Ayurvedic treatments for ailments such as cholera, diabetes, dysentery, enteritis, gastritis, malaria, pneumonia, pyelonephritis, and rabies. The leaf juice serves various purposes, including acting as an alterative (gradually restoring body functions), a tonic, pain and stomach distress reliever, parasite expeller, bile flow promoter, fever reducer, as well as an antiseptic, antispasmodic, and laxative. In traditional household remedies, it is used to address snake and insect bites, diabetes, dysentery, fever, and malaria. The roots and leaves primarily aim to reduce fever, improve stomach tone, stimulate appetite, and enhance overall well-being (Tan *et al*., 2016). *Andrographis paniculata* also serves as an astringent, bacteria-fighting agent, pain reliever, fever reducer, and treatment for worms. It is recommended for use in cases of leprosy, gonorrhea, scabies, boils, skin eruptions, and both chronic and seasonal fevers due to its perceived "blood purifying" properties. In China, this herb derived from the leaves or aerial parts is known as Chuanxinlian, Yijianxi, or Lanhelian. It is characterized as bitter and cold, considered to have antipyretic, detoxifying, anti-inflammatory, and detumescent properties, and is believed to eliminate "pathogenic heat" from the blood. *Andrographis paniculata* is used to treat conditions like

pharyngolaryngitis, diarrhea, dysentery, cough with thick sputum, carbuncles, sores, and snake bites. Various preparations and compound formulas involving this herb have been employed to manage infectious and non-infectious diseases, with significant effectiveness reported for conditions such as epidemic encephalitis B, suppurative otitis media, neonatal subcutaneous annular ulcers, vaginitis, cervical erosion, pelvic inflammation, herpes zoster, chickenpox, mumps, neurodermatitis, eczema, and burns (Wasman *et al*., 2011).

2.1.7 Antibacterial Activity

According to Humnabadkar *et al*. (2012), the acetone and alcohol extracts from *Andrographis paniculata* exhibit strong inhibitory activity against Bacillus subtilis, Staphylococcus aureus, and Proteus mirabilis. Another study evaluated the antibacterial properties of the ethanolic extract of *Andrographis paniculata* using the disc diffusion method. The results indicated notable inhibitory effects, particularly against *Staphylococcus aureus* (26 mm), *Escherichia coli* (21 mm), *Klebsiella pneumonia* (23 mm) and *Pseudomonas aeruginosa* (19 mm). Although, no antimicrobial activity was observed against *Proteus mirabilis*, this research also identified the presence of various compounds in the ethanolic extract, including oils, resins, phenols, flavonoids, tannins, terpenoids, and steroids, further supporting the antibacterial potential of *Andrographis paniculata* (Rajeswari *et al*., 2018). Andrographolide, a component of *Andrographis paniculata*, has demonstrated antibacterial effects against a wide range of bacteria and is considered a promising candidate for the development of new antibacterial drugs. In a study involving 100 urine samples from patients with suspected infections, 47 isolates of bacteria including *Escherichia coli*, *Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* were identified. The ethanolic extract of andrographolide was evaluated for its antibacterial activity using the disc diffusion method.

2.1.8 Anti-inflammatory properties

Research has indicated that *Andrographis paniculata* extract possesses anti-inflammatory properties by inhibiting the expression of various inflammatory markers. Specifically, it reduces the expression of iNOS, TNF-α, IL-1β, IL-6, and IL-12, as well as the production of NO (nitric oxide) by downregulating the p38MAPKs signaling pathways (Wang *et al*., 2014). Andrographolide, a component of the plant, also exhibits strong anti-inflammatory effects. It effectively modulates the secretion of pro-inflammatory cytokines like TNF-α, IL-6, IL-1β, and IL-10 in human blood in a concentration-dependent manner. This modulation occurs through the selective downregulation of cytokines and cytokine receptors, chemokines, JAK/STAT signaling, toll-like receptor signaling, and nuclear factor kappa B (NF-κB) (Parichatikanond *et al*., 2010). Gout, an ancient inflammatory arthritis condition, is initiated by elevated uric acid levels in the blood and the deposition of MSU (monosodium urate) crystals in the joints. MSU crystals play a critical role in gouty inflammation, acting as pro-inflammatory stimuli by activating cells through toll-like receptor signaling. These crystals can interact with various synovial cell types, including neutrophils, monocytes/macrophages, and fibroblast-like synoviocytes. In monocytes, microcrystals stimulate the synthesis of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), and prostaglandin E2. MSU-induced cell activation often leads to inflammation, with long-term inflammation resulting in tophi, joint deformities, and thickening of synovial walls (Shi *et al*., 2012).

2.1.9 Antifungal properties

One of the antifungal compounds identified in the plant extract, *Andrographis paniculata* is Andrographolide. This plant possesses the potential to serve as a valuable source of antifungal compounds (Eugene *et al*., 2015). It is used in the treatment of skin infections in India, China and Malaysia by folk

medicine practitioners.

2.1.10 Cytotoxic properties

Andrographolide, a bioactive component present in *Andrographis paniculata* has demonstrated the ability to impede the growth of different types of cells, including those associated with leukemia, breast cancer, lung cancer, and melanoma. Additionally, it exhibits potent anticancer effects against human colorectal carcinoma LoVo cells by disrupting the progression of the cell cycle (Joselin and Jeeva., 2014).

2.1.11 Antihelmintic properties

An examination of the *Andrographis paniculata* extract's phytochemical composition identified the presence of alkaloids, flavonoids, tannins, and steroids. Notably, tannins were found to exhibit anthelmintic properties, demonstrating effectiveness comparable to the standard drug Albendazole (Padma *et al.,* 2011).

2.1.12 Antioxidant properties

The aqueous and ethanol extracts of *Andrographis paniculata* possess the highest levels of free radical scavenging and superoxide dismutase activities which indicates its greatest antioxidant potential.for protecting cells from oxidative stress and reduce inflammation (Sheeja *et al.,* 2006).

2.1.13 Wound Healing properties

Histologically, when wounds were treated with extracts from *Andrographis paniculata*, they exhibited substantially reduced scar width and displayed extensive fibroblast proliferation. Compared to a blank placebo, the wounds treated with a 10% concentration of *Andrographis paniculata* also exhibited more collagen, reduced angiogenesis, and the absence of inflammatory cells. In summary, the use of

Andrographis paniculata extracts significantly accelerated the rate of wound healing in rats, as indicated by the study (Al-Bayaty F.H *et al*., 2012).

2.1.14 Haemostatic properties

In vitro assessments of the methanolic extract from both the leaves and roots of *Andrographis paniculata* (MAP) revealed its neutralizing effects against various enzymes present in N.N venom. These enzymes include phosphomonoesterase, phosphodiesterase, acetylcholine esterase, phospholipase A2, hyaluronidase, L-amino acid oxidase, ATPase, and fibrinolytic activity (Sivakumar *et al*., 2015). Furthermore, in vivo experiments using MAP demonstrated its anti-snake venom properties by reducing the formation of edema and hemorrhage. It also extended the survival time of male Swiss albino mice that were orally administered the ethanolic extract of *Andrographis paniculata* (S. Jhon *et al*., 2011). Additionally, MAP exhibited the capacity to reverse hemostatic abnormalities (Robert A. *et al*., 2014).

