PROFILING OF PHYTOCHEMICALS IN THE LEAVES OF *ASYSTASIA GANGETICA* (L) T. ANDERSON

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this final year project work was carried out by **ELAKHE DEBORAH EVELYN (MAT NO. PHA1707036)** of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, in partial fulfilment for the award of Doctor of Pharmacy(Pharm.D) degree in Pharmacy.

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Dr. E.E. Odion Date

(Project Supervisor)

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Dr. Imieje Vincent, Date

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty for his strength; wisdom and understanding that brought this work to fulfilment and also to my late Father who made it his life duty to ensure that my siblings and I get only the best out of life.

ACKNOWLEDGMENT

This research project is a complex entity and emerges from the contribution of many people. First and foremost, praises and thanks to God Almighty, for his showers of blessings and faithfulness throughout the preparation for my undergraduate research project.

Special acknowledgement to my wonderful and studious Project supervisor, Dr. E.E. Odion for taking out time to be of utmost help despite his busy schedule, for guidance, advice, corrections and fatherly love. I also thank the head of the department, Dr. Imieje Vincent, and to all my lecturers in the faculty of Pharmacy, I say a huge thank you. My sincere and undiluted gratitude goes to my parents, Mr. and Mrs. Elakhe for their love and believe in me and for their moral, financial, and spiritual support all through my years in school, not forgetting my siblings Ernest, Alice and Eunice.

Finally, I am forever grateful for the friends I made during the course of my study, likes of Michael, Stephanie, Johnson, Joshua, Tracy, Nathaniel, Imade, Oluwamayowa, Oluwatobiloba and a host of others for their love and support.

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ABSTRACT

Asystasia gangetica is a perennial herb that have naturalized in Africa and Asia, where it is use in treatment of varying ailments. The presence of phytochemicals in this plant maybe responsible for its acclaimed effects. This study aims to identify the phytoconstituents in the leaves of *Asystasia gangetica*. Preliminary phytochemical screening was achieved by standard methods, gas chromatography mass spectrometry (GC-MS) and high pressure liquid chromatography (HPLC) analysis were utilized to determine volatile and non-volatile compounds. Phytochemicals identified include alkaloid, tannin, steroid, saponin, flavonoid, and glycoside. GC-MS analysis identified 2,3-dihydroxypropyl elaidate and HPLC evaluation reported aphyllidine, flavone and spartein as the most prominent compounds. Identification of these compounds with documented evidence of pharmacological activities, thus validate the use of *Asystasia gangetica*, in many disease conditions.

CHAPTER ONE

INTRODUCTION

1.1 Botanical Description and Distribution

Asystasia gangetica is native to tropical and subtropical regions of Asia and Africa. It has a wide distribution and can be found in countries such as India, China, Japan, Indonesia, Malaysia, and various African nations. The stems of *Asystasia gangetica* are herbaceous, slender, and can either be prostrate or semi-erect. They exhibit a quadrangular cross-section, which is a characteristic feature. Leaves are opposite, simple, and possess petioles. They are generally ovate to lanceolate in shape with serrated margins. The leaf surface is often pubescent, and the leaves may vary in size but are commonly 5-15 cm long. The inflorescence is a terminal spike, featuring clusters of tubular flowers. The arrangement is typically one-sided, creating a spike at the tip of the stem (Saeedi *et al.,* 2020). *This* inflorescence structure contributes to the distinctive appearance of the plant. The tubular flowers are bilabiate, with a two-lobed upper lip and a three-lobed lower lip.

The corolla may exhibit shades of violet, pink, or white. The flowers attract pollinators, and the two-lipped structure is a common characteristic of the Acanthaceae family. The plant produces small, ovoid capsules that contain numerous tiny seeds. These capsules split open when mature, dispersing the seeds for reproduction. *Asystasia gangetica* is adaptable to various habitats, including grasslands, forest margins, riverbanks, and disturbed areas. It can thrive in both open and shaded environments. The plant is often associated with disturbed sites, and its ability to grow in different conditions contributes to its status as a widespread and adaptable species (Mathew *et al.,* 2017). *Asystasia gangetica* has been recognized as invasive in certain regions outside its native range. Its ability to spread quickly and establish itself in diverse ecosystems has raised concerns in areas where it has been introduced.

1.2 Taxonomy

Kingdom: Plantae	Subclass: Eudicotyledonae
Subkingdom: Viridiplantae	Superorder: Asteranae
Infrakingdom: Streptophyta	Order: Lamiales
Superdivision: Embryophyta	Suborder: Lamianae
Division (Phylum): Tracheophyta	Family: Acanthaceae
Subdivision: Spermatophytina	Genus: Asystasia
Class: Angiospermae	Species: gangetica

Source: Suzuki *et al.* (2019).

1.3 Etymology

Asystasia means 'Inconsistency' and is correlated with the more or less regular corolla of the plant. Gangetica means 'The Ganga river' where this species supposed to be grown in India (Ojiako *et al.,* 2015).

1.4 Traditional, Unani and Other Ethnobotanical Uses

The plant is used traditionally as a decoction for the cure of rheumatism, stomachache, and heart pain. In East African countries, like Kenya, the decoction of leaves is used as a vermifuge to cure intestinal worms. Leaves are prevalently employed in the management of asthma in Nigeria. In India, it is used as astringent, stomachic, and diaphoretic. It is claimed that the decoction of leaves is extremely effective in the treatment of asthma (anti-inflammatory). In Cameroonian traditional medicine, it is used in the treatment of bone fracture, bone diseases, diarrhoea, and woman infertility (Ramesar *et al.,* 2018).

1.5 Phytoconstituents Investigated

Preliminary phytochemical analysis: n-hexane, ethyl acetate, and methanol extract of the plant have been investigated for the presence of phytoconstituents which revealed the presence of sugar, steroid, flavonoid, glycoside, anthraquinone in n-hexane extract; saponin, sugar, steroid, flavonoid, glycoside, anthraquinone in ethyl acetate extract; saponin, sugar, flavonoid, glycoside, anthraquinone in methanol extract. The whole plant sample was dried, extracted with different solvents, and then tested for the presence of constituents. The plant is reported by Adeyemi *et al.* (2021) to possess steroids (in ethanol, chloroform, petroleum ether, and benzene extract), sugars (in aqueous, ethanol, petroleum ether, benzene extract), phenols (in ethanol, chloroform, petroleum ether, benzene extract), flavonoids (in aqueous, petroleum ether, benzene extract), saponins (in chloroform, benzene extract), tannins (in petroleum ether, benzene extract) and amino acid (in aqueous, chloroform, benzene extract).

