LIVER FUNCTION STATUS INDICES OF MALE *WISTAR* RATS ADMINISTERED *EMILIA PRAETERMISSA* LEAF EXTRACT

BY

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CERTIFICATION

This is to certify that this project was carried out by MAROH OGHENEYOMA EMMANUELA with matriculation number LSC2103766, in the department of BIOCHEMISTRY in thr faculty of LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, in partial fulfilment of the requirements for the award of bachelor of science degree (B.Sc hons) in Biochemistry.

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DEDICATION

This project work is humbly dedicated to the Almighty God, whose grace and guidance made this work a reality, making it possible for us to begin this project and also bring the project to a successful end. I also dedicate it to my beloved parents and siblings for their unwavering love and support and their endless support both financially and spiritually. It is also dedicated to my project group members for their active participation and encouragement during the course of the practical work.

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ABSTRACT

The liver is crucial for metabolism, detoxification, and homeostasis, making its functional status vital for physiological health. This study investigated the liver function status of *Wistar* rats after they were administered an extract of *Emilia praetermissa* leaf, a medicinal plant traditionally used for its antimicrobial, gastrointestinal, and cardiovascular activity. Nine Wistar rats were divided into control and experimental groups, with the latter receiving varying doses of the *Emilia praetermissa* leaf extract over a defined period. Liver function markers, specifically total protein (TP), albumin (ALB), and bilirubin levels (including total bilirubin (T.BIL) and direct bilirubin (D.BIL)) were assessed to evaluate liver functionality. The toxicity of the leaf extract was also studied in the rats, and no toxicity was observed. The results showed dose-related changes in liver function parameters of rats treated with *Emilia praetermissa* local gin and aqueous extracts. Total protein and albumin levels were highest in Group 2 and Group 4 respectively, while ALT and AST levels were markedly elevated in Group 2, suggesting possible liver stress. Bilirubin levels showed mild increases in Groups 4 and 7, and ALP activity peaked in Group 6. Overall, the variations indicate that the treatments caused biochemical alterations in liver function, suggesting potential hepatic effects that warrant further investigation. These findings offer insight into the potential therapeutic or toxicological effects of *Emilia praetermissa* on liver health, contributing to its pharmacological evaluation for safe medicinal use.

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CHAPTER ONE

1.1. Introduction

Emilia praetermissa Milne-Redh, commonly referred to as yellow thistle or tassel flower, is a member of the *Asteraceae* (daisy) family. It is widely distributed in tropical regions, particularly in Africa, where it is commonly observed in both disturbed and semi-natural habitats (Olorode *et al*., 1973). This species thrives in a variety of environments including roadsides, swamps, gardens, forest edges, agricultural fields, lawns, and secondary forests. Its ecological flexibility and resilience to environmental stressors contribute to its successful colonization across diverse landscapes (Olorode *et al*., 1973).

Despite being widely regarded as a weed, *E. praetermissa* plays an important ecological role and poses significant implications for agricultural productivity and nutritional benefits (Ifediora *et al*., 2024). It is frequently found in major crop systems, especially in banana and oil palm plantations, where its rapid growth and competitive nature can lead to reduced crop yields. Its impact on crop systems, biodiversity, and ecosystem function makes it a subject of interest for both ecological and agricultural research (Adedeji *et al*., 2006)

Furthermore, the species exh ibits morphological similarities with other members of the *Emilia* genus—such as *E. sonchifolia*, *E. coccinea*, and *E. fosbergii*—which often leads to misidentification. Accurate differentiation based on floral color, leaf margin morphology, and other diagnostic traits is therefore essential to ensure reliable field identification and scientific study.

Research into the biology, distribution, and ecological interactions of *E. praetermissa* is vital for understanding its invasive characteristics and potential health applications. *E. praetermissa can be used to treat various illness, such as; gastric ulcer, hypertension, fever, and heart problems* (Lebeau, 2021), (Ebhohon 2025), and (Ighomena 2025).

In summary, *Emilia praetermissa* represents a plant of notable pharmacological and ecological significance. Previous investigations have demonstrated that the species is rich in secondary metabolites such as tannins, saponins, flavonoids, polysaccharides, and phenols, (Lebeau, 2016). These phytochemical constituents provide a scientific basis for its traditional and potential therapeutic applications. Empirical studies conducted by Anaka *et al*., (2013)

revealed that oral administration of aqueous leaf extract of *E. praetermissa* is associated with significant modulation of lipid profiles, including reductions in plasma total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and atherogenic index of plasma (AIP), alongside an elevation of high-density lipoprotein (HDL). Notably, co-administration of the extract with atorvastatin produced an enhanced lipid-modulating effect, suggesting a possible synergistic interaction (Anaka, 2013). These findings support the plant's promising role as an alternative or complementary therapeutic agent in the management of hyperlipidemia (Anaka, 2013).

1.2. Background of Study

Emilia praetermissa is a herbaceous annual in the *Asteraceae*, native to tropical Africa. Unlike most *Emilia* species (which are diploid, 2n=10), *E. praetermissa* is a natural allotetraploid (2n=20) of probable hybrid origin (Olorode *et al.* 1973). Classic crossing experiments between *E. coccinea* and *E. sonchifolia* (both diploids) produced synthetic tetraploids morphologically identical to *E. praetermissa*, suggesting that *E. praetermissa* originated from an ancient hybridization of these relatives. This polyploid origin is reflected in its intermediate morphology and chromosome count (Olorode *et al*. 1973).

E. praetermissa thrives in tropical West Africa, especially Nigeria, where it often occurs as a common weed in disturbed habitats (Chung *et al*. 2009). It has been reported from Sierra Leone through Nigeria and across the Guinean forest–savanna mosaic. In these regions it favors open or degraded sites, disturbed fields, sunny environments, and open and moist grasslands (Ebhohon 2025). Dumbardon-Martial and Delblond (2019) note that in West Africa, *E. praetermissa* characteristically grows "in crops, on roadsides and in diverse disturbed areas". Its ability to colonize such disturbed ground (often with no special soil requirements) explains why it can be weedy and widely distributed in Nigeria and neighboring countries. It has also become naturalized beyond Africa, e.g. in tropical America, but its core range and ecological niche is West African tropical zones.

Local populations in Nigeria and West Africa have long recognized *E. praetermissa* as both a food and medicine. In several countries of the region (Nigeria, Cameroon, Gabon, D.R. Congo, etc.), the young leaves are eaten as a green leafy vegetable, either raw or cooked in salads. For example, Lebeau *et al.* (2016) report that in West Africa and D.R. Congo the

leaves of *E. praetermissa* (and its close relative *E. lisowskiana*) “are occasionally eaten as a vegetable”.

Ethnomedical surveys note that the leaves and sap are used as remedies for a wide range of ailments. In Nigeria, Cameroon and Gabon, leaf preparations are applied to treat eye infections and filarial complaints (Green 2007). In Gabon the crushed leaves are taken for heart problems (Lebeau 2017). In Nigeria a leaf decoction serves as a febrifuge (fever reducer) (Lebeau 2016). In Congo, the leaf juice is applied to skin lesions (abscesses, ulcers, leprosy) and to treat lice, ringworm and other dermal conditions (Lebeau 2016). Other traditional uses include remedies for hernia, backache, syphilis, convulsions, skin infection spleen enlargement, vertigo, menstrual problems, and gastric ulcer (Lebeau, 2021), (Ebhohon 2025), and (Ighomena 2025). These diverse uses are commonly attributed to the plant’s pharmacologically active compounds (notably its antioxidant constituents) and reflect a belief in its detoxifying and tonic effects (Ebhohon 2025).

Phytochemical screening and analysis show that *E. praetermissa* leaves are rich in bioactive plant chemicals. Qualitative tests on the ethanol leaf extract reveal the presence of phenolic and flavonoid compounds (tannins, flavonoids, phenols), as well as alkaloids, terpenoids, saponins and oxalates (Ikezu 2023). The presence of polyphenols and flavonoids is significant, as these classes are known antioxidants. Indeed, Alli-Smith (2021) notes that *E. praetermissa* contains “biologically active constituents” that can neutralize free radicals. The high content of antioxidant compounds (flavonoids, tannins, phenols) underlies much of the plant’s reputed medicinal activity.

1.3. Justification of Study

Medicinal plants continue to play a crucial role in primary health care, particularly in regions where access to modern pharmaceuticals is limited or where cultural traditions favor natural remedies. *Emilia praetermissa* Milne-Redhead, a member of the family Asteraceae, has gained scientific interest due to its widespread ethnomedicinal applications and its emerging pharmacological profile. Traditionally, the plant has been utilized in the treatment of various ailments such as bacterial diarrhea, respiratory tract infections, wound healing, and the prevention of neonatal skin infections, making it an important resource in community health management (Ighomena and Odion, 2025).

Beyond its ethnobotanical relevance, several studies have provided evidence supporting the plant's bioactive potential. Investigations into its chemical composition have revealed the presence of compounds with notable antibacterial properties. For instance, spectroscopic and chromatographic analyses of partially purified dichloromethane fractions from *E. praetermissa* identified molecules with significant antibacterial activity against pathogenic microorganisms, thereby supporting its traditional use in the treatment of bacterial infections (Ighomena and Odion, 2025). Similarly, the antimicrobial properties of different solvent extracts of the plant have been documented, with methanolic, hot water, and cold water extracts showing inhibitory effects against bacteria and fungi commonly associated with otitis media, suggesting its potential as a source of novel bioactive compounds for drug development (Afolayan *et al*., 2017).

The therapeutic potential of *E. praetermissa* is not limited to antimicrobial activity. Its ethanolic extract has demonstrated remarkable cytoprotective and anti-ulcer effects in experimental models, significantly promoting the healing of chronic gastric ulcers induced by glacial acetic acid and ethanol/aspirin, thus highlighting its gastroprotective role (Lebeau et al., 2016). In addition, studies have revealed that the plant exhibits haemopoietic potential. Extracts of *E. praetermissa* improved haematological indices and reduced biochemical markers of liver injury in stress-induced ulcerated *Wistar* rats, thereby demonstrating both stress-mitigating and hepatoprotective properties (Ebunlomo *et al*., 2012).

Recent investigations have further expanded its pharmacological scope to cardiovascular health. The aqueous leaf extract of *E. praetermissa* was shown to exert significant antihypertensive effects in salt-induced hypertensive male *Wistar* rats, improving cardiovascular function, lipid profile, and antioxidant enzyme activity while reducing blood pressure and cardiac fibrosis (Ebhohon *et al*., 2025). These findings not only validate the plant's folkloric use in hypertension management but also open avenues for its integration into therapeutic strategies for cardiovascular diseases.

The chemical constituents responsible for these pharmacological activities highlight the plant's relevance as a potential reservoir of novel bioactive compounds. Its demonstrated safety profile, with an oral acute toxicity (LD50) exceeding 5000 mg/kg, further strengthens the argument for its exploration in modern drug discovery (Ebhohon *et al*., 2025).

In conclusion, a systematic investigation into its chemical composition and medicinal properties provides a scientific basis for its ethnomedicinal uses, to identify pharmacologically active compounds, and to explore its potential role in addressing global health challenges such as antimicrobial resistance, gastrointestinal disorders, stress-induced metabolic disruptions, and hypertension. Ultimately, this study aims to bridge the gap between traditional knowledge and modern pharmacological research, thereby contributing to the development of new plant-based therapeutic agents.

1.4. Aim and Objectives of the Study

1.4.1. Aim of the Study

The main aim of this study is to **evaluate the effects of the aqueous and local gin extracts of** *Emilia praetermissa* **on liver function indices and assess its potential toxicological effects in *Wistar* rats.**

1.4.2. Objectives

- **i.** **To determine the effects** of aqueous and local gin extracts of *Emilia praetermissa* on key liver function enzymes such as **Alanine transaminase (ALT)**, **Aspartate transaminase (AST)**, **Alkaline phosphatase (ALP)**, and **Gamma-glutamyl transferase (GGT)** in *Wistar* rats.
- **ii.** **To evaluate the influence** of *Emilia praetermissa* extracts on **bilirubin metabolism**, including **total bilirubin**, and **direct (conjugated)**, bilirubin levels, as indicators of hepatic excretory function.
- **iii.** To a**ssess changes** in **serum total protein**, **albumin**, and **globulin levels**, to determine the effects of the extracts on the liver’s synthetic capacity.
- **iv.** **To compare the biochemical outcomes** between rats administered the aqueous extract and those given the local gin extract, to determine the influence of the extraction medium on the plant’s toxicity or bioactivity.
- **v.** To e**stablish a dose–response relationship** between *Emilia praetermissa* extract concentrations and the extent of alterations in liver function parameters.

1.5. Literature Review

1.5.1. Botanical description of *Emilia. praetermissa*

The genus *Emilia* (Cass.) Cass. consists of approximately 100 species widely distributed across tropical and subtropical regions of the world, with East Africa noted as a major center of diversity (Nordenstam, 2007). The identification of species within this genus is often straightforward when observing living specimens, as diagnostic traits such as plant habit, robustness, involucral shape, and floret coloration are more distinct. However, these distinguishing features tend to diminish once the specimens are dried, thereby complicating taxonomic classification within the genus (Fosberg, 1972).

Emilia praetermissa is an annual herbaceous plant that can attain a height of up to 140 cm. The stems are usually erect or ascending and may be simple or branched from the base, with surfaces ranging from glabrous to pilose. The basal internodes typically measure 0.6–2 cm in length, whereas the upper internodes can extend to about 9 cm. The lower leaves are broad-ovate, subcordate, with attenuate petioles; margins are strongly dentate, measuring around 4 × 4.5 cm, with petioles 1.5–2.5 cm long, wingless, and not auriculate. The second and third leaves from the base are also broad-ovate and subcordate but differ in having winged petioles (2–3 cm long) with broad dentation and moderate pilosity. Leaves located at the middle part of the stem are morphologically similar but exhibit strongly auriculate bases with broadly dentate auricles. The uppermost leaves are sessile, auriculate-cordate, and deltoid in shape, with moderately pilose midribs on the lower surface (Chung *et al.*, 2009).