2.1.15 Antidiabetic Properties

Andrographis paniculata is reported to possess antidiabetic properties that is attributed to increase glucose metabolism. The promotion of glucose metabolism was found when treating diabetic rat with this plant extract. This plant exerts its effect by inhibiting disaccharide metabolism and/or glucose metabolism (Dai., 2006).

2.1.16 Hepatorenal Properties

This plant could be exploited in the management of arsenic-induced hepato-renal toxicity. Arsenic is a metalloid and environmental toxicant characterized by a high atomic weight with a proportion relatively absorbed by the gastrointestinal tract and diffused via the blood tissue primarily to the hepatic, renal, bladder and muscular system (Joselin and Jeeva., 2014).

2.2 Cultivation and Propagation

Andrographis paniculata is typically grown from seeds and thrives in various soil types when enriched with adequate organic matter. This versatile plant can be cultivated in a range of environments, including flat lands, open fields, hilly terrain, humid habitats, farms, and alongside roads. Alternatively, it can be propagated through tissue culture methods. It's noteworthy that the leaves tend to contain higher levels of andrographolide during the plant's growth phase. A successful harvest can typically be achieved within a span of 90 to 100 days. Cultivating this plant during the rainy season is considered optimal (Nitave and Koumaravelou., 2016).

2.3 Phytochemicals

Andrographis paniculata contains certain phytochemicals like diterpenes, lactones, and flavonoids that play major roles in it growth and development. Flavonoids mainly exist in the root, but have also been isolated from the leaves. Its aerial parts contain alkanes, ketones, and aldehydes. Although it was initially thought that the bitter substance in the leaves was the lactone andrographolide, later investigations showed that the leaves contained two bitter principles – andrographolide and a compound named kalmeghin. Four lactones – chuanxinlian A (deoxyandrographolide), B (andrographolide), C (neoandrographolide) and D (14-deoxy-11, 12-didehydroandrographolide) – were isolated from the aerial parts in China. Diterpene glucoside (deoxyandrographolide-19beta-D-glucoside) has been detected in the leaves14 and six diterpenoids of the ent-labdane type, two diterpene glucosides and four diterpene dimers (bis-andrographolides A, B, C, and D) have been isolated from aerial parts. Two flavonoids identified as

5,7,2',3'-tetramethoxyflavanone and 5-hydroxy-7,2',3'-trimethoxyflavone were isolated from the whole plant,16 while 12 new flavonoids and 14 diterpenoids have been reported from the aerial parts. Two new flavonoid glycosides and a new diterpenoid (andrographic acid) were recently reported, and two new diterpenoid glycosides were isolated from the aerial parts.

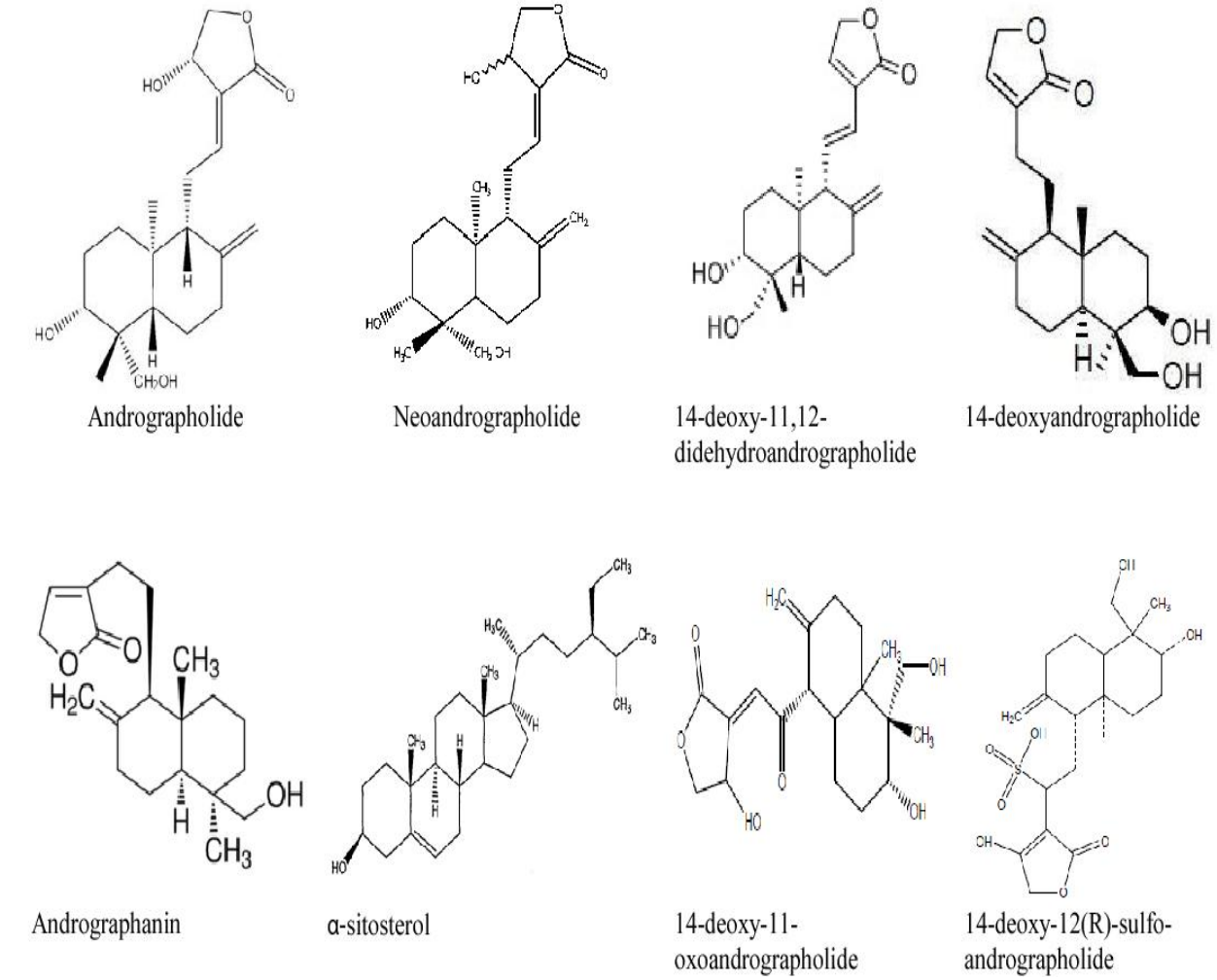


Figure 4 : An Image showing the bioactive compounds with therapeutic potencies in *Androphagis paniculata*