Leaves extract (ethanol) is reported to possess alkaloids, saponins, flavonoids, tannins. Phytochemical isolated/Characterised: The ethylacetate fraction from A. gangetica ethanol (80%) extract yielded a glycoside, luteolin-7-oneohesperidoside (1) which was confirmed byphysical and chemical analysis. Two chalcones, isosalipurposide (2) and cernuoside (3) has been reported from the yellow coloured flower petals of A. gangetica. The plant especially leaves encompass tremendous quantity of amino acids (thiamine), fibers, proteins, sugars, minerals, etc., thus it is considered as an important source of food. A methanol extract of the aerial part of *A. gangetica* was defatted with diethyl ether and subjected to silica gel chromatography to obtain 8 compounds, including a new compound; asysgangoside is also known as 5, 11-epoxymegastigmane glucoside(4).Other constituents are; apigenin 7-O-neohes-peridoside (5); apigenin 7-O-β-D-glucopyranoside (6); benzyl β-D-gluco-pyranoside (7); (6S,9R)-roseoside (8);

ajugol (9); salidroside (10); and apigenin 7-O-b-D-gluco-pyranosyl (1-6)-β-D-gluco-pyranoside (11) (Adeyemi *et al.*, 2021).

From the methanol extract (ethyl acetate fraction) of yellow portion of the *A. gangetica* flowers, a yellow solid compound has been isolated and was characterized by UV, NMR, MS study as apigenin 7-O-glucosyl(3'-6")luteolin7"-O-glucoside (12). Aerial, seed and root portion from *A. gangetica* was collected, crushed, and volatile oil was extracted using Clevenger apparatus to give 0.10%, 0.56% & 0.51% yields respectively. These volatile oils were subjected to GC-MS analysis (Mugabo and Raji, 2013).

1.6 Pharmacologic Inactivity Documented By Researchers

1.6.1 Anti-inflammatory Activity:

A. gangetica extract (80% ethanol) and its isolated glycoside, luteolin-7-o-neohesperidoside when subjected to hypotonicity induced haemolysis. It produced dose-dependent %inhibition and exhibited biphasic activity. Antiinflammatory activity of luteolin-7-o-neohesperidoside was assessed by carrageenan induced rat paw oedema method in albino rats and the effect was found to be significant and comparable to phenyl butazone (Marim, 2016).

1.6.2 Anti-asthmatic Activity

A. gangetica leaves possess significant anti-asthmatic property in Guinea pigs by relaxing histamine-pre-contracted tracheal strips. The ethyl acetate extract is found to be more potent than its hexane & methanol extract. These extracts especially methanol, also showed an anti-inflammatory response against egg albumininduced acute inflammation in Albino rats (Janakiraman *et al.*, 2021).

1.6.3 Antihypertensive Activity:

Methanolic extract of leaves of *A. gangetica* exhibited Angiotensin-1Converting Enzyme (ACE-1) inhibitory activity in vitro with 51% inhibition and thus possessed antihypertensive properties. *A. gangetica* decreased the diastolic, systolic, and mean arterial BP significantly ($p<0.01$) and dose-dependently (10-400 mg/kg). It also produces a drop-in heart rate which was significant ($p<0.05$) but not dosedependent. A mixture of infusion of either angiotensin I or angiotensin II with *A. gangetica* (200 mg/kg) significantly ($p<0.001$ and $p<0.01$ respectively) restrained their hypertensive effect, and this was also accompanying by drops in heart rate (Akah *et al.,* 2013).

1.6.4 Anti-diabetic and Hypolipidemic Action:

Administration of ethanolic extract (100 and 200mg/kg, p.o.) of *A. gangetica* to diabetic rats for 4 weeks, significantly reduced blood glucose, restored lipid levels, thus held significant antidiabetic action. The effect may be due to the antioxidant activity of extract. *A. gangetica* leaves, at three dose level (25%, 50% and 75% juice), suppress the raised blood glucose concentration and improved body weight in the diabetic rat (alloxaninduced). The levels of TC, TG were significantly ($P < 0.05$) reduced in all treated groups. The flowers of A. gangetica were extracted with ethanol and acidified using citric acid to obtained anthocyanins extract, which was assayed in vitro against α-amylase and α-glucosidase.

Anthocyanins extract possessed significant inhibitory activity (71.46 ± 1.21% and 76.85 ± 0.75%, respectively) and IC50 value (260 ml/ml and 244 ml/ml, respectively) at the concentration of 400 mg/ml. Thus, the enzyme inhibition may be the possible mechanism of anti-diabetic activity. Aqueous and alcoholic extract of leaves of *A. gangetica* lowered down the fasting blood glucose level by 48.47 ± 1.01 and 48.46 ± 0.93% respectively, in type I diabetic wistar rats (alloxan

induced). Leaf extract of *A. gangetica* in the form of herbal formulation with leaf extracts of *Hibiscus rosasinensis, Emilia coccinea*, and *Acanthus montanus* significantly improved hyperglycaemia as well as dyslipidaemia in alloxan-induced diabetic male rats (Hamid *et al.,* 2021).

1.6.5 Anti-oxidant Activity:

70% ethanolic extract of *A. gangetica* leaves (100 mg/kg) along and in combination with 90% ethanolic extract of Morusindica (400 mg/kg) possessed significant antioxidant and antidiabetic action in diabetic albino rats (alloxan induced). Alcoholic extract of leaves upsurges the levels of glucose-6-phosphate dehydrogenase (G-6-PDH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), reduces glutathione (GSH), and declines lipid peroxidation (thiobarbituric acid reactive substances) level; thus, possessed anti-oxidant property. Free radical scavenging activity has been performed on isolated iridoid glycoside viz. 6β-hydroxyantirrhide; angeloside; 6-O-α-L-rhamnopyranosyl-catapol; 6-O-α-(3”-O-trans-caffeoyl)-Lrhamnopyranosyl-catapol; ajugol. In DPPH assay, iridoid glycoside, 6-O-α-(3”-O-trans-caffeoyl)-Lrhamnopyranosyl-catapol exhibited the potent scavenging activity with SC50(half maximal scavenging activity) value. 3 mM with respect to the other iridoid glycosides. In the ORAC assay by Sudhakar *et al.* (2016), 6-O-α-(3”-O-trans-caffeoyl)-L-rhamnopyranosylcatapol was more potent than the positive control. The *A. gangetica* alone or in combination with *Hibiscus rosasinensis, Emilia coccinea* and *Acanthus montanus* showed the free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydrogen peroxide (HP), hydroxyl radical (HR), nitric oxide radical (NOR), and superoxide radicals (SOR) using invitro models 35. All these studies suggested the use of plant as anti-oxidant. Antimicrobial: Hexane, ethyl acetate and methanol extracts of *A. gangetica* at conc. 25 to 200

mg/ml inhibited (dose dependent) the growth of 12 pathogenic microorganisms, including 6 bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonellae typhi, Bacillus subtilis* and *Klebsiellae pneumonae*) and 6 fungi (*Epidermophyton floccosum, Rhizopus stolon, Aspergillus niger, Penicillum notatum, Candida albicans and Tricophyton rubrum*) to different degrees using agar diffusion pour plate method (Kokwaro, 2016).