The inflorescence is characterized by erect, discoid capitula occurring singly or in clusters of up to seven within loose corymbs. The bracts are narrowly lanceolate with attenuate apices, measuring 8–11 mm in length and moderately pilose. Capitula during anthesis are about 20 mm long and 12 mm in diameter, with tubular involucres approximately 10 mm long, 4 mm wide at the base, and 3 mm at the middle. The phyllaries are uniseriate, numbering 9–12, and moderately pilose. Each capitulum typically contains about 80 tubular florets, which are 5-lobed and may appear yellowish, orange, or cream-white; corolla lobes are often purple or orange-tinged, measuring about 8 mm in length with lobes around 2 mm. Anthers are dark orange, 1.7 mm long, and appendiculate at the apex. Style branches are orange, recurved, and approximately 1.2 mm in length. The pappus is delicate, about 7 mm long, and finely

barbellulate. Achenes are truncate-elliptic, measuring 3 mm in length, 5-ribbed, and hispid along the midribs. The species has a chromosome number of 2n = 20 (Chung, 2009).

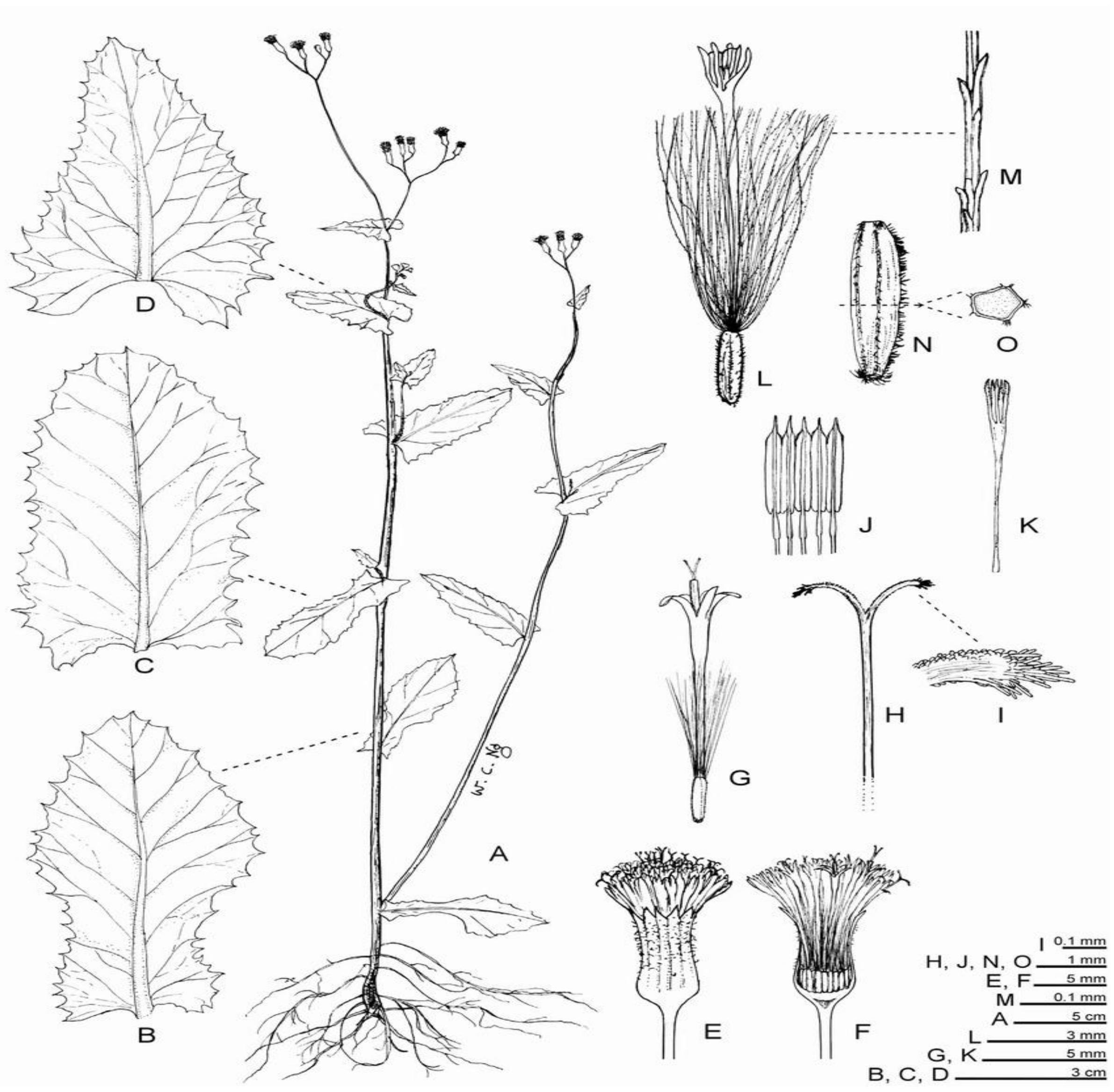


Fig 1*:* *Emilia praetermissa* Milne-Redh. A: Habit. B: Lower leaf. C: Middle leaf. D: Upper leaf. E: Capitulum. F: Capitulum, longitudinal section. G: Floret. H: Style branch. I: Stigma, showing hirtellous papillate appendages. J: Stamens. K: Corolla of withered floret. L: Achene with pappus and corolla. M: Barbellate bristle of pappus. N: Achene. O: Achene, cross section (Chung *et al*., 2009).

1.5.2. Origin and distribution

A research conducted by Adedeji (2006) revealed that *E. praetermissa* was found to be closely allied to the diploid species *E. coccinea* and *E. sonchifolia*. Morphologically and ecologically, *E. praetermissa* displayed intermediate characteristics between the two, suggesting a hybrid origin. Specific features such as bloom and corolla tube coloration and pedicel length were especially indicative of its intermediate status.

Experimental hybridization between *E. coccinea* (n = 5) and *E. sonchifolia* (n = 5) yielded diploid hybrids that, apart from differences in size-related traits (overall plant height, head size, and leaf dimensions), closely resembled natural *E. praetermissa* (n = 10). Subsequent artificial chromosome doubling of these diploid hybrids produced allotetraploids that were morphologically indistinguishable from *E. praetermissa* (Olorode 1973).

Emilia praetermissa is an allotetraploid weed of hybrid origin that grows widely in disturbed, humid environments. It is integrated into local ethnobotany both as a leafy vegetable and a multi-use medicinal herb (Olatokunbo 2020). Phytochemical analysis shows it to be rich in antioxidant flavonoids and phenolics, and available data (by analogy to related species) suggest it also provides modest nutrition as a green vegetable (Shehu 2023). These findings align with its traditional uses and underscore the importance of exploring *E. praetermissa* further as a source of natural antioxidants and nutrients.

Direct analyses of *E. praetermissa*'s nutrient composition are limited, but by analogy with related leafy *Emilia* species, its nutritional profile is expected to resemble that of common leafy vegetables. *E. praetermissa* leaves, when consumed as a vegetable, supply hydration, fiber, some protein and micronutrients (minerals and vitamins), although detailed compositional data for this species are not yet available (Shehu 2023). Nutrient-rich weeds like *E. praetermissa* may thus help meet dietary needs where conventional vegetables are scarce.

1.5.3. Habitat and cultivation

Emilia praetermissa is widely distributed across tropical West Africa, with its presence particularly notable in Nigeria, where it frequently occurs as a common weed in disturbed habitats (Chung *et al*., 2009). Its natural range extends from Sierra Leone through Nigeria

and into the Guinean forest–savanna mosaic. Within these regions, the species demonstrates a clear preference for open or degraded environments, thriving in disturbed agricultural fields, sunny landscapes, moist grasslands, and other habitats where vegetation cover is reduced (Ebhohon, 2025).

From an ecological standpoint, *E. praetermissa* exhibits traits that make it both resilient and potentially invasive. Its ability to establish and persist outside its native range, coupled with its adaptability as a habitat generalist, allows it to colonize a wide range of ecological niches. This adaptability, particularly in environments undergoing disturbance, suggests a high degree of ecological plasticity.

1.5.4. Taxonomy

Domain: Eukaryota
Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Emilia
Species: Emilia praetermissa (Sandoval *et al*., 2020).

Plate 1*:* Leaf of *Emilia praetermissa* Milne-Redh.

Source: iNaturalist

1.5.5. Pharmacological properties of *Emilia praetermissa* Milne-Redh:

Emilia praetermissa is traditionally used in managing conditions such as hypertension, gastric ulcers, skin infections, diarrhea, and stress-related disorders (Ebhohon *et al*., 2025). Scientific studies have validated these uses by demonstrating broad pharmacological activities. Antimicrobial investigations revealed inhibitory effects against bacterial and fungal pathogens, (Odion and Ighomena, 2025; Afolayan *et al*., 2017). Its aqueous extract also produced significant antihypertensive and cardioprotective effects in salt-induced hypertensive rats by lowering blood pressure, enhancing antioxidant defenses, and preserving cardiac, renal, and hepatic structures (Ebhohon *et al*., 2025).

In addition, ethanolic extracts demonstrated strong gastroprotective and ulcer-healing properties in chronic ulcer models, and moderate acid-neutralizing effects (Lebeau *et al*., 2021). The plant also improved hematological parameters and reduced stress-induced elevations of liver enzymes, indicating both haemopoietic and hepatoprotective potential (Ebunlomo *et al*., 2012).

Collectively, these findings provide scientific evidence that *E. praetermissa* possesses antimicrobial, cardiovascular, gastrointestinal, hepatoprotective, and anti-hyperlipidemic activities.

1. Antimicrobial Activity

Several studies have reported on the antimicrobial potential of *Emilia praetermissa*, highlighting its phytochemical constituents and biological activities against pathogenic microorganisms. Gradient elution chromatography has been employed to obtain partial purification of its bioactive compounds, followed by characterization through gas chromatography–mass spectrometry (GC–MS) and Fourier transform infrared (FTIR) spectroscopy (Ighomena and Odion, 2025). The eluent fraction designated EP1 was found to contain fourteen distinct compounds, including phthalate derivatives such as di-sec-butyl phthalate (46.92%) and bis(2-ethylhexyl) phthalate (6.62%), long-chain alkanes like pentadecane, heptacosane, heptadecane, nonadecane, eicosane and heneicosane, as well as 2,4-di-tert-butylphenol (7.90%) and 2-ethyl-2H-benzotriazole (4.51%). Functional groups such as O–H, N–H, C–H, C–C and C=C were also identified, confirming the chemical diversity of the compounds present.

Susceptibility testing using the agar well diffusion assay revealed that several eluents (EP1–EP11) at a concentration of 10 mg/mL inhibited the growth of clinical bacterial isolates including *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*, with the exception of EP4, EP5 and EP10 (Ighomena and Odion, 2025). These results provide evidence that the compounds present in *E. praetermissa* possess antibacterial activity against pathogens associated with skin infections and diarrheal diseases, thus supporting its traditional medicinal use. According to Odion and Ighomena (2025), the observed antibacterial effect could be linked to the presence of long-chain alkanes, benzotriazole, phthalate and butylphenol derivatives, acting individually or through synergistic and additive effects.

Further investigations by Afolayan *et al*. (2017) also demonstrated the antimicrobial potential of *E. praetermissa*. Their study revealed that both hot and cold aqueous extracts of the plant inhibited the growth of fungi and bacteria. The cold water extract exhibited the highest activity against *Aspergillus flavus* with a zone of inhibition of 12.33 mm, while the lowest was observed against *Candida albicans* with 5.33 mm. For the hot water extract, *Aspergillus niger* showed the highest susceptibility (12.67 mm), whereas *Candida albicans* again demonstrated the least (3.33 mm). The minimum inhibitory concentrations (MICs) of the extracts ranged between 3.125 mg/mL and 12.5 mg/mL. Interestingly, the antimicrobial efficacy of the extracts was comparable to that of standard commercial antibiotics. This finding suggests that *E. praetermissa* could be a promising source of novel bioactive compounds with potential applications in drug development, particularly for conditions such as otitis media infections (Afolayan, Onifade and Akindele, 2017).

Overall, these studies provide strong evidence that *E. praetermissa* contains diverse secondary metabolites with significant antimicrobial activities. The correlation between its phytochemical profile and antimicrobial efficacy not only validates its traditional application in the treatment of infectious diseases but also underscores its potential as a candidate for the discovery of new therapeutic agents.

2. Cardiovascular Activity of *Emilia praetermissa*

Emilia praetermissa has a long history of traditional use for the management of hypertension. However, scientific validation of this practice has only begun to emerge in recent years. A study by Ebhohon *et al*. (2025) investigated the antihypertensive potential of the plant's aqueous leaf extract using a salt-induced hypertension model in male Wistar rats. Seven

experimental groups were designed, including controls, salt-loaded hypertensive groups, treatment groups receiving the extract (100 mg/kg), and comparison groups administered standard antihypertensive agents such as captopril (50 mg/kg) and hydrochlorothiazide (10 mg/kg).

Oral administration of the extract for two weeks significantly reduced systolic, diastolic, and mean arterial blood pressures in hypertensive rats ($p \leq 0.05$). Importantly, the safety profile of the extract was highlighted by its high oral acute toxicity threshold ($LD_{50} > 5000$ mg/kg), suggesting it is well tolerated. Beyond blood pressure control, the extract also improved markers of liver and kidney function, regulated lipid profiles, and enhanced antioxidant enzyme activities in cardiac tissue. These findings suggest that *E. praetermissa* exerts systemic protective effects in addition to its antihypertensive action (Ebhohon *et al*., 2025).

