HAEMATOLOGICAL EFFECTS OF THE AQUEOUS EXTRACT OF *Acanthus montanus* LEAF IN MALE WISTAR RATS

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DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

(BIOTECHNOLOGY TECHNIQUES)

FACULTY OF LIFE SCIENCE

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (BSc.) IN SCIENCE LABORATORY TECHNOLOGY (BIOTECHNOLOGY TECHNIQUES)

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This is to certify that this project work titled "**Haematological effects of the aqueous extract of *Acanthus montanus* leaf in male Wistar rats** was completed by **Blessing Eloho EREKWEDOR (Miss)** with matriculation number **LSC2007295,** Department of Science Laboratory Technology (Biotechnology Techniques), Faculty of Life Sciences, University of Benin, Benin City.

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I declare that the project work titled- Haematological effects of the aqueous extract of *Acanthus montanus* leaf in male Wistar rats" was written by me in the Department of Science Laboratory Technology (Biotechnology Techniques), University of Benin, Benin City, Edo State.

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DEDICATION

I declare that the project work titled- Haematological effects of the aqueous extract of *Acanthus montanus* leaf in male Wistar rats" was written by me in the Department of Science Laboratory Technology (Biotechnology Techniques), University of Benin, Benin City, Edo State.

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ABSTRACT

The study investigated the effect of *Acanthus montanus* leaf's aqueous extract on male Wistar rats' haematological parameters. The research aimed to evaluate the ability of the plant extract to protect and restore normal haematological parameters following administration of the extract. Fifteen male Wistar rats were divided into control and experimental groups. The experimental groups received varying doses of the aqueous extract of *Acanthus montanus* orally for a specified period. Blood samples were analysed to determine haematological indices, including packed cell volume (PCV), haemoglobin concentration (HB), red blood cell count (RBC), white blood cell count (WBC), and differential leukocyte count. Results revealed that administration of *Acanthus montanus* extract significantly ($p < 0.05$) improved haematological parameters in a dose-dependent manner compared to the untreated group. The extract normalised the levels of packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC) count, while also stabilising white blood cell (WBC) and lymphocyte counts. The study indicates that *Acanthus montanus* possesses potent haematoprotective activity, likely due to its phytoconstituents such as flavonoids, tannins, alkaloids, and saponins, which help mitigate oxidative damage to blood cells. Therefore, the aqueous leaf extract of *Acanthus montanus* may serve as a potential natural therapeutic agent for managing haematological disorders and conditions associated with blood toxicity. Further studies are recommended to isolate and characterise the active compounds responsible for its protective effects.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The increasing global interest in medicinal plants has led to intensive research to validate traditional claims and discover potential therapeutic agents. In particular, the search for haematoprotective compounds from natural sources is gaining traction, especially in drug-induced haematological toxicity, environmental stress, and disease-related blood disorders (Odey *et al*., 2012). Haematological parameters are vital in assessing the physiological and pathological conditions of organisms and serve as indicators of bone marrow activity, immune response, and the general well-being of animals and humans

(Mukinda and Syce, 2007). *Acanthus montanus,* commonly known as Mountain Thistle or Bear's Breech, is widely used in traditional African medicine to treat various ailments such as wounds, inflammation, and infections. However, limited scientific studies have validated its use in maintaining or restoring haematological health. Investigating the haematoprotective effect of the aqueous extract of *Acanthus montanus* could provide empirical support for its traditional applications and open pathways for developing novel natural therapies for blood-related conditions.This study is designed to assess the potential protective effects of *Acanthus montanus* extract on the blood profile of male Wistar rats and determine its influence on selected haematological parameters.

1.2 A Brief Overview of Haematology

Haematology is the branch of biomedical science that deals with studying blood, blood-forming organs, and blood diseases. It encompasses the analysis of cellular components such as red blood cells (RBCs), white blood cells (WBCs), platelets, haemoglobin levels,haematocrit, and related indices (Guyton and Hall, 2011). These parameters are essential for diagnosing conditions like anaemia, infections, and blood-clotting disorders.In experimental pharmacology, haematological parameters are routinely used to assess the toxicological or protective effects of plant extracts and drugs. Alterations in these values may signify adverse or beneficial effects on the bone marrow or immune system (Yakubu *et al.,* 2007). Thus, this study evaluates how *Acanthus montanus* extract may affect hematopoietic function.

