PHYTOCHEMICAL COMPOSITION OF *Emilia praetermissa* Milne-redh Leaf.

BY

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DEPARTMENT OF BIOCHEMISTRY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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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 BACHELORS OF SCIENCE DEGREE (B. Sc. HONS) IN BIOCHEMISTRY

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CERTIFICATION

This is to certify that this report was carried out by **EKECHUKWU ONYEDIKACHI PRINCE** with matriculation number **LSC2103731** under the supervision of PROF. E. CHUKWU ONYENEKE and has been read and approved as meeting the requirement of Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This work is dedicated to almighty God for his grace and mercy on me throughout this study period, and to my family and well-wishers who supported me every step of the way.

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I am deeply grateful to the Almighty for His guidance, wisdom, and protection throughout my research journey.

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ABSTRACT

This study comprehensively characterized the phytochemical profile of *Emilia praetermissa* Milne-Redh leaves, a plant traditionally used in West Africa for its medicinal properties. Qualitative phytochemical screening revealed the presence of tannins, cardiac glycosides, sterols, terpenoids, and phenols. Quantitative analysis showed that cardiac glycosides had the highest concentration (0.26 ± 0.00), followed by terpenoids (0.21 ± 0.00) and phenols (0.12 ± 0.00), while sterols (0.05 ± 0.00) and tannins (0.11 ± 0.00) were present in relatively lower amounts. The identified phytochemicals are known to possess various biological activities, including anticancer, anti-inflammatory, antioxidant, and antimicrobial properties, which may contribute to the plant's medicinal uses. The findings of this study provide valuable insights into the phytochemical composition of *Emilia praetermissa* and support its potential as a source of essential nutrients and therapeutic agents for nutraceutical and pharmaceutical applications. Further research is needed to isolate and characterize the specific bioactive compounds responsible for the plant's medicinal properties and to explore their potential uses in the development of new drugs and nutraceuticals.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Traditionally, medicinal plants continue to play a vital role in meeting the healthcare needs of many cultures worldwide, and notably, about 25% of modern medicines have their roots in plants used by indigenous communities for generations, showcasing the enduring influence of traditional knowledge on contemporary medicine (Adotey, *et al.*, 2012).

Emilia praetermissa , commonly called Tassel flower or Wild lettuce, is a herbaceous plant native to West Africa (Maunda, *et al.*, 2009). It was originally described from Sierra Leone and Nigeria, and was subsequently discovered in other parts of West Africa including Ghana, Liberia, Cameroon, Côte d'Ivoire and Guinea (Sandoval, 2020). Other names of *Emilia praetermissa* Milne-Redh. includes: Tassel flower (United Kingdom); Nti-ene (Nigeria, Igbo); Odundun-odo (Nigeria, Yoruba); Ndogbo (Sierra Leone, Mende); Konami-abien (Mali, Manding-Mandinka); Kluapo (Côte d'Ivoire, Kru); Dein blih su (Liberia , Mano) (Ebhohon *et al.*,2025; and Olorode,1973). *Emilia praetermissa* can be found growing as a weed in disturbed places, lawns, gardens, swamps, secondary forests, forest gaps and margins, agricultural lands and along roadsides (Mapaya and Cron, 2016). It is an erect annual plant usually growing up to 1 metre tall but occasionally 1.5 metres (Sandoval, 2020).

Emilia praetermissa is a herb that belongs to the family of *Asteracea* and is well known for its medicinal properties and well utilized in West Africa. It is used traditionally to treat several diseases including gastric ulcers, and the ethanol extract has shown cytoprotective anti-ulcer effects (Lebeau, *et al.,* 2021). In Nigeria, the plant is considered a children's medicine, used as poultice to heal the navel of newborn, the leaf sap is also rubbed over patients with epilepsy and vertigo (Okafor, 2002). Among the Igbo people of Southeastern Nigeria, the leaves of *Emilia praetermissa* are pounded up with *Piper guineense* (uziza) and are useful in managing sour throat and backache (Okafor, 2002).

Emilia praetermissa is used as vegetable in most parts of West Africa and Central America, besides its medicinal uses (Anaka *et al*., 2013). *Emilia praetermissa* spreads by seed propagation. The seeds are mostly dispersed by wind, but they can be secondarily dispersed by water, as a contaminant in soil and seed crops and adhered to vehicles and agricultural machinery (Sandoval, 2020).

Emilia praetermissa thrives in areas with moist, well-drained soil and full sunlight, typically found at elevations up to 1,700 metres (Smith, 2021).

1.1.1 Background of study

Medicinal plants have therapeutic properties which rely on bioactive compounds known as phytochemicals. Phytochemicals are secondary plant metabolites naturally occurring in plants and confers a protective function to plants against bacteria, fungi, viruses, and damage by free radicals, insects and herbivores that feed on them (Yemisi, 2021).

Researchers have examined medicinal plants to discover secondary metabolites with healing properties, including compounds like alkaloids, flavonoids, saponins, tannins, and terpenes (Njoya *et al*., 2018). Plant derived substances have become of great interest due to their versatile applications (Yemisi, 2021). *Emilia praetermissa* is one of these plants

Tropical and subtropical regions of Africa boast an estimated 40,000 to 45,000 plant species, many with potential applications, particularly in medicine (Seukep *et al*., 2013). These diverse plant species offer promising opportunities for various uses (Van-Wyk, 2008). Of these diverse species, approximately 5,000 are currently used for medicinal purposes (Van-Wyk, 2008). The rich botanical diversity provides a valuable source for natural health remedies, scientific research and sustainable development, as many of these plants contain unique compounds that can help treat various health conditions (Van-Wyk, 2008). In Nigeria and most West African countries, *Emilia praetermissa* Milne- Redh., is said to have medicinal properties that are effective in the management of ailments (Nwaefulu *et al*., 2016).

Emilia praetermissa Milne- Redh. of the family *Asteraceae* is a medicinal plant of high importance. In traditional African medicine, it is valued for its healing properties. The plant's leaves and roots are used to make teas and decoctions to treat various ailments such as gastric ulcer and hyperlipidemia, and the leaves are also consumed as a cooked vegetable (Sandoval, 2020). The crushed green leaves of the plant are applied externally to treat conditions like sores and sinusitis, and are also used as a poultice to aid in wound healing (Ebhohon, *et al.*, 2025).

The leaves of *Emilia praetermissa* are particularly valued for their ability to lower lipid levels and are used as cardioprotective agent (Michel *et al*., 2020). They are believed to contain

bioactive compounds that help reduce blood pressure, and in Nigeria, people often chew the fresh leaves for this purpose (Ebhohon, *et al.*, 2025).

The therapeutic properties of *Emilia praetermissa* are largely attributed to its rich phytochemical profile. The plant parts contain various secondary metabolites like tannins, saponins, steroids, terpenoids and alkaloids (Nwankwo *et al.*, 2025). The presence of these secondary metabolites suggests potential therapeutic benefits (Mohanta *et al*., 2023). Tannins have astringent properties and can protect against microbial infections. Saponins possess antimicrobial and anti-inflammatory properties, while steroids are known for their anti-inflammatory and analgesic effects, reducing sugars are essential for metabolic activities, and terpenoids are known for their broad spectrum of biological activities, including antimicrobial and anti-inflammatory effects (Nwankwo *et al.*, 2025).

One of the most significant discoveries about *Emilia praetermissa* is its potent antimicrobial activity (Afolayan *et al.*, 2017). Studies has shown that extracts from the plant's leaves—whether in methanol, hot water, or cold water—can effectively combat a range of pathogens. For instance, methanol extracts have demonstrated powerful inhibition against *Staphylococcus aureus*, a bacteria known for causing infections ranging from skin diseases to pneumonia, and hot water extracts in addition, have shown effectiveness against *Streptococcus pneumoniae*, which is responsible for respiratory infections like bronchitis and pneumonia (Anselem, 2024).

Researchers have been studying *Emilia praetermissa* to validate its traditional medicinal uses, and several reports have shed light on the pharmacological properties of the various parts of the plant including anti-inflammatory activity (Nwankwo *et al.*, 2025), anti-microbial activity

(Anselem, 2024), antioxidant activity and analgesic activity (Smith, 2021), including antidiabetic activities and its potency in improving hyperlipidemic conditions (Mahanta, *et al.*,2023).

1.1.2 Justification of study

Plants like vegetables, packed with fiber, minerals, and vitamins, contain compounds called phytochemicals that can effectively treat various health conditions (Baghbanna *et al*., 2014). These naturally occurring substances are generally considered safe because they come from plants , but , however, some studies suggest that certain commonly consumed plants may carry risks, including the potential to cause cancer (Bode and Dong, 2015).

In contrast to primary compounds (lipids, carbohydrates, proteins, etc.), the secondary metabolites are characterized because they are store in low concentration in plant tissues and play an important role in the survival of the plant in the environment (Bourgaud *et al*., 2001). Secondary metabolites are a rich source of bioactive compounds that are biodegradable into nontoxic products (Ebadollahi, 2013). Phenolics, terpenes and alkaloids are considered the main groups of secondary metabolites and they are classified according to their biosynthetic pathways (Bernabé-Antonio *et al.*, 2014).

1.1.3 Aim and specific objective of the study

1.1.3.1 Aim of the study

To characterize comprehensively the phytochemical profile of *Emilia praetermissa* Milne-redh. leaves, evaluating their potential as a source of essential nutrients for nutraceutical and therapeutic applications.

1.1.3.2 Specific Objectives of Study

To identify and quantify the various phytochemicals present in the leaves of *Emilia praetermissa*, and to evaluate their concentrations and potential therapeutic activities.

1.2 Literature Review

1.2.1 Plant description

Emilia praetermissa Milne-Redh , a specie in the genus *Emilia* is an erect herb to 1 m high, of open ground in the forest zone from Sierra Leone to southern Nigeria, and a common plant in brackish Avicenna swamp. It belongs to the family of *Asteraceae* (Chung *et al.*, 2009).

The genus *Emilia* consists of about hundred species, predominantly found in tropical and subtropical regions, with East Africa having the highest species diversity (Beentje and Lansdown, 2018). Specifically, *Emilia praetermissa* was first identified in Sierra Leone and Nigeria (Ifediora *et al.*,2024) and later discovered in other West African countries, including Cameroon, Côte d'Ivoire, Ghana, Guinea and Liberia (Ifediora *et al.*,2024).

Emilia praetermissa is an erect annual plant, usually growing up to 1 metre tall but occasionally to 1.5 metres. The leaf margins strongly sinuate dentate, florets white , pale yellow or pale orange ; corollas distinctly exceeding involucre (Ebhohon *et al.,* 2025).

The leaves of *Emilia praetermissa* vary in shape and size along the stem: the lower leaves have petioles 1.5-3 cm and are broadly ovate , 4-6 x 4.5-6 cm , with dentate margins, while the upper leaves are sessile and smaller. The plant produces clusters of flower heads (capitula) with cream to orange-colored florets 8 mm, that exceed the involucres. The achenes , 3 mm , are small and hairy, with a pappus (modified calyx) that's about 7 mm long (Sandoval, 2020).

This plant has erect or ascending stems that can be simple or branched, with varying hairiness, and produces solitary or clustered flower heads (capitula) with yellowish, orange, or cream-colored florets that have purple or orange-tinged lobes (Beentje and Lansdown, 2018). The anthers are dark orange, and the style branches are orange and recurved, the seeds (achenes) are small, 5-ribbed, and hairy, with a finely barbellulate pappus, and has a chromosome number of 2n = 20 (Chung, *et al.*,2009).