The study further reported that treatment with the extract resulted in a significant reduction in circulating levels of angiotensin II (AngII) and aldosterone (ALD), two hormones central to hypertension pathophysiology, while increasing 6-keto-prostaglandin F1α, a vasodilatory metabolite ($p \leq 0.05$). Histopathological analysis confirmed that the extract mitigated myocardial fibrosis, reduced tunica media thickness of both the heart and aorta, and prevented ultrastructural damage in the liver and kidneys of hypertensive rats.

Collectively, these results provide compelling evidence that *E. praetermissa* possesses cardioprotective and antihypertensive activity, validating its ethnomedicinal application in the management of hypertension. The observed effects appear to be mediated through modulation of the renin–angiotensin–aldosterone system, improvement of antioxidant defense, and preservation of vascular and organ integrity (Ebhohon *et al*., 2025).

3. Hyperlipidemic Activity of *Emilia praetermissa*

The potential of *Emilia praetermissa* in regulating lipid metabolism and protecting against cardiovascular disorders has been scientifically investigated. In a study using adult albino Wistar rats, hyperlipidemia was experimentally induced to assess the effects of the plant's aqueous leaf extract on plasma lipid profiles (Anaka *et al*., 2013). Administration of the extract produced a significant decrease ($p < 0.05$) in plasma total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and atherogenic index of plasma (AIP), while significantly increasing high-density lipoprotein cholesterol (HDL-C)

compared to the hyperlipidemic group. Co-administration of the extract with atorvastatin, a standard antihyperlipidemic drug, further enhanced these effects, leading to a more pronounced increase in HDL-C and a decrease in LDL-C and AIP levels.

Elevated plasma TG levels are recognized as both independent and synergistic risk factors for cardiovascular diseases and are commonly associated with conditions such as hypertension, abnormal lipoprotein metabolism, obesity, insulin resistance, and diabetes mellitus (Shepherd, 1998; Zicha et al., 1999; Krauss *et al*., 2006; McBride, 2007; Brunzell et al., 2008). Since there is an inverse relationship between plasma TG and HDL-C levels, the significant reduction of TG by *E. praetermissa* correlates with the observed increase in HDL-C. Low plasma HDL-C levels are considered a risk factor for cardiovascular diseases, whereas higher levels are protective (Rang *et al*., 2005).

The study also reported a significant decrease in LDL-C levels following extract administration. High plasma LDL-C and very-low-density lipoprotein cholesterol (VLDL-C) are strongly linked to cardiovascular disease and are often associated with hypertension, obesity, and diabetes (Shepherd, 1998; Zicha *et al*., 1999; Ademuyiwa et al., 2005; Lichtennstein *et al*., 2006). Furthermore, the extract significantly lowered the atherogenic index of plasma (AIP), an important predictor of cardiovascular risk. AIP values below 0.1 are classified as low risk, values between 0.1–0.24 indicate moderate risk, while values above 0.24 reflect a high risk of cardiovascular disease (Dobiasova, 2004).

These findings suggest that *E. praetermissa* may protect against the development of atherosclerosis and coronary heart disease by modulating lipid profiles and improving cardiovascular health. The plant extract's lipid-lowering properties also indicate potential benefits in managing dyslipidemia associated with obesity, hypertension, diabetes mellitus, and metabolic syndrome. Consequently, *E. praetermissa* may serve as a natural supplement for hyperlipidemic patients, either alone or in combination with conventional drugs such as atorvastatin (Anaka *et al*., 2012).

4. Gastrointestinal Activity of *Emilia praetermissa*

Emilia praetermissa has long been valued in traditional medicine for the management of gastrointestinal disorders, particularly gastric ulcers. Scientific studies have provided evidence supporting its use. Lebeau *et al.* (2021) investigated the gastroprotective and ulcer-

healing potential of the plant's ethanolic extract using two experimental models of chronic gastric ulcer induction: glacial acetic acid and ethanol/aspirin solution. The results demonstrated a marked healing effect, with the extract producing 85.92% ulcer healing in the acetic acid model and complete (100%) healing in the ethanol/aspirin-induced model at a dose of 500 mg/kg.

To further clarify its mode of action, the study assessed the extract's neutralizing capacity in vitro and its cytoprotective effects in vivo. The extract displayed mild antacid properties, as shown by its ability to increase the pH of gastric juice from 2.90 to 3.15 at 25 °C and from 2.06 to 4.07 at 37 °C, compared to the stronger neutralizing effect of sodium bicarbonate, which elevated gastric juice pH to above 8.0 under similar conditions. These findings suggest that *E. praetermissa* exerts a moderate acid-buffering capacity, which may contribute to its gastroprotective role (Lebeau *et al*., 2021).

The study also evaluated the influence of nitric oxide in the plant's mechanism of action. Pretreatment of rats with L-NAME, a nitric oxide synthase inhibitor, resulted in decreased nitrite levels in gastric mucus and increased ulcer index, indicating exacerbation of gastric damage. However, administration of the extract reversed these effects, suggesting that its gastroprotective activity may, at least in part, be mediated by nitric oxide-dependent pathways and enhancement of cytoprotective mechanisms (Lebeau *et al*., 2021).

Overall, these findings highlight the strong gastrointestinal benefits of *E. praetermissa*, demonstrating both anti-ulcerogenic and cytoprotective activities. Its healing effect against chemically induced chronic gastric ulcers, coupled with its ability to buffer gastric acidity and support nitric oxide-mediated protection, validates its ethnomedicinal application in ulcer management and indicates potential for development into gastroprotective therapies.

5. Liver Enzyme Modulation of *Emilia praetermissa*

The effect of *Emilia praetermissa* on liver function and hematological indices has also been examined in stress-related ulcer models. Ebunlomo *et al*. (2012) investigated its activity using cold water immersion to induce stress ulcers in Wistar rats. Twenty animals were divided into four groups: a control group, a saline-treated ulcer group, a post-treatment group administered *E. praetermissa* extract (500 mg/kg) for seven days, and a pre-treatment group given the extract for fourteen days before stress induction.

The study revealed that stress significantly depressed most hematological parameters, except for total white blood cell and platelet counts, when compared with the control. Interestingly, treatment with *E. praetermissa* not only reversed these stress-induced changes but also elevated the hematological parameters beyond control levels, suggesting a stimulatory effect on blood cell formation (Ebunlomo *et al*., 2012).

In terms of biochemical parameters, cold water stress markedly elevated serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are key markers of hepatic injury. However, administration of *E. praetermissa* extract significantly ($p < 0.05$) lowered both ALT and AST levels, indicating hepatoprotective activity through modulation of liver enzymes. This reduction implies that the extract may help maintain hepatic integrity under stress conditions by mitigating oxidative or inflammatory damage associated with ulceration.

Collectively, these findings demonstrate that *E. praetermissa* possesses both haemopoietic and hepatoprotective properties. Its ability to restore hematological parameters while reducing stress-induced elevations of ALT and AST supports its potential as a natural therapeutic agent for improving liver function and protecting against hepatic damage (Ebunlomo *et al*., 2012).

1.6. Toxicity Studies of *Emilia praetermissa*

The toxicological evaluation of *Emilia praetermissa* is crucial for ensuring its safe application in herbal medicine and pharmacological research. As traditional medicinal plants continue to gain scientific validation, assessing their toxicity profiles helps define safe therapeutic limits, identify organ-specific risks, and promote rational use in both traditional and modern healthcare systems.

1.6.1. Safety Assessments

Comprehensive safety assessments of *Emilia praetermissa* have been performed through both acute and sub-chronic toxicity studies in laboratory animals. Findings indicate that aqueous and ethanolic extracts exhibit a wide margin of safety when administered within traditional dosage ranges (Edeoga *et al*., 2005; Ajayi *et al*., 2014).

Acute toxicity evaluations have generally reported high median lethal dose (LD_{50}) values, suggesting low acute toxicity. Sub-chronic toxicity studies have further shown that the plant's extracts do not produce significant adverse effects on hematological or biochemical parameters at moderate doses (Akinmoladun *et al*., 2015).

A study by Lebeau *et al*. (2016) assessed the acute and sub-chronic toxicity of the ethanolic leaf extract of *Emilia praetermissa* in male and female rats. Acute toxicity results showed that doses up to 2000 mg/kg caused no mortality or changes in body weight, behavior, or respiratory rate. In the sub-chronic stage, female rats showed no significant changes in body or organ weights, except slight kidney variations. However, males treated with 500 and 1000 mg/kg doses exhibited significant increases in liver and kidney weights, likely due to heightened liver metabolic activity and inflammation. Also hematological tests revealed a significant increase ($p<0.001$) in total white blood cell (WBC) counts in males, suggesting inflammatory reactions or immune stimulation. This persisted in male satellite groups but not females. Platelet counts also rose significantly, indicating no risk of coagulation problems. Further biochemical findings showed a decrease in plasma HDL cholesterol at 1000 mg/kg, which may increase cardiovascular disease risk if taken in high doses. Bilirubin levels remained normal across all groups, suggesting no adverse effects on hemoglobin metabolism. Creatinine levels increased significantly at 1000 mg/kg and persisted after treatment, indicating possible irreversible kidney damage and impaired filtration at high doses.

Overall, the extract was safe at lower doses but at higher doses (500–1000 mg/kg) caused significant liver, kidney, and lung toxicity, especially with prolonged use.

Long-term exposure at high doses may produce mild alterations in liver and kidney functions, implying potential hepatotoxic and nephrotoxic effects if misused. Hence, the safety of *E. praetermissa* largely depends on dosage, extraction method, and duration of administration. Standardization of extract preparation is recommended to minimize variations and ensure safety across studies.

1.6.2. Dose-dependent Effects

Toxicological and pharmacological evaluations of *Emilia praetermissa* demonstrate clear dose-dependent responses. At lower concentrations, the plant exhibits beneficial effects such as antioxidant, anti-inflammatory, and antimicrobial activities (Owolabi *et al*., 2013). However, excessive dosages have been associated with oxidative stress in liver tissues and mild gastrointestinal irritation in animal studies (Afolayan *et al.*, 2018).

Defining the therapeutic window is therefore essential ensuring that doses used for medicinal purposes maximize efficacy while minimizing adverse outcomes. Such information is critical for developing standardized dosage guidelines for human use.

1.6.3. Side Effects and Contraindications

Reported side effects of *E. praetermissa* are generally mild and reversible, including nausea, mild diarrhea, and transient fatigue when consumed in large quantities (Ajayi *et al*., 2014). Nonetheless, specific populations such as pregnant and lactating women, infants, and individuals with chronic liver or kidney diseases should exercise caution, as safety data in these groups remain limited.

Due to its phytochemical constituents particularly alkaloids and flavonoids prolonged or excessive intake without professional supervision may lead to cumulative toxicity. Therefore, the use of *E. praetermissa* in such individuals should be restricted until further safety validations are conducted.

1.6.4. Drug Interactions

The potential for herb-drug interactions is an important consideration in the clinical use of *Emilia praetermissa*. Its bioactive compounds, including saponins and flavonoids, may interfere with drug-metabolizing enzymes, particularly hepatic cytochrome P450 systems (Owolabi et al., 2013).

Caution is advised when used concurrently with medications that undergo hepatic metabolism, such as antihypertensives, anticoagulants, and antidiabetic drugs. These interactions could alter plasma concentrations or therapeutic responses of co-administered drugs, warranting professional supervision during combined use.

1.6.5. Monitoring and Risk Management

Effective monitoring and risk management strategies are vital for ensuring the safe use of *Emilia praetermissa*. Healthcare practitioners and researchers are encouraged to conduct periodic assessments of hepatic and renal function during prolonged use.

Documentation of adverse reactions should be standardized to aid in establishing comprehensive toxicological databases for the species. Moreover, attention should be given to inter-individual differences such as age, health status, and concurrent drug use, as these factors may influence toxicity outcomes (Akinmoladun *et al*., 2015).

1.6.6. Future Research Needs

Despite promising evidence of safety at moderate doses, extensive toxicological evaluations, particularly chronic and reproductive toxicity studies are still required for *Emilia praetermissa*. Future research should focus on identifying and quantifying specific toxic phytochemicals, elucidating their mechanisms of action, and assessing long-term organ-specific safety in both animal models and clinical settings.

Standardizing extraction and dosage parameters will also improve reproducibility and reliability across different studies.

1.6.7. Recommendations for Clinical Practice

Based on current findings, *Emilia praetermissa* can be considered relatively safe within traditional dosage limits. However, clinical application should include proper patient screening, dose optimization, and continuous monitoring to detect potential adverse effects early.

Healthcare practitioners are advised to integrate traditional knowledge with scientific evidence in order to guide safe and effective use. Additionally, open communication with patients about dosage, potential interactions, and the importance of reporting side effects is essential for maintaining patient safety and improving therapeutic outcomes.

1.7. Anatomy and Functions of the Liver

The liver is the largest internal organ and a central metabolic hub. It sits in the right upper quadrant of the abdomen, beneath the diaphragm, and performs dozens of essential roles such as: metabolic processing and storage, plasma protein synthesis, bile production and excretion, detoxification of endogenous and exogenous compounds, and important immunologic functions. Structurally and functionally the liver is organized to expose hepatocytes to blood from the gut (portal vein) and oxygenated arterial blood (hepatic artery) while routing bile out to the biliary tree.