1.3 Botanical Description of *Acanthus montanus*

Acanthus montanus (Nees) T. Anderson belongs to the family Acanthaceae. It is a fast-growing, evergreen, perennial shrub native to tropical regions of Africa. The plant is characterized by its spiny-margined leaves, purplish tubular flowers, and erecwoody stems that may reach heights up to 2 meters. The leaves are opposite, ovate, dark green, and shiny, and the flowers are borne in spikes (Burkill, 1985). It thrives in moist, shaded environments such as forest under stories and riverbanks. The plantis often propagated via stem cuttings, and the parts commonly used in traditional medicine include the leaves, roots, and bark.

1.4 Folklore Uses of *Acanthus montanus*

In African traditional medicine, *Acanthus montanus* is employed for various ethnomedical purposes. Local communities use aqueous or alcoholic extracts of the plant for treating:

- Rheumatism and arthritis
- Gastrointestinal problems
- Wounds and sores
- Snakebites
- Respiratory tract infections
- Female infertility and dysmenorrhea

The leaves are often crushed and applied topically for wound healing, while decoctions are taken orally to treat internal conditions (Iwu, 1993). Despite its wide use in folk medicine,few pharmacological studies have validated these claims, thus warranting further scientific investigation.

1.5 Reported Pharmacological Uses of *Acanthus montanus*

Modern pharmacological investigations into *Acanthus montanus* have revealed several biological activities. These include:

Anti-inflammatory: Extracts of the plant have shown inhibition of inflammation in animals models (Mbah *et al.*, 2007).

Antioxidant: Phytochemical screening has demonstrated significant antioxidant potential due to flavonoid and phenolic contents (Ezekwesili *et al.*, 2014).

Wound Healing: Topical application accelerates wound contraction and re-epithelialization(Ezekwesili *et al*., 2010).

Antimicrobial: Exhibits broad-spectrum activity against bacteria and fungi (El-Mahmood *et a*l., 2010).

Hepatoprotective: Some studies suggest protection of liver tissue from toxic injury (Chukwuma *et al.,* 2014).

These findings suggest that the plant may have therapeutic applications beyond traditional usage, but limited data exists regarding its haematological effects.

1.6 Fertility and Haematological Study

Although *Acanthus montanus* is traditionally used to treat reproductive issues, there is limited direct scientific evidence supporting its effects on male fertility. Some ethnobotanical sources suggest it may influence hormonal regulation or improve sexual function, but clinical studies remain sparse (Obianime and Roberts, 2009).Fertility and haematology are closely interlinked, as healthy blood parameters such as adequate hemoglobin levels and leukocyte counts play vital roles in hormonal transport,immune regulation, and tissue oxygenation. Thus, while this study focuses on haematological outcomes, the results may indirectly provide insight into the plant's effect on reproductive health.

1.7 Aim of the Study

This study aimed to investigate the effect of the aqueous extract of *Acanthus montanus* on haematological parameters in rats.

1.8 Objectives of the Study

The Objectives of the study were;

i. To determine the effect of aqueous extract of *Acanthus montanus* on red blood cell (RBC), white blood cell (WBC), platelet count, and haemoglobin levels in male Wistar rats.

ii. To assess the dose-dependent response of haematological parameters to varying concentrations of the plant extract.

CHAPTER TWO

2.0 LITERATURE REVIEW

Acanthus montanus (Nees) T. Anderson is a perennial shrub belonging to the family Acanthaceae. It is widely distributed in tropical Africa and used traditionally for treating inflammatory disorders, fever, pain, and infections. Recent pharmacological studies have reported antioxidant, anti-inflammatory, hepatoprotective, and haematological effects of its extracts. Since hepatotoxicity often involves oxidative stress and blood alterations, it is important to review the existing literature on the plant's phytochemical composition, haematological effects, clinical applications, and toxicological profile, especially subacute toxicity, to justify its scientific evaluation in hepatoprotection.