FLAVONOIDS

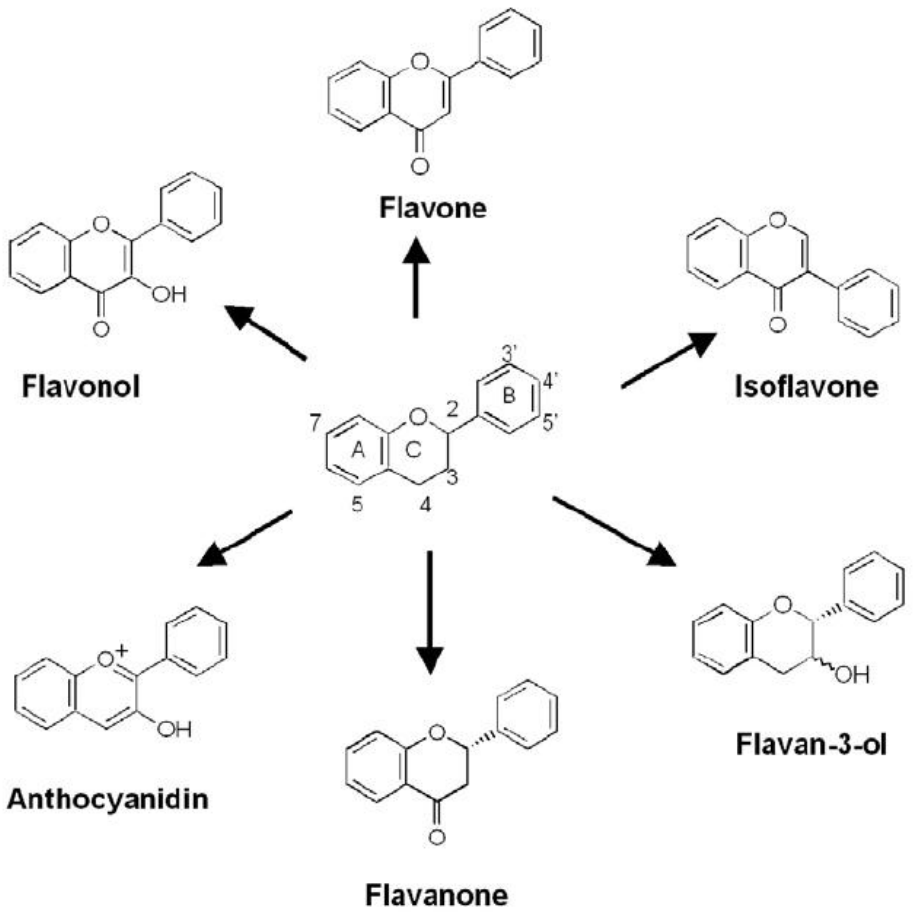


Figure 5: A image representing the structure of Flavonoids

Source: Frontier in Bioscience (2011).

Flavonoids are a class of naturally occurring polyphenolic compounds found in *Andrographis paniculata*, characterized by its unique structure of two benzene rings linked by a three carbon chain forming a closed oxygenated heterocycle. They serve as secondary metabolites that play a crucial role in the plant's defense mechanisms against herbivores and pathogens. They can deter herbivores, inhibit microbial growth, and protect the plant from environmental stressors. They are also known for their antioxidant properties to scavenge harmful free radicals in the plant's tissues, protecting cellular components from oxidative damage thus maintaining the plant's overall health and vitality. They possess anti-inflammatory, immunomodulatory, and antiviral effects, making them valuable in the treatment of various ailments.

ALKALOIDS

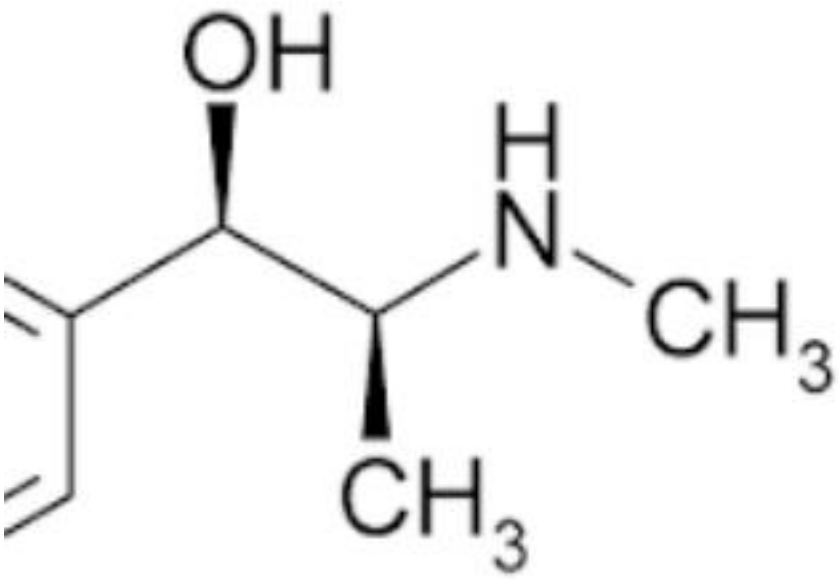


Figure 6 : The structure of an Alkaloid

Source: International Union of Pure and Applied Chemistry (1995).

Alkaloids are a diverse group of naturally occurring organic compounds, often containing nitrogen and are characterized by its alkaline properties due to the presence of a basic nitrogen atom. Therapeutically, they are well known for its anaesthetics, cardioprotective, anti-inflammatory, immunomodulatory, and antiviral activities in *Andrographis paniculata.*