Anti-snake Venom: Administration of methanol extract of leaves of *A. gangetica* at 1g/kg i.p., 30min before administration of Najamelanoleuca' svenomto mice provided 60% protection which is significant ($p<0.05$) compared to control group. On the other hand, its polyphenolic fraction (flavonoids, tannins, saponin) each at 1 g/kg i.p. provide 60%, 80%, and 60% protection, respectively, against venom, which is significant ($p<0.05$) compared to control group. Anthelmintic Activity: Methanol extract (12.5, 25, 50 mg/ml) of fresh leaves of *A. gangetica* exhibited significant ($P<0.05$) and conc. dependent reduction in death time against Nsukkadrilus be compared to the piperazine 37. Methanolic extract (10-200 mg/ml) of the plant exhibited dose-dependent decline in time taken for paralysis and subsequently death against Pheretima posthuma. 200 mg/ml conc. showed the most potent effect against earthworms comparable to albendazole (Ngueguima *et al.*, 2012).

1.6.6 Anti-arthritic activity:

Dose-dependent (10-1000 µg/ml) inhibition of protein denaturation was reported in the methanolic extract of *A. gangetica* for anti-arthritic activity. The highest % inhibition (78.94%) was noted in 1000 µg/ml conc. compared to diclofenac sodium (84.47%) (Ezike *et al.,* 2018).

1.6.7 Anti-platelet

Methanolic extract of the plant revealed a dose-dependent (100-500µg/ml) inhibition of aggregation for anti-platelet activity with the highest inhibition was noted down at 500 µg/ml conc. compared with the aspirin (Saunders, 1958).

1.6.8 Effect of Blood Viscosity

In blood, methanolic extract and its flavonoid fraction displayed a dosedependent (100-500 µg/ml) reduction in viscosity in 90 minutes spam. Flavonoid fraction exhibited a higher reduction in viscosity than methanol extract (Saunders, 1958).

1.7 Phytochemicals

Phytochemical composition of *Asystasia gangentica*

The leaves, stem bark and roots are reported to contain steroids, flavonoids, phlobatannins, tannins and saponins (Sonibare *et al.* 2021; Altemimi *et al.* 2017). These secondary metabolites are said to be responsible for the pharmacological activity of the plant (Amonkan *et al.*, 2018). Various studies have been done to isolate the particular active constituents belonging to these secondary metabolites that are believed to be directly responsible for the activity of *Asystasia gangentica* (Adebayo *et al.*, 2019).

1.7.1 Tannins

Tannins are a class of astringent polyphenolic biomolecules that bind and precipitate proteins and other organic compounds, including amino acids and alkaloids (Boralle *et al*., 2018)

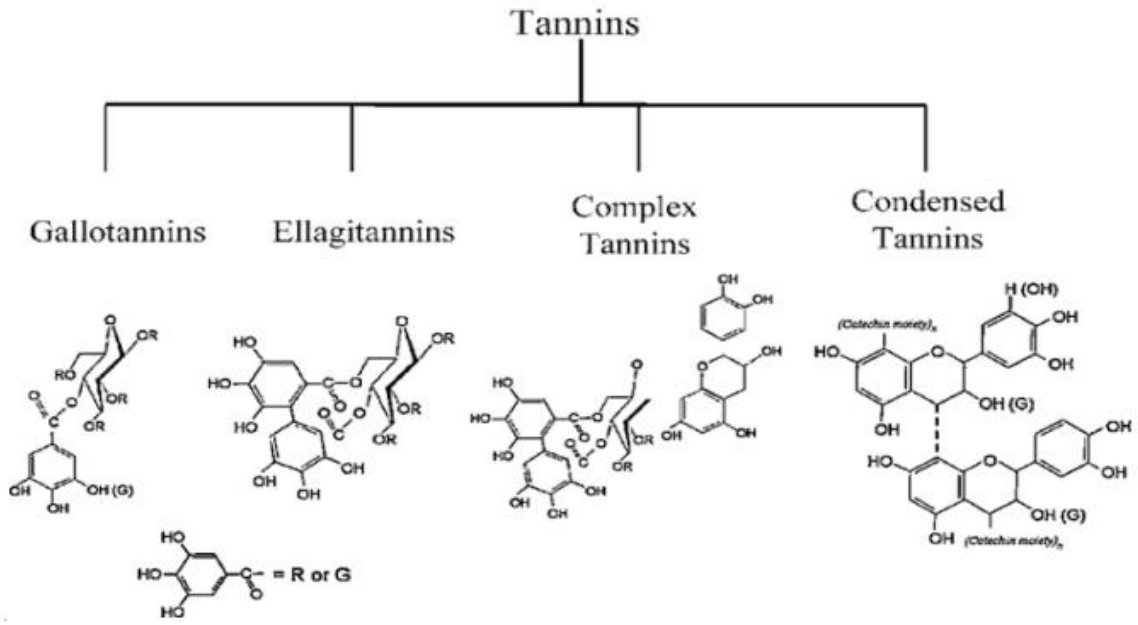


FIGURE 1: TYPE OF TANNIN

Source: Amonkan *et al.,* 2018.

Hydrolysable tannin found in *Asystasia gangentica*, has previously been demonstrated to effectively suppress the growth of colorectal tumors in an ethylene glycol rat model. Hydrolyzable tannins are potentially toxic to ruminant as pyrogallol, a hepatotoxin and nephrotoxin, is a product of HT degradation by ruminal microbes (Reed, 2020). Problems in the analysis of tannins are that sample processing and drying decrease extraction and reactivity, suitable standards are unavailable, and quantitative analytical methods are poorly correlated with enzyme inhibition, protein precipitation, and nutritional effects.

Tannin compounds are widely distributed in many plant species. They protect plants from predation (as insecticides) and may help regulate plant growth (Scalbert, 2022). The astringent taste of tannins can dry out the mouth after consuming raw fruit, red wine, or tea (Kadam *et al.*, 2020). Likewise, the breakdown or alteration of tannins over time plays an important role in determining the moment of harvest.

1.7.2 Flavonoids

Chemically, flavonoids have a general structure of a 15-carbon skeleton consisting of two benzene rings (A and B) and a heterocycle (C, the ring containing the complete oxygen) (Galeotti *et al.*, 2018). This carbon structure can be abbreviated as C6-C3-C6. According to the IUPAC (Souza *et al.*, 2021) nomenclature, they can be divided into:

• Flavonoids or bioflavonoids

• Isoflavones derived from the structure of 3-phenylchroman-4-one (3-phenyl-1,4-benzopyrone)

• Flavonoids derived from the structure of 4-phenylcoumarin (4-phenyl-1,2-benzopyrone)

Several flavonoids have shown renal protective effects against many nephrotoxic agents that frequently cause acute colorectal injury (ACI) or chronic colorectal disease (CCD), such as LPS, gentamycin, alcohol, nicotine, lead or cadmium. Many of them also exert renoprotective actions that may be of interest in diseases such as glomerulonephritis, diabetic nephropathy, and chemically-induced colorectal insufficiency. The three flavonoids mentioned above are all ketone-containing compounds, thus anthocyanins (flavonoids and flavonols) (Bate-Smith and Swan, 2022).