Emilia praetermissa has daisy-like flower heads that are white-pink in color. Its seeds are small, black, and oval-shaped, while the seedlings feature thin, green stems with small, oval-shaped leaves (Edgar, 1951). Plate 1.1 shows a picture of Emilia praetermissa Milne-redh plant.

Plate 1.1 Emilia praetermissa Milne-redh. plant

1.2.2 Origin and distribution

Emilia praetermissa originated from West Africa , specifically first identified in Sierra Leone and Nigeria (Ifediora *et al.*,2024). It has since been found in other West African countries, including Cameroon, Côte d'Ivoire, Ghana, Guinea, and Liberia (Ifediora *et al.*,2024). Additionally, it has been naturalized in several locations outside its native range, including Taiwan, St. Lucia, St. Vincent, and Martinique (Chung, *et al.*,2009).

Emilia praetermissa have also been found native to some Central African countries including Gabon, D.R. Congo, and Equatorial Guinea , where it is found inhabiting open or disturbed habitats (Lisowski, 1991).

1.2.3 Habitat and cultivation

Emilia praetermissa thrives in open forest grounds and is also commonly found in brackish mangrove swamps. It grows in various environments, including disturbed areas, lawns, gardens, swamps, secondary forests, agricultural lands, and along roadsides, often behaving like a weed (Sandoval, 2020). It also grows in areas like dry grasslands, disturbed habitats, and open woodlands (Edgar, 1951).

Emilia praetermissa, an annual plant, grows best in conditions with full sun and well-drained soil (Rao, 2017). For successful propagation, seeds are sown in a flat and lightly covered with soil , and after seeding, the seedlings are thinned out to approximately 6 inches apart to allow for proper growth and well watered for successful establishment (Edgar, 1951). The fertilized florets produce single-seeded fruits within two weeks (Kumar *et al*., 2015). When mature and

dry, the achenes disperse along with the connected phyllaries (Kumar *et al*., 2015). If there is not much wind, these achenes typically fall near the parent plants (Shrivastava and Jain, 2014). However, during strong winds, they can spread far away (Rao, 2017). The best conditions for achene dispersal are sunny days with strong winds, additionally, because the connected phyllaries with achenes can stick to hair or clothing, humans also play a role in dispersing them (Mbagwu *et al*., 2009).

1.3 Taxonomy

Kingdom : Plantae

Phylum : Angiosperm

Class : Dicotyledonae

Order : Asterales

Family : Asteraceae

Genus : Emilia

Specie : praetermissa

(Ndukwu and Agbagwa, 2006).

1.4 Chemical and bioactive components of *Emilia praetermissa* Milne-redh.

Emilia praetermissa is rich in bioactive compounds that contribute to its medicinal properties and physiological effects (Isah *et al*., 2023). Gas Chromatography Flame Ionization Detector

(GC-FID) analysis of the leaves confirmed the presence of various phytocompounds, including ammodendrine, phytate, hydroxylupanine, sapogenin, tannin, cardiac glycoside, ephedrine, anthocyanin, flavones, flavonone, proanthocyanidin, cyanogenic glycoside, and naringenin (Ikezu, 2023). *Emilia praetermissa* extract contains flavonoids, polysaccharides, and phenols, which are compounds known for their antioxidant properties (Lebeau *et al*., 2016). The presence of these phytocompounds in *Emilia praetermissa* leaf extract supports the plant's traditional medicinal uses (Banni and Jayaraj, 2024).

Table 1 shows the biological activity of some compounds isolated from *Emilia praetermissa* Milne- redh.

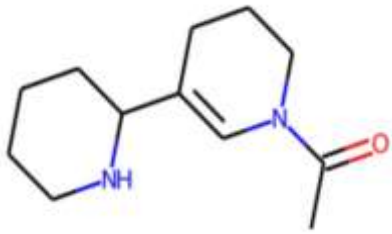
TABLE 1.1: Biological activities of some compound isolated from *Emilia praetermissa* Milne-redh

COMPOUND	BIOLOGICAL ACTIVITY	REFERENCES
Ammodendrine	It has been shown to cause adverse consequences in embryo development, leading to birth defect.	Green *et al*., 2013
Phytate (chemically known as myoinositol -1,2,3,4,5,6-hexa kisphosphate)	1. It prevents calcifications in diabetes, cancer and dental medicine. 2. As an antioxidant, can reduce the oxidative stress formed due to the effect of free radicals 3. They serve as chelating agents for storage minerals, particularly for Cu2+ and Zn2+ cations, due to their negative charge at physiological pH	Omoruyi *et al*., 2013; Pallem *et al*., 2020. Pires *et al*., 2023 Raboy, 2003.
Hydroxylupanine	1. It possesses antibacterial activity. 2. It possesses antimicrobial and anti-fungal activities.	Romeo *et al*., 2018 Osama *et al*., 1992
Sapogenin	1. It is capable of reducing reactive oxygen species 2. It has anti-fungal, antiviral and immunomodulatory activities.	Suresh *et al*., 2021 Majumder and Annegowda, 2021

Tannins	1. They have been known for their antioxidant, cardioprotective and anti-inflammatory properties. 2. They exhibit antimicrobial activities by inhibiting the growth of fungi, yeast , bacteria and viruses	Cosme *et al*., 2025 Chung *et al*., 1998
Cardiac glycosides	1. They increase the output force of the heart and increase its rate of contraction. 2. They act as cardiotonic agents commonly used for various cardiac diseases due to inhibition of Na+/K+-ATPase.	Fu *et al*., 2019 Škubník *et al*., 2021
Ephedrine	1. It acts by both direct stimulation of α and β-adrenergic receptors and indirectly by increasing the release of norepinephrine. 2. It also has mild central nervous system stimulating effects, such as increased alertness and reduced fatigue 3. ephedrine acts as a bronchodilator, making it useful in treating conditions like asthma and bronchitis	Roth and Brody, 2020 Ghosh *et al*., 2022 Brunton *et al*., 2018
Anthocyanin	1. It has an anticancer effect through a wide range of anti-inflammatory and antioxidant activities.	Liu *et al*., 2021

Flavones	2. They possess a wide range of biological activities, including antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer properties	Leonte *et al*., 2023
Flavanones	They show anti-inflammatory, antiviral , and antimicrobial activities.	Bellocco *et al*., 2009
Proanthocyanidin	It shows various biological activities, including antioxidant, anti-inflammatory, anti-cancer , anti-atherosclerosis, hypoglycemic, and anti hypertensive effects.	Yu *et al*., 2025.
Cyanogenic glycosides	They show antimicrobial and anti-fungal activity	Harenca *et al*., 2021.
Naringenin	1. It has antidiabetic, anticancer, antimicrobial, antiobesity, and gastroprotective activities 2. It shows immunomodulatory cardioprotective, nephroprotective, and neuroprotective properties.	Uçar and Göktas , 2023 Alam *et al*., 2014

1.4.1 Structures of some compounds isolated from *Emilia praetermissa* Milne-redh.



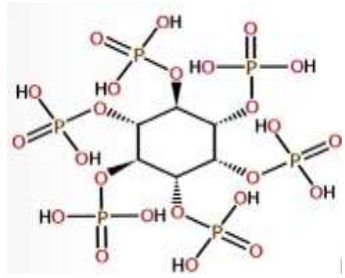
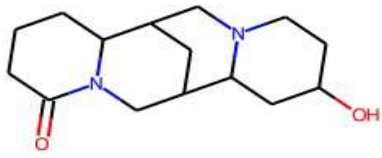


Figure 1.1 : Ammodendrine (Murakoshi *et al*., 1991). **Figure 1.2:** Phytate (Lim *et al.*, 2000)



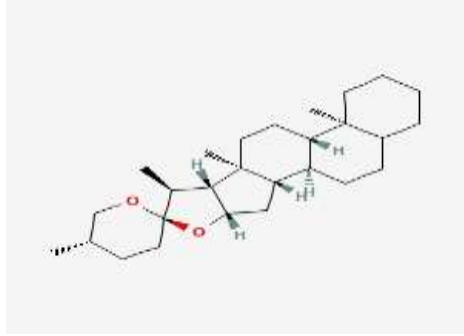
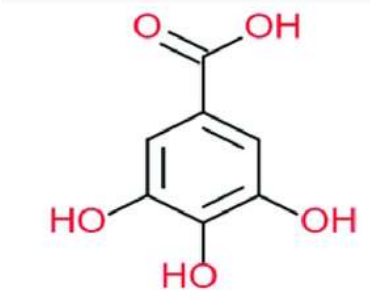


Figure 1.3: Hydroxylupanine (Esumi *et al*., 2009). **Figure 1.4:** Sapogenins (Lobo *et al*., 2010).



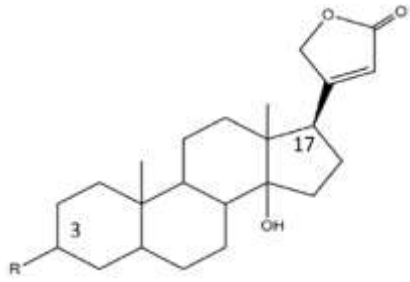


Figure 1.5: Tannins (Chung *et al.*, 1998). **Figure 1.6:** Cardiac glycosides (Fariba and Fariborz, 2024).

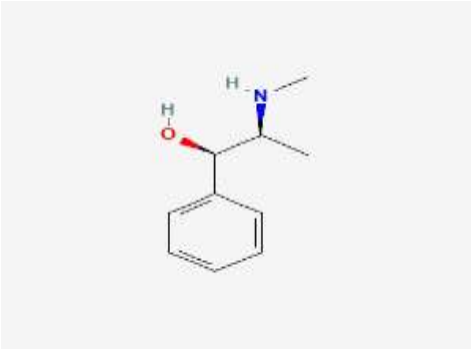


Figure 1.7: Ephedrine (Chen and Schmidt ,1924)

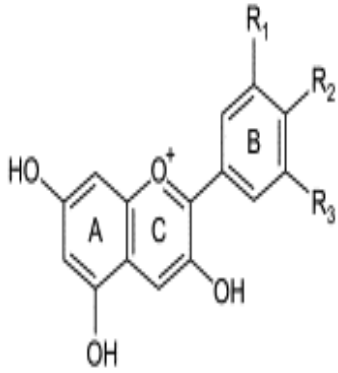


Figure 1.8: Anthocyanins (Mattioli *et al*., 2020)

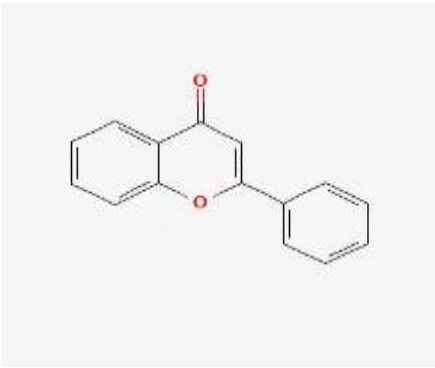


Figure 1.9: Flavones (Hostetler *et al*., 2017).