TANNINS

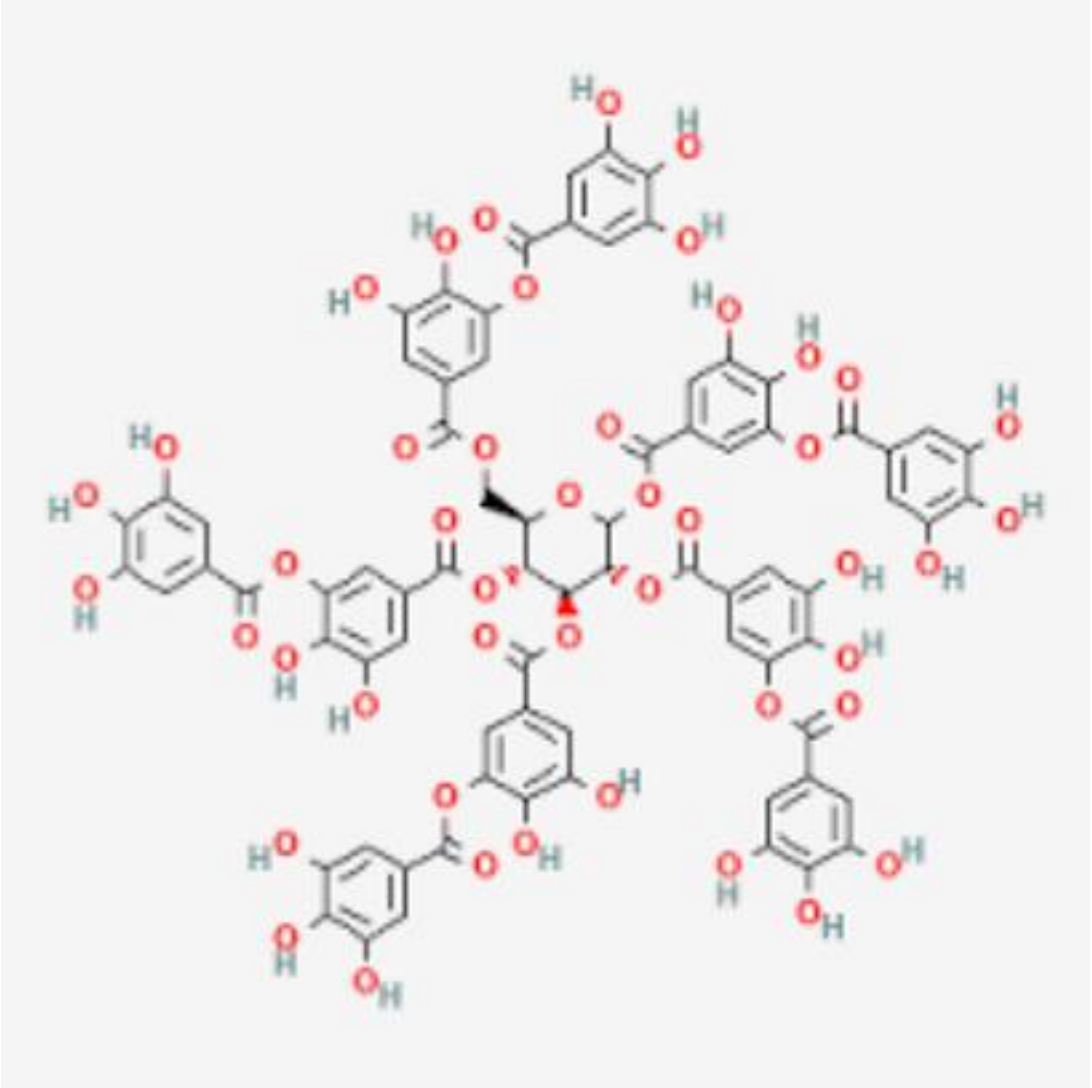


Figure 7: The structure of Tannins

Source: Review on the Effect of Tannins on Mediterranean Ruminant Ingestive Behaviour 92011).

Tannins are polyphenolic compounds with a unique chemical structure characterized by multiple hydroxyl groups. They can be classified into two main groups: hydrolyzable tannins and condensed tannins. *Andrographis paniculata* contains primarily hydrolyzable tannins. They are known for their astringent taste and have antioxidant and anti-inflammatory properties.

PHENOLS

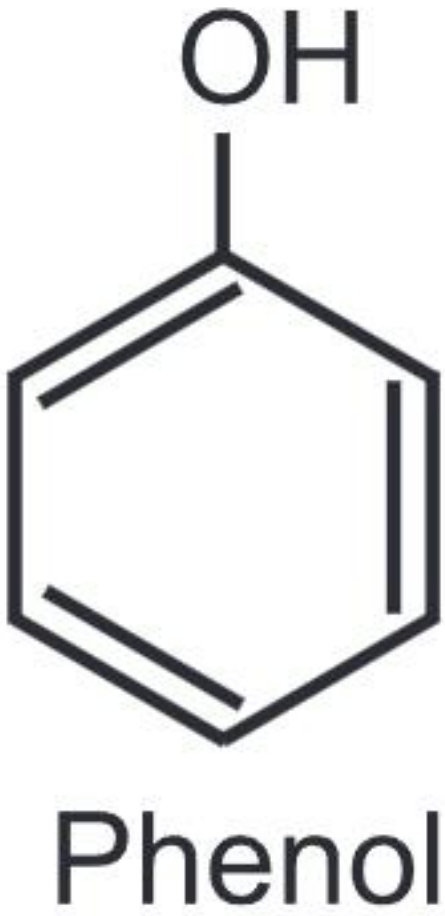


Figure 8: An Image of the structure of Phenol

Source: National Center for Biotechnology Information (2023).

Phenols are a diverse group of organic compounds characterized by a hydroxyl (-OH) group attached to an aromatic ring. They can vary in structure and function. Phenolic compounds in *Andrographis paniculata* may contribute to its medicinal uses.

TERPENOIDS

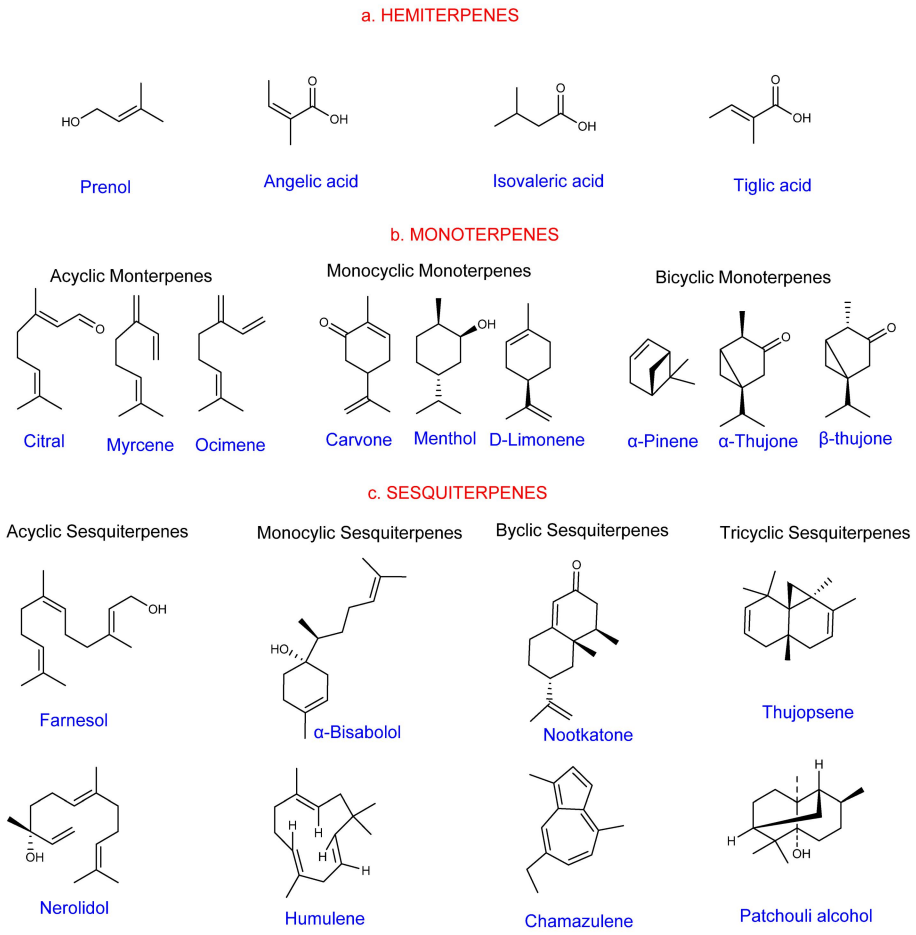


Figure 9: Terpenoids

Source: *International Journal of Molecular Science* (2021)

They are also known as terpenes. They are a diverse group of compounds derived from isoprene units with a wide range of structures and functions. They are often associated with cancer chemo preventive effects, antimicrobial, antifungal, antiviral, anti-hyperglycemic, anti-inflammatory and anti-parasitic properties.

CARDIAC GLYCOSIDES

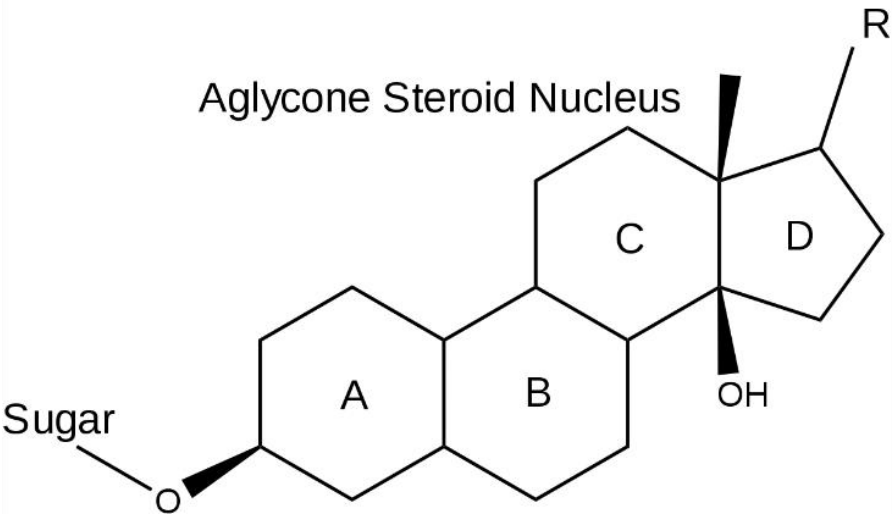


Figure 10: Cardiac Glycosides

Source: Review on Comprehensive Natural Products Chemistry, (1999).