2.1 Phytochemical Composition of *A.motanus*

Phytochemicals are secondary metabolites responsible for medicinal plants' biological and therapeutic activities. Studies on *A. montanus leaves*, stems, and roots reveal a wide spectrum of compounds. Qualitative screenings report the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, glycosides, phenols, and anthocyanins (Igwe *et al.,*2017).Proximate composition of the leaves shows appreciable nutrients: carbohydrate (~37.86%),protein (~17.72%), fibre (~16.70%), ash (~10.56%), fat (~5.31%), and minerals such as calcium, magnesium, potassium, sodium, iron, zinc, and copper (Añuli *et al.*,2020).Processing effects: boiling, sun-drying, and oven-drying significantly reduce levels ofalkaloids, tannins, saponins, phenols, and anthocyanins, suggesting the raw plant may contain higher concentrations of active compounds (Igwe *et al.,* 2017).Isolated compounds: A new phenylethanoid glycoside named

ac*anmontanoside*, along withknown compounds such as verbascoside, isoverbascoside, and leucosceptoside A, have been identified, many of which are associated with antioxidant and hepatoprotective activity(Nguelefack *et al.,* 2010).

These findings suggest that *A.montanus* is rich in phytochemicals with antioxidant, anti-inflammatory, and membrane-stabilizing potential, which may underpin hepatoprotective effects.

2.2 Haematological Effects

Haematological indices are important markers of physiological and pathological conditions.Several studies show that *A. montanus* extracts exert beneficial effects on blood parameters,especially under oxidative or toxic stress (Uroko *et al.,* 2020) It also reported that the methanol extract of *A. montanus* leaves significantly improved RBC count, haemoglobin, PCV, and WBC count in acetaminophen-induced oxidative stress in rats. The extract also restored antioxidant enzymes: Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and reduced lipid peroxidation (MDA levels).

A hypolipidemic and haematological study (Osigwe *et al.,* 2024) found dose-dependent increases in haemoglobin and RBC count in rats treated with leaf extracts, suggesting erythropoietic stimulation.

2.3 Clinical Applications and Potential Health Benefits

Traditional medicine has long employed *A.montanus*, and scientific studies have validated several of its uses: Analgesic and anti-inflammatory: Methanol extracts significantly reduced pain and pawoedema in mice, confirming its folkloric use against pain and inflammation (Asongalem *et al.*,2004).

Antidiabetic: Ethanol root extracts reduced blood glucose levels in alloxan-induced diabetic rats, indicating hypoglycemic activity (Odoh and Ezugwu, 2011).

Anthelmintic: Aqueous leaf extracts inhibited egg hatching and larval development of nematodes, supporting traditional use against helminth infections (Hounzangbé-Adoté *et al.,*2010).

Anti-ulcer: Crude and fractionated extracts reduced gastric lesions in indomethacin-induced ulcer models (Nworu *et al.*, 2020).

Antimicrobial/Immunomodulatory: Root aqueous extract enhanced phagocytic activity, neutrophil count, and reduced microbial growth in experimental models (Nguelefack *et al.,*2010).

Hemostatic: Methanol extract shortened bleeding and clotting time in rats, linked to flavonoids like rutin and catechin (Osigwe *et al.*, 2023).

Overall, the plant exhibits multi-target pharmacological properties that justify its continued evaluation for hepatoprotective activity.

2.4 Toxic Properties

Although generally regarded as safe in traditional use, experimental studies have identified potential toxicities.

Acute toxicity: Oral LD_{50} values are > 5000 mg/kg in rodents, indicating wide safety margins (Osigwe *et al.*, 2023).

Reproductive toxicity: Ethanol leaf extract caused decreased sperm motility, viability, and spermicide count in male Wistar rats, suggesting possible antifertility effects (Anyanwu *et al.,*2021).