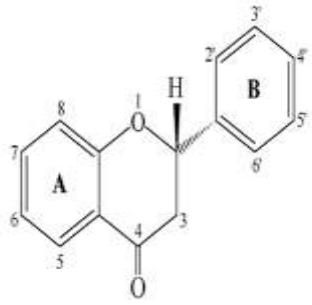


Figure 2.0: Flavonones (Belloccо*et al*., 2009)

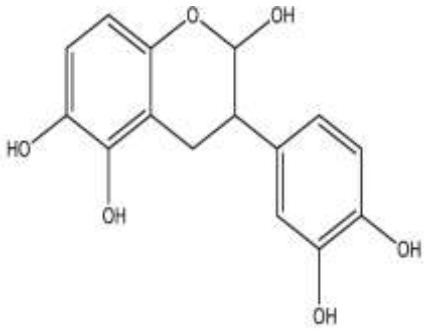


Figure 2.1: Proanthocyanidin (Yu *et al*., 2025)

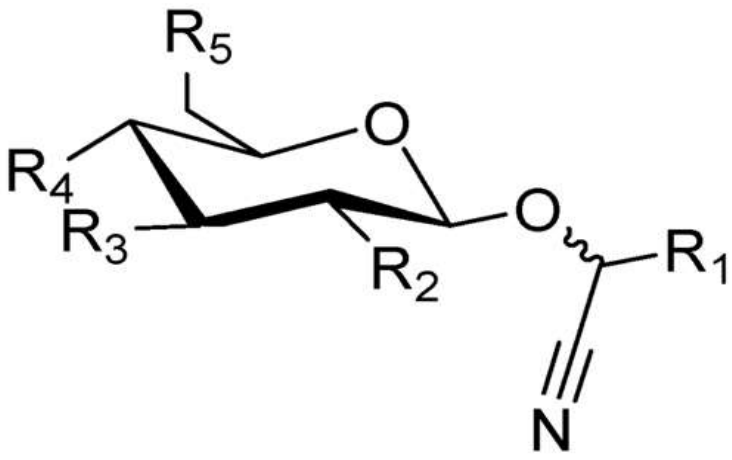


Figure 2.2: Cyanogenic glycosides (Harenca *et al*., 2021)

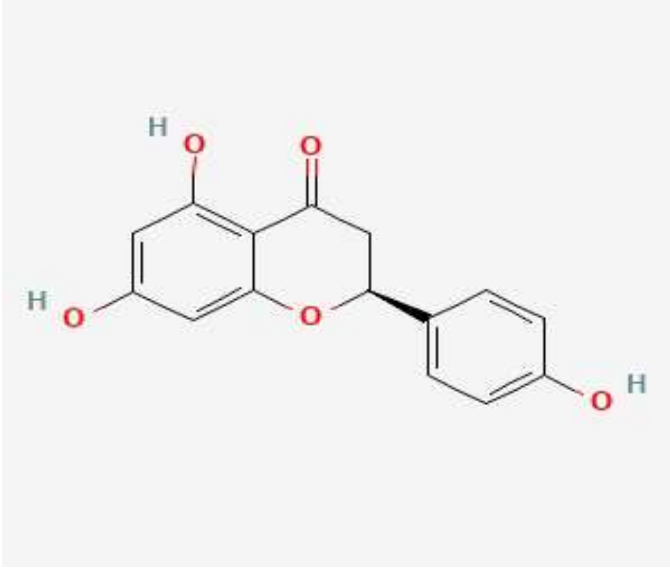


Figure 2.3: Naringenin (Uçar and Göktas , 2023)

1.5 Traditional and medicinal uses of *Emilia praetermissa* Milne-redh.

Emilia praetermissa is used in folk medicine particularly in Africa and the efficacy of its leaves in treatment of different illnesses ethnomedically can be attributed to the presence of numerous phytochemicals (Ikezu, 2023).

The plant parts of *Emilia praetermissa* have been traditionally used to treat various ailments including diarrhea and gastric ulcer, diabetes, fever, convulsion in children, worms, fibroid, syphilis, eye inflammation, night blindness and sore throat etc in Nigeria and other African countries (Ikezu, 2023; Okafor , 2002)

In China , leaves of *Emilia praetermissa* is used for the treatment of looseness of the bowels, roundworm infestations, wounds and abscesses, flu, burns and snake bites (Naraganan *et al*., 2023).

Emilia praetermissa is documented in ethnomedicine to exert antibacterial effects and used in relieving the aches and pains associated with malaria fever (Ighomena and Odion, 2025). The leaves of this plant in West Africa are ground and administered to heal ulcers , hypertension and swelling (Edeoga *et al*., 2005). Traditionally, *Emilia praetermissa* is used as an anti-inflammatory agent (Ikezu, 2023).

Among the Igbo people of Southeastern Nigeria , the leaves of *Emilia praetermissa* are pounded up with *Piper guineense* (uziza) and are useful in managing sour throat and backache (Okafor , 2002). The plant has been documented in the Nigerian folk medicine for the treatment of epilepsy in infants (Medabalimi *et al* ., 2017). It has been used as an imperative

medicinal plant in tropical and subtropical countries comprising the South-South region of Akwa Ibom State, Nigeria (Hussain *et al*., 2023).

1.6 Biochemical/ physiological function of phytochemicals

Phytochemicals are naturally occurring secondary metabolites in plants with diverse structures and functions (González-Vallinas *et al*., 2013).

These plant-derived bioactive substances play various roles, such as providing coloration and scent, serving as phytoalexins for disease resistance, acting as antifeedants and toxins to deter insects, and functioning as allelochemicals to protect against herbivores (Yancui *et al.*, 2016). Additionally, they support plant reproduction, including insect pollination, and regulate growth and signaling pathways. These compounds can be sourced from fruits, vegetables, grains, nuts, and herbs (Kumar *et al*., 2023).

Key examples of phytochemicals include carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides etc (Kumar *et al*., 2023).

1.6.1 Flavonoids

Flavonoids are a category of naturally occurring compounds characterized by varying phenolic structures. They are commonly found in a wide range of plant-based foods such as fruits, vegetables, grains, tea, bark, roots, and wine, and are recognized for their health-promoting benefits (Panche *et al*., 2016). As a major class of plant secondary metabolites, flavonoids are known for their broad biological activities and play key roles in nutraceutical, medicinal, cosmetic, and pharmaceutical applications (Panche *et al*., 2016).

Structurally, flavonoids are defined by a 15-carbon skeleton, made up of two aromatic rings linked by a three-carbon bridge (Tsukasa, 2000).

Flavonoids are further categorized based on the position at which the B-ring connects to the central C-ring. If it attaches at carbon 3, the compound is classified as an isoflavone; if it attaches at carbon 4, it is termed a neoflavonoid.

At the second carbon position, flavonoids can be further categorized into groups like flavones, flavanols, flavanones, flavanonols, catechins, anthocyanins, and chalcones. This classification also depends on how saturated or oxidized the C-ring is (Panche *et al*., 2016).

Flavonoids serve multiple functions in plants, acting as antioxidants, antimicrobials, and photoreceptors. They also play roles in attracting pollinators through flower color and scent, repelling herbivores, and filtering light (Panche *et al*., 2016). Various studies have shown that flavonoids possess important biological properties, including anti-allergic, antiviral, anti-inflammatory, and blood vessel-dilating effects (Pietta, 2000).

1.6.2 Tannins

Tannins, often called tannic acid, are water-soluble polyphenols found in a wide range of plants (Chung *et al*., 2010). These complex compounds can reduce intestinal absorption due to their low bioavailability (Hoque *et al*., 2024). They typically have large molecular weights (500kDa–3000kDa) and can bind strongly with proteins, starch, cellulose, and other macromolecules (Aguilar *et al*., 2007; Frutos *et al*., 2004).

Tannins help protect plants by disabling microbial enzymes, making them less prone to infections. However, some microorganisms have adapted to survive in their presence and continue to grow despite this defense mechanism (Govindarajan *et al*., 2016).

1.6.3 Cardiac glycosides

These are naturally occurring plant compounds with a steroid core. This core is usually linked to a sugar molecule at the third carbon and an unsaturated lactone ring at the seventeenth carbon position. They possess ionotropic properties and some of them are used as a treatment for congestive heart failure (Mazumdar *et al*., 2018).

1.6.4 Terpenoids

These are also known as isoprenoids and represent a class of natural compounds such as terpenes, sesquiterpene, limonoids, ubiquinone, menthol, and camphor. Their hydrocarbons are arranged in specific patterns, therefore they are organic compounds (Kumar *et al*., 2023).

They are produced in diverse genera of plants, algae, and sponges. The biosynthesis of terpenoids starts with three (3) acetyl-CoA units which form the central intermediate mevalonic acid, the precursor for isopentyl pyrophosphate (IPP) and its double bond isomer dimethylallyl pyrophosphate (DMAPP). These building blocks can be considered as active isoprenes.

condenses with IPP to form geranyl pyrophosphate (GPP) which after reaction with another DMAPP forms farnesyl pyrophosphate (FPP). In this fashion, the biosynthetic pathway

continues stepwise to the tetraterpenes (carotenoids) and eventually to polyisoprene (Rolf and Eckehard, 2016).

Traditionally, terpenoids gotten from plant sources have been used by humans in the pharmaceutical, food and chemical industries and recently in the development of biofuel (Tholl, 2014) while ecologically, terpenoids has gained increased popularity in developing strategies for sustainable pest control and abiotic stress protection (Tholl, 2014).

1.6.5 Alkaloids

Alkaloids are a class of secondary metabolites predominantly composed of nitrogen-containing compounds derived from amino acids (Ali *et al.*, 2019). These nitrogen bases typically have hydrogen atoms substituted with various side chains within the peptide ring structure (Ali *et al*., 2019). Alkaloids exhibit basic chemical properties and are alkaline in nature, as evidenced by their ability to turn red litmus paper blue. Their basicity is mainly due to the presence of nitrogen atoms in forms such as primary, secondary, or tertiary amines, with the level of basicity influenced by the molecular structure and the type and position of functional groups (Sarker and Nahar, 2007).

When alkaloids interact with acids, they form crystalline salts without releasing water (Firn, 2010). These compounds are generally very bitter in taste. Functionally, they serve as a defense mechanism in plants, protecting against herbivores and pathogens. Due to their potent biological effects, alkaloids are extensively utilized in medicine as pharmaceuticals,

stimulants, narcotics, and even poisons. Naturally, they are abundant in plant seeds and roots, often existing alongside organic acids (Mamta *et al*., 2013).

Pharmacologically, alkaloids are applied in various therapeutic areas, including as anesthetics, central nervous system (CNS) stimulants, antidiabetic agents, analgesics, mood-altering and addictive substances, anticancer agents, and antimalarial drugs (Mamta *et al*., 2013).

1.6.6 Glycosides

Glycosides are formed by the condensation of sugars (such as polysaccharides) with various organic hydroxyl compounds, and occasionally with thiol groups. These compounds are typically colorless, crystalline, and composed of carbon, hydrogen, and oxygen—though some may also contain nitrogen and sulfur. They are water-soluble phytochemicals commonly found in plant cell sap. Structurally, glycosides consist of a sugar portion (like glucose) linked to a non-sugar component called an aglycone or genin (Firn, 2010). Classification of glycosides is based on their sugar type, the chemical structure of the aglycone, or their pharmacological properties such as antioxidant activity, vasoconstriction, stimulation of the central nervous system (CNS), anti-cancer effects, and benefits for heart health (Xiao *et al*., 2015).