They are a group of compounds with a characteristic structure consisting of a steroid or steroidal-like nucleus attached to one or more sugar molecules. They are known for their effects on the heart and cardiovascular system. They inhibit the sodium-potassium pump (Na+/K+ ATPase) in heart muscle hence aid in the therapeutic treatment of congestive heart failure.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 Materials and Apparatus/Equipment

250mL Beaker

Burette

Andrographis paniculata leaves and stems

Air tight container

Glass jar

Micropipette

Muslin cloth

Precision weighing balance

water bath

Spectrophotometer

Stirrer

Volumetric flask

Incubator

3.1.2 Chemicals and Reagents

Ascorbic acid

Local gin

Ammonium anhydride

Vanallin

Distilled H2O

Wagner's Reagent

99.5% Acetic acid

N-1-Nagrthylenediamine dihydrochloride

Hydrogen peroxide

Quercetin dihydrate

Follin Dennis Reagent

Acetic Anhydride

Methanol

Sodium phosphate dibasic dehydrate (97%)

Sodium dihydrogen Orthophosphate dihydrate (98%)

1 Chloro204-dinitrobenzene (99%)

L-Adrenaline (98.5%)

2,4,6-Tri(Pyridyl)-1,3,5-triazine

Folin Ciocalteu's test

3.1.3 Plant sample and Identification

Fresh leaves of *Andrographis paniculata* was harvested from Ota in Ogun state, Warri in Delta state and Ugbowo in Edo state and taken to the Department of Plant Biology and Biotechnology, University of Benin's Herbarium for identification and authentication with voucher number

UBH-C036.

3.1.4 Preparation of *Andrographis paniculata* extracts

Freshly leaves and stems of *Andrographis paniculate* was collected and air dried under room temperature. It was then milled in pharmacognosy department into fine powder. 539.4g of the pulverized leaves was weighed and separated into equal halves : 269.7g respectively. 1348.5 mL of Local gin and Aqueous water (1:5v/v) was measured respectively . For the **Aqueous extract** (1348.5mL of distilled water mixed with 269.7g of milled samples), Under low heat and constant monitoring , it was boiled for 15 minutes immediately the extract started boiling. It was allowed to air dry, after which it was stirred for a while and sieved using muslin bag. The filtrate was boiled and allowed to cool, hence extract was gotten

For the **Ethanol extract,** It was (the measured milled sample mixed with local gin) soaked for 72 hours. During the course of 72 hours, It was stirred occasionally and finally boiled on the third day under low temperature. The ethanol extract after it has been cooled, was gotten,

3.1.5 Phytochemical constituents of *Andrographis paniculata*

3.1.5.1 Qualitative phytochemical screening

The phytochemical content of the extract using a standard chemical tests was ascertained (Stahl, 1973; Sofoworn,1982; Trease and Evans, 2002). A total of 2g of the extracts was gotten and dissolved separately in 20mL of methanol and filtered. The filtrates obtained was used in the following tests.

3.1.5.1.1 Test for Saponins

Frothing test

The filtrate and distilled water in the ratio of 1/10 v/v was mixed and shook vigorously for one minute. A persistent frothing was observed.

Emulsion test

Two (2) drops of olive oil was added to 1mL of the filtrate and shook for the observation of emulsion.

Lieberman Burchard's test for steroidal saponins or phytosterol

Five Hundred (500) nano litres of chloroform with acetic anhydride (few drops) and added to 1mL of the filtrate. Two drops of concentrated Sulfuric acid was added to the final mixture and a gradual transition of color from violet to blue and finally to green was observed, which indicated the presence of phytosterol.

3.1.5.1.2 Test for Alkaloids

Two millitres (2mL) of Rangeomorphs' reagent to of the filtrate. Reddish-brown precipitate was observed. 1mL of 1% v/v HCL was added to 1mL of the extracts which was used for the following tests;

(i) Two (2) drops of Mayer's reagent was added to 1mL of the extract and a creamy precipitate was observed.

(ii) Two (2) drops of Wagner's reagent was added to 1mL of the extract. A reddish-brown precipitate indicated the presence of alkaloids in the extract.

3.1.5.1.3 Test for Tannin

Equal volume of gelatin solution in 10% Nacl was added to the filtrate. There was a formation of a precipitate that indicated the presence of tannins

Lead Acetate test

In a test tube containing 3mL of the extract, few drops of 1% solution of lead acetate was added. The formation of yellow precipitate observed indicated the presence of tannins.

3.1.5.1.4 Test for Phenols

Folin Ciocatleau's test

Ten percentage (10%) of Folin Ciocalteau's reagent was added to 5mL of the filtrate. 7% NaCo3 was also added to the mixture. There was a formation of intense purple coloration which indicated the presence of phenols.

3.1.5.1.5 Test of Flavonoids

Lead Acetate test

Two to three drops of lead acetate solution was added to 2mL of the filtrate. There was a formation of yellow coloration in the course of this experiment

Aluminum chloride test

Four (4) millitres of the filtrate were shaken with 1% of dilute 1mL of aluminum chloride. Yellow coloration was observed afterwards (Awe and Sodipo, 2001).

3.1.5.1.6 Test for Cardiac Glycosides

Two (2) millitres of the extract, 3mL of glacial acetic acid and 1 drop of 5% of ferric chloride were integrated in a test tube. 0.5mL of concentrated sulphuric acid was integrated by the side of the test tube. There was a formation of blue colouration in the acetic acid layer indicating the presence of cardiac glycosides (Soforowa, 1993)

3.1.5.1.7 Test for Anthraquinones

Ten (10) millitres of chloroform was added to 2mL of the extract. It was then heated for 5 minutes in a steam bath and filtered while cold. To the filtrate, There was an addition of equal volume of ammonia solution (10mL in 100mL distilled water v/v and shaken), but there was no formation of bright-pink colouration at the upper aqueous layer, hence anthraquinone was not present.

3.1.5.1.8 Test for Terpenoids

Salkowski test

Four Hundred (400) millitres of chloroform to 2mL of extract, followed by drops of concentrated H2SO4 which formed a layer. There was a formation of reddish-brown colour between two layers.

