Thesis on

Phytochemical Screening and Biological Tests of Methanol Extract of *Ruellia simplex*

[In the partial fulfillment of the requirements for the degree of Masters of Pharmacy]

Submitted To

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January 2024

APPROVAL

This project paper, **"Phytochemical Screening and Biological Tests of Methanol Extract of *Ruellia simplex*",** submitted to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Masters programmed and approved as to its style and contents.

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DECLARATION

I hereby declare that this thesis report **"Phytochemical Screening and Biological Tests of Methanol Extract of *Ruellia simplex*",** is done by me under the supervision of Mr. Pollob Ahmed Shuvo Senior Lecturer (On leave) Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University. I am declaring that this thesis is my original work. I also declare that neither this project nor any part therefore has been submitted elsewhere for the award of Masters or any degree.

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ACKNOWLEDGEMENT

I might want to communicate my profound applause to the All-powerful God who has given me the capacity to finish my undertaking work and the chance to concentrate in this subject.

I'm a lot of thankful to my honorable thesis supervisor Mr. Pollob Ahmed Shuvo Senior Lecturer (On leave) Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University for her brilliant direction and steady oversight just as for giving essential data in regards to the task and furthermore for her help in finishing the project.

I also wish to offer my respect to all of the teachers of Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University and thankful to other members for their excellent cooperation with us.

Finally, I would like to express my gratitude towards my parents and other family members for their kind cooperation and encouragement which helped me in completion of this project.

Dedication……

My Parents

The persons who always encourage me in every sphere of my life.

Abstract

The phytochemical composition and possible biological activities of Ruellia simplex, a medicinal herb that has conventional uses, were studied. Plant material was extracted using methanol, and the resultant extract was thoroughly screened for the existence of several secondary metabolites using phytochemical analysis. Alkaloids, flavonoids, Saponin, Glycoside, and tannins were found in the results of the research, indicating the plant's varied chemical composition. The biochemical properties of the Ruellia simplex methanol extract were then assessed using a battery of in vitro experiments and in-vivo anti-diarrheal, in-vitro thrombolytic activity, in vitro antioxidant & in vivo anthelminthic activity. Ruellia Simplex methanol extract showed adequate thrombolytic effectiveness (69%), according to this investigation. Standard ascorbic acid's DPPH inhibitory effect displays the IC50 (9.072 μg/ml) as the positive control. The Methanol extract of Ruellia Simplex has a notable DPPH inhibitory effect, with an IC50 of 20.487 μg/ml when compared to standard. The findings of this research emphasize Ruellia simplex's potential uses in medicine and healthcare by providing significant details on the phytochemical makeup of the plant and its biological activities. The results highlight the significance of looking for novel therapeutic compounds in natural sources and may open the door for future studies.

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Chapter one
Introduction

1. Introduction

"Ruellia simplex," commonly known as Mexican petunia, is a captivating flowering plant that belongs to the Acanthaceae family. Native to Mexico, this perennial herb has found its way into gardens and landscapes around the world due to its striking appearance and adaptability. Renowned for its vibrant trumpet-shaped flowers and lush green foliage, Ruellia simplex is a popular choice among garden enthusiasts and landscapers alike [1]. The plant's botanical name, Ruellia simplex, pays homage to the French botanist Jean Ruel, and its common name, Mexican petunia, draws parallels to the petunia flower due to the similarity in appearance. With its ability to thrive in a variety of environments, from full sun to partial shade, and its resistance to pests, Ruellia simplex has become a resilient and low-maintenance option for those seeking to enhance the beauty of their outdoor spaces [2]. In this introduction, we will explore the key features, cultivation tips, and ecological significance of Ruellia simplex, shedding light on why this plant has become a favorite among horticulturists and nature enthusiasts alike [3].

Figure 1: Ruellia simplex

1.1 Medicinal plant

In the intricate tapestry of nature, medicinal plants stand as invaluable contributors to the well-being of humanity. For centuries, these botanical treasures have played a pivotal role in traditional healing practices, offering a plethora of therapeutic compounds that address a myriad of health

concerns. Defined by their ability to synthesize and accumulate bioactive substances, medicinal plants have been harnessed by diverse cultures across the globe, forming the foundation of herbal medicine systems [4]. The historical significance of medicinal plants transcends geographical boundaries, with civilizations from ancient China to the indigenous tribes of the Amazon rainforest recognizing the healing potential embedded within the leaves, roots, flowers, and seeds of various plant species. As a testament to their enduring relevance, many of these botanical remedies have seamlessly integrated into contemporary medicine, serving as sources of inspiration for the development of pharmaceutical drugs [5]. Beyond their role in treating ailments, medicinal plants also play a crucial role in maintaining ecological balance. As key components of biodiversity, they contribute to the health of ecosystems and serve as habitats for various species. However, the rapid pace of environmental change poses a threat to many medicinal plant species, highlighting the need for sustainable practices in their cultivation and preservation. In this exploration of medicinal plants, we delve into the rich tapestry of botanical diversity, examining the cultural, historical, and scientific dimensions that underscore their significance [6]. From traditional healing practices to modern pharmacology, the study of medicinal plants not only offers insights into the complex relationship between humans and the natural world but also holds the promise of unlocking new frontiers in healthcare and sustainable living. Join us on this journey through the lush landscapes of medicinal flora, where ancient wisdom converges with contemporary scientific inquiry to illuminate the extraordinary potential of these botanical healers [7].

1.2 Chemical Composition of Ruellia simplex

Ruellia simplex leaf extract was used by Ukwubile CA et al. (2023) to isolate a novel fatty acid called 2,4-PHPBEa, which has antinociceptive (analgesic), anti-inflammatory, and antidiabetic