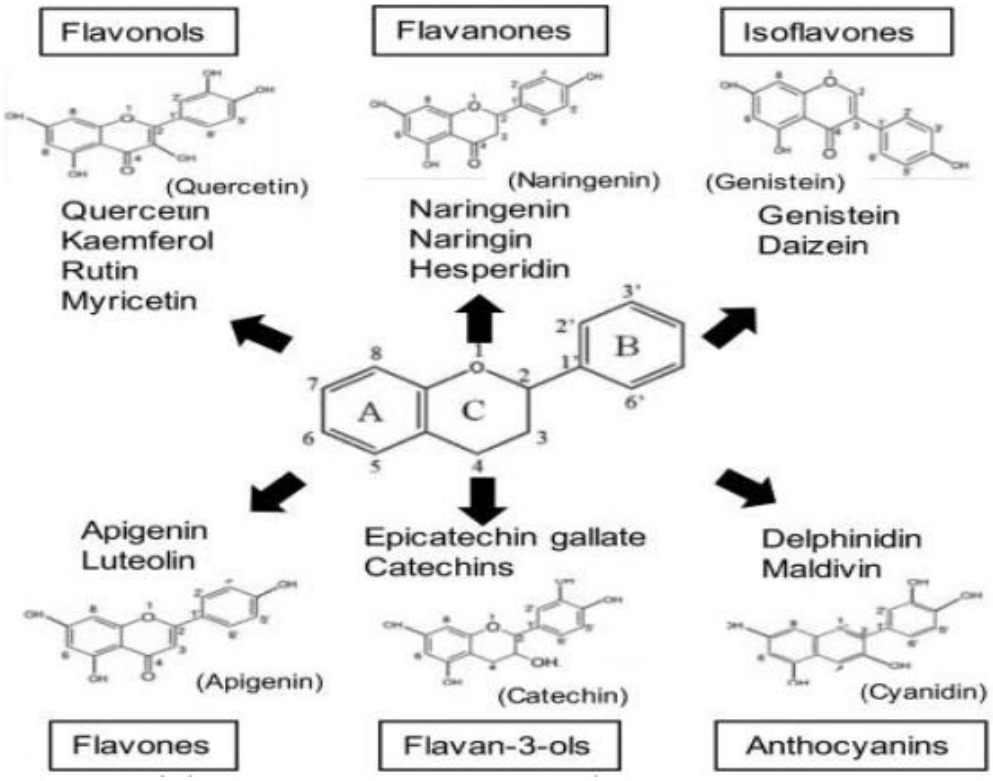
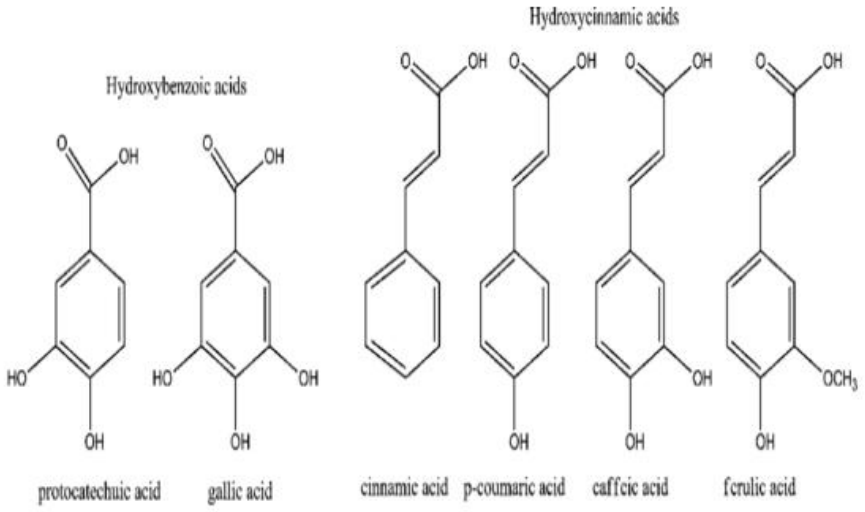


FIGURE 2: MOLECULAR STRUCTURE OF THE FLAVONE BACKBONE (2-PHENYL-1, 4-BENZOPYRONE)

Source: Amonkan *et al.,* 2018.

Phenolic acids have carboxylic acids in their chemical structure. Hydroxycinnamic and hydroxybenzoic acids are the mainstay of phenolic acids (Naczk *et al.*, 2021). Moreover, scientists point out that p-coumaric acid, caffeic acid, ferulic acid and sinapic acid are the main components of hydroxycinnamic acid (Anokwuru *et al.*, 2021; Koffi *et al.*, 2020).



Phenolic acid

FIGURE 4: PHENOLIC

Source: Amonkan *et al.,* 2018.

1.7.3 Proanthocyanidin

Proanthocyanidins are a class of polyphenols found in many plants, such as the banana tree. Chemically, they are oligomeric flavonoids. More complex polyphenols with similar polymeric structural units form tannins (Hatano *et al.*, 2022), (Merghem *et al.*, 2019).

Proanthocyanidins are considered to be non-toxic because they are not absorbed, but they are associated with lesions of the gut mucosa (Qa'Dan *et al.*, 2021). Proanthocyanidins are often associated with the consumption of cranberries, grape seeds, or red wine to prevent UTIs, but their effectiveness in preventing or treating UTIs has not been proven (Bogs *et al.*, 2022).

1.8 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) is a hybrid analytical technique that couples the separation capabilities of GC with the detection properties of MS to provide a higher efficiency of sample analyses. While GC can separate volatile components in a sample, MS helps fragment the components and identify them on the basis of their mass (Cheriyedath, 2017). GC-MS provides enhanced sample identification, higher sensitivity, an increased range of analyzable samples, and faster results, which enable a whole new range of applications for GC-MS in several areas (Cheriyedath, 2017).

FIGURE 5: EXAMPLE OF A GC-MS MACHINE

Source: Wang *et al.,* (2019).

1.8.1 Medicine

GC-MS is used in screening tests for the detection of several congenital metabolic diseases. It detects trace levels of compounds present in the urine of patients with genetic metabolic disorders. It can also detect the presence of oils in ointments, creams, and lotions. This tandem technique combines the separation capabilities of gas chromatography with the molecular identification and quantification capabilities of mass spectrometry, making it an invaluable tool for medical research, diagnostics, and drug development. In this write-up, we will explore the various applications of GC-MS.

1.8.2 Drug Analysis and Pharmacokinetics

GC-MS is extensively used to analyze pharmaceutical drugs, both for quality control and in pharmacokinetic studies (Smith *et al.*, 2017). It helps identify and quantify drugs and their metabolites in biological samples, aiding in understanding drug absorption, distribution, metabolism, and excretion (Wang *et al.,* 2019).