3.1.6.1 Quantitative Phytochemical analysis

3.1.6.1.1 Determination of total Flavonoids

The flavonoid content of the extracts was ascertined following the procedure outlined by Ebraahimzadeh *et al*. (2008). Specifically, 1.5mL of methanol was mixed with 0.5mL of the extract (1 mg/mL). Then, 100nl of 10% aluminum chloride, followed by 100nl of 1M potassium acetate, and finally, 2.8mL of distilled water were added and allowed to stand at 25°C for 30 minutes. The absorbance was measured using a spectrophotometer at 415nm. Quercetin was treated in six different concentrations (10, 25, 50, 75, 100, and 150 mg/l) in the same manner to create a standard curve. The results were expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

3.1.6.1.2 Determination of Total Phenols

The phenolic content of the extract was quantified using the method outlined by Folin and Ciocalteau (1927). First, there was an addition of 100nl of extract to 4.5mL of deionized distilled water, followed by 0.5mL of Folin Ciocalteau's reagent (pre-diluted with water 1:10 v/v). The solution was mixed and kept it at 25°C for 5 minutes. Then, another addition of 5mL of 7% sodium carbonate and 2mL of deionized distilled water, thoroughly mixed. The samples were incubated for 90 minutes at 25°C and the absorbances measured at 750nm using a spectrophotometer.

3.1.6.1.3 Determination of Total Proanthocyanidin content

Proanthocyanidin was quantified using the protocol of Sun *et al*. (1998). 500 microliters of the extract (1.0 mg/mL) with 1.5mL of vanillin-methanol solution (4g in 100mL w/v) and 0.75mL of concentrated Hydrogen Chloride (HCL). After incubating the mixture for 15 minutes, its absorbance was read at 500nm to create the standard curve for ascorbic acid with varied concentrations (10, 25, 50, 75, 100, and 150 mg/l). The results were expressed as milligrams of tannic acid equivalent in one gram of extract (mg TAE/g extract).

3.1.6.1.5 Determination of Total Tannins

Tannin content in the extract of *Andrographis paniculata* was determined in accordance with AOAC (1970) with some modifications. Folin Dennis reagent (1.25mL) was added to 500nl of the extract (1 mg/mL). Afterward, 2.5mL of sodium carbonate (10%) was mixed with the solution and incubated for 30 minutes at 25°C. Finally, the absorbance was read at 760nm.. The results were expressed as milligrams of tannic acid equivalent in one gram of extract (mg TAE/g extract).

3.2 DATA ANALYSIS

Statistical Package for Social Sciences (SPSS), a software, was used to analyze quantitative data for the phytochemicals between aqueous and ethanol extract thus, determining its significant differences. Microsoft Excel was used also for the determination of the mean and Standard deviation of the phytochemicals for quantitative analysis. Microsoft word was also used for inputting the result of this research.

CHAPTER FOUR

RESULTS

4.1 Qualitative phytochemical screening of ethanol and aqueous extract of *Andrographis paniculata*

Results of phytochemical analyses revealed the presence of flavonoids, phenols, saponins, terpenoids, tannins, cardiac glycoside and alkaloids. (Table 1)

Phytochemical	Aqueous Extract	Ethanol Extract
Saponins	++	+
Phytosterols	-	-
Tannin	+	++
Phenol	++	+
Flavonoids	++	+
Cardiac Glycosides	+	+
Anthraquinone	-	-
Terpenoid	++	+

[+]: presence of constituent, [++]: moderately high concentration of constituent and [-]: shows absence of constituents.

On the basis of the result obtained by qualitative analysis of *Andrographis paniculata*, it is evident

that Saponins, Phenol, Flavonoids and Terpenoids are moderatly high in all the fractions of aqueous extract compared to ethanol extract while phytosterol and anthraquinone are absent all fractions of aqueous and ethanol extract of *Andrographis paniculata.*

4.2 Quantitative phytochemical screening of ethanol and aqueous extract of *Andrographis paniculata*

Table 2: Quantitative Analysis of Phytochemical Constituents of *Andrographis paniculata*

Phytochemical	**Aqueous Extract *A. paniculata***	**Ethanol Extract *A. paniculata***
Total Phenol (mg GAE/g Extract)	77.53 ± 3.73^{a}	38.34 ± 2.47^{a}
Total Flavonoids (mg QE/g Extract)	65.95 ± 5.36^{b}	35.12 ± 1.49^{b}
Total Tannins (mg TAE/g Extract)	54.15 ± 1.51^{c}	38.34 ± 2.47^{c}
Total Proanthocyanidins (mgAAE/g Extract)	174.44 ± 3.33^{d}	180.74 ± 5.01^{d}

Data represent the mean concentration ± standard deviation (n=3) of phytochemicals in aqueous and ethanol extract of *Andrographis paniculata* .Values with the same superscript in a row are not significantly different from each other ($P \geq 0.05$).

Based on the qualitative analysis of phytochemicals, quantitative analysis was also done on major phytochemicals like, flavonoids, tannins, phenol and proanthocyanidin. Both aqueous and ethanol extract expressed different values of phytochemicals. Highest amount of phenol, flavonoids and tannins were recorded in aqueous extract of *Andrographis paniculata* while proanthocyanidin was only found higher in ethanol extract.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

From the research carried out, the result revealed the scientific basis for the traditional uses of the plant extracts in which phytochemical analysis revealed the presence of flavonoids, tannins, phenols and proanthocyanidin in its quantitative screening and the presence of further phytochemicals like saponin, alkaloid, proanthoquinones, cardiac glycosides, phenols and terpenoids. Both the extracts were positive for alkaloids, phenols, tannins, hydrolysable tannins, flavonoids, terpenoids and saponins which were earlier reported to be important for antiviral activity, antibacterial and antidiabetic activity. (Arbab *et al*., 2017). The results of quantitative analysis could be due to its extraction process or the plant itself, *Andrographis paniculata*. There was no significant difference between aqueous and ethanol extract of *Andrographis paniculata.* From the qualitative analysis, Saponin which plays a major role in hormone regulation, binding of cholesterol molecule to reduce hypercholesterolemia was moderately high in aqueous extract than ethanol extract. Saponins also aid in detoxifying toxins out of the body. Phytosterol, a phytochemical that reduces risk of cardiovascular diseases by limiting the amount of cholesterol able to enter the body, was absent in both the ethanol and aqueous extract of *Andrographis paniculata..* Phenols and Flavonoids were more present in aqueous extract of the plant than its ethanol form. They play major role as reducing agents, free radical scavenger in antioxidant activity to prevent cell aging and diseases. Tannins and Cardiac

glycosides were also present in both aqueous and ethanol extract of *Andrographis paniculata..*at considerable amount. Their major role is in treatment of pulmonary arterial hypertension in humans.

5.2 CONCLUSION

Healthy phytochemicals are abundant in *Andrographis panicuata*. These phytochemicals have been reported to possess therapeutic properties against infections and diseases that tends to affect majorly the pancreas, liver and heart. After the phytochemical screenings carried out in this plant, Aqueous solvent used in extracting this plant has been depicted to be more therapeutic in its medicinal uses compared to when ethanol solvent was used for extraction of *Andrographis panicuata*