1.6.7 Phenols

Phenolic compounds—also known as phenolics or polyphenols—are chemical substances that serve as natural pigments in fruits, giving them their color. These compounds are vital to plant survival due to their diverse roles. Notably, they play a major role in plant defense against

pathogens and herbivores and have been used in treating human infectious diseases (Puupponen-Pimia *et al*., 2008). Phenols are grouped into three categories: phenolic acids, flavonoid polyphenolics (such as flavonones, flavones, xanthones, and catechins), and non-flavonoid polyphenolics. Among them, caffeic acid is the most prevalent in plants, followed by chlorogenic acid, which is associated with allergic skin reactions in humans (Kar, 2007). Phenolic compounds are known for their strong antioxidant properties and are often used as nutraceuticals. Found in apples, green tea, and red wine, they have been recognized for their cancer-fighting potential, protective effects against heart disease, and anti-inflammatory properties (Puupponen-Pimiä *et al*., 2008). Other notable examples include flavones, rutin, naringin, hesperidin, and chlorogenic acid.

1.6.8 Saponins

Saponins are naturally occurring compounds extracted from plants like *Saponaria vaccaria* and *Quillaja saponaria*, both known for their rich saponin content. Historically, they were used as soap due to their ability to create foam in water, exhibiting surfactant-like behavior (Bora, 2014). Saponins are broadly classified into two main categories: steroidal saponins and triterpenoid saponins. They dissolve well in water but not in ether and, like glycosides, can be hydrolyzed to yield aglycones (Bora, 2014).

Toxicologically, saponins are known to cause red blood cell lysis and have been linked to livestock poisoning. They have a characteristically bitter and irritating taste, especially affecting mucous membranes. Despite their toxicity, saponins also offer therapeutic benefits. They exhibit lipid-lowering (hypolipidemic) and anticancer properties (Rani and Kumar,

2021), and are crucial in the functioning of cardiac glycosides. The two major steroidal sapogenins—diosgenin and hecogenin—play a key role in synthesizing steroid hormones (Kar, 2007). Notably, diosgenin is used industrially in the production of progesterone.

1.6.9 Anthraquinones

Anthraquinones, also known as anthracenediones or dioxoanthracenes, are derivatives of both phenolic and glycosidic structures and are commonly located in various parts of plants such as roots, rhizomes, fruits, and flowers (Chen *et al*., 2016). They originate exclusively from anthracene and exist in several oxidized forms, including anthrones and anthranols (Firn, 2010). Besides being utilized as natural dyes, anthraquinones display important biological effects, such as anti-inflammatory (Chen *et al*., 2016), antifungal (Wuthi-udomlert *et al*., 2010), and antioxidant activities (Dave and Ladwani, 2012).

1.6.10 Terpenenes

Terpenes are unsaturated hydrocarbons that are volatile and found primarily in the essential oils of plants, especially conifers and citrus species (Gershenzon, 2007). They form the largest and most diverse group of naturally occurring compounds. Terpenes are classified into mono-, di-, tri-, tetra-, and sesquiterpenes based on the number of isoprene units. They are abundant in plant essential oils and are responsible for the aroma, taste, and coloration of many plants. Pharmacologically, terpenes have several applications including antioxidant, antihyperglycemic, anti-inflammatory, antimicrobial, anticancer, analgesic, and antifungal properties (Ngoci *et al*., 2011; Wang *et al*., 2012).

1.6.11 Anthocyanidin

Anthocyanidins are water-soluble plant pigments that are chiefly responsible for the red, blue, and purple hues found in fruits, vegetables, flowers, and various plant tissues (Mazza *et al*., 2004). These pigments are typically found in the skin of fruits, although in certain red fruits such as cherries and strawberries, they are also present in the flesh (D'Archivio *et al*., 2007). Structurally, anthocyanidins are based on the flavylium ion (Anderson and Jordheim, 2006). To date, researchers have identified at least 31 different monomeric forms of anthocyanidins in living organisms. They are also found in some animals (D'Archivio *et al*., 2007). Pharmacologically, they have been reported to possess antioxidants, anticancer, anti-inflammatory, cardiovascular, antidiabetic, neuronal diseases etc (He *et al*., 2011).

1.6.12 Sterols

Sterols are plant-derived compounds that closely resemble cholesterol in both structure and biological activity (Berger *et al*., 2004). They are characterized by the presence of additional functional groups such as methyl, ethyl, or double bonds (Berger *et al*., 2004).

These compounds impact membrane fluidity and permeability under both laboratory and biological conditions (Hannich *et al*., 2011). Additionally, they serve as precursors for various signaling molecules like brassinosteroids, bile acids, and vitamin D (Hannich *et al*., 2011). The most common plant sterols include β-sitosterol, stigmasterol, and campesterol (Valitova *et al*., 2016).

1.6.12.1. β-Sitosterol

β-Sitosterol is a type of phytosterol classified as stigmast-5-ene, with a β-hydroxyl group attached at the third carbon position (National Center for Biotechnology Information, 2025).

It functions as an inhibitor of sterol methyltransferase and serves as an anti-cholesteremic agent, antioxidant, and plant-derived metabolite (National Center for Biotechnology Information, 2025). Research has demonstrated that β-sitosterol modulates several cellular signaling pathways, affecting processes such as the cell cycle, programmed cell death (apoptosis), metastasis, and cellular proliferation (Khan *et al*., 2022).

Furthermore, it has been found to induce apoptosis in prostate cancer cells in both experimental and living systems (Macoska, 2023).

1.6.12.2 Stigmasterol

Stigmasterol, also known as stigmasterin or Wulzen anti-stiffness factor, is an unsaturated sterol found in many medicinal plants (Kaur *et al*., 2011). It belongs to the tetracyclic triterpene class and is commonly present in plant-derived substances like vegetable oils, seeds, pollen, and soybeans (Bakrim *et al*., 2022; Huo *et al*., 2024). This plant sterol is known for its anti-cancer, anti-inflammatory, and antioxidant effects (Huo *et al*., 2024). It also helps manage diabetes by lowering fasting blood sugar, improving insulin levels, and enhancing glucose tolerance (Bakrim *et al*., 2022).

1.6.12.3 Campesterol

Campesterol is a plant sterol naturally found in many plants. It plays a key role in maintaining membrane structure and function. It is also a biological precursor for brassinosteroids, a group of plant hormones (Xu *et al*., 2023). Campesterol has been linked to lowering cholesterol and reducing the risk of cancer (Uttu *et al*., 2023)

1.7 Pharmacological properties of *Emilia praetermissa* Milne-redh.

Emilia praetermissa holds significant pharmacological importance due to its diverse array of phytochemicals, such as polyphenols, phenolic acids, flavonoids, acetylenes, and triterpenes (Isah *et al*., 2023).

The plant in several reports has shown to possess a wide range of activities including anti-inflammatory activity (Ikezu, 2023; Edeoga *et al*., 2005), antimicrobial activity (Afolayan *et al*., 2017; Ebhohon *et al*., 2025) , antioxidant activity (Ebunlomo *et al*., 2012; Elusiyan *et al*., 2018) etc . The leaves are used to reduce swelling (Edeoga *et al*., 2005) and for the treatment of rheumatic pains, muscular pains and hypertension (Ebhohon *et al*, 2005).

1.7.1 Anti-inflammatory activity

The results obtained from a study carried out by Smith (2021), suggests that the phytochemicals identified in *Emilia praetermissa* may be the bioactive constituents

responsible for the efficacy of the leaves of the plant . The presence of bioactive compounds such as terpenoids, saponins, tannins, and phenolics may account for the anti-inflammatory activity of the leaves, as reported by Okoli *et al* (2006). High concentrations of phytate (14.50%), sapogenin (15.65%), and tannin (2.55%) were detected, all of which are known to play key roles in controlling inflammation (Ikezu, 2023).

Scientific evidence indicates that antioxidants such as phenolics, phenolic acids, and flavonoids present in *Emilia praaetermissa* exhibit cytotoxic effects against various cancer cells (Ganesan and Xu, 2017). In addition to their antioxidant activity, they also demonstrate anti-inflammatory effects, and together, these properties contribute to their potential in managing degenerative diseases associated with oxidative stress and inflammation (Lopez-Corona *et al*., 2022).

A study revealed that the ethanol extract of *Emilia praetermissa* leaves exhibited strong inhibitory and stabilizing effects on heat-induced protein denaturation and hypotonicity-induced hemolysis of human red blood cells (HRBCs) (Odion *et al.,* 2024). Protein denaturation, which occurs when protein lose their natural structure due to heat or other stressors, is a well-documented contributor to various inflammatory processes in the body and since inflammation is often driven by the denaturation of tissue proteins, drugs capable of preventing this process could be highly beneficial in managing inflammatory conditions (Gupta *et al.,* 2013).

Inhibition of cyclooxygenase (COX), an enzyme essential for prostaglandin synthesis involved in inflammation, is a key target in the development of anti-inflammatory drugs (Hussain *et al*., 2023). As a result, the suppression of COX isoforms ,COX-1 and COX-2, is actively being explored (Hussain *et al*., 2023). Molecular docking studies have shown that doronine, a pyrrolizidine alkaloid present in *Emilia praetermissa*, binds specifically to COX-2, indicating its potential as a novel alternative to existing anti-inflammatory agents (Hussain *et al.*, 2020).

1.7.2 Antioxidant activity

Antioxidants prevent oxidation by interrupting free radical chain reactions, which is done by donating their own electrons to neutralize free radicals, yet remain stable and do not become free radicals themselves (Onyeneke *et al*., 2022).

Report of the antioxidant property of *Emilia praetermissa* showed that the aqueous extract of the leaves was richer in phenolic and flavonoids than the other extracts (Smith, 2021). Plant-derived natural antioxidants like flavonoids and phenolic acids help counteract oxidative stress by neutralizing free radicals, preventing lipid peroxidation, and acting through various protective biochemical mechanisms (Masella *et al.*, 2005). Antioxidant nutrients like vitamin E, vitamin C, carotenoids, and various plant-based polyphenols directly neutralize reactive oxygen species (ROS) (Muscolo *et al*., 2024). Because of this, they are believed to serve as part of the body's internal defense system against oxidative damage (Pham-Huy *et al*., 2008). Plant-derived antioxidants can significantly enhance overall antioxidant activity, making them

valuable in the prevention and management of various clinical conditions linked to oxidative stress (Michalak, 2022).

Phenolic and flavonoids are the polyphenolic compounds which have been found to have free radical scavenging activity (Mayakrishnan *et al.,* 2013). These compounds function as antioxidants by donating hydrogen atoms to neutralize free radicals (Lü, *et al*., 2010). Findings show that the aqueous extract contains higher levels of phenolics and flavonoids compared to the methanol and ethanol extracts; however, all three extracts demonstrate potential antioxidant activity (Smith, 2021).

The extracts of *Emilia praetermissa* exhibited significant free radical scavenging activity in a dose-dependent manner against DPPH, ABTS, hydrogen peroxide, nitric oxide, and hydroxyl radicals. When compared to the standard antioxidant (Ascorbic Acid) at various concentrations, the extracts consistently showed notable antioxidant potential (Smith, 2021).