1.8.3 Clinical Toxicology

GC-MS plays a crucial role in toxicology by detecting and quantifying drugs, toxins, and their metabolites in blood, urine, and tissue samples (El-Masri *et al*., 2018). This aids in diagnosing poisoning cases and monitoring patients undergoing toxicological treatment.

1.8.4 Disease Biomarker Discovery

GC-MS is used to identify volatile organic compounds (VOCs) in bodily fluids or breath, which can serve as biomarkers for various diseases, including cancer and diabetes (Amal *et al.,* 2020). Early disease detection through VOC analysis is an active area of research.

1.8.5 Metabolomics

GC-MS is a cornerstone technology in metabolomics, allowing researchers to profile and quantify small molecules in biological samples. It aids in understanding metabolic pathways, biomarker discovery, and the impact of nutrition on health (Dunn *et al*., 2021).

1.8.6 Forensic Medicine

In forensic toxicology, GC-MS is employed to detect drugs, alcohol, and poisons in post-mortem samples, helping to determine the cause of death (Musshoff *et al*., 2017). It ensures accurate and reliable results in legal investigations.

1.8.7 Environmental Medicine

GC-MS is used to analyze environmental pollutants and toxins in air, water, and soil samples (Xiao *et al.*, 2019). This information is essential for assessing environmental health risks.

1.8.8 Pharmacogenomics

GC-MS can identify genetic variations affecting drug metabolism, enabling personalized medicine approaches (D'Andrea, 2016). Tailoring drug treatments based on an individual's genetic profile can enhance therapeutic outcomes.

1.8.9 Research on Metabolic Disorders

GC-MS is vital in studying metabolic disorders like phenylketonuria and maple syrup urine disease by analyzing metabolic byproducts in patient samples (Adam *et al.*, 2018). It aids in disease understanding and treatment development.

1.8.10 Environmental Monitoring

Monitoring environmental pollutants is a major application of GC-MS. It is widely used in the detection of dibenzofurans, dioxins, herbicides, sulfur, pesticides, phenols, and chlorophenols in air, soil, and water.

1.8.11 Food and Fragrance Analysis

Aromatic compounds such as fatty acids, esters, aldehydes, alcohols, and terpenes present in food and beverages can be easily analyzed using GC-MS. The technique can also be used to detect c spoilage or contamination of food. The analysis of a wide range of oils such as lavender oil, olive oil, spearmint oil, and essential oils, perfumes, fragrances, allergens, menthol, and syrups is also possible using GC-MS.

1.8.12 Pharmaceutical Applications

In the pharmaceutical industry, GC-MS is used in research and development, production, and quality control. It is used in identification of impurities in active pharmaceutical ingredients. In medicinal chemistry, GC-MS is used in the synthesis and characterization of compounds and in pharmaceutical biotechnology.

1.8.13 Forensic Applications

Using GC-MS, fire debris analysis can be performed as per the American Society for Testing Materials (ASTM) standards. GC-MS is widely used in forensic toxicology to identify poisons and steroids in biological specimens and in anti-doping labs to detect performance enhancing drugs such as anabolic steroids.

1.8.14 Biological Analysis

GC-MS can be used for the bioanalysis of body fluids to detect narcotics, barbiturates, alcohols, and drugs such as anticonvulsants, anesthetics, antihistamines, sedative hypnotics, and anti-epileptic drugs. It is also useful in detecting pollutants and metabolites in serum and in fatty acid profiling in microbes.

1.8.15 Chemical Warfare

Explosive detection systems in public places use GC-MS technique for the analysis and detection of chemical warfare agents.

1.8.16 Geochemical Research

Due to its structurally significant mass spectral peaks, extended range of analyzable low volatility samples, enhanced molecular ions, and valuable isotope ratio information, GC-MS is a powerful tool for geochemical applications. GC-MS has been used to analyze the atmosphere of Venus and has also been used by the Viking program on Mars. Additionally, a chiral GC-MS system has been used by the Rosetta mission to analyze the materials in the comet 67P/Churyumov-Gerasimenko.

1.8.17 Industrial Applications

GC-MS is ideal for the analysis of inorganic gases and aromatic solvents, detection of impurities and allergens in cosmetics. It is also used in the synthesis of cellulose acetate, polyethylene, polyvinyl, and synthetic fibers. Therefore we may conclude that automated GC-MS systems offer rapid and reproducible results in several applications.

1.9 Justification of Study

There has been an exponential growth in plant-based products during the last three-decade for the treatment of various ailments. According to literature, plants are the important source of secondary metabolite and constitute about 25% of all prescribed drugs globally. The family Acanthaceae comprises medicinal plants and phytoconstituents with an extensive range of biological activities. From this family, the genus *Asystasia* comprising of nearby 70 species, mainly scattered in sub-Saharan tropical Africa, Arabia,and tropical Asia4where; they were used up as vegetable (Banoth and Thatikonda, 2020).

One such species is Asystasia gangetica, also known as Chinese violet, coromandel, creeping foxglove, *Ganges primrose*, etc. In India, it is also known as kaligharani (Gujrati), lavangavalli, lavanavalli (Kannada), Valli-upu-dal (Malayalam), lavanavalli (Marathi), Miti-kirai (Tamil), etc. *A. gangetica* is a beautiful ornamental herb, which is rapidly growing, perennial, scattering groundcover, and is grown from 0.30-0.60 m in height. Stems (slightly hairy) develop adventitious roots easily at the nodes when it comes in contact with wet soil. Greenish leaves (up to 8cm long and 4 cm wide) were elliptical or cordate in shape possesses ovate outline occurring in opposite pairs (Ghabru and Rana, 2019).

A. gangetica is a beautiful ornamental herb, which is rapidly growing, perennial, scattering groundcover, and is grown from 0.30-0.60 m in height. Stems (slightly hairy) develop adventitious roots easily at the nodes when it comes in contact with wet soil. Greenish leaves (up to 8cm long and 4 cm wide) were elliptical or cordate in shape possesses ovate outline occurring in opposite pairs. The flowers (up to 3.5 cm long and 3 cm wide) are white-cream coloured with

tessellated purple markings. Fruits are elongated, club-shaped, contain 4 seeds splitting from tip to base, and is of green coloured, which is converted to brown on maturity. Bone coloured flat seeds are 0.5 cm long and 0.1 cm wide. It is hard and young plant that requires protection in areas of heavy frost and can grow wildly in tropical areas (Okudu, 2018).

This study is crucial as it scientifically examines *Asystasia gangetica'*s chemical composition, laying the groundwork for targeted anticancer and antifungal applications. It bridges knowledge gaps and holds promise for developing novel drugs with enhanced efficacy against these health challenges.