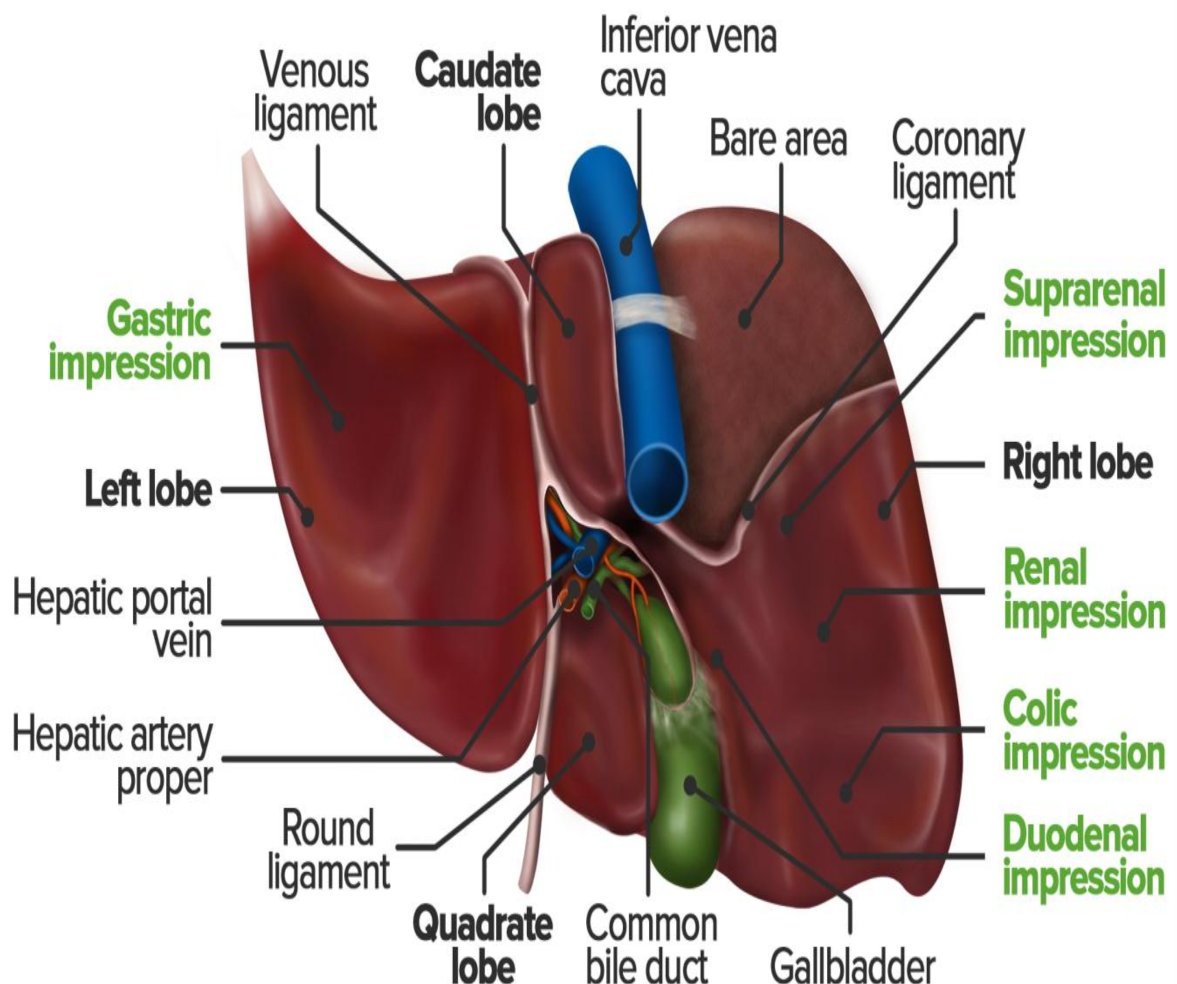


PLATE 2 Inferior view of the visceral surface of the liver.

Lecturio, (2024).

1.7.1. Structure of the Liver

Macroscopic anatomy (surface and ligaments)

i. The liver has a diaphragmatic (anterior, smooth) surface and a visceral (inferior) surface that contacts the stomach, right kidney, and colon.
ii. It is tethered by ligaments (falciform ligament, coronary ligaments, ligamentum teres) and receives the portal triad at the porta hepatis (portal vein, hepatic artery, bile ducts).

Lobes and segments

i. Traditionally the liver is described as right and left lobes (plus caudate and quadrate lobes on the visceral surface). Modern surgical/anatomical descriptions use Couinaud's segmental classification (8 functional segments) based on portal and hepatic venous flow; each segment has its own vascular inflow and biliary drainage, which is important for resections.

Microscopic architecture (functional unit)

i. The lobule (classic hexagonal lobule) is the liver's functional repeating unit. At each hexagon corner is a portal triad (branch of portal vein, hepatic artery, bile duct). Blood flows from the triad area through sinusoids toward a central vein; bile flows in the opposite direction from hepatocytes to bile canaliculi and then to bile ducts.
ii. Hepatocytes are polarized epithelial cells organized in plates one to two cells thick. Important non-parenchymal cells include Kupffer cells (resident macrophages), sinusoidal endothelial cells (fenestrated), and hepatic stellate (Ito) cells (vitamin A storage and fibrosis-mediating cell when activated). Hepatocyte function varies by position in the lobule (zone 1 periportal → zone 3 pericentral), which affects vulnerability to hypoxia and to toxins that require enzymatic activation.

1.7.2. Parts of the liver

i. **Right lobe: this is the** largest, receives most portal flow.
ii. **Left lobe**: it is smaller, and extends across midline.
iii. **Caudate and quadrate lobes: it is** on visceral surface and is defined by porta hepatis and gallbladder landmarks.

iv. **Porta hepatis**: it is the entry/exit for portal vein, hepatic artery, bile ducts, lymphatics and nerves.
v. **Couinaud segments (I–VIII)**: it is clinically important for surgical planning (each segment has separate inflow/outflow and biliary drainage).

1.7.3. Function of the liver

1) Metabolism and homeostasis:

i. **Carbohydrate**: Glycogen synthesis and storage, glycogenolysis and gluconeogenesis to maintain blood glucose during fasting.
ii. **Lipid**: Fatty-acid β-oxidation, ketogenesis, synthesis of cholesterol, bile acids, and lipoproteins (VLDL).
iii. **Protein and nitrogen**: Deamination of amino acids, urea cycle to remove ammonia, synthesis of plasma proteins (albumin, transferrin) and most coagulation factors (except factor VIII produced partly elsewhere).

2) Synthesis and secretion:

i. **Plasma proteins**: Albumin (oncotic pressure, carrier), coagulation factors (fibrinogen, prothrombin, most vitamin K–dependent factors) and many binding proteins.
ii. **Bile**: Production and secretion of bile (bile salts, bilirubin excretion, cholesterol elimination) essential for fat digestion/absorption and removal of certain waste products.

3) Detoxification and biotransformation

Two-phase drug metabolism: Phase I (oxidation/reduction, often cytochrome P450 enzymes) and **Phase II** (conjugation, glucuronidation, sulfation, acetylation) convert lipophilic compounds to water-soluble metabolites for excretion in bile or urine. This system also activates or detoxifies many xenobiotics and endogenous compounds (e.g., steroid hormones). Dysfunction increases drug toxicity risk.

4) Storage and micronutrient handling:

Stores glycogen, fat-soluble vitamins (A, D, E, K), vitamin B12, and minerals (iron as ferritin). Regulation of iron and copper is partly hepatic.

5) Immunologic roles:

Kupffer cells clear bacteria and particulate matter from portal blood; the liver contributes to immune tolerance (important in oral tolerance and systemic immune responses).

1.7.4. Maintaining a healthy liver

Prevention and lifestyle measures reduce liver disease risk and slow progression:

i. **Avoid or limit alcohol: alcohol is broken down by the liver. While moderate amount can be easily broken down, excessive alcohol use can cause liver damage** and risk for alcoholic liver disease and cirrhosis.
ii. **Maintain healthy weight and manage metabolic syndrome:** nonalcoholic fatty liver disease (NAFLD/NASH) is increasing globally with obesity and insulin resistance. Weight loss (5–10% body weight) improves steatosis and inflammation.
iii. **Vaccination and infection control:** hepatitis B vaccination and screening/treatment for hepatitis B and C prevent chronic viral liver disease; safe injection practices, blood safety and harm-reduction reduce transmission.
iv. **Medication safety**: avoid chronic or excessive use of hepatotoxic drugs (acetaminophen overdose is a common cause of acute liver failure), and follow dosing adjustments for people with liver disease.
v. **Balanced diet and exercise**: reduce sugar/refined carbohydrate, saturated fat; increase physical activity to lower risk of NAFLD.
vi. **Regular medical care and screening**: monitor at-risk individuals (chronic viral hepatitis, heavy alcohol use, metabolic syndrome) with periodic liver tests and imaging as indicated.
vii. **Public health measures:** wider vaccination, alcohol policy, obesity prevention are also high-impact strategies to reduce liver-related morbidity and mortality.

1.7.5. Liver Function Tests

Liver function tests (LFTs) is a term for groups of laboratory tests that reflect hepatocellular injury, cholestasis, synthetic function, and protein status. Common components: ALT, AST, ALP, total and direct bilirubin, albumin, total protein, and prothrombin time/INR; globulin

(and the albumin/globulin ratio) is also informative. Interpretation requires clinical context because tests are not disease-specific. The subsections below follow your TOC.

1. Alanine transaminase (ALT) test

ALT (alanine aminotransferase) is an intracellular enzyme concentrated in hepatocytes; serum ALT rises when hepatocytes are injured and leak cytosolic enzymes into the blood.

Clinical use and interpretation:

i. ALT is a sensitive marker of hepatocellular injury (viral hepatitis, toxin-induced injury, ischemic injury, autoimmune hepatitis). Very high ALT (often hundreds to thousands U/L) suggests acute hepatocellular injury (e.g., acute viral hepatitis, ischemic hepatitis, toxin overdose). Mild–moderate elevations may occur in chronic liver diseases (e.g., chronic viral hepatitis, NAFLD).
ii. ALT is more specific for liver injury than AST because AST is found in other tissues (muscle, heart). However, absolute values and patterns matter; labs use their own reference ranges so report-specific norms should be checked. Typical adult reference ranges are on the order of ~7–56 U/L (varies by lab).

Limitations: ALT elevation does not indicate the cause; very advanced chronic liver disease can have near-normal ALT despite severe dysfunction.

2. Aspartate aminotransferase (AST) test

AST (aspartate aminotransferase) is present in liver mitochondria and cytoplasm but also in heart, skeletal muscle, kidney and red blood cells.

Clinical use and interpretation:

i. AST rises with hepatocellular injury but is less liver-specific than ALT.
ii. AST:ALT ratio can be diagnostically useful. An AST/ALT ratio >2 is classically associated with alcoholic liver disease (but is not definitive), while ratios <1 are more typical of viral hepatitis and NAFLD in many cases. Also, marked mitochondrial AST release (very high AST) is seen in severe or ischemic injury. Typical adult AST reference ranges are approximately 8–33 U/L (lab-dependent).

Limitations: Because of extrahepatic sources, elevated AST should be interpreted with CK (creatine kinase) and clinical context if muscle injury is suspected.

3. Alkaline phosphatase (ALP) test

ALP is an enzyme in bile duct epithelium (cholangiocytes) and bone (osteoblasts), intestine and placenta. In the liver, ALP rises when there is cholestasis or obstruction of bile flow.

Clinical use and interpretation:

1. A cholestatic pattern is characterized by disproportionate elevation of ALP and gamma-glutamyl transferase (GGT) with or without bilirubin elevation — suggests extrahepatic bile duct obstruction, cholangiopathies (e.g., primary biliary cholangitis), or intrahepatic cholestasis.
2. Because bone disease can elevate ALP, accompanying GGT (which is more liver-specific) helps determine hepatic origin. Typical adult ALP reference ranges vary by lab but are often ~44–147 U/L (age and lab dependent).

Limitations: ALP alone does not localize the cause (bone vs liver) , also use isoenzyme testing or GGT.

4. Total Protein

The **total protein test** measures the overall concentration of proteins present in the blood serum. Proteins are essential biomolecules that perform numerous functions, including maintaining osmotic pressure, transporting hormones and drugs, immune defense, and acting as enzymes and hormones (Kalra, 2023). The test provides critical information about the synthetic ability of the liver, since most plasma proteins are synthesized in the liver, except for immunoglobulins. **Normal range of total Protein is between** 6.0 – 8.3 g/dL.

Composition and Role

Serum proteins are broadly divided into **albumin** and **globulins**:

i. **Albumin:** Constitutes about 60% of total serum protein and is synthesized exclusively in the liver. It maintains colloid osmotic pressure and transports various endogenous and exogenous substances.

ii. **Globulins:** Represent the remaining 40% and include α, β, and γ-globulins. They play key roles in immunity (immunoglobulins), transport (transferrin), and enzymatic activity (ceruloplasmin).

Therefore, measuring total protein gives a comprehensive overview of **liver synthetic function**, **nutritional status**, and **immunological health** (Lala *et al*., 2023).

Clinical Significance

Abnormal total protein levels can indicate several pathological conditions:

i. **Low Total Protein (Hypoproteinemia):** May result from liver disease (impaired synthesis), malnutrition, nephrotic syndrome (protein loss in urine), or gastrointestinal protein loss.
ii. **High Total Protein (Hyperproteinemia):** May occur in chronic infections, inflammatory disorders, or certain malignancies such as multiple myeloma, where there is excessive production of immunoglobulins.

In liver disease, **decreased total protein** is often accompanied by **reduced albumin** and **elevated globulin** levels, altering the **albumin-to-globulin (A/G) ratio** (usually between 1.0 – 2.2 in healthy individuals).