According to a study by Yemisi (2021), the aqueous extracts of *Emilia praetermissa* demonstrated the highest free radical scavenging activity, followed by the ethanolic extracts, while the methanolic extracts exhibited the least scavenging capacity.

Ferric reducing ability is broadly used to estimate the antioxidant activity of polyphenols (Debnath *et al*., 2013). This effect is linked to the presence of reductones, which exhibit antioxidant properties by interrupting free radical chain reactions and donating hydrogen atoms (Lubna *et al*., 2019). The reducing capacity of a compound is connected to its ability to

transfer electrons, making it a useful indicator of its potential antioxidant activity (More and Makola, 2020).

1.7.3 Antimicrobial activity

Studies have shown that extracts of *Emilia praetermissa* exhibit broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as some fungal strains (Ogunleye and Fakorede, 2020). The zones of inhibition observed in agar diffusion assays varied depending on the solvent used for extraction (e.g., aqueous, methanol, ethanol) and the specific microorganism tested (Peter and Olugbenga, 2022). For instance, aqueous extracts have been reported to show greater antimicrobial efficacy than methanol and ethanol extracts, possibly due to the higher solubility and availability of polar bioactive compounds in water (Akinmoladun *et al*., 2021).

The antimicrobial activity of *Emilia praetermissa* is thought to be linked to the presence of phytochemicals that disrupt microbial cell walls, interfere with protein synthesis, or inhibit enzyme systems (Afolayan *et al*., 2017). For example, tannins are known to precipitate microbial proteins, while flavonoids may inhibit nucleic acid synthesis (Peter and Olugbenga, 2022). These compounds likely work synergistically, enhancing the overall antimicrobial effect (Akinmoladun *et al*., 2021).

The extracts have also demonstrated dose-dependent inhibition of pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Peter and Olugbenga, 2022). Higher concentrations of the extracts yielded larger inhibition zones, indicating greater

effectiveness (Ogunleye and Fakorede, 2020). This suggests that *Emilia praetermissa* could be a valuable source of antimicrobial agents, particularly in settings where antibiotic resistance is a growing concern (Akinmoladun *et al*., 2021).

1.7.4 Anti-fungal activity

Recent studies have shown that extracts from this *Emilia praetermissa* possess notable antifungal properties, especially against common pathogens such as *Candida albicans* and *Aspergillus niger* (Nwankwo *et al*., 2025).

Laboratory-based investigations have demonstrated that ethanol and aqueous extracts of *Emilia praetermissa* produce clear zones of inhibition when tested against fungal organisms, supporting its broad-spectrum antifungal potential (Ikezu, 2023). Nwankwo *et al*. (2025) reported that ethanol leaf extract inhibited fungal growth in a dose-dependent manner, with defined minimum inhibitory concentrations (MICs) recorded against *Candida albicans* and *Aspergillus niger* (Nwankwo *et al*., 2025). This validates its use in ethnomedicine and points to its therapeutic promise (Nwankwo *et al*., 2025).

1.7.5 Anti-hypertensive activity

Hypertension, commonly known as high blood pressure, is a major global health concern linked to cardiovascular diseases, stroke, and kidney failure (Ajayi *et al*., 2019).

Phytochemical screening of *Emilia praetermissa* has revealed the presence of flavonoids, saponins, alkaloids, tannins, phenols, and glycosides (Ogunleye *et al*., 2023). These bioactive compounds are known for their vasodilatory and cardioprotective effects, which contribute to

blood pressure regulation (Ajayi *et al*., 2019). For instance, flavonoids can enhance nitric oxide (NO) production, leading to vascular smooth muscle relaxation and decreased peripheral resistance, which ultimately lowers blood pressure (Ajayi *et al*., 2019).

A study by Peter *et al*. (2021) evaluated the anti-hypertensive activity of aqueous extracts of *Emilia praetermissa* in salt-induced hypertensive rat models , the results showed a significant reduction in systolic and diastolic blood pressure following oral administration of the extract. The hypotensive effect was dose-dependent and comparable to that of standard antihypertensive drugs such as nifedipine (Peter, 2021).

Moreover, the plant's alkaloid content may exert a direct inhibitory effect on angiotensin-converting enzyme (ACE), an enzyme responsible for converting angiotensin I to the vasoconstrictor angiotensin II (Olaleye and Ologunde, 2020). ACE inhibition is a common pharmacological target in managing hypertension, and preliminary in vitro assays suggest that *Emilia praetermissa* may act similarly (Olaleye and Ologunde, 2020).

Table 1.2 shows some pharmacological potentials of some parts of *Emilia praetermissa* Milne-Redh.

TABLE 1.2: Pharmacological potentials of *Emilia praetermissa* Milne-redh

S/N	PLANT PART	COMPOUND	FORMULATION	MEDICINAL USES	REFERENCE
1.	Leaf	Doronine	Decotion	Antihypertensive	Roder and Helmut, 2013
2.	Leaf	Naringenin	Decotion	Antimicrobial, antioxidant	Uçar and Göktas, 2023
3.	Leaf	Proanthocyanidin	Decotion		Yu *et al*., 2025.
4.	Leaf	Anthocyanin	Decotion	Anti-inflammatory	Liu *et al*., 2021
5	Leaf	Hydroxylupanine	Decotion	Antibacterial	Romeo *et al*., 2018
6.	Leaf	Sapogenin	Decotion	Antioxidant	Suresh *et al*., 2021
7	Leaf	Flavanone	Decotion	Antiviral	Bellocco *et al.*, 2009
8	Stem	Resveratrol	Decoction	Antioxidant, anticancer, antiangiogenic	Meng *et al*., 2023.
9	Stem	Charyophyllene oxide	Decoction	Antifungal	Kuame *et al*., 2025

10	Stem	Ellagic acid	Decoction	Anticancer, antioxidant, anti-inflammatoy	García-Niño *et al*., 2015.
11	Root	Senkirkine	Decoction	Anti-inflammatory, analgesics effects	Lebada *et al*., 2000
12	Root	Pyrrolizidine alkaloids	Decoction	Hepatotoxic, cytotoxic, tumorigenic	Schramm *et al*., 2019

.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample collection and authentication

The fresh leaves of *Emilia praetermissa* Milne-Redh were collected at University of Benin and at Okhun community, both in Ovia North East Local Government Area, Benin City, Edo State, Nigeria.

The leaves were identified at the Department of Plant Biology and Biotechnology, Herbarium Unit, University of Benin, Benin City by Prof. Henry Adewale Akinnibosun and Voucher code UBH-E407 given for reference purposes.

2.1.2 Chemicals	**Manufacturers, Model, Country/Town.**
1. Acetic anhydride	Sigma-Aldric
2. Ammonia	Yara, Brazil
3. Ammonium chloride	Hibrett Purate
4. Benzene	Sinopec Group, China
5. Chloroform	Fisher Chemical
6. Distilled water	BCH Research Lab, UNIBEN
7. Ferricchloride	Haihang Industry, Co. Ltd, China.

8. Gallic acid	Dalian Chem, China
9. Glacial acetic acid	Fengbai, China
10. Hydrochloric acid (concentrated)	Brenntag Chemicals Ltd, Nigeria.
11. Local gin	Uselu, Benin City, Nigeria
12. Methanol	BDH, England
13. Olive oil	Goya, Spain
14. Sodium carbonate.	Danto Chemicals Ltd, Nigeria
15. Sodium	Citrique Belge, Tienen, Belgium
16. Sodium hydroxide	Brenntag Chemicals Ltd, Nigeria
17. Sulphuric acid (concentrated)	British Drug House Chemicals Ltd, Poole, England.

2.1.3 Equipment and Instruments	**Manufacturers, Model, Country/Town.**
1. Analytical weighing balance (PA213)	OMAUS Corp. Pioneer, U.S.A
2. Beakers (10ml, 50ml, 100ml, 250ml)	Pyrex, England
3. Burette (100ml,200ml,500ml,1000ml)	Pyrex, England
4. Conical flask (100ml,200ml,500ml)	Pyrex, England

5.	Cotton wool	Royal lint, England
6.	Dessicator	England
7.	Disposable gloves	Unigloves, Nigeria.
8.	Eppendorf tubes	Tower Group Ltd, Nigeria
9.	Foil paper	Tower Group Ltd, Nigeria.
10.	GC-FID	Thermo Fisher Scientific, USA
11.	GC-MS	Thermo Fisher Scientific, USA
12.	Hammer mill machine	Varahi Industries, India.
13.	Hot plate	Infiniti forever, INF-HP8070.
14.	Magnetic stirrer (79-1)	JINOTECH Instruments, China
15.	Measuring cylinder	Pyrex, England
16.	Micro pipette	Erba Lachemas Brno, Czech Republic
17.	Mortar and pestle	United Scientific, USA
18.	Muffle furnace	Nabertherm, Lilienthal, Germany
19.	Muslin cloth	Jante Textile, Turkey
20.	Oven(DHG 90 30A)	Hinoteck, China.

21. Pasteur pipette	Alpha Laboratories Ltd, U.K.
22. Pipette	Pyrex, England
23. Plastic containers (4L calibration)	OK plastics, Nigeria
24. Refrigerator (HA-137)	Haier Thermocool, Nigeria
25. Separating funnel	Pyrex, England
26. Soxhlet extractor	Pyrex, England
27. Spectrophotometer (20D S23A)	Teckmel and Teckmel, U.S.A
28. Test tubes	Pyrex, England
29. Test tubes racks	Equitron Medica Instruments, India
30. Thimble	Dritz Corporation, South Carolina
31. UV-visible spectrophotometer	Thermo Fisher Scientific, USA
32. Vortex XH-B	Hinoteck China
33. Whatman filter paper	Whatman Int'l, England

2.1.4 Preparation of Aqueous and local Gin Extract

After obtaining the dried and finely powdered *Emilia praetermissa* plant material, 150g of the powder was weighed and soaked separately in 3500mL of distilled water and in 3500mL

of locally sourced gin. Each mixture was stirred thoroughly and allowed to macerate at room temperature for 48 hours with occasional shaking to enhance extraction. The mixtures were then filtered using muslin cloth followed by Whatman No. 1 filter paper. The filtrates were concentrated by gentle evaporation at room temperature and stored in sterile containers at 4°C until use.

2.2 Methods

2.2.1 Extraction

Fresh *Emilia praetermissa* leaves were cleaned properly of sand and dried at ambient temperature (24°C - 27°C) for five (05) days in the open in the laboratory. The dried leaves were pulverized using a dry hammer mill (Varahi Industries, India). An exact amount (150g) of the pulverized sample (plant material) was weighed and macerated for 48 hours with 3.5L of distilled water while it was intermittently stirred. The resultant extract was filtered using Muslin cloth, followed by whatman No. 1 filter paper, and then placed in a sanitized plastic container. Another ground sample was again mixed in 3.5L of local gin and proceeded as for water. The filtrates were then concentrated by means of rotary evaporator and subsequently freeze dried. The extracts were then weighed. There after, the extracts were stored in sterile containers at 4°C until use.

2.3 Phytochemical Screening:

2.3.1 Preliminary phytochemical screening:

The evaluations were carried out strictly adhering to recognized standard protocols (Harborne 1998; Trease and Evans, 1989; Sofowora 1993).

1. Flavonoid detection test:

Exactly five milliliters (5mL) of dilute ammonia were introduced to a 1mL aliquot of each extract. Following this, 1mL of concentrated H_2SO_4 was added. The observation of a yellow coloration indicated the presence of flavonoids.