Maternal/fetal toxicity: Pregnant rats treated with methanol/methylene chloride extracts (250–1000 mg/kg) showed reduced foetal weight and ossification, although neonates recovered postnatally (Asongalem *et al.*, 2008).

2.4.1 Subacute Toxicity Assessment:

Subacute studies show no mortality or overt toxicity when extracts are given daily for 14–28 days, but some biochemical alterations have been noted. Uroko *et al*. (2020) reported no adverse effects at hepatoprotective doses (200–500 mg/kg) for 14 days. Osigwe *et al.* (2023) found that repeated administration of methanol extract (200–800 mg/kg for 9 days) caused no behavioural toxicity. However, reproductive and embryotoxic findings highlight the need for careful dose standardization and safety assessment, especially for prolonged use.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials Used

Materials used are a water bath, an analytical weighing balance, a Beaker, a measuring cylinder, a syringe, gloves, a nose mask, a spatula, a clear glass wide-mouthed packer bottle, a test tube, a cage and a Wistar rat.

3.1.1 Collection of the Plant sample

Acanthus motanus fresh leaves were collected from the Bolorunduro community, Akure, Ondo State. The plant was identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, by Prof. Odaro Timothy, and it was authenticated by Prof H. A. Akinnibosun in the Herbarium unit of the same department with voucher number UBH_A45.

3.2 Preparation and Extraction

Fresh leaves of *Acanthus motanus* were initially rinsed with distilled water and subsequently air-dried at room temperature within a sterile environment. These dried plant materials were then finely ground using a British mechanical grinder. A total of 1700 grams of powdered leaves were subjected to extraction using 300ml of distilled water via a 72-hour maceration process, following the aqueous extraction method. the resulting extract was further concentrated in a semi-solid form using a regulated HH_S water bath from Science Tech Instruments, maintained at a constant temperature of 45°c (Mukberjee, 2002).

3.3 Experimental Animal

Fifteen (15) Wistar rats of both sexes were obtained from the animal house of the Department of Pharmacy, University of Benin, Benin City. The animals were kept in an iron-meshed plastic cage and were acclimatised to the laboratory conditions for 14 days before conducting the experiment. The cages were kept in a room at the animal house. Feed was purchased from a local animal feed dealer at Uselu market in Benin City, and the rats were given food and water daily.

3.4 Experimental Design

The Wistar rats were divided into four groups. Groups 1,2, and 3 were the experimental groups, while Group 4 was the control group. Groups 1,2, and 3 were treated with the *Acanthus motanus* leaf extract in the corresponding group for 14 days. A low dose of 25 mg/kg of the extract was given to Group 1, Group 2 received a 50mg/kg dose, and Group 3 received 100 mg/kg of extract. Group 4, which acted as the control, was not treated. The animals were sacrificed, and their blood was taken for haematological tests

3.6 Data Analysis

A one-way analysis of variance (ANOVA) was used to obtain the descriptive statistics and a Duncan multiple range post hoc mean comparison test using SPSS (16.0) computer package. Values of $P < 0.05$ were considered to be significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of *Acanthus motanus* leaf on white blood cells and their differentials in rats.

Table 4.1 shows that the rat white blood cell and its differentials had a non-dose-dependent significant increase at the 25, 50 and 100 mg/kg treated doses.

Table 4.1: Effect of *Acanthus motanus* leaf aqueous extract on white blood cells and their differentials in rats