1.10 Aim and Objectives of Study

This research endeavors to comprehensively analyze the chemical constituents of *A. gangetica* and identify its bioactive compounds. By investigating its potential anticancer and antifungal properties, the study aims to delineate specific mechanisms of action and establish a scientific foundation for the development of pharmaceutical interventions harnessing the therapeutic potential of *A. gangetica.*

CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection, Identification and Preparation

AG plant was collected in the month of September in Ugbowo, Benin City with latitude of 6° 21' 1''N and longitude 5° 36' 36''E. It was identified in the Department of Plant Biology and Biotechnology by H.A. Akinnibosun (Prof) with herbarium number UBH-460 and the sample specimen was deposited in the Departmental herbarium.

The leaves were detached, air-dried undershade for two weeks and then pulverized by electric milling machine to powder. One hundred gram of the powder was macerated with 400 mL of methanol (99 %) for 72 hours. This was concentrated in vacuum at 50 °C and extract kept at 4 °C until used.

2.2 Phytochemical Screening of the Powdered Leaves of *Asystasia gangetica*

Phytochemicals in the leaves of AG were determined by methods described by Sofowora and Trease and Evans. They include alkaloids, tannins, steroids, saponins, flavonoids, glycosides and triterpenoids (Sofowora, 1993; Evans & Trease, 2009).

Detection of Alkaloids: The powdered vines (0.5 g) was dissolved in dilute hydrochloric acid, filtered and tested for the presence of alkaloids.

Mayers test: To the filtrate (1 mL) in a test tube, 4 drops of Mayers reagent was added. A yellow cream precipitate formation indicates the presence of alkaloids.

Wagner's test: Wagner's reagent (4 drops) was added to 1 mL of the filtrate, if a brown-reddish brown formation is observed, and it indicates the presence of alkaloids.

Detection of Flavonoids: The powdered vines (0.5 g) was dissolved in distilled water , boiled for 5 min., filtered and tested for the presence of flavonoids and saponins.

Lead acetate test: A few drops of lead acetate solution was added to the filtrate (1 mL). A yellow-colour precipitate indicates the presence of flavonoids.

Detection of Saponins: Filtrate (1 mL) was mixed vigorously with 1 mL of distilled water. The formation of frothing indicates the presence of saponins.

Detection of Tannins: Powdered vines (0.5 g) are mixed with a few millilitres of distilled water and heated on a water bath, then the mixture was filtered. Ferric chloride was added to the filtrate. The dark green colour indicates the presence of tannins.

Detection of Steroids: A few drops of acetic anhydride are added to the filtrate (methanolic) and the formation of violet to blue to green in some samples indicates the presence of steroids.

Detection of Terpenoids: Salkowski's Test: Powdered vines (10 mg) was mixed with 2 mL chloroform and 3 mL concentrated sulfuric acid added carefully to form a layer. A reddish-brown colour indicates the presence of terpenoids.

Detection of glycosides: Bontrager's Test: About 5 mg of the powdered vines was boiled with 10 % HCl for a few minutes on a water bath, filtered and allowed to cool. An equal volume of chloroform is added to the filtrate. A few drops of 10 % ammonia are added to the mixture and heated. The formation of pink colour indicates the presence of glycosides.

2.3 Gas Chromatography-Mass Spectrometric analysis of the methanol extract of *Asystasia gangetica*

GC-MS QP2010 SE model (Schmadzu, Japan) was utilized to analyze methanol extract of AG. Phases in the equipment include phenymethyllsiloxane (stationary pahse) and helium (mobile phase). Column (DB 5MS) of measurements (0.25 mm x 30 mm x 0.10 μm) and sample size of 1

μm was injected in the split mode. The operating conditions: inlet temperature 250 °C, oven temperature 60 °C for 3.4 min which was remped for 12 °C/min to 240 °C. Rate of increase was maintained until temperature changed to 290 °C and kept for 2 min.

Electron impact mode with ionization energy of 70 eV was used for the mass spectrometer and scanned within 45-700 dalton. Chemstation software was used to acquire data and compounds were identified by comparing the fragmentation patterns produced by each compound with data from the National Institute of Standard Technology (Odion *et al*., 2020).

2.3 High Pressure Liquid Chromatography Analysis of methanol extract of AG

Analysis (HPLC) of the methanol extract of AG was done using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. C-12 normal phase column (Phenomenex, Gemini 5 μ, 200 mm length × 4.8 mm internal diameter) was utilized for the analysis. Mobile phase consisting of solvent A and B, Solvent A is made of acetic acid-acidified deionized water at pH 2.8, while solvent B is acetonitrile at 0.8 mL/min flow rate. Solvent B (5 %) was used to equilibrate the column for 20 min post injection of each sample. Temperature of the column was set at 38 °C, volume of injection was 20 μL and wavelength set at 280 nm, Compounds were identified and quantified by comparison of the retention times and peak areas with standard (pure) compounds by plotting calibration plot of external standards.

Gradient elution: 0-5 min, 5-9 % solvent B; 5-15 min, 9 % solvent B; 15-22 min, 9-11 % solvent B; 22-38 min, 11-18% solvent B; 38-43 min, 18-23 % solvent B; 43-44 min 23-90 % solvent B; 44-45 min, 90-80 %, solvent B; 45-55 min (Kaisoon *et al*., 2011).

CHAPTER THREE

3.0 RESULTS

Preliminary phytochemical screening show the presence of alkaloids, steroids, saponins, tannins, triterpenoids, flavonoids and glycosides (Table 3.1) in the leaves of AG.

Table 3.1:Phytoconstituents of the powdered leaf of *Asystasia gangetica*

Phytochemical	Inference
Alkaloid	+ve
Saponin	+ve
Steroid	+ve
Tannin	+ve
Terpenoid	+ve
Flavonoids	+ve
glycoside	+ve

Twenty-eight compounds were identified from the methanol leaves extract of AG (Table 3.2), most which are esters, fatty acids, alcohols and imidazole derivatives.

Table 3.2:Phytoconstituents of methanol leaves extract of *Asystasia gangetica*

S/N	Compounds	RT (min)	% Area	MF	MW
1	Ribitol	2.257	4.23	$C_5H_{12}O_5$	152.15
2	Tetradecanoic acid, propyl ester	2.454	2.17	$C_{17}H_{34}O_2$	270.45
3	cis-9-Hexadecenoic acid	2.623	1.05	$C_{16}H_{30}O_2$	254.41
4	Ribitol, 1,3:2,4-di-O-benzylidene-d-threitol	2.764	1.08	$C_{18}H_{18}O_4$	298.30
5	5-Octadecene, (E)-	2.961	3.48	$C_{18}H_{36}$	252.50
6	Eicosanoic acid	3.299	0.88	$C_{20}H_{40}O_2$	312.54
7	1-Thia-2-azacyclopenta[a]anthracene-3,6,11-trione	3.384	2.92	$C_{15}H_7NO_3S$	281.28
8	Tricosane, 2-methyl-	3.637	0.88	$C_{24}H_{50}$	338.65
9	Octadecanoic acid	3.722	2.70	$C_{18}H_{36}O_2$	284.48
10	Methyl 21-methyl-hexacosanoate	3.975	0.96	$C_{28}H_{56}O_2$	424.70
11	15-Hydroxypentadecanoic acid	4.651	2.63	$C_{15}H_{30}O_3$	258.40
12	Octadecane, 1-(ethenyloxy)-	4.933	8.77	$C_{20}H_{40}O$	296.53
13	Octadecane, 6-methyl-	5.271	1.65	$C_{19}H_{40}$	268.5
14	Tetraethyl 1,1'-(1,8-naphthylene)bis(1,2,3-triazole-4,5-dicarboxylate)	5.891	0.59	$C_{26}H_{26}N_6N_8$	550.5
15	15-Hydroxypentadecanoic acid	6.060	11.94	$C_{15}H_{30}O_3$	258.40
16	n-Decanoic acid	6.792	3.30	$C_{10}H_{20}O_2$	172.26
17	Dodecanoic acid, methyl ester	7.017	2.46	$C_{14}H_{28}O_2$	228.37