2. Tannin detection test:

Each extract (1mL) was boiled in 2 mL of water in a test tube, followed by filtration. A few drops of 0.1% ferric chloride were then added, and the sample was visually inspected for a brownish-green to blue-black coloration.

3. Cardiac glycosides test (Keller-Killiani Method):

Exactly one milliliter (1mL) of each extract was treated with glacial acetic acid, containing a single drop of ferric chloride solution. This mixture was carefully overlaid with 1mL of concentrated H_2SO_4. The appearance of a distinct brownish ring at the interface signified the presence of a deoxysugar, characteristic of cardenolides, thus confirming cardiac glycosides.

4. Saponin detection test (Foaming Test):

The ability of saponins to produce persistent foam in aqueous solutions was leveraged as a screening method. A 0.1mL sample of each extract was combined with 5mL of distilled water and vigorously shaken. The formation of a stable, long-lasting froth served as an affirmative indication of saponin presence.

This was further confirmed through the addition of 3 drops of olive oil and vigorous agitation, after which the visible formation of an emulsion ensued.

5. Sterol detection test:

Exactly two milliliters (2mL) of acetic anhydride were introduced to 0.5g of each extract, followed by the addition of 2mL of H_2SO_4. A color shift from violet to blue or green served as confirmation for the presence of steroids.

6. Terpenoid detection test (Salkowski Test):

Exactly one milliliter (1mL) aliquots of each extract, placed in separate test tubes, were combined with 2mL of chloroform and 3mL of concentrated H_2SO_4. The development of a reddish-brown coloration at the liquid interface confirmed the existence of terpenoids.

7. Phenol detection test:

Drops of a 10% aqueous $FeCl_3$ solution were added to a test tube containing 5mL of each extract. The emergence of a blue or green coloration signaled the presence of phenols.

8. Phlobatanin detection test:

A sample of each extract, diluted with 3mL of methanol, received 2mL of 1% HCl, and the mixture was subsequently boiled. The appearance of a red precipitate provided conclusive evidence of phlobatanins.

9. Coumarin detection test:

A 0.1mL volume of each extract, placed in individual test tubes, was augmented with 3mL of 10% NaOH solution. The subsequent observation of a yellow coloration indicated the presence of coumarins.

10. Alkaloid detection test (Meyer's reagent test):

A known volume (1mL) of each extract was combined with 3 drops of Meyer's reagent. The subsequent formation of a cream-colored precipitate confirmed the presence of alkaloids.

11. Anthraquinone detection test:

Exactly five milliliters (5mL) of benzene were added to 1mL of extracts in a test tube, and the mixture was vigorously shaken with 2.5mL of NH_3. The emergence of a pink-red coloration in the lower phase was indicative of the presence of free anthraquinone.

2.3.2. Quantitative Phytochemical Assessment:

Following the preliminary phytochemical screening to confirm the presence of specific compounds, the samples proceeded to quantitative evaluation to determine the precise concentration of the secondary metabolites. The total phenolic, tannins, alkaloids, flavonoid

e.t.c contents were meticulously quantified according to established methods (Madu *et al.*, 2016; Selvakumar *et a*l., 2019).

I. Quantification of total alkaloid content:

Method: BCG (Bromocresol Green) colorimetric method (Fazel *et al.*, 2008).

Principle: Alkaloids react with bromocresol green in acidic medium to form a yellow complex that is extractable in chloroform. The intensity of the color, measured at 760nm, is proportional to alkaloid concentration and expressed as atropine equivalents.

Reagent composition:

Bromocresol green (BCG) solution: 0.01% (w/v)

Phosphate buffer: 0.1M, pH 4.7

Chloroform (analytical grade)

Atropine stock solution (1 mg/mL)

Procedure: Approximately 1mL of the extract was transferred to a separating funnel. Here, 5mL of bromocresol green (0.01% w/v) solution and 5mL of phosphate buffer (0.1M, pH 4.7) were introduced. The mixture underwent vigorous shaking with sequential additions of 1, 2, 3, and 4mL of chloroform.

The chloroform layers were then collected in a 10 mL volumetric flask and topped up to the mark with chloroform. A set of atropine reference standard solutions (20, 40, 60, 80, and 100

µg/mL), derived from the atropine stock solution, were prepared in an identical manner to the samples.

The absorbance of both the standard and test solutions was measured against a reagent blank at 760nm using a UV/Visible spectrophotometer. All experimental procedures were replicated three times. The total alkaloid content was expressed as atropine equivalents . The reagent blank, devoid of extract, was prepared similarly.

Calculation:

The total alkaloid concentration was obtained from the atropine calibration curve (20–100 µg/mL). Absorbance values of the samples were converted to atropine-equivalent concentrations (µg/mL) using the regression equation of the standard curve. The total alkaloid content was expressed as µg atropine equivalents per mg of dried extract.

12. Quantification of total phenolic content:

Principle: Phenolic compounds react with the Folin–Ciocalteu reagent under alkaline conditions to form a blue-colored complex. The intensity of the color, measured at 760nm, is directly proportional to the concentration of phenolics in the sample.

Reagent composition:

Folin–Ciocalteu reagent (10% v/v)

Sodium carbonate solution (7.5% w/v)

Gallic acid stock solution (standard, 20–100 µg/mL)

Procedure: The concentration of phenolic compounds within the extracts was estimated using a slightly modified Folin-Ciocalteu method (Singleton and Rossi, 1965) . In brief, 1mL of the ethanol extract solution was combined with 2mL of 10% Folin-Ciocalteu reagent. After a 5-minute interval, 3mL of 7.5% sodium carbonate was added, and the volume was then adjusted to 10mL with distilled water. A series of gallic acid standard solutions (20, 40, 60, 80, and 100 µg/mL) derived from the stock solution were prepared identically, and the test tubes were incubated for 90 minutes at ambient temperature in a dark environment. All experiments were performed in triplicate. Absorbance readings were taken at 760nm. The results for the total phenolic content were reported as gallic acid equivalents (µg GA/mg of dried extract). The reagent blank was prepared in the same manner, but without the inclusion of extracts.

Calculation:

Total phenolic content was determined from the gallic acid standard calibration curve (20–100 µg/mL) and expressed as µg gallic acid equivalents (GAE) per mg of dried extract.

iii. **Concentration of total flavonoid content:**

Principle: Flavonoids are polyphenol compounds contained in plants with benzo-γ-pyrone structures. The total flavonoid content in the extracts can be determined by colorimetry using aluminum chloride reagent. The principle of determining the flavonoid content with aluminum chloride method is the formation of a complex between aluminum chloride with

keto clusters on a C-4 atom and hydroxy clusters on C-3 or C-5 atom, neighbouring from the group of flavones and flavonols, forming a yellow stable compound. The compound used as a standard in this experiment was quercetin. This is because quercetin is a flavonoid from flavonol group which has keto clusters on a C-4 atom and hydroxyl clusters on neighboring C-3 and C-5 atom. (Sumiayah *et al* 2018).

Reagent composition: 5% Sodium nitrate, 10% Aluminium chloride, 1M Sodium hydroxide, quercetin stock solutions (Standard 20, 40, 60, 80, and 100 µg/mL).

Procedures:

A colorimetric assay employing aluminum chloride was utilized to determine the total flavonoid content. For this reaction, 1mL of the test sample was combined with 4mL of distilled water in a test tube, followed by the addition of 0.30mL of 5% sodium nitrite. After 5 minutes, 0.30mL of 10% aluminium chloride was introduced. Subsequently, following a 6-minute incubation period at room temperature, 2mL of 1M sodium hydroxide were added to the reaction mixture, and the final volume was immediately diluted to 10ml with distilled water. A set of quercetin standard solutions (20, 40, 60, 80, and 100 µg/mL), obtained from the stock solution, were prepared using the same methodology as described earlier. The absorbance for both test and standard solutions was measured against a reagent blank at 510nm wavelength using a UV/Visible spectrophotometer. The total content of flavonoid was expressed as quercetin equivalents (µg QAE/mg of dried extract). The blank reagent, devoid of extracts, was prepared identically. All experiments were conducted in triplicate.

iv. Concentration of total tannin content:

Principle: The Folin-Ciocalteu (F-C) method determines the total phenolic content in a sample by measuring its reducing capacity. The method relies on the reduction of the F-C reagent, a mixture of phosphotungstic and phosphomolybdic acids, by phenolic compounds in an alkaline solution. This reduction causes a color change from yellow to blue, and the intensity of the blue color, measured spectrophotometrically, is proportional to the concentration of phenolic compounds in the sample. The principle of the F–C assay is the reduction of the Folin–Ciocalteu reagent (FCR) in the presence of phenolics resulting in the production of molybdenum–tungsten blue that is measured spectrophotometrically at 760nm and the intensity increases linearly with the concentration of phenolics in the reaction medium (Swain and Hillis 1959).

Reagent composition: Folin phenol reagent, 35% sodium carbonate, tannic acid stock solutions (Standard 20, 40, 60, 80, and 100 µg/mL)

Procedure: The total tannin content was quantified using the Folin Ciocalteu method, incorporating minor adjustments. Approximately 0.5mL of the local gin extract was combined with 3.75mLof distilled water in a test tube. Subsequently , about 0.25mL of Folin phenol reagent and 0.5mLof 35% sodium carbonate solution were added , and the mixture was diluted to 10mL with distilled water. A series of tannic acid standard solutions (20, 40, 60, 80, and 100ug/ml) were prepared following the same protocol as outlined previously. The absorbance for test and standard solutions was read at 725nm wavelength using a

UV/Visible spectrophotometer. The overall tannin content is presented as tannic acid equivalents (ug TA/mg of dried extract). The reagent blank was prepared identically, but without extract. All experimental measurements were carried out in triplicate.

Reagents: 0.25mL Folin phenol reagent, 10mL distilled water, 0.5mL 35% sodium carbonate

CHAPTER THREE

RESULTS

3.1 Phytochemical screening

This chapter presents the results of the phytochemical screening of the leaves of *Emilia praetermissa* Milne-redh, which includes the qualitative and quantitative analysis. The qualitative result shows the phytochemicals present in the leaf extract while the quantitative result shows the amount of the phytochemicals identified.

3.1.1 Qualitative

The result of the phytochemical screening (qualitative) of *Emilia praetermissa* Milne-redh identified five (5) compounds present in the leaf extract. These phytochemicals have been reported to possess biological and pharmacological activity. Results are shown in Table 3.1 , where arithmetic signs (-, +, ++,) are used to show the presence of phytochemical in varying concentrations.

Table 3.1 Phytochemical screening (qualitative) of *Emilia praetermissa* Milne-redh.

Phytochemical	Results
Flavonoids	-
Tannins	+
Cardiac glycosides	+
Saponins	-
Sterols	++
Terpenoids	+
Phenols	++
Phlobatannins	-
Coumarin	-
Alkaloids	-
Anthraquinone	-

KEY:

- = Absent

+ = present (Low Conc)

++ = Present (High Conc)

3.1.2 Quantitative

Table 3.2 shows the amount of the identified phytochemicals present in the leaves of *Emilia praetermissa* . The concentration determination were done in triplicates, and the results shown in tabular form, where the data are expressed as mean ± standard error of mean (SEM).