Clinical Interpretation

i. A **low total protein with low albumin** suggests **liver failure** or **protein-energy malnutrition**.
ii. A **high total protein with increased globulin** suggests **chronic inflammation** or **autoimmune disease**.
iii. Persistent **abnormal A/G ratio** can indicate **chronic liver disease** or **monoclonal gammopathies**.

5. Globulin test (GLB)

Globulin usually refers to the total serum globulin fraction, the sum of non-albumin serum proteins (α1, α2, β, and γ globulins). Total globulin is commonly calculated as:

total protein − albumin = globulin.

More detailed characterization (serum protein electrophoresis, immunofixation) separates the globulin classes and detects monoclonal spikes.

Why is the Globulin Test Important?

Globulins include immunoglobulins (gamma globulins) and numerous transport and acute-phase proteins. Measuring globulins (and patterns on electrophoresis) helps detect immune activation, chronic inflammation, monoclonal gammopathies (e.g., multiple myeloma), and some liver and kidney disorders. The globulin fraction also influences the albumin/globulin (A/G) ratio, an additional interpretive tool.

How is the Globulin Test Performed?

i. **Basic test:** Total serum protein and albumin are measured with the formula below:

globulin = total protein − albumin (automated chemistry analyzers).

ii. **Further testing:** If abnormalities appear, **serum protein electrophoresis (SPEP)** shows patterns (polyclonal broad increases vs monoclonal spikes). **Immunofixation** and quantitative immunoglobulin measurements (IgG, IgA, IgM) further characterize abnormalities. Blood is drawn via venipuncture like other serum chemistries.

Normal range for the Globulin Test

Typical reported globulin ranges vary by lab and units. A common adult reference range for total globulin is approximately **2.0–3.5 g/dL** (20–35 g/L), but values depend on the laboratory method and population. Always compare to the lab’s reference interval on the report.

Interpreting Globulin Test Results

i. **Elevated globulin (hyperglobulinemia)**: look at whether the increase is polyclonal (broad-based increase on SPEP → chronic inflammation, infection, autoimmune disease) or monoclonal (sharp “M-spike” on SPEP → monoclonal gammopathy, multiple myeloma, Waldenström macroglobulinemia).
ii. **Low globulin (hypoglobulinemia)**: may reflect reduced immunoglobulin production (e.g., primary or secondary immunodeficiency), protein-losing states (nephrotic

syndrome, protein-losing enteropathy), or severe liver synthetic failure (because many globulins are produced outside the liver; albumin is made in the liver but some globulins are made by plasma cells). Correlate with clinical features and other labs (albumin, total protein, A/G ratio, SPEP).

Caues of elevated globulin levels may indicate:

i. **Chronic infection** (e.g., chronic viral hepatitis, parasitic infections)
ii. **Autoimmune diseases** (e.g., autoimmune hepatitis, rheumatoid arthritis, SLE)
iii. **Chronic inflammatory states** (e.g., chronic liver disease with immune stimulation)
iv. **Monoclonal gammopathies / hematologic malignancies** (e.g., multiple myeloma — needs SPEP and hematology workup)
v. **Hypergammaglobulinemia in certain cholestatic liver diseases** (e.g., primary biliary cholangitis typically shows elevated IgM; autoimmune hepatitis often shows high IgG).

Possible causes of low globulin levels are: primary/secondary immunodeficiency (reduced immunoglobulin production), protein-losing conditions (nephrotic syndrome, severe enteropathy), malnutrition, or laboratory artifact (e.g., dilution). In advanced fulminant liver failure, total protein and albumin fall; globulin patterns vary because many immunoglobulins are produced by plasma cells, not hepatocytes.
Clinical correlation and further testing (quantitative immunoglobulins, urine protein) are crucial.

6. Albumin

Albumin is the **most abundant plasma protein**, synthesized exclusively by hepatocytes at a rate of about 10–15 g/day.
It constitutes approximately **60% of total serum protein**, with normal concentrations between **3.5–5.0 g/dL**.

Physiological Functions

i. **Maintains plasma oncotic pressure:** Prevents fluid from leaking into interstitial spaces; low albumin leads to edema or ascites.
ii. **Transport protein:** Binds and carries bilirubin, free fatty acids, hormones (thyroxine, cortisol), calcium, and drugs.

iii. **Buffer system:** Contributes to acid-base balance.
iv. **Antioxidant and scavenger role:** Binds toxic compounds and reactive species.

Clinical Significance

Because albumin is synthesized only in the liver, its plasma concentration reflects **hepatic synthetic function**, but it changes slowly (half-life ≈ 20 days). Thus, it indicates **chronic liver disease** rather than acute injury.

Causes of Hypoalbuminemia

i. **Chronic liver disease:** (cirrhosis, hepatitis), decreased synthesis.
ii. **Protein-losing states** (nephrotic syndrome, enteropathy, burns).
iii. **Malnutrition or malabsorption** (inadequate amino acid supply).
iv. **Inflammation:** albumin is a negative acute-phase reactant; levels fall during systemic inflammation.
v. **Dilutional states:** overhydration, pregnancy.

Clinical Significance

i. Marker of **hepatic synthetic capacity:** low levels suggest advanced liver dysfunction or chronic liver disease.
ii. Used in **disease severity scoring systems** (e.g., Child-Pugh classification for cirrhosis).
iii. Assists in evaluating **nutritional status**.

Forms part of the **albumin-to-globulin (A/G) ratio**, which further differentiates causes of altered serum protein levels.

Abnormal albumin-to-globulin (A/G) ratio

A:G ratio = albumin ÷ globulin. A normal A:G ratio is **slightly >1** (more albumin than globulins), but normal ranges vary by lab.

Low A:G ratio (<1): common causes are increased globulins (chronic inflammation, multiple myeloma, autoimmune disease) or decreased albumin (chronic liver disease, nephrotic syndrome, malnutrition). A low ratio prompts evaluation of both albumin and globulin fractions.

High A:G ratio (>normal): may reflect low globulins (e.g., immunodeficiency) or elevated albumin (dehydration). Clinical context guides further workup.

A single abnormal A:G ratio is a screening clue SPEP, quantitative immunoglobulins, and tests for liver synthetic function (albumin, INR/prothrombin time) help narrow the cause.

7. Gamma-Glutamyl Transferase (GGT)

Gamma-glutamyl transferase (GGT) is a membrane-bound enzyme involved in **the transfer of γ-glutamyl groups** between peptides and amino acids. It plays a central role in **glutathione metabolism**, helping maintain intracellular antioxidant defense and detoxification capacity.
It is predominantly found in **hepatocytes and bile duct epithelial cells**, but also occurs in the pancreas, kidneys, and intestinal lining.

Because GGT is highly concentrated in the **bile canalicular and microsomal membranes** of hepatocytes, elevated serum levels are typically associated with **cholestasis** and **bile duct obstruction**. However, GGT is **more sensitive than specific**, and elevations can occur in various systemic and metabolic conditions.

Adult serum GGT reference range: approximately **9–48 U/L** (values vary slightly by lab and gender).

Physiological Function

i. Participates in the **gamma-glutamyl cycle**, enabling amino acid transport across membranes and regeneration of intracellular glutathione (GSH).
ii. In the liver, GGT helps protect cells against oxidative stress by supporting the recycling of GSH, the body's major antioxidant defense molecule.

Causes of Elevated GGT

i. **Cholestatic liver disease** (e.g., gallstones, biliary atresia, primary biliary cholangitis)
ii. **Alcoholic liver disease:** GGT is induced by chronic alcohol consumption. Elevations often precede other enzyme abnormalities.

iii. **Drug-induced enzyme induction:** phenytoin, barbiturates, carbamazepine, and certain herbal products.
iv. **Non-alcoholic fatty liver disease (NAFLD)** and **metabolic syndrome**
v. **Hepatocellular carcinoma or metastatic liver disease**
vi. **Pancreatic or renal disease** (mild elevations possible)

Clinical Significance

i. **Differentiation aid:** When ALP is elevated, simultaneous elevation of GGT confirms **hepatic origin** rather than bone or placental origin.
ii. **Monitoring alcohol abstinence:** GGT levels gradually normalize after 2–6 weeks of alcohol cessation, making it a useful adherence marker in rehabilitation.
iii. **Drug hepatotoxicity screening:** GGT rises early in hepatic microsomal enzyme induction.

Clinical Interpretation

i. **Isolated elevation:** Often reflects enzyme induction from alcohol or drugs, not structural damage.
ii. **Elevated with ALP:** Indicates cholestasis or biliary obstruction.
iii. **Elevated with ALT/AST:** Suggests mixed hepatocellular and cholestatic pattern.

7. 5′-Nucleotidase (5′-NT)

5′-Nucleotidase is a **cell membrane enzyme** that catalyzes the hydrolysis of nucleotides, removing phosphate groups from 5′-nucleotides (e.g., AMP → adenosine + phosphate).
It is present in many tissues, **especially the liver, kidney, heart, and brain,** but is particularly **abundant in hepatocytes and biliary epithelial cells**, where it contributes to nucleotide metabolism. Typical adult reference range: **2–17 U/L.**

5′-Nucleotidase is a **sensitive marker of cholestasis** and biliary tract disease.
Like GGT, it increases when bile canaliculi are obstructed or bile flow is impaired, but unlike GGT, it is **not induced by alcohol or drugs**, making it a **more specific indicator of hepatic or biliary dysfunction**.

Physiological Function

i. Facilitates the **catabolism of nucleotides** during cellular metabolism and injury.
ii. Plays a role in maintaining **nucleotide balance** and supports the salvage pathways for purine and pyrimidine synthesis.

Causes of Elevated 5′-Nucleotidase

i. **Intrahepatic or extrahepatic cholestasis** (e.g., gallstones, tumors, biliary cirrhosis)
ii. **Infiltrative liver diseases** (e.g., metastases, amyloidosis)
iii. **Hepatitis** (viral, autoimmune, or toxic)
iv. **Primary sclerosing cholangitis**
v. **Granulomatous liver disease (e.g., sarcoidosis)**

Clinical Utility

i. Confirms **hepatic origin of elevated ALP** when GGT is inconclusive.
ii. Useful for distinguishing **bone vs. liver ALP elevations**, as 5′-NT is not found in bone tissue.
iii. Serves as a **specific cholestatic enzyme**, especially valuable when alcohol or drug induction complicates GGT interpretation.

Clinical Interpretation

i. **Elevated ALP + Elevated 5′-NT:** Hepatic or biliary cause (cholestasis).
ii. **Elevated ALP + Normal 5′-NT:** Suggests bone or placental origin.
iii. **Elevated 5′-NT with mild ALT/AST rise:** Indicates early or partial bile duct obstruction.

8. Total and Direct (Conjugated) Bilirubin

Bilirubin is a yellowish pigment that results from the normal breakdown of heme, a component of hemoglobin found in red blood cells. The liver plays a central role in bilirubin metabolism, and measuring bilirubin levels in the blood is an important diagnostic tool for assessing liver function and identifying possible liver or biliary tract diseases (Ruiz *et al.*, 2021).

When old or damaged red blood cells are destroyed by macrophages in the spleen and bone marrow, the heme portion of hemoglobin is converted into biliverdin by the enzyme **heme oxygenase**, and subsequently reduced to bilirubin by **biliverdin reductase**. This bilirubin, which circulates in the blood bound to albumin, is **unconjugated** or **indirect bilirubin**, and is **water-insoluble**.

Upon reaching the liver, unconjugated bilirubin is taken up by hepatocytes and conjugated with glucuronic acid by the enzyme **UDP-glucuronyl transferase**, forming **conjugated (direct) bilirubin**, which is **water-soluble**. This conjugated bilirubin is then excreted into the bile and passes into the intestine, where it is further converted to **urobilinogen** and **stercobilin**, giving feces their characteristic brown color (McPherson, 2021).

The **Total Bilirubin** level in the blood represents the **sum of both unconjugated (indirect)** and **conjugated (direct)** bilirubin. The **Direct (Conjugated) Bilirubin** test specifically measures the amount of bilirubin that has undergone conjugation in the liver.

An elevated **Total Bilirubin** level may result from increased production (as seen in hemolytic anemia), impaired hepatic uptake, defective conjugation, or obstruction of bile flow. On the other hand, an isolated increase in **Direct Bilirubin** often indicates **cholestasis**, **biliary obstruction**, or **hepatocellular injury** that impairs bile excretion (Creeden *et al.*, 2021). **Normal ranges for total Bilirubin is** 0.2 – 1.2 mg/dL, while d**irect (Conjugated) Bilirubin is** 0.1 – 0.3 mg/dL.

Clinical Significance

i. **Elevated Total Bilirubin** may suggest liver dysfunction, hemolysis, or disorders such as Gilbert's syndrome.
ii. **Elevated Direct Bilirubin** typically indicates obstruction of bile ducts, viral hepatitis, cirrhosis, or cholestatic liver disease.
iii. **Low Bilirubin Levels** are usually of little clinical concern but can occur with increased antioxidant activity or in individuals with high vitamin C intake (Gowda *et al*., 2009).

Interpretation of Results

In clinical diagnostics, the ratio of **direct to total bilirubin** aids in determining the nature of liver dysfunction:

i. **Predominantly Unconjugated Hyperbilirubinemia:** suggests pre-hepatic causes such as hemolysis or defective conjugation (e.g., Gilbert’s or Crigler-Najjar syndrome).

ii. **Predominantly Conjugated Hyperbilirubinemia:** suggests intrahepatic or post-hepatic causes such as hepatitis, bile duct obstruction, or Dubin–Johnson syndrome.