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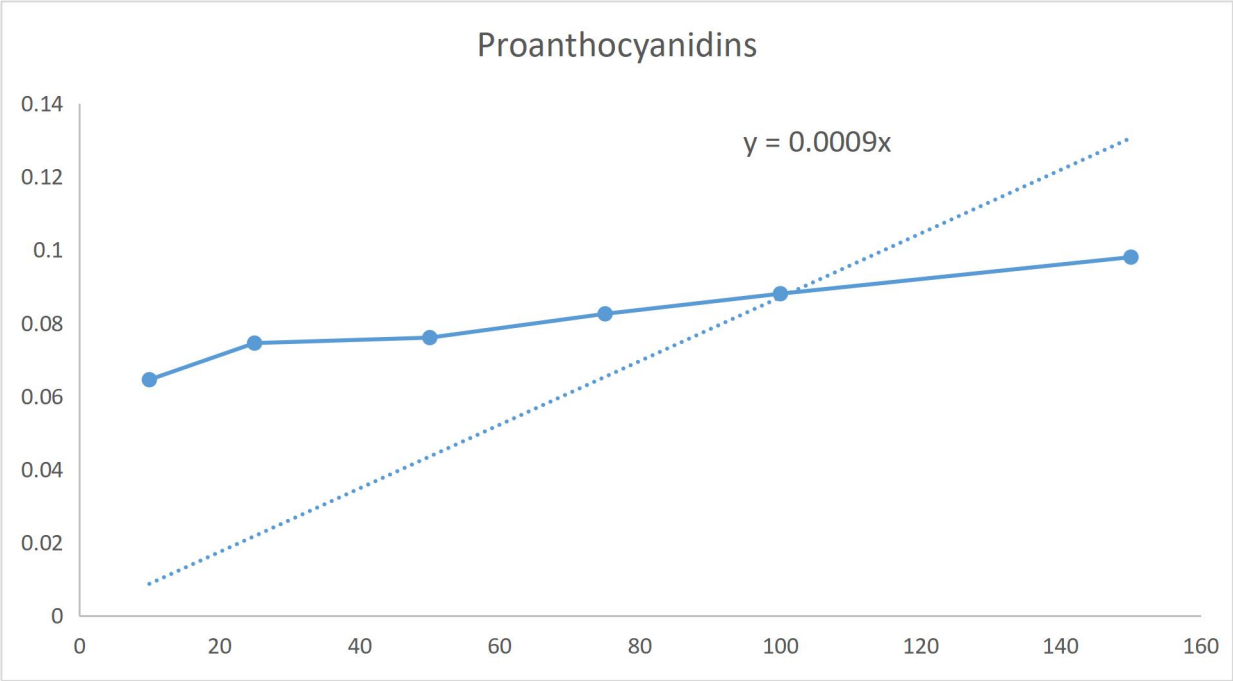
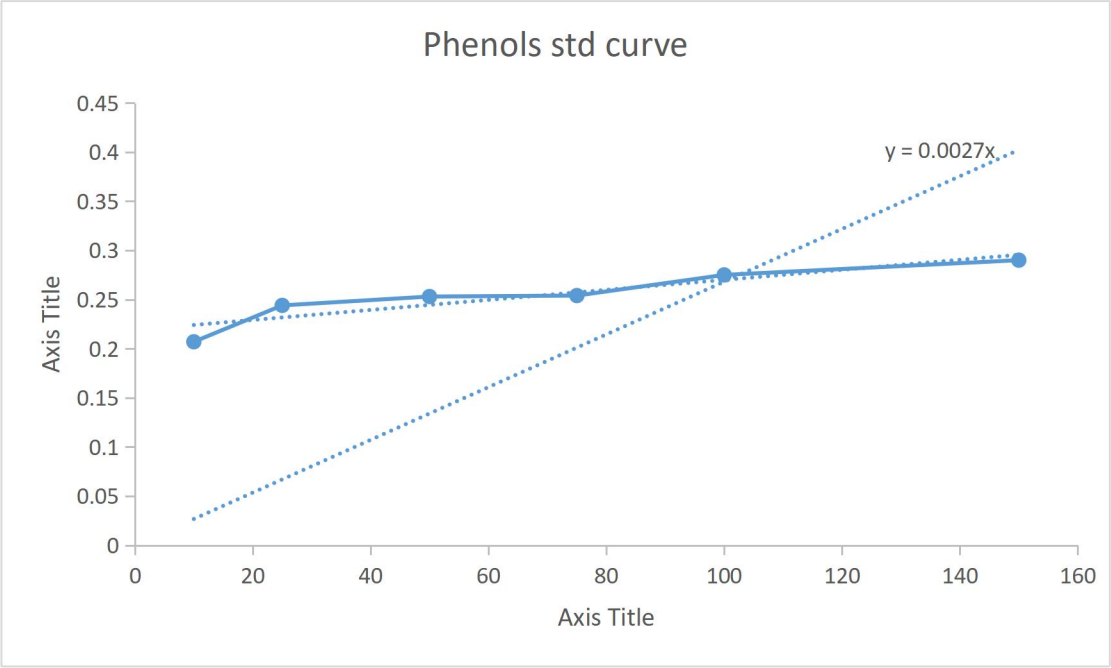
APPENDICES



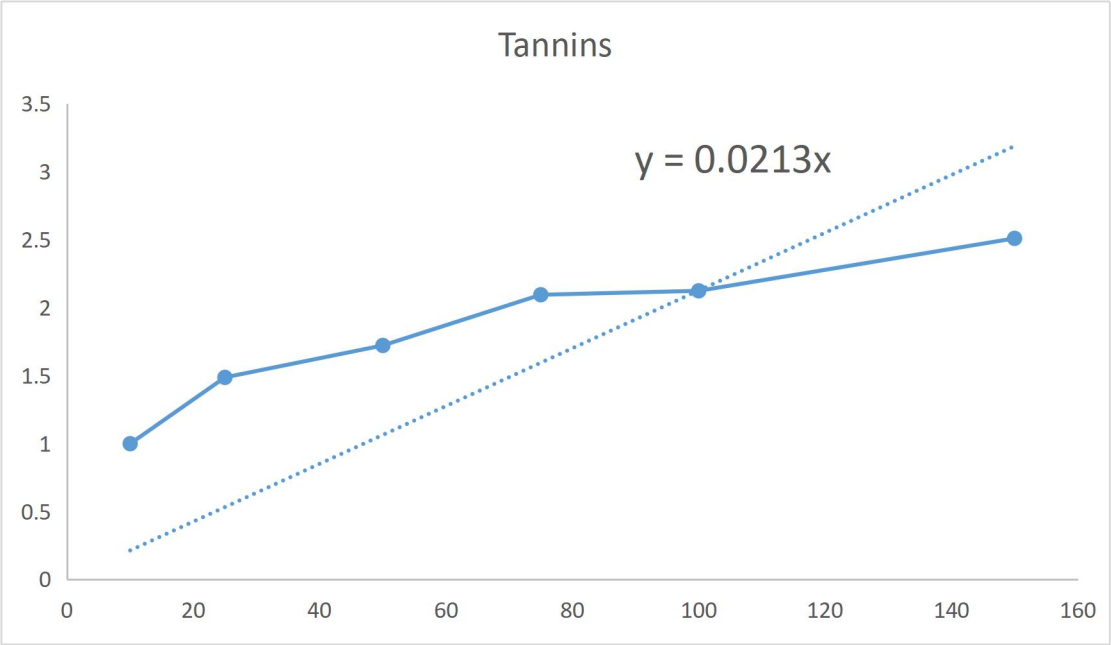
Proanthocyanidins						
Sample ID	Absorbance	Mean Absorbance	Concentration		STD DEV	MEAN CONCENTRATION
Aq1	0.154		171.1111			
Aq2	0.16		177.7778			

Aq3	0.157	0.157	174.4444	Aq	3.333333	174.4444
Eth 1	0.157		175.5556			
Eth 2	0.167		185.5556			
Eth 3	0.163	0.162667	181.1111	Eth	5.010278	180.7407