18	Z-(13,14-Epoxy)tetradec-11-en-1-olacetate	7.609	8.72	$C_{16}H_{28}O_3$	268.39
19	2,3-Dihydroxypropyl elaidate	9.130	1.85	$C_{21}H_{40}O_4$	356.5
20	17-Pentatriacontene	10.060	0.51	$C_{35}H_{70}$	490.90
21	Oleic Acid	11.158	0.69	$C_{18}H_{34}O_2$	282.5
22	D-erythro-Pentose, 2-deoxy-	11.581	1.12	$C_5H_{10}O_4$	134.13
23	Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranoside	12.285	0.53	$C_{11}H_{22}O_6$	250.00
24	13-Docosenoic acid, methyl ester	13.102	1.64	$C_{23}H_{44}O_2$	352.60
25	cis-Vaccenic acid	13.750	10.20	$C_{18}H_{34}O_2$	282.46
26	Cycloheptano[d]imidazolidine, 1,3-dihydroxy-2-methyl-	14.088	9.18	$C_9H_{18}N_2O_2$	186.25
27	7-Octenoic acid	17.750	3.71	$C_{10}H_{18}O_2$	170.25
28	Trehalose	17.835	4.81	$C_{12}H_{22}O_{11}$	342.30

Key: RT=Retention Time, % Area=Percentage Area, MF=Molecular Formula, MW=Molecular Weight

HPLC analysis revealed nineteen compounds with Spartein (7.64 %), cyangenic glycoside (10.51 %), aphylidine(10.33 %) and flavone (7.51 %) as the prominent compounds (Table 3.3). The compounds identified can be grouped into flavonoids (kaempferol, naringenin, proanthocyanidine (oligomeric flavonoid), anthocyanin, flavone, flavonone), alkaloids (spartein, ribalinidine, ammodendrine, epehidrine, aphyllidine, dihydrocytisine), steroids, tannins, glycoside (cyanogenic) and saponins.

Table 3.3: Phytoconstituents from the HPLC analysis of the methanol leaf extract of *Asystasia gangetica.*

S/N	Compound	Retention Time (min)	Percentage Area	Concentration (µg/ml)
1	Kaempferol	0.226	1.29	1.8214
2	Steroids	2.223	5.56	4.6952
3	Proanthocyanidine	3.950	6.69	13.9885
4	Anthocyanin	6.893	3.68	5.7657
5	Naringenin	10.593	3.55	5.5688
6	Dihydrocytisine	13.300	4.03	6.3206
7	Cyanogenic glycoside	15.783	10.51	8.9749
8	Aphylidine	19.573	10.33	0.8215
9	Ammodendrine	22.293	3.90	2.3273
10	Tannin	26.000	5.58	3.6986
11	Flavonone	28.566	4.75	9.9310
12	Cardiac glycoside	29.490	3.65	5.5250
13	Flavone	34.176	7.51	11.3604
14	Ribalinide	37.260	5.38	8.1357
15	Spartein	38.326	7.64	11.5595
16	Phytate	39.590	3.42	5.1781
17	Oxalate	40.930	2.59	1.1912

18	Epehidrine	42.090	4.70	7.0800
19	Sapogenin	42.943	5.26	7.9606

CHAPTER FOUR

4.1 DISCUSSION

Phytochemical Screening

Study carry out on leaves sample of AG collected from Obio/Akpor in River state showed the presence of tannins, cyanogenic glycosides and saponins (Chukwu & Chukwu, 2019). This is in agreement with our study even though the samples were collected in different locations. Location, altitude of the area, seasonal variation and exposure to pollution are important determinant in phytochemicals produced in a plant (Gololo, 2019). Similarly, flavonoids, alkaloids, glycosides, saponins and tannins have been reported in the flower of AG (Sama *et al*., 2013), implying that different parts of the plant could produce similar classes of compound, with similar pharmacological activity. Related phytochemicals have been reported in *Asystasia variabilis* (Wijerathna *et al*., 2016), indicating that the phytochemicals are not specific to AG but also seen in other species of the same genus.

GC-MS analysis

This method of analysis is use in the determination of the volatile contents of AG. Previous analysis of *Asystasia* gangetica revealed ten compounds from the ethanol extract of the whole plant, out of which 2,6,10-Dodecartrien-1-ol and 9-hexylheptadecane was identified (Komalavalli *et al*., 2014). Also quercetin, ungeremine, lucenin, isoquinoline and cervnomycin were identified from the root extract of AG (Tamilselvan *et al.*, 2014) 2,3-Dihydroxypropyl elaidate initially identified in Tiger Milk mushroom which was shown to have significantly reduce IgE in serum and IL-13, IL-4 and IL-5 in bronchioalveolar lavage fluid (Jonathan *et al*., 2016).

HPLC analysis

Kaempferol, isorhamnetin, quercetin and luteolin have previously been reported in the whole plant by Gopal and co-workers (Gopal *et al*., 2013). Kaempferol is a 3,4',5,7-tetrahydroflavone, a natural flavonol, that have been identified in several plants. Possesses antioxidant, anti-inflammatory, neuroprotective, cardiovascular, chemopreventive and antimicrobial potentials (Archoo *et al*., 2022; Lee *et al*., 2014). Exert it chemopreventive action by blocking DNA damage at early stage (initiation step) or through arrest or reversal of the process at the progression and promotion steps (Kelloff *et al*., 1999). Anthocyanins are water soluble vacuolar pigment, produced from the phenylpropanoid pathway. They produce characteristic colours (red or blue) in vegetables, like other polyphenols they possess the ability to scavenge for reactive oxygen and nitrogen species (Mattioli *et al*., 2020). Naringenin is a 2,3-dhydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one. It is a flavanone that is derive from narirutin hydrolysis, apart from its ability to scavenge for free radicals, it modulate immune response potential (Salehi *et al.*, 2019). Proanthocyanidine oligomeric flavonoid, derived from the condensation of two flavan-3-ol subunits by one single or double bond. In plants, they act as biochemical defense against external aggressors, making it effective against fungi (Mannino *et al*., 2021).