Table 3.2 Quantitative results of the phytochemicals present in *Emilia praetermissa* Milne-redh.

s/n	Phytochemical	Mol.w (g/mol)	mean±SEM	Biological activity
1.	Terpenoids	414.71	0.21±0.00	Anticancer
2.	Cardiac glycosides	508.48	0.26±0.00	Cytotoxicity, antiviral, enzyme inhibitory.
3.	Sterols	412.69	0.05±0.00	Anticancer, anti-arthritis, anti-inflammatory immunomodulatory
4.	Tannins	1701.20	0.11±0.00	Antioxidant, antibiotic, anti-inflammatory.
5.	Phenols	170.12	0.12±0.00	Antimutagenic, antioxidant, anticancer, anti-inflammatory

Among the identified phytochemicals, cardiac glycosides had the highest mean concentration, followed closely by terpenoids and phenols , while sterols and tannins were present in relatively lower amounts.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

4.1.1 Phytochemical screening of *Emilia praetermissa* Milne-redh leaves

The phytochemical screening of *Emilia praetermissa* Milne-redh leaves revealed the presence of various bioactive compounds, including terpenoids, cardiac glycosides, sterols, tannins, and phenols. These findings are consistent with previous studies that have reported the presence of these phytochemicals in *Emilia praetermissa* (Ikezu, 2023; Ebhohon *et al*., 2025). The presence of these compounds suggests that *Emilia praetermissa* may possess medicinal properties, which could be attributed to the individual or synergistic effects of these phytochemicals.

4.1.2 Quantitative analysis of phytochemicals

The quantitative analysis of the phytochemicals present in *Emilia praetermissa* leaves showed that cardiac glycosides had the highest mean concentration, followed closely by terpenoids and phenols. Sterols and tannins were present in relatively lower amounts. The high concentration of cardiac glycosides is notable, as these compounds are known for their cardiotonic and cardioprotective effects (Mazumdar *et al*., 2018). The presence of terpenoids, which have been reported to possess antimicrobial and anti-inflammatory properties (Tholl, 2014), suggests that *Emilia praetermissa* may be useful in the treatment of various diseases.

4.1.3 Pharmacological significance of identified phytochemicals

The identified phytochemicals in *Emilia praetermissa* leaves have been reported to possess various pharmacological activities. Terpenoids have been shown to exhibit anticancer properties (Gershenzon, 2007), while cardiac glycosides have been used in the treatment of congestive heart failure (Mazumdar *et al*., 2018). Sterols have been reported to possess anti-inflammatory and immunomodulatory effects (Hannich *et al*., 2011), while tannins have been shown to exhibit antioxidant and antimicrobial properties (Chung *et al*., 2010). Phenols have been reported to possess antioxidant, anticancer, and anti-inflammatory effects (Puupponen-Pimiä *et al*., 2008).

4.1.4 Implications for medicinal use

The presence of various bioactive compounds in *Emilia praetermissa* leaves suggests that the plant may possess medicinal properties, which could be useful in the treatment of various diseases. The identified phytochemicals have been reported to possess pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. These findings support the traditional use of Emilia praetermissa in folk medicine for the treatment of various ailments, including gastric ulcers, fever, and convulsion in children (Ikezu, 2023; Okafor, 2002).

4.2 Conclusion

In conclusion, the phytochemical screening of *Emilia praetermissa* leaves revealed the presence of various bioactive compounds, including terpenoids, cardiac glycosides, sterols,

tannins, and phenols. The quantitative analysis showed that cardiac glycosides had the highest mean concentration, followed closely by terpenoids and phenols. The identified phytochemicals have been reported to possess various pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. These findings support the traditional use of *Emilia praetermissa* in folk medicine and suggest that the plant may possess medicinal properties, which could be useful in the treatment of various diseases. Further studies are needed to isolate and characterize the bioactive compounds present in *Emilia praetermissa* and to evaluate their pharmacological activities in more detail.

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APPENDIX

STANDARD

CALIBRATION GRAPH

VALUES (100ug/mL)

Beta-Sitosterol

Abs @ 548nm	0	0.158	0.23	0.345	0.468	0.565
Amount (ug)	0	20	40	60	80	100

Absorbance

test values for

Terpenoids

(Extract)

SAMPLE	Abs @ 548nm					
ID	SAMPLE A (Y)	CONST	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**
A1	1.083	0.0053	0.0376	**211.4339623**	1000	**0.211433962**
A2	1.09	0.0053	0.0376	**212.754717**	1000	**0.212754717**
A3	1.094	0.0053	0.0376	**213.509434**	1000	**0.213509434**

STANDARD

CALIBRATION

GRAPH

VALUES

(100ug/mL)

Securidaside

Abs @ 495nm	0	0.068	0.135	0.21	0.275	0.341
Amount (ug)	0	20	40	60	80	100

Absorbance

test values for

Cardiac

glycosides

(Extract)

SAMPLE	Abs @ 495nm				
ID	SAMPLE A(Y)	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**
A1	0.879	0.0034	**258.5294118**	1000	**0.258529412**
A2	0.88	0.0034	**258.8235294**	1000	**0.258823529**
A3	0.876	0.0034	**257.6470588**	1000	**0.257647059**

STANDARD

CALIBRATION

GRAPH

VALUES

(100ug/mL)

Stigmasterol

Abs @ 780nm	0	0.059	0.131	0.245	0.367	0.466
Amount (ug)	0	20	40	60	80	100

Absorbance test values for **Steroids** (Extract)

SAMPLE	Abs @ 780nm					
ID	SAMPLE A (Y)	CONST	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**
A1	0.202	0.0053	0.0614	**49.69811321**	1000	**0.049698113**
A2	0.201	0.0053	0.0614	**49.50943396**	1000	**0.049509434**
A3	0.204	0.0053	0.0614	**50.0754717**	1000	**0.050075472**

CALIBRATIO

N GRAPH

VALUES

(100ug/mL)

Tannic Acid.

Abs @ 725nm	0	0.161	0.319	0.395	0.49	0.542
Amount (ug)	0	20	40	60	80	100

Absorbance test values for tannins (Extract)

SAMPLE	Abs @ 780nm					
ID	SAMPLE A (Y)	CONST	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**
A1	0.436	0.0047	0.1015	**114.3617021**	1000	**0.114361702**
A2	0.437	0.0047	0.1015	**114.5744681**	1000	**0.114574468**
A3	0.439	0.0047	0.1015	**115**	1000	**0.115**

STANDARD

CALIBRATIO

N GRAPH

VALUES

(100ug/mL)