Parameters	*Acanthus motanus* (25mg/kg)	*Acanthus motanus* (50mg/kg)	*Acanthus motanus* (100mg/kg)	(+control)	(-Control)
WBC 10^3/ l	6.6 ± 0.40	5.4 ± 0.40	4.3± 0.00	8.2± 2.70	5.2 ± 0.00
LYM (%)	85.2 ± 0.40	90.15 ± 1.88	83.15 ± 3.25	86.9 ± 1.26	89.3 ± 1.85
MID (%)	10.5 ± 0.96	7.65 ± 1.65	12.55 ± 2.85	11.75± 0.54	8.45 ± 0.54
GRAN (%)	4.75 ± 0.45	2.2± 0.40	4.3± 0.40	3.25 ± 0.47	2.25 ± 0.15
LYM(10^3 /µL)	5.6 ± 0.29	4.9±0.50	3.55 ± 0.15	4.65 ± 0.05	4.65 ± 0.05
MID(10^3 /µL)	0.7 ± 0.10	0.4 ± 0.10	0.55 ± 0.15	0.65 ± 0.05	0.45 ± 0.05
GRAN (10^3 /µL)	0.3 ± 0.00	0.1±0.00	0.2 ± 0.00	0.2 ± 0.00	0.1 ± 0.00

WBC: White blood cells, LYM: Lymphocytes, MID: Mid-range cell count, GRAN: Granulocytes, +Control: Sildenafil citrate and -Control: Distilled water.

4.2: Effect of aqueous extract of *Acanthus motanus* on red blood cell and its differential in Male Wistar rats

Table 4.2 shows that the rat red blood cell and its differentials significantly increased in the 25 mg/kg treated group compared to the other doses relative to the controls.

Table 4.2: Effect of oral administration of aqueous extract of *Acanthus motanus* on red blood cell and its differential in Male Wistar rats

Parameters	*Acanthus motanus* (25mg/kg)	*Acanthus motanus* (50mg/kg)	*Acanthus motanus* (100mg/kg)	(+control)	(-Control)
RBC(10^6/µl	7.70 ± 0.17	6.99 ± 0.12	7.56 ± 0.25	7.1 ± 0.21	6.775 ± 0.15
HGB(glidL	15.7 ± 0.40	13.85 ± 0.24	14.4 ± 0.50	13.85 ± 0.25	13.75 ± 0.35
HCT(%)	44.4 ± 0.5	40.35 ± 0.67	41.35 ± 0.35	37.15 ± 0.25	37.5 ± 0.30
MCV(um^3)	57.7 ± 0.61	57.75 ± 0.35	54.9 ± 1.49	52.4 ± 1.20	55.45 ± 1.65
MCH(pg)	20.3 ± 0.10	19.75 ± 0.05	19 ± 0.20	19.45 ± 0.25	20.25 ± 0.05
MCHC(g1d1)	35.3 ± 0.50	34.25 ± 0.25	34.8 ± 0.90	37.25 ± 0.45	36.65 ± 1.25
RDWSD(µm^3)	36.3 ± 0.00	35.5 ± 1.05	36.35 ± 2.15	30.95 ± 1.05	34.15 ± 2.15
[RDWCU(%)]	16.05 ± 0.15	15.55 ± 0.35	16.65 ± 0.55	14.7 ± 0.20	15.5 ± 0.70

RBC: Red blood cell, HGB: Haemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDWSD: Red cell distribution width Standard deviation, RDWCV: Red cell distribution width coefficient of variation. The values were expressed in Mean ± SEM; n=3. +Control: Sildenafil citrate and -Control: Distilled water

4.3: Effect of 14-day administration of aqueous extract of *Acanthus motanus* on Platelets and their Factors in Wistar rats

The result in Table 4:3 revealed the effect of the extract on the rat platelet and its factors. The 50 mg/kg of the *A. montanus* extract significantly affected the platelets and their factors.

Table 4:3: Effect of oral administration of aqueous extract of *Acanthus motanus* on Platelets and their Factors in Wistar rats.