CHAPTER TWO

2.1 Materials and Methods

2.1.1 Equipment and apparatus

The following equipment and apparatus were used for this study and their make:

EQUIPMENTS	MODEL/MANUFACTURERS
Analytical weighing balance (Mettler Toledo)	OMAUS Corp. Pioneer, U.S.A
Beakers (10ml, 50ml, 100ml, 250ml)	Pyrex, UK
Centrifuge	Thermo Fisher, Inc. USA
Conical flask (100ml,200ml,500ml)	Pyrex, UK
Measuring cylinder	Pyrex, England
Micropipettes	Gilson, US
PH meter	Mettler H8, UK
Refrigerator (HA-137)	Haier Thermocool, Nigeria
Spectrophotometer (20D S23A)	Teckmel and Teckmel, U.S.A
Test tubes	Pyrex, UK
Water bath	Ametek, Inc. USA

2.2 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 Easth 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.

2.2 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 using rotary evaporator (local gin extract) and freeze drying (aqueous extract). The extracts were then weighed. There after, the extracts were stored in sterile containers at 4°C until use.

2.3 Animal Studies

2.3.1 Experimental animals

Adult male *Wister* rats (100-150g) were used for this study of *Emilia praetermissa* extracts. The animals were housed under standard laboratory conditions (12-hour light/dark cycle, 20–26°C), with free access to food and water and were acclimatized for 7 days prior to the experiment. Doses of the extracts were administered orally, and the animals were observed for 28 days for signs of toxicity or mortality, following OECD guidelines.

2.3.1. Animal grouping

The rats were randomly divided into four groups of six animals each (n=6). Group 1 served as the control and received only the vehicle solution. Groups 2, 3, and 4 received escalating oral doses of the *Emilia praetermissa* extract (e.g., 300 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight, respectively). This grouping allowed for the assessment of dose-dependent effects on anti-oxidant potential and toxicity parameters. Each group was observed

individually to monitor clinical signs, behavioural changes, and mortality over a period of 28 days.

2.3.2 Measurement of body weight

Each rat's body weight was recorded weekly after the acclimatization period. This helped to monitor any weight changes due to the administration of *Emilia praetermissa* extracts and to determine the appropriate dosage of the extract.

2.4 Acute Toxicity Studies

Acute toxicity assessment was conducted in two stages, adhering to the modified Lorke's methodology (1983):

Phase 1: Six rats per extract type (local gin and water) were categorized into three groups of two animals each, receiving 10mg/kg, 100mg/kg and 1000mg/kg body weight of extract via oral administration. Animals were observed for a 24-hour period for indications of toxicity and mortality.

Phase 2: Based on Phase 1 outcomes, six rats per extract type were administered 1600mg/kg, 2900mg/kg and 5000 mg/kg body weight of extract orally. The animals were observed for 24hrs for behaviour and mortality.

Calculation:

$LD_{50} = \sqrt{D_0 \times D_{100}}$

Where;

LD_{50} = Lethal dose

D_0 = highest dose that gave no mortality

D_{100} = lowest dose that produced mortality

2.4.1 Experimental animals

Adult male *Wistar* rats, weighing between 100-150g, were obtained from the institutional animal facility. The animals were housed in standard cages under controlled laboratory conditions (temperature 22-25°C, 12-hour light/dark cycle, relative humidity 45-55%). All animals underwent acclimatization for two-weeks prior to experimentation and had unrestricted access to standard rodent feed and water *ad libitum*.

2.4.2 Experimental design

The investigation was performed over four weeks utilizing the aqueous and local gin extracts. Rats were randomly allocated into four groups:

Group Control: Received vehicle used in dissolving the extract (distilled water)

Group A: Received 100 mg/kg body weight of the extract

Group B: Received 250 mg/kg body weight of the extract

Group C: Received 500 mg/kg body weight of the extract

Body weights were recorded weekly throughout the study duration.

2.4.3 Blood collection

Blood samples were collected at weeks 1, 2, 3 and 4 via cardiac puncture under light anesthesia. Samples were separated into two portions: one for haematological analysis and the other centrifuged at 3000rpm for 15 minutes to obtain serum for liver function tests. Blood samples for haematological analysis was collected into containers while that for biochemical analysis into plain tubes .

2.4.4 Organ collection

At the conclusion of each experimental period, animals were euthanized under anesthesia. The liver, heart, and kidneys were meticulously excised, cleaned of adhering tissues, and weighed. Relative organ weights were computed as a percentage of body weight.

2.5 Biochemical Assays

2.5.1 Liver function status indices

2.5.2 Assay for alanine aminotransferase (ALT) activity

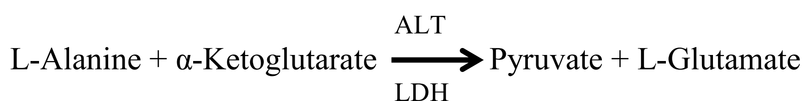
Method:

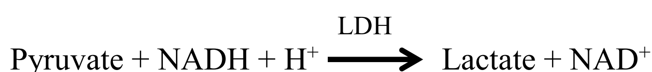
The activity of Alanine Aminotransferase (ALT) was determined using the method of Reitman and Frankel (1957), as specified by Agappe Diagnostics.

Principle:

Alanine Aminotransferase (ALT) catalyzes the transfer of an amino group from L-alanine to 2-oxoglutarate, forming pyruvate and L-glutamate.

Pyruvate then reacts with NADH in the presence of Lactate Dehydrogenase (LDH) to yield lactate and NAD^+. The rate of oxidation of NADH to NAD^+, measured as a decrease in absorbance at 340 nm, is directly proportional to ALT activity.





The rate of decrease in absorbance at 340 nm is proportional to ALT activity.

Reagent Composition:

Reagent 1 (R_1)**:** Tris buffer (pH 7.4) containing L-Alanine and LDH

Reagent 2 (R_2)**:** α-Ketoglutarate solution

Procedure:

Exactly 1.0 mL of Reagent 1 (containing Tris buffer, L-alanine, and LDH) was dispensed into two clean test tubes labeled as *blank* and *sample*. To the blank tube, 0.1 mL of distilled water was added, while 0.1 mL of serum sample was added to the sample tube. Thereafter, 0.25 mL of Reagent 2 (α-ketoglutarate solution) was added to both tubes. The mixture was gently mixed and incubated at 37°C for one minute. The initial absorbance of the sample was

read at 340 nm against the blank using a spectrophotometer, followed by readings taken at one-minute intervals for three minutes. The mean change in absorbance per minute ($\Delta A/min$) was obtained, and the enzyme activity was also calculated.

Protocol:

Tube	Reagent 1	Sample/distilled water	Reagent 2
Blank (mL)	1.0 ml	0.1 mL distilled water	0.25 mL
Sample (mL)	1.0 ml	0.1 mL serum	0.25 mL

Calculation:

ALT Activity (U/L) = $\Delta A/min \times F$

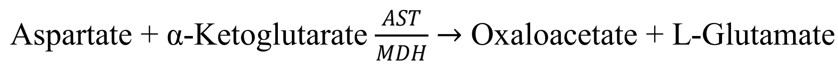
Where **F** is the factor provided by the manufacturer (1745).
One unit (U) of ALT activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADH per minute at 37°C.

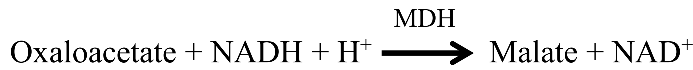
2.5.3 Assay for aspartate aminotransferase (AST) activity

Method:
The activity of Aspartate Aminotransferase (AST) was determined using the method described by Reitman and Frankel (1957), as adopted by Agappe Diagnostics.

Principle:
Aspartate Aminotransferase (AST) catalyzes the reversible transfer of an amino group from L-aspartate to 2-oxoglutarate, forming oxaloacetate and L-glutamate.
Oxaloacetate subsequently reacts with NADH in the presence of Malate Dehydrogenase

(MDH) to yield malate and NAD^+. The rate of oxidation of NADH to NAD^+ is directly proportional to AST activity and is measured as a decrease in absorbance at 340 nm.


Aspartate + α-Ketoglutarate $\frac{AST}{MDH}$ → Oxaloacetate + L-Glutamate


Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+

The rate of decrease in absorbance at 340 nm is proportional to AST activity.

Reagent Composition:

Reagent 1 (R_1)**:** Tris buffer (pH 7.8) containing L-Aspartate, LDH, and MDH

Reagent 2 (R_2)**:** α-Ketoglutarate solution

Procedure:

Exactly 1.0 mL of Reagent 1 (containing Tris buffer, L-aspartate, LDH, and MDH) was pipetted into two clean test tubes labeled as *blank* and *sample*. To the blank tube, 0.1 mL of distilled water was added, while 0.1 mL of serum sample was added to the sample tube. Then, 0.25 mL of Reagent 2 (α-ketoglutarate solution) was added to both tubes. The contents were mixed gently and incubated at 37°C for one minute. After incubation, the initial absorbance of the sample was measured at 340 nm against the blank using a spectrophotometer. Subsequent absorbance readings were taken at one-minute intervals for three minutes. The mean change in absorbance per minute (ΔA/min) was determined, and the enzyme activity was calculated using the manufacturer's factor.

Protocol:

Tube	Reagent 1	Sample/distilled water	Reagent 2
Blank (mL)	1.0 ml	0.1 mL distilled water	0.25 mL
Sample (mL)	1.0 ml	0.1 mL serum	0.25 mL

Calculation:

AST Activity (U/L) = $\Delta A/min \times F$

Where **F** is the factor provided by the manufacturer (1745).
One unit (U) of AST activity is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of NADH per minute at 37°C.

2.5.4 Assay of alkaline phosphatase (ALP) activity

Method:
The activity of ALP was measured using the method of Englehardt (1970).
Principle:
Alkaline phosphatase (ALP) hydrolyzes p-nitrophenyl phosphate in the presence of diethanolamine to form p-nitrophenol. Under alkaline conditions, p-nitrophenol is converted into p-nitrophenoxide ions, which exhibit a yellow coloration. The intensity of this color is directly proportional to the enzyme activity. The absorbance is measured at 405 nm.
pNPP + H_2O → pNP + Phosphate. (The reaction takes place in the presence of ALP).
The rate of increase in absorbance at 405nm is proportional to ALP activity.
Reagent composition:
Reagent 1a: Diethanolamine buffer (1mol/L, pH 9.8) + $MgCl_2$ (0.5mmol/L)
Reagent 1b: p-Nitrophenyl phosphate (10mmol/L)
Note: Reagent 1a and 1b were mixed to prepare the working reagent.
Procedure:
In a blank tube, 0.02mL of distilled water and 1mL of the prepared working reagent were added.
In a sample tube, 0.02mL of serum and 1mL of the prepared working reagent were added.
The spectrophotometer was zeroed at 405nm using the blank. The initial absorbance of the sample at 405nm was then measured. Subsequent absorbance readings were taken at 1, 2, and 3 minutes.

Protocol:

Tube 1 (Blank)	Tube 2 (Sample)	Condition
0.02 mL distilled water + 1.0 mL working reagent	0.02 mL serum + 1.0 mL working reagent	Mix gently
Immediately measure initial absorbance at 405 nm (0 min)		
Record absorbance again at 1, 2, and 3 minutes		
Use Blank tube to zero the spectrophotometer	Compare absorbance change (ΔAbs/min) with sample tube	Enzyme activity is proportional to increase in absorbance

Calculation:

The activity of ALP is determined based on the rate of absorbance change over time. ALP was calculated using the formula:

ALP activity(U/L)=ΔA/min×F

The enzyme activity is usually expressed in units per liter (U/L), where one unit (U) is defined as the amount of enzyme that hydrolyzes 1 micromole of pNPP per minute at 37°C.

2.5.5 Total protein

Total protein was estimated using the Bradford protein assay method. Bradford protein assay is used to measure the concentration of total protein in the sample (Bradford, 1976).

Principle: The principle of this assay is based on the binding of protein molecules to **Coomassie Brilliant Blue G-250 dye** under acidic conditions, which causes a color change from **reddish-brown to blue** and a spectral shift in the dye's absorbance maximum from ~ 465 nm to ~ 595 nm. The dye binds primarily to **basic amino acid residues** (especially arginine, lysine, and to a lesser extent histidine, as well as aromatic residues) through electrostatic and hydrophobic interactions. The intensity of the blue color formed is **proportional** to the protein concentration in the sample and is measured at **595 nm**.

Reagent Composition:

Reagent 1a: Coomassie Brilliant Blue G-250 dye

Reagent 1b: Phosphoric acid (acidic medium)

Reagent 1c: Methanol or ethanol (to help solubilize the dye and stabilize it)

Procedure: protein standards were prepared with five to eight dilutions of a protein standard - bovine serum albumin (BSA) with a concentration range of 5 to 100 µg protein. Unknown protein samples were diluted to contain 5-100 µg protein in 30 µl. 30 µl of each standard solution or unknown protein sample were added to appropriately labeled test tubes. Two blank tubes were set up: one with 30µl H_2O for the standard curve and one with 30µl protein preparation Tris-HCl buffer (pH 7.4–8.0) buffer for unknown samples. Protein solutions were assayed in triplicate. 1.5ml of Bradford reagent was added to each tube and mixed well. Incubation was done at room temperature for at least 5 minutes but not more than 1 hour. Absorbance was measured at 595nm.

Calculation:

Protein concentration of unknown (µg/ml) =

$$\frac{(\text{Asample} - \text{c})}{m}$$

where:

Asample = absorbance of the unknown sample at 595 nm

m = slope of the standard curve

c = intercept of the standard curve

2.5.6 Albumin (ALB)

Principle:

This method relies on **specific binding** of the **bromocresol green (BCG)** dye to albumin in acidic conditions, producing a green-blue complex whose absorbance is measured, typically around **628–630 nm**.