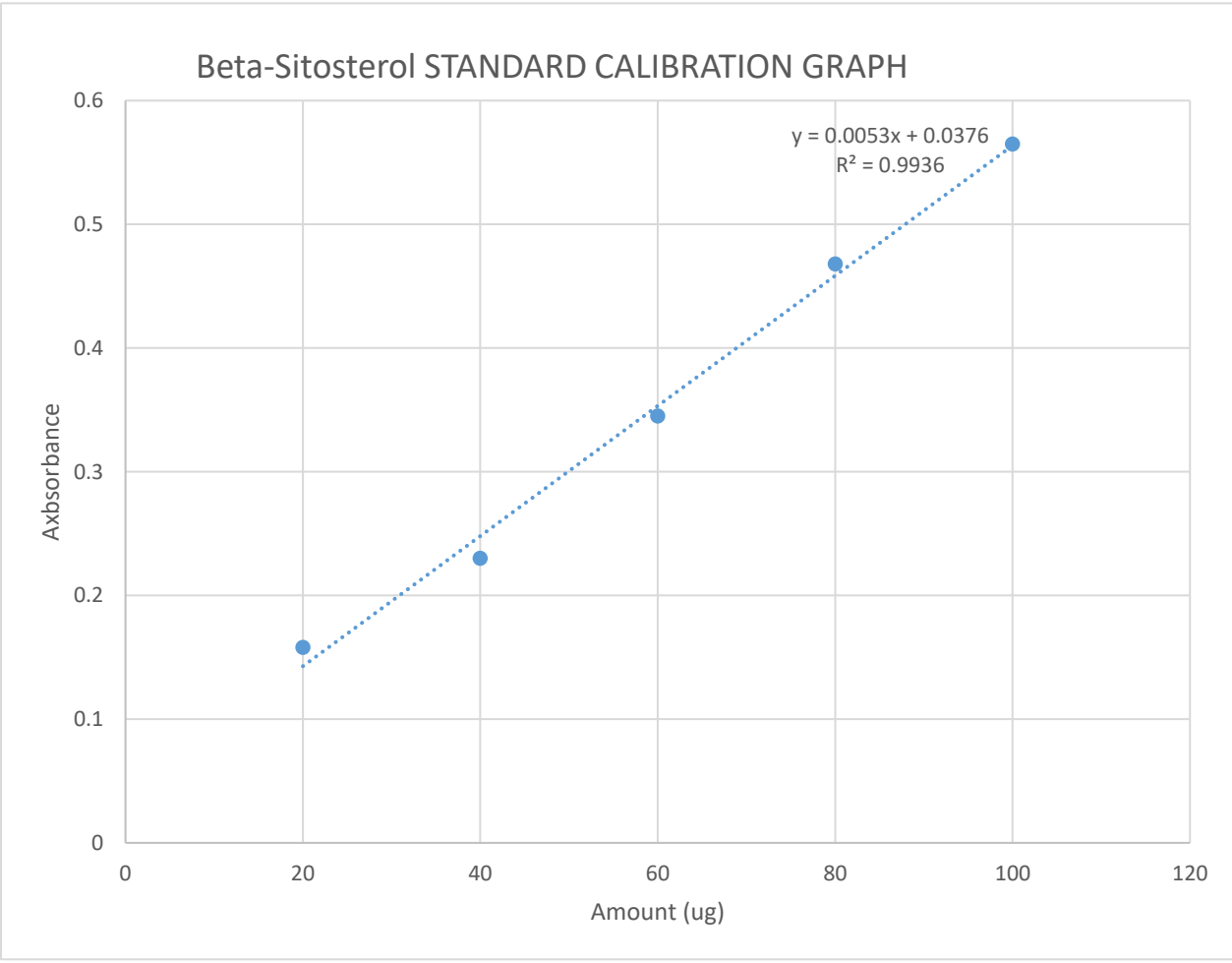
Gallic Acid

Abs @ 760nm	0	0.181	0.267	0.334	0.407	0.556
Amount (ug)	0	20	40	60	80	100

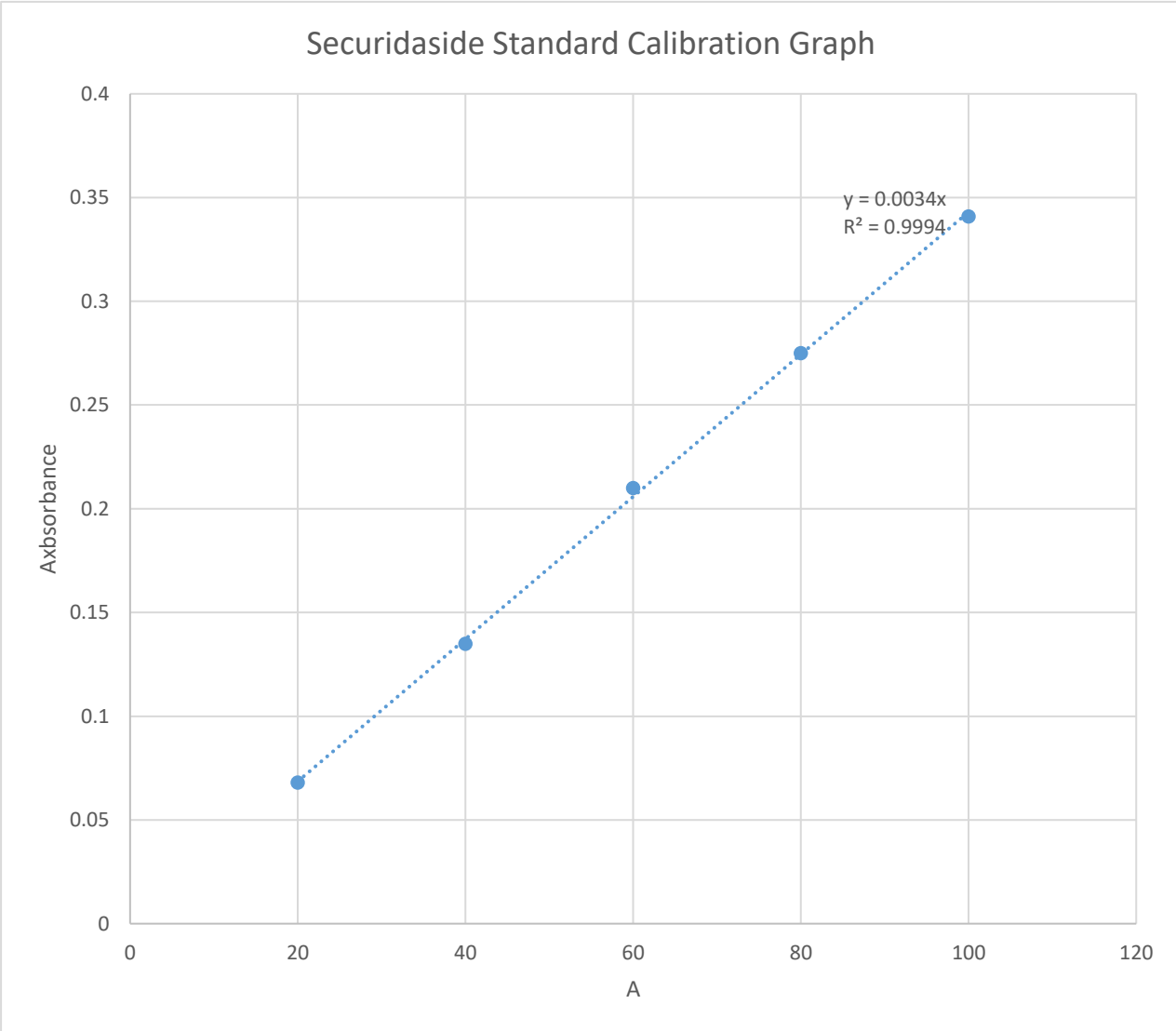
Absorbance of test values for Phenol (Extract)

SAMPLE	Abs @ 780nm					
ID	SAMPLE B(Y)	CONST	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**
A1	0.453	0.0047	0.1015	**117.9787234**	1000	**0.1179787 23**
A2	0.457	0.0047	0.1015	**118.8297872**	1000	**0.1188297 87**
A3	0.458	0.0047	0.1015	**119.0425532**	1000	**0.1190425 53**

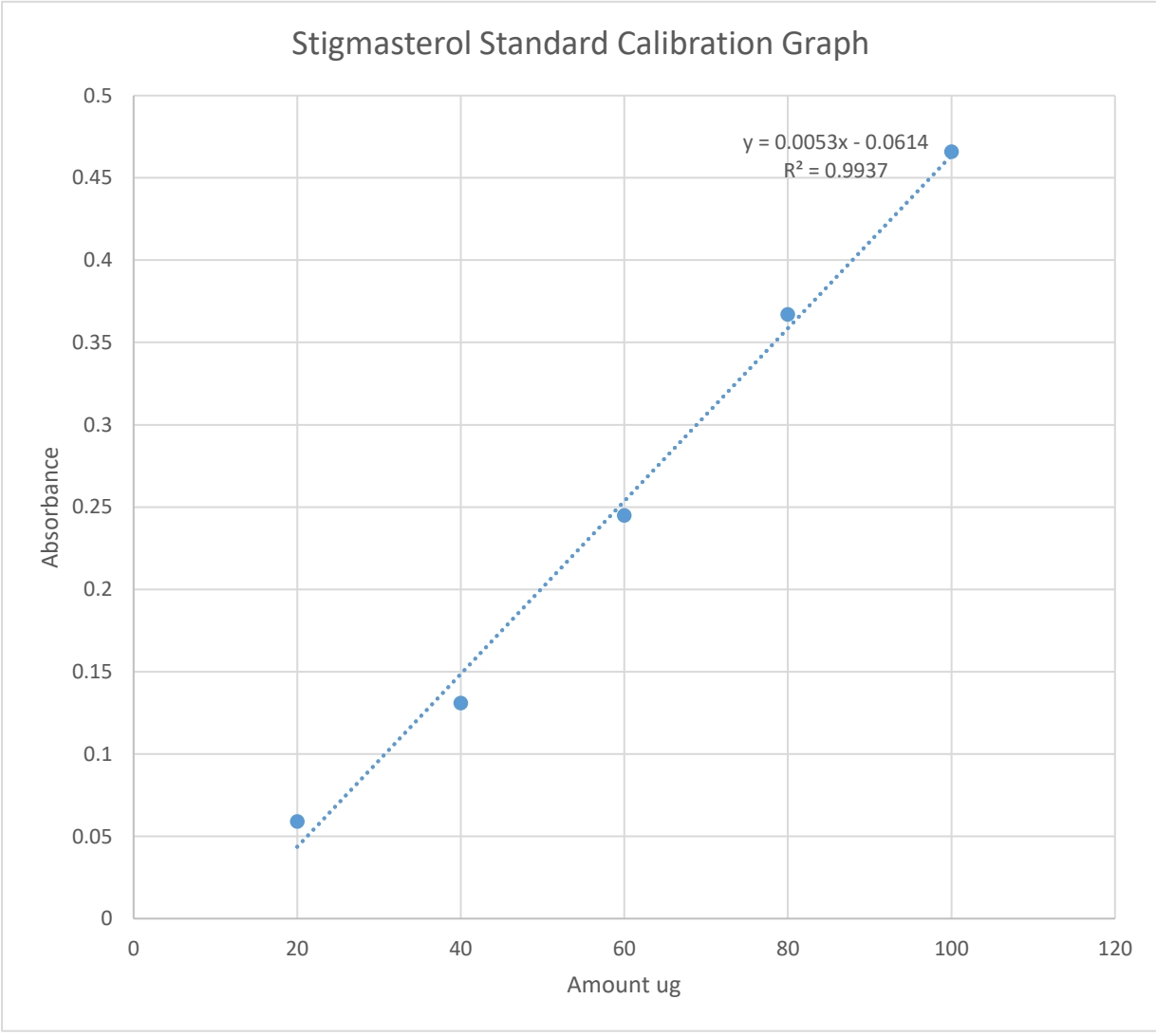
STANDARD CALIBRATION CURVE FOR BETA- SITOSTEROL



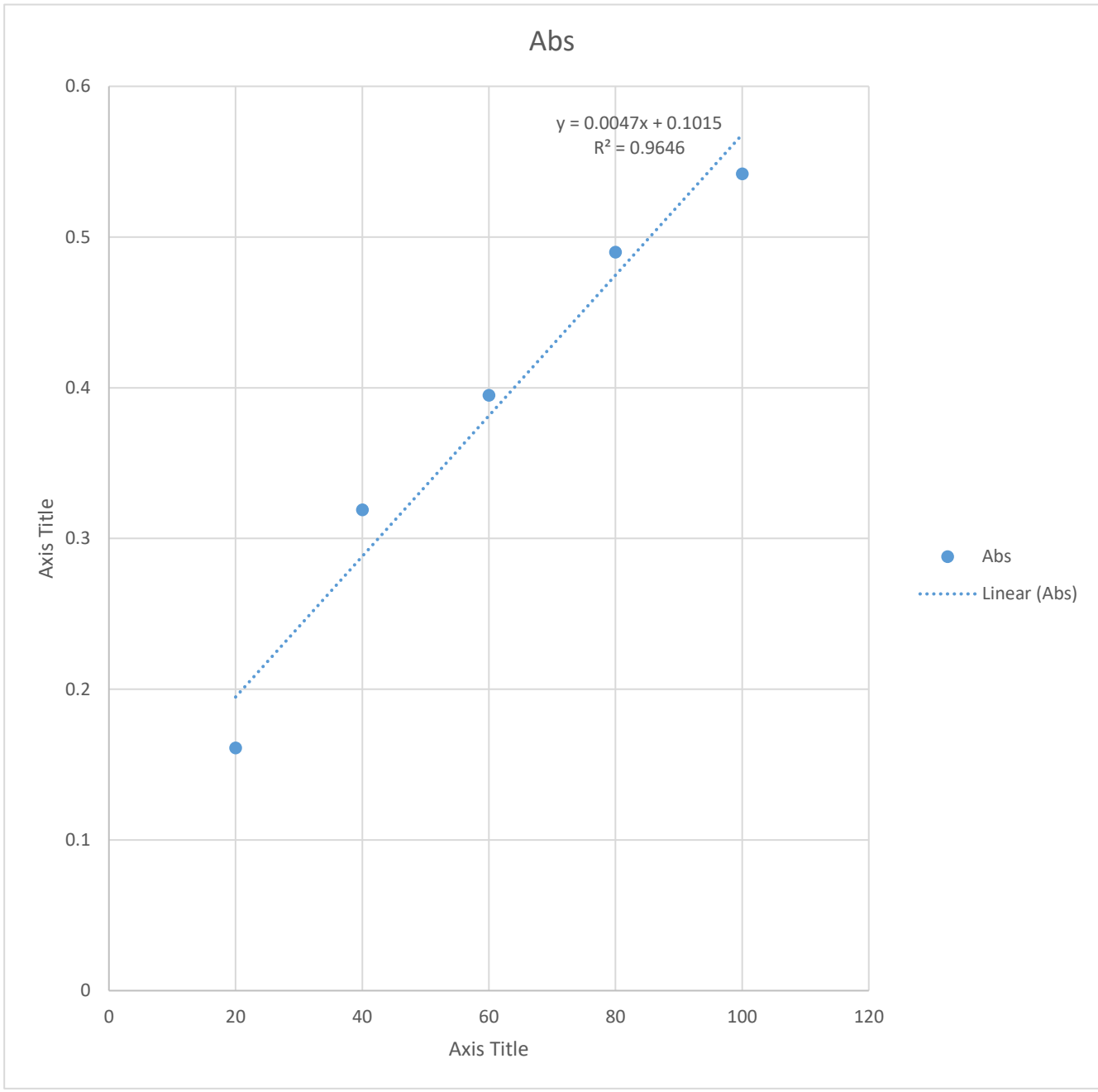
STANDARD CALIBRATION CURVE FOR SECURIDASE



STANDARD CALIBRATION CURVE FOR STIGMASTEROL



STANDARD CALIBRATION CURVE FOR TANNINS



STANDARD CALIBRATION CURVE FOR PHENOLS