These flavonoids are important to the plant either by impacting colour, flavour or fragrance. In human, their could alter vital biochemical pathways in the body, thus improving pathological conditions. However, they could act as preventive molecules or cause reversal of a debilitating condition.

Sparteine is a quinolizidine alkaloid cause slight analgesia, reduce motility and act as anticonvulsant against acute seizure and status epilepticus (Villalpando-Vargas & Medina-Ceja, 2016). Other effects associated with sparteine include reduction in cardiac conductivity, respiratory arrest, circulatory collapse and stimulation of uterine motility (Aaronson, 2016). Ribalinidine is a tertiary alkaloid with 4-quinolone framework have shown radical scavenging potential (Rhamani & Sukari, 2010). Ammodendrine is a piperidine alkaloid known as 1-[5-[(2R)-piperidin-2-yl]-3,4-dihydro-2H-pyridin-1-yl]ethanone with acute murine toxicity (Lee *et al.*, 2005). Ephedrine is a central nervous system stimulant, use to treat narcolepsy, asthma and obesity (ASHSP, 2017). Worthy of note, is that the ethno-medicinal usage of the plant could be due to the synergistic or additive effects of the individual compounds.

CHAPTER FIVE

5.1 CONCLUSION

Asystasia gangetica powdered leaves contain phytochemicals, which were identified based on their volatility and the use of standard analytical methods. These phytochemicals contains invivo and invitro biological activities. The study thus validate some of the ethnomedicinal usage of *Asystasia gangetica.*

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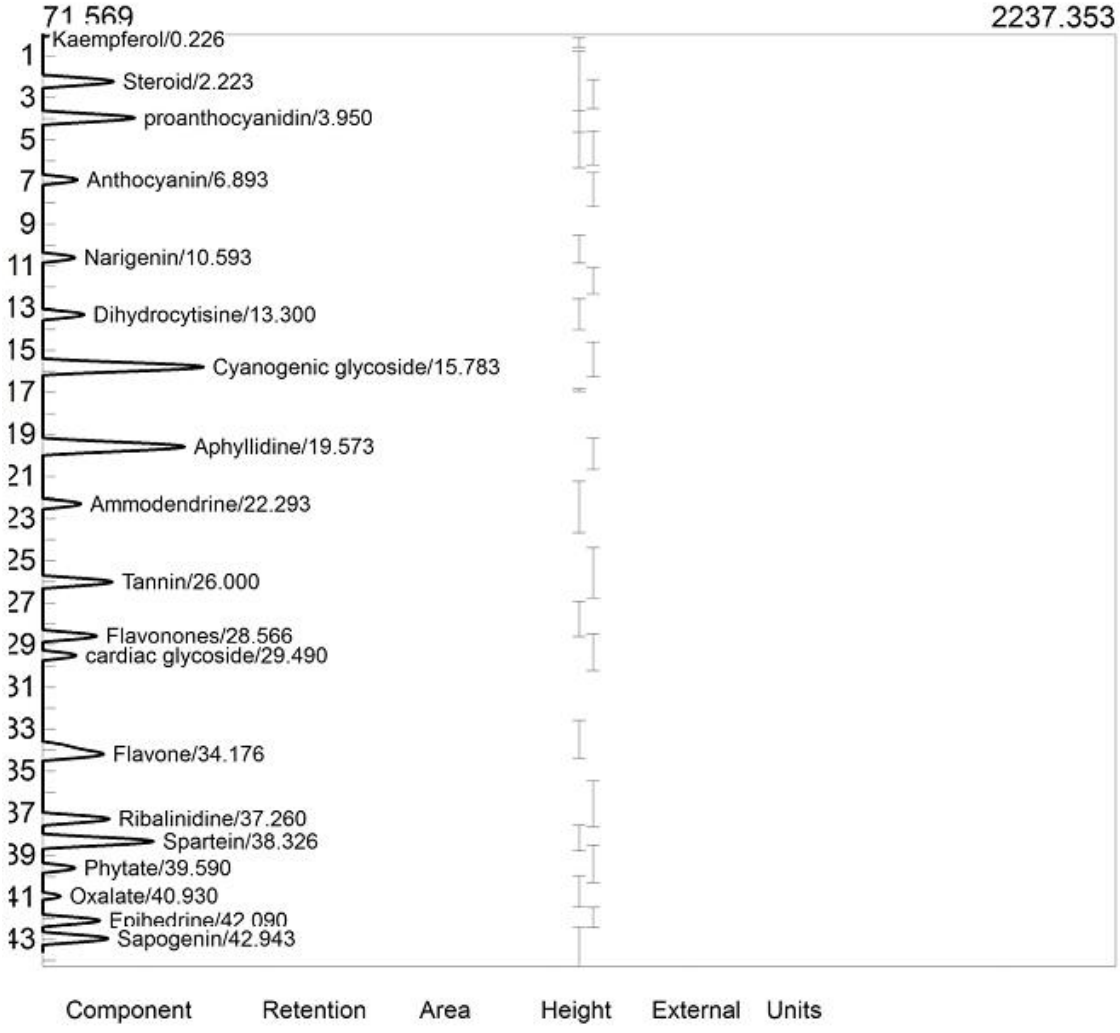

Phytochemical Analysis of the Methanolic Extract of *Asystasia gangetica* leaves

Kaempferol	0.226	1567.7301	71.377	1.8214	ug/ml
Steroid	2.223	6781.4592	212.138	4.6952	ug/ml
proanthocyanidin	3.950	8155.3112	255.541	13.9885	ug/ml
Anthocyanin	6.893	4481.9072	140.323	5.7657	ug/ml
Narigenin	10.593	4332.5197	135.220	5.5688	ug/ml
Dihydrocytisine	13.300	4913.1862	154.031	6.3206	ug/ml
Cyanogenic glycoside	15.783	12807.2396	395.766	8.9749	ug/ml
Aphyllidine	19.573	12590.5678	358.644	0.8215	ug/ml
Ammodendrine	22.293	4750.4508	148.987	2.3273	ug/ml
Tannin	26.000	6794.9023	212.169	3.6986	ug/ml
Flavonones	28.566	5789.7791	180.678	9.9310	ug/ml
cardiac glycoside	29.490	4450.4074	139.936	5.5250	ug/ml
Flavone	34.176	9150.8176	195.436	11.3604	ug/ml
Ribalinidine	37.260	6555.3447	204.731	8.1357	ug/ml
Spartein	38.326	9314.0338	293.230	11.5595	ug/ml
Phytate	39.590	4172.2344	135.004	5.1781	ug/ml
Oxalate	40.930	3158.2206	104.042	1.1912	ug/ml
Epihedrine	42.090	5702.9629	183.801	7.0800	ug/ml
Sapogenin	42.943	6412.2832	203.007	7.9606	ug/ml
		121881.3578		121.9041	

Phytochemical results from the Methanolic Extract of *Asystasia gangetica* leaves

Figure 1: ***Asystasia gangetica*** **leaves**