The absorbance of this complex is **proportional to the albumin concentration**. However, note that the BCG method can suffer interference from **α_1- and α_2-globulins**, especially in disease states (e.g. systemic inflammation), leading to positive bias in measured albumin.

Reagent Composition:

i. Bromocresol Green dye
ii. Acid buffer (commonly succinate buffer, pH ~ 4.20)
iii. Distilled water or buffer diluents

The spectrophotometer was set at wavelength 630 nm (with a range of 600-650 nm), cuvette had a 1 cm light path, and temperature was between 15-25 °C. The instrument was adjusted to zero with distilled water. Reagents were pipetted into a test tube with specifications including 1 mL blank, 1 mL sample, 1 mL standard, and 5ml of the BCG dye reagent. The mixture was mixed and incubated for 10 minutes at room temperature (15-25 °C). The absorbance (A) of the samples and standard were read against the blank. The colour was stated to be stable for 60 minutes at room temperature.

Protocol:

Tube 1 (Blank)	**Tube 2 (Sample)**	**Condition**
1.0 mL distilled water + 5.0 mL BCG reagent	1.0 mL serum + 5.0 mL BCG reagent	Mix gently
Incubate for 10 minutes at room temperature (15–25 °C)		
Use Blank tube to zero the spectrophotometer at 630 nm	Measure absorbance of sample against Blank	Absorbance of the green-blue complex is proportional to albumin concentration. The colour remains stable for 60 minutes at room temperature

Calculation:

(As) Sample
(As) Standard
X 5 (Standard conc) = g/dL albumin

Albumin concentration (g/dL) = $\frac{\text{Astandard}}{\text{Asample}}$ × (Concentration of standard in g/dL)

Albumin (g/dL) = $\frac{\text{A(STD)}}{\text{A(S)}} \times 5$

2.5.7 Globulin

Globulin levels are often calculated rather than directly measured . The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration.

Calculation:

Globulin = Total protein concentration - Albumin concentration

2.5.8 Total Bilirubin

Bilirubin is a breakdown product of hemoglobin. It is transported from the spleen to the liver and excreted into bile (Hall and Hall, 2020).

Principle: Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. In the determination of indirect bilirubin, the direct is also determined, the results correspond to total bilirubin. The intensity of the color formed is proportional to the bilirubin concentration in the sample (Burtis *et al*., 2012).

Reagent Composition:

R_1 (Total Bilirubin Reagent): Contains diazotized sulfanilic acid

R_2 (Total Bilirubin Nitrite Reagent): Contains sodium nitrite solution

Procedure: The wavelength of the instrument was set at 555nm (530-580)nm and temperature of 15-25 °C. The instrument was adjusted to zero with distilled water. Total bilirubin reagents (R_1) were pipetted into a cuvette with specifications including 1.5 mL blank, 1.5 mL sample. Total bilirubin, nitrite reagent (R_2) were pipetted with specifications including 50 µL blank and 100 µL sample, and 100 µL sample added to the blank . The mixture was mixed and incubated for 10 minutes at room temperature of 15-25°C. The absorbance (As) of the samples and standard was read against the blank.

Protocol:

Reagent	Blank (mL)	Standard (mL)	Sample (mL)
R_1	1.5	1.5	1.5
R_2	0.05	0.05	0.10

Calculation:

With calibrator:

(As)Sample - (As) Sample Blank x Conc. Calibrator = mg/dL

(As) Calibrator - (As)Calibrator Blank with Factor:

bilirubin (As)Sample - (As) Sample Blank x 19.1 = mg/dL bilirubin in the sample

Conversion factor: mg/dL x 17.1 = µmol/L

2.5.9 Direct bilirubin

The calorimetric method was used in the determination of direct bilirubin (Horsfall and Chapman, 1997).

Principle: Bilirubin is converted to coloured diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin glucuronide and free bilirubin loosely bound to albumin. only the former reacts directly in aqueous solution (bilirubin direct) (Doumas *et al*., 1973).

Reagent Composition:

R_1(Direct Bilirubin Reagent): Contains diazotized sulfanilic acid

R_2 (Direct Bilirubin Nitrite Reagent): Contains sodium nitrite solution

Procedure: Direct bilirubin reagent (R_1) were pipetted with specifications including 1.5µL blank and 1.5mL sample. Direct bilirubin Nitrite reagent (R_2) were pipetted with specifications including 50µL sample, and 100µL sample added to the blank. The mixture was mixed and incubated for 5 minutes at temperature of 20 - 25°C. The absorbance (As) of the samples and standard was read against the blank at 546nm (530-580nm).

Protocol:

Reagent	Blank (mL)	Standard (mL)	Sample (mL)
R_1	1.5	1.5	1.5
R_2	0.05	0.05	0.10

Calculation:

{(As)Sample - (As) Sample blank} x Factor = mg/dL

Direct bilirubin factor = 14

Conversion Factor = mg/dl x 17.1 = µmol/L

CHAPTER THREE

3.0. RESULTS

This chapter presents the findings on the toxicity studies and effects of *Emilia praetermissa* Milne-redh extracts on body weight. Also contains the result for the liver function status (analytes) of male *Wistar* rats administered *Emilia praetermissa*. The results are reported as means ± SEM and include statistical significance where applicable ($p < 0.05$).

3.1. ACUTE TOXICITY STUDIES IN MALE *WISTAR* RATS ADMINISTERED *Emilia praetermissa* Milne-redh LEAF EXTRACTS (PHASE 1 and 2)

(Table 3.1 and Table 3.2) The acute toxicity studies were conducted in two phases. In Phase 1, two different extracts (local gin, and aqueous) were administered at doses of 10, 100, and 1000 mg/kg body weight. No mortality or observable signs of toxicity were recorded across all groups. In Phase 2, higher doses of 1600, 2900, and 5000 mg/kg body weight were administered, and similarly, no mortality or signs of toxicity were observed. This indicates that the LD_{50} of *Emilia praetermissa* Milne-redh leaf extracts is greater than 5000 mg/kg body weight.

3.2. MEAN BODY WEIGHT OF *WISTAR* RATS ADINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

Table 3.3 presents the body weights of rats administered different doses of local gin and aqueous extract over two weeks. The control group showed the highest initial body weight (225.04 ± 0.00 g), while Group 6 (250 mg/kg bw aqueous extract) showed the lowest initial weight (105.46 ± 0.00 g). By week 2, all treatment groups showed slight variations in body weight, with Group 2 (500 mg/kg bw local gin extract) maintaining the highest slight weight among treated groups (206.02 ± 0.00 g).

3.3. PERCENTAGE CHANGE IN BODY WEIGHT OF *WISTAR* RATS ADMINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS.

Table 3.4 shows the percentage change in body weight of *wistar* rats administered *Emilia praetermissa* Milne-redh leaf extracts. The acclimatized *wistar* rats were placed in groups (7 groups of 6 rats each). The control (group 1) received no administration. The week 1 and week 2 (group 2 to 7) rats were administered *Emilia praetermissa* extract for two weeks respectively. The body weights of the rats in the different groups were determined before and after administration and the percentage change in body weight calculated.

3.4. ORGAN BODY WEIGHT OF WISTAR RATS ADMINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

Table 3.5 shows the weight of the liver, heart, right and left kidneys respectively. The control was sacrificed on the day zero, the week 1 rats after one week and the week 2 rats after two weeks. The weight of the organs was determined.

3.5. ORGAN BODY WEIGHT RATIO OF WISTAR RATS ADMINISTRATION *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

Table 3.6 shows the organ body weight ratio of the control and the weeks 1 and 2 *wistar* rats administered *Emilia praetermissa* extract.

3.6. LIVER FUNCTION INDICES (ANALYTES) OF WISTAR RATS ADMINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

Table 3.7 shows the results of the liver function analytes (total protein, albumin, globulin, total bilirubin, direct bilirubin) present in the serum of control, group two to group 7 animals respectively.

Table 3.1 ACUTE TOXICITY STUDIES IN WISTAR RATS ADMINISTERED *Emilia praetermissa* Milne-redh LEAF EXTRACTS

PHASE 1

DOSE	EXTRACT	NO. OF ANIMALS	MORTALITY
10mg/kg bw		03	Nil
100mg/kg bw	LOCAL GIN	03	Nil
1000mg/kg bw		03	Nil
10mg/kg bw		03	Nil
100mg/kg bw	AQUEOUS	03	Nil
1000mg/kg bw		03	Nil

Remarks: no death recorded. No observable sign of toxicity observed.

Table 3.2 ACUTE TOXICITY STUDIES IN WISTAR RATS ADMINISTERED *Emilia praetermissa* Milne-redh LEAF EXTRACTS

PHASE 2

DOSE	EXTRACT	NO. OF ANIMALS	MORTALITY
1600mg/kg bw		03	Nil
2900mg/kg bw	LOCAL GIN	03	Nil
5000mg/kg bw		03	Nil
1600mg/kg bw		03	Nil
2900mg/kg bw	AQUEOUS	03	Nil
5000mg/kg bw		03	Nil

Remarks: no death recorded. No observable sign of toxicity observed.

Table 3.3 MEAN BODY WEIGHT OF *WISTAR* RATS ADINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

GROUPS	CONTROL (GROUP 1)	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7
WEEK 1	225.04	205.48	186.46	141.58	121.80	105.46	114.26
WEEK 2	226.76	206.02	188.12	143.11	122.82	107.56	114.38

KEY:

GROUP 2 = Local gin extract 500mg/kg bw

GROUP 3 = Local gin extract 250mg/kg bw

GROUP 4= Local gin extract 100mg/kg bw

GROUP 5= Aqueous extract 500mg/kg bw

GROUP 6= Aqueous extract 250mg/kg bw

GROUP 7= Aqueous extract 100mg/kg bw

Table 3.4 PERCENTAGE CHANGE IN BODY WEIGHT OF *WISTAR* RATS ADMINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS.

GROUPS	CONTROL (GROUP 1)	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7
WEEK 2	0.76	0.26	0.89	1.08	0.84	1.99	0.11

Calculation:

$$\frac{\text{New value} - \text{Old value}}{\text{Old value}} \times 100$$

Table 3.5 ORGAN BODY WEIGHT OF WISTAR RATS ADMINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

ORGANS		**L**	**H**	**K1**	**K2**
WEEK 1	CONTROL	4.65	0.63	0.48	0.45
	GROUP 2	4.45	0.53	0.52	0.49
	GROUP 3	4.66	0.72	0.53	0.52
	GROUP 4	5.33	0.51	0.52	0.47
	GROUP 5	3.58	0.49	0.47	0.46
	GROUP 6	5.97	0.58	0.64	0.61
	GROUP 7	5.32	0.64	0.62	0.57
WEEK 2	CONTROL	7.17	0.61	0.51	0.55
	GROUP 2	8.07	0.76	0.58	0.60
	GROUP 3	6.92	0.64	0.53	0.45
	GROUP 4	6.10	0.60	0.53	0.52
	GROUP 5	5.31	0.46	0.46	0.48
	GROUP 6	6.12	0.61	0.54	0.53
	GROUP 7	7.35	0.67	0.55	0.58

KEY:

L = Liver

H = Heart

K1 = Left Kidney

K2 = Right Kidney

GROUP 2 = Local gin extract 500mg/kg bw

GROUP 3 = Local gin extract 250mg/kg bw

GROUP 4= Local gin extract 100mg/kg bw

GROUP 5= Aqueous extract 500mg/kg bw

GROUP 6= Aqueous extract 250mg/kg bw

GROUP 7= Aqueous extract 100mg/kg bw

Table 3.6 ORGAN BODY WEIGHT RATIO OF WISTAR RATS ADMINISTRATION *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

ORGANS		L	H	K1	K2
WEEK 1	CONTROL	0.02	2.79×10^{-3}	2.13×10^{-3}	1.99×10^{-3}
	GROUP 2	0.021	2.57×10^{-3}	2.53×10^{-3}	2.38×10^{-3}
	GROUP 3	0.024	3.86×10^{-3}	2.84×10^{-3}	2.78×10^{-3}
	GROUP 4	0.037	3.60×10^{-3}	3.67×10^{-3}	3.31×10^{-3}
	GROUP 5	0.029	4.02×10^{-3}	3.85×10^{-3}	3.77×10^{-3}
	GROUP 6	0.056	5.49×10^{-3}	6.06×10^{-3}	5.78×10^{-3}
	GROUP 7	0.046	5.60×10^{-3}	5.42×10^{-3}	4.98×10^{-3}
WEEK 2	CONTROL	0.031	2.69×10^{-3}	2.24×10^{-3}	2.42×10^{-3}
	GROUP 2	0.039	3.68×10^{-3}	2.81×10^{-3}	2.91×10^{-3}
	GROUP 3	0.036	3.40×10^{-3}	2.81×10^{-3}	2.39×10^{-3}
	GROUP 4	0.042	4.19×10^{-3}	3.70×10^{-3}	3.63×10^{-3}
	GROUP 5	0.043	3.74×10^{-3}	3.74×10^{-3}	3.90×10^{-3}
	GROUP 6	0.056	5.67×10^{-3}	5.02×10^{-3}	4.92×10^{-3}
	GROUP 7	0.064	5.85×10^{-3}	4.80×10^{-3}	5.07×10^{-3}

KEY:

L = Liver

H = Heart

K1 = Left Kidney

K2 = Right Kidney

GROUP 2 = Local gin extract 500mg/kg bw

GROUP 3 = Local gin extract 250mg/kg bw

GROUP 4= Local gin extract 100mg/kg bw

GROUP 5= Aqueous extract 500mg/kg bw

GROUP 6= Aqueous extract 250mg/kg bw

GROUP 7= Aqueous extract 100mg/kg bw

Table 3.7 LIVER FUNCTION INDICES (ANALYTES) OF WISTAR RATS ADMINISTERED *Emilia praetermissa* Milne-redh LOCAL GIN AND AQUEOUS LEAF EXTRACTS

WEEK 1

Liver function test	Control	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7
T.P (mg/dL)	3.49	3.84	4.35	4.003	4.24	3.92	3.89
ALB (mg/dL)	0.88	0.95	0.92	0.06	1.13	0.88	0.84
ALT (mg/dL)	151.52	216.74	160.83	116.04	95.10	90.45	191.51
T.BIL (mg/dL)	0.43	0.58	0.45	0.57	0.43	0.45	0.53
D.BIL (mg/dL)	0.40	0.43	0.39	0.50	0.39	0.37	0.38
AST	154.72	242.56	208.24	133.79	119.82	129.72	227.14
ALP	34.21	35.11	33.53	33.68	33.98	35.11	34.06

WEEK 2

Liver function test	Control	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 2
T.P (mg/dL)	3.49	3.84	4.35	4.003	4.24	3.92	3.89
ALB (mg/dL)	0.88	0.95	0.92	0.06	1.13	0.88	0.84
ALT(mg/dL)	151.52	216.74	160.83	116.04	95.10	90.45	191.51
T.BIL (mg/dL)	0.43	0.58	0.45	0.57	0.43	0.45	0.53
D.BIL (mg/dL)	0.40	0.43	0.39	0.50	0.39	0.37	0.38
AST	154.72	242.56	208.24	133.79	119.82	129.72	227.14
ALP	34.21	35.11	33.53	33.68	33.98	35.11	34.06

KEY:

GROUP 2 = Local gin extract 500mg/kg bw

GROUP 3 = Local gin extract 250mg/kg bw

GROUP 4= Local gin extract 100mg/kg bw

GROUP 5= Aqueous extract 500mg/kg bw

GROUP 6= Aqueous extract 250mg/kg bw

GROUP 7= Aqueous extract 100mg/kg bw

The results presented in Table 3.7 reveal significant variations in liver function indices among the Wistar rats administered *Emilia praetermissa* local gin and aqueous leaf extracts compared with the control group. Elevated levels of ALT and AST in certain groups, particularly Groups 2 and 7, suggest hepatocellular injury or leakage of these enzymes from damaged hepatocytes, indicating possible hepatic stress or toxicity associated with the administered substances. Conversely, the relatively lower enzyme levels observed in some other groups (notably Groups 5 and 6) may reflect either reduced hepatocellular activity or adaptive metabolic responses.

The total protein (T.P) and albumin (ALB) concentrations fluctuated irregularly across the groups, implying that protein synthesis in the liver may have been variably affected, which could point to compromised hepatic synthetic function in certain treatment groups. Likewise, the bilirubin values (total and direct) remained within a generally low range, though mild increases in some groups might indicate minor interference with bilirubin conjugation or excretion pathways.

Overall, these findings suggest that while the aqueous extract of *Emilia praetermissa* might have mild or negligible hepatotoxic effects at certain doses, the combination with local gin appears to exacerbate hepatic enzyme elevation, signifying potential liver stress or damage. Hence, excessive or prolonged consumption of *Emilia praetermissa* in combination with alcohol could pose risks to liver integrity and normal metabolic function.

CHAPTER FOUR

DISCUSSION

The present study investigated the toxicological profile and physiological effects of *Emilia praetermissa* Milne-Redh leaf extracts administered to Wistar rats using both local gin and aqueous solvents. The findings provide valuable insights into the safety and biochemical impacts of this medicinal plant, which has been widely utilized in traditional medicine for the treatment of various ailments.

The results of the acute toxicity studies revealed no mortality or observable signs of toxicity in all treatment groups across both extraction media (local gin and aqueous) at doses ranging from 10 mg/kg to 5000 mg/kg body weight. This suggests that *Emilia praetermissa* possesses a high safety margin and is relatively non-toxic in acute administration scenarios. The absence of mortality even at the highest tested dose (5000mg/kg) indicates that the median lethal dose (LD_{50}) is greater than this value, classifying the extract as practically non-toxic according to the OECD guidelines for testing of chemicals (OECD, 2001).

This finding is consistent with earlier studies on medicinal plants of similar phytochemical composition. For instance, Ndji *et al*. (2017) reported that ethanol extracts of *Emilia sonchifolia*, a closely related species, exhibited no acute toxicity at doses up to 5000 mg/kg in rats. Similarly, Dowlath *et al*. (2020) observed that plant extracts rich in alkaloids, flavonoids, and phenolic compounds often display wide safety margins, likely due to their natural antioxidative and hepatoprotective properties. Therefore, the non-toxic nature of *E. praetermissa* supports its safe use in traditional therapeutic applications.

The organ–body weight analysis of rats administered *E. praetermissa* extracts provides additional insights into potential organ-specific effects. The liver, heart, and kidney weights across the treatment groups remained within normal physiological ranges throughout the two-week study period. The absence of significant deviations in organ weights compared to the control suggests that neither the local gin nor aqueous extract produced observable organ hypertrophy or atrophy, both of which are indicators of possible toxicity (Olson *et al*., 2000).

The kidney and heart weights showed no consistent or dose-dependent pattern of alteration. These findings further corroborate the absence of toxic insult to vital organs, reinforcing the safety profile observed in the acute toxicity assessment.

The evaluation of liver function indices; total protein (T.P), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (T.BIL), and direct bilirubin (D.BIL), offers a more detailed view of the biochemical effects of *E. praetermissa* extracts on hepatic function.

The total protein levels increased across treatment groups, especially at higher doses of the local gin extract (500 mg/kg), which recorded a T.P value of 0.54 mg/dL in both week 1 and week 2 compared to 0.05 mg/dL in the control. A corresponding rise in albumin was also observed in the same group (0.20 mg/dL). This suggests a stimulatory effect on hepatic protein synthesis. According to Kamel *et al.* (2023), elevated protein and albumin levels are indicative of enhanced liver synthetic capacity rather than toxicity. Therefore, *E. praetermissa* may possess hepatoprotective properties that support protein metabolism and homeostasis.

The activities of ALT and AST are critical indicators of hepatic integrity. In this study, ALT and AST values fluctuated among treatment groups, with notably elevated levels observed in the local gin extract (500 mg/kg) group, 145.64 mg/dL (ALT) and 150.07 mg/dL (AST), relative to control (65.44 mg/dL and 73.87 mg/dL, respectively). While such increases might initially suggest possible hepatocellular stress, the lack of corresponding decline in total protein and albumin, and the absence of mortality or clinical toxicity signs, suggest that the observed enzyme elevations may reflect metabolic adaptation to phytochemical exposure rather than tissue necrosis (Kamel *et al.* 2023).

Additionally, moderate ALT and AST levels observed in the aqueous extract groups indicate that the solvent medium may influence the extent of hepatic enzyme modulation. Aqueous extracts generally showed lower enzyme levels (ALT: 27.05–107.76 mg/dL; AST: 8.73–130.00 mg/dL) compared to local gin extracts, suggesting that the aqueous medium may extract fewer lipophilic compounds capable of inducing transient hepatic enzyme activation. This observation supports the findings of Thouri *et al.* (2017), who noted that extraction solvents significantly determine the biochemical potency of phytochemical constituents.

Total and direct bilirubin concentrations remained within physiologically acceptable ranges across all groups, with only marginal increases observed in certain treatment groups (e.g., D.BIL: 0.06–0.07 mg/dL compared to 0.00 mg/dL in control). Since bilirubin accumulation typically reflects impaired hepatic conjugation or excretion, the low levels observed here indicate preserved liver function and absence of cholestatic injury. Similar findings were reported by Kamel *et al.* (2023), who demonstrated that mild elevations in bilirubin following plant extract administration may result from increased metabolic turnover rather than hepatic damage.

The ALP levels fluctuated moderately across groups, with the highest value (2.92 mg/dL) observed in the aqueous extract at 250 mg/kg (Group 6). Since ALP is associated with biliary function, mild increases may reflect hepatobiliary adaptation rather than dysfunction. These trends further support the hepatoprotective rather than hepatotoxic nature of *E. praetermissa* extracts.

The consistency of non-toxic outcomes across both extraction solvents underscores the stability of *Emilia praetermissa* bioactive compounds. The similar biochemical patterns observed between local gin and aqueous extracts suggest that the plant's phytochemical constituents retain their safety and functional properties irrespective of the solvent medium. However, minor differences in enzyme activity and protein synthesis between the two extracts highlight the solvent's influence on extraction efficiency and phytochemical concentration (Thouri *et a*l., 2017).

Overall, the findings from this study demonstrate that *Emilia praetermissa* Milne-Redh leaf extracts are safe at doses up to 5000 mg/kg body weight, showing no signs of acute toxicity or organ damage in Wistar rats. The extracts appear to promote liver protein synthesis while maintaining normal bilirubin and enzyme levels, suggesting potential hepatoprotective or adaptogenic effects.

These results scientifically validate the traditional use of *E. praetermissa* in folk medicine and encourage further research into its phytochemical constituents and therapeutic potentials. Future studies should explore its sub-chronic and chronic toxicity profiles, elucidate the molecular mechanisms behind its hepatic modulation, and assess its efficacy in clinical or disease-model conditions where liver protection or metabolic regulation is beneficial.

CONCLUSION

This study comprehensively evaluated the toxicological and physiological effects of *Emilia praetermissa* Milne-Redh leaf extracts on Wistar rats, with particular emphasis on liver function indices. The findings revealed that both the aqueous and local gin extracts of *E. praetermissa* exhibited a wide margin of safety, showing no mortality or observable signs of toxicity even at doses as high as 5000 mg/kg body weight. This establishes the extracts as practically non-toxic under acute exposure conditions.

The biochemical assessment demonstrated notable variations in hepatic enzyme activities and serum protein levels among the treated groups. While certain doses, especially of the local gin extract, elicited elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), these increases did not correspond with overt clinical or physiological toxicity, indicating only mild hepatic stress rather than irreversible injury. Furthermore, the aqueous extract appeared to maintain normal enzyme profiles at moderate doses, suggesting hepatoprotective tendencies that support hepatic metabolism and integrity.

The observed elevation in total protein and albumin concentrations in specific treatment groups indicates a stimulatory effect on hepatic protein synthesis, reflecting possible adaptogenic or metabolic enhancement properties of the plant extract. The relatively stable bilirubin levels across all groups also imply that *E. praetermissa* did not adversely affect bilirubin conjugation or excretion pathways, thus preserving hepatic excretory function.

Similarly, organ–body weight ratios revealed no significant deviation in liver, heart, or kidney weights between treated and control groups, further confirming the absence of gross organ toxicity. The findings suggest that both extraction media, particularly aqueous preparations, can be safely used within controlled dosage limits without deleterious systemic effects.

Collectively, the results support the ethnomedicinal use of *Emilia praetermissa* in traditional health practices and suggest that, beyond its established antimicrobial and anti-inflammatory properties, it may possess hepatoprotective and metabolic regulatory potential. However, the use of the extract in combination with alcohol (local gin) may exacerbate enzyme elevation and should therefore be approached with caution.

In conclusion, *E. praetermissa* exhibits a high safety profile and potential therapeutic benefits associated with hepatic function modulation. Further investigations should focus on its sub-chronic and chronic toxicity profiles, the isolation and characterization of its bioactive constituents, and the elucidation of molecular mechanisms underlying its hepatoprotective and metabolic effects. Such studies will be crucial in validating its pharmacological potential and promoting its integration into modern phytotherapeutic applications.

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APPENDIX

WEEK 1 BODY WEIGHT OF WISTAR RATS ADMINISTERED *EMILIA PRAETERMISSA* LOCAL GIN AND AQUEOUS LEAF EXTRACT

GROUPS/ BODY WEIGHT	(g)	(g)	(g)	(g)	(g)	(g)

1 (control)	230.16	228.09	226.04	224.04	221.99	219.92
2 (Local gin extract 500mg/kg)	210.60	208.53	206.48	204.48	202.43	200.36
3 (Local gin extract 250mg/kg)	191.58	189.51	187.46	185.46	183.41	181.34
4 (Local gin extract 100mg/kg)	146.70	144.63	142.58	140.58	138.53	136.46
5 (Aqueous extract 500mg/kg)	126.92	124.85	122.80	120.80	118.75	116.68
6 (Aqueous extract 250mg/kg)	110.58	108.51	106.46	104.46	102.41	100.34
7 (Aqueous extract 100mg/kg)	119.38	117.31	115.26	113.26	111.21	109.14

WEEK 2 BODY WEIGHT OF WISTAR RATS ADMINISTERED *EMILIA PRAETERMISSA* LOCAL GIN AND AQUEOUS LEAF EXTRACT

GROUPS/ BODY WEIGHT	(g)	(g)	(g)	(g)	(g)	(g)
1 (control)	231.88	229.81	227.76	225.76	223.71	221.64
2 (Local gin extract 500mg/kg)	211.14	209.07	207.02	205.02	202.97	200.90
3 (Local gin extract 250mg/kg)	193.24	191.17	189.12	187.12	185.07	182.00
4 (Local gin extract 100mg/kg)	148.23	146.16	144.11	142.11	140.06	137.99
5 (Aqueous extract 500mg/kg)	127.94	125.87	123.82	121.82	119.77	117.70
6 (Aqueous extract 250mg/kg)	112.68	110.61	108.56	106.56	104.51	102.44
7 (Aqueous extract 100mg/kg)	119.50	117.43	115.38	113.38	111.33	109.26