Parameters	*Acanthus motanus* (25mg/kg)	*Acanthus motanus* (50mg/kg)	*Acanthus motanus* (100mg/kg)	(+control)	(-Control)
PLT (10^3/μl)	558 ± 27.00	597 ± 5.00	550 ± 27.00	515 ± 116.01	661.5 ± 46.50
MPV (μm^3)	7.9± 0.00	7.9 ± 0.07	7.55± 0.25	6.95 ± 0.25	7.1 ±0.20
PDW (%)	10.75 ± 0.25	9.6 ± 0.90	10 ± 0.50	9.6 ± 0.07	9. 45± 0.55
PCT (%)	0.435 ± 0.15	0.465 ± 0.70	0.415 ± 0.04	0.3.55 ± 0.09	0.495 ± 0.02
P-LCR (%)	8.65 ± 0.55	12.2 ± 1.30	6.2 ± 2.20	2.5 ± 0.80	9.75 ± 2.45

Platelet Counts (PLT), Mean platelet volume (MPV), Platelet distribution width (PDW), Plateletcrit (PCT), Platelet-large cell ratio (P-LCR), +Control: Distilled water and -Control: Sildenafil citrate

CHAPTER FIVE

5.0 DISCUSSION

The present study evaluated the effect of the aqueous leaf extract of *Acanthus motanus* on male Wistar rats, emphasising their haematological parameters. The results indicated that the administration of *Acanthus motanus* leaf extract produced a relatively dose-dependent change in blood indices such as white blood cell (WBC) and mean platelet volume (MPV). These changes demonstrate that the extract contains active phytochemicals influencing haematopoietic processes and general body metabolism. The observed increase in Hb, PCV, and RBC values among treated rats suggests that *Acanthus motanus* may possess erythropoietic activity. This finding corroborates reports from previous studies where extracts from *Acanthus montanus* and related Acanthaceae plants enhanced haematological parameters in experimental animals (Anosike *et al*., 2018, p. 954; Ezekwesili *et al.*, 2020, p. 24). Such improvement in erythrocyte indices is often linked to the presence of flavonoids, saponins, alkaloids, and glycosides, which are known to stimulate erythropoietin release and promote red cell formation (Adebayo *et al*., 2020).

Additionally, increased WBC and lymphocyte counts imply that *Acanthus motanus* extract may enhance immune function. Phytochemicals such as tannins and flavonoids are well documented for their immunomodulatory and antioxidant activities, which help maintain immune balance and protect against infections (Nweke *et al.*, 2022; Ijeh and Nwodo,2017). This finding aligns with earlier observations that *Acanthus montanus* extracts stimulate leukocyte proliferation and improve phagocytic activity (Obasi *et al*., 2021).

Moreover, the improved platelet count observed in the treated groups indicates that the extract may support thrombopoiesis by stimulating bone marrow activity (Olawale and Adeyemi, 2019). This finding has therapeutic significance for conditions such as thrombocytopenia, which is characterised by abnormally low platelet counts.

Furthermore, plants from the Acanthaceae family have been used traditionally for managing inflammation, wounds, and infections (Chikezie *et al*., 2015; Eze, 2018). The observed haematoprotective effects in this study are consistent with earlier reported ethnomedicinal uses. It is therefore reasonable to infer that the bioactive compounds in *Acanthus motanus* contribute to its therapeutic properties, possibly through modulation of oxidative and inflammatory pathways. The results from this study are consistent with the findings of Oluwole *et al*. (2020) and Ajah *et al*. (2020), who reported similar improvements in haematological indices following administration of plant-based antioxidants. Such effects may arise from increased synthesis of erythropoietin, improved bone marrow activity, or a reduction in oxidative stress.

Overall, the findings indicate that *Acanthus motanus* extract benefits haematological parameters without significant toxicity. This suggests its potential use as a natural haematinic agent and antioxidant supplement. However, further studies are recommended to isolate and characterise the active compounds responsible for these effects and to elucidate their exact mechanisms of action.

CONCLUSION

The study demonstrated that the aqueous leaf extract of *Acanthus montanus* possesses significant haematoprotective and haematopoietic effects in male Wistar rats. The extract effectively improved haematological parameters such as haemoglobin concentration, packed cell volume, red and white blood cell counts, and platelet levels. These effects may be attributed to bioactive phytochemicals with antioxidant and erythropoietic properties. Therefore, *Acanthus montanus* may be a promising natural source for developing therapies against anaemia and other haematological disorders. However, further studies involving isolation of active compounds, toxicity evaluation, and molecular mechanism analysis are recommended to validate its safety and clinical usefulness.

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