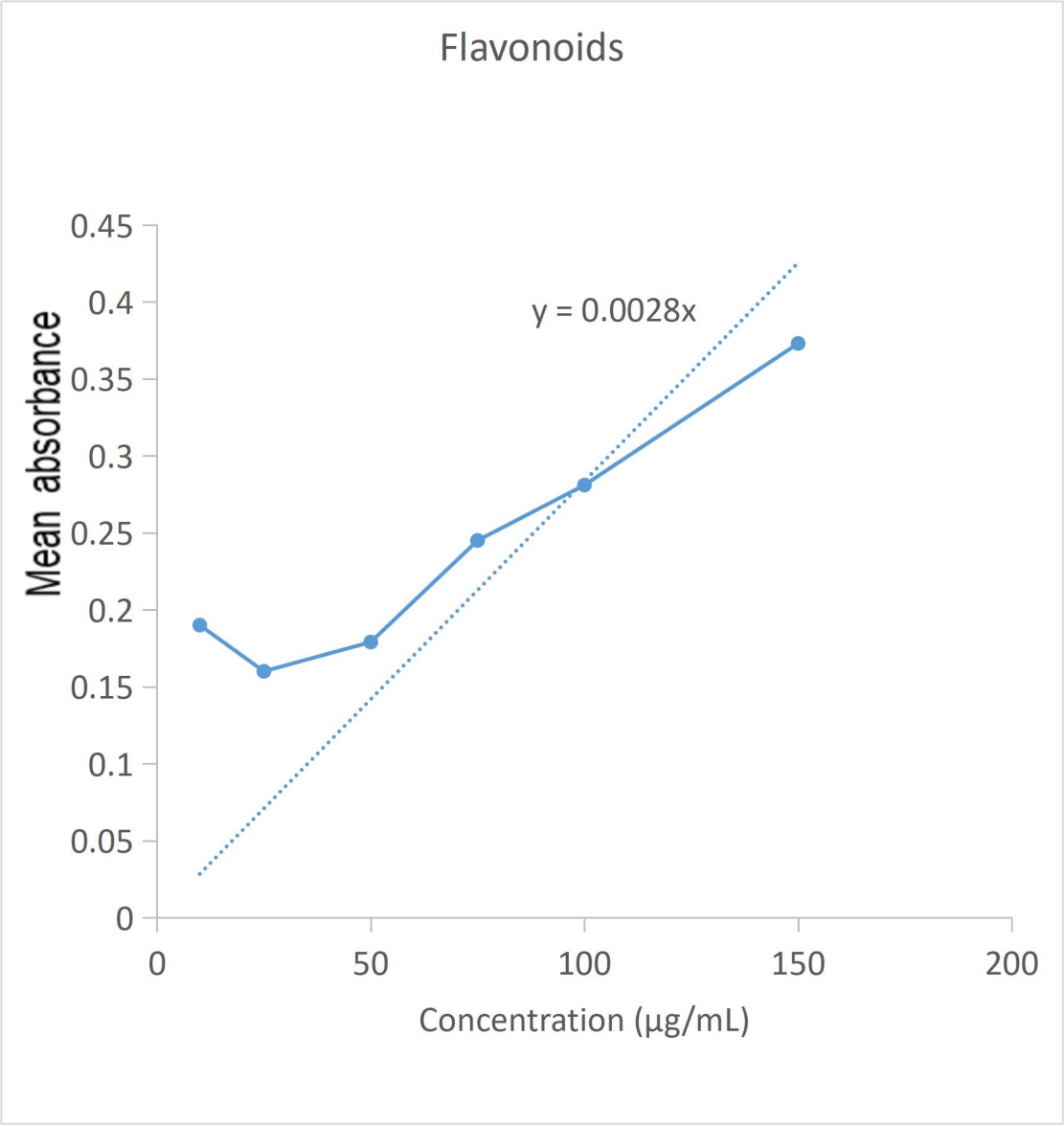


Phenols					
Sample ID	Absorbance	Mean Abs	Concentration	STD DEV	MEAN CONC

Aq1	0.22		81.48148		
Aq2	0.208		77.03704		
Aq3	0.2	0.209333	74.07407	71.48148	77.53086
Eth1	0.184		68.14815		
Eth2	0.194		71.85185		
Eth3	0.193	0.190333	71.48148	2.039841	70.49383



Tannin						
Sample ID	Absorbance	Mean Absorbance	Concentration		STD DEV	Mean Concentration
Aq1	1.165		54.69484			
Aq2	1.178		55.30516			
Aq3	1.117	1.153333	52.44131	Aq	1.508448	54.1471
Eth1	0.847		39.76526			
Eth2	0.847		39.76526			
Eth3	0.847	0.816667	35.49296	Eth	2.466614	38.34116



FLAVONOIDS						
Sample ID	absorbance	Mean abs	concentration		STD DEV	Mean concetration
Aq1	0.184		65.71429			
Aq2	0.2		71.42857			
Aq3	0.17	0.184667	60.71429	Aq	5.36111	65.95238
Eth1	0.095		33.92857			
Eth2	0.097		34.64286			
Eth3	0.103	0.098333	36.78571	Eth	1.486904	35.11905

Quantitative phytochemical analysis

Phytochemical	Aqueous extract *A. paniculata*			Ethanol extract *A. paniculate*		
	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance 1	Absorbance 2	Absorbance 3
Proanthocyanidins	0.154	0.160	0.157	0.158	0.167	0.163
Flavonoids	0.184	0.200	0.170	0.095	0.097	0.103
Phenols	0.22	0.208	0.200	0.184	0.194	0.193
Tannin	1.165	1.178	1.117	0.847	0.939	0.756

ANOVA

				Sum of Squares	df	Mean Square
Proanthocyanidin	Between Groups	(Combined)		103.244	1	103.24
		Linear Term	Contrast	103.244	1	103.24
	Within Groups			56.428	4	14.10
	Total			159.671	5	
Flavonoids	Between Groups	(Combined)		1426.041	1	1426.04
		Linear Term	Contrast	1426.041	1	1426.04
	Within Groups			61.904	4	15.47
	Total			1487.945	5	
Tannins	Between Groups	(Combined)		374.639	1	374.63
		Linear Term	Contrast	374.639	1	374.63
	Within Groups			16.763	4	4.19
	Total			391.402	5	
Phenol	Between Groups	(Combined)		74.272	1	74.27
		Linear Term	Contrast	74.272	1	74.27
	Within Groups			36.119	4	9.03
	Total			110.391	5	

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidenc Lower Bound
Proanthocyanidin	Aqueous Extract	3	172.4444	1.76381	1.01834	168.062
	Ethanol Extract	3	180.7408	5.01028	2.89268	168.294
	Total	6	176.5926	5.65104	2.30703	170.662
Flavonoids	Aqueous Extract	3	65.9524	5.36109	3.09523	52.634
	Ethanol Extract	3	35.1190	1.48690	.85846	31.425
	Total	6	50.5357	17.25077	7.04260	32.432
Tannins	Aqueous Extract	3	54.1471	1.50845	.87090	50.399
	Ethanol Extract	3	38.3433	2.47106	1.42667	32.204
	Total	6	46.2452	8.84762	3.61203	36.960
Phenol	Aqueous Extract	3	77.5300	3.72922	2.15307	68.266
	Ethanol Extract	3	70.4933	2.03780	1.17653	65.431
	Total	6	74.0117	4.69875	1.91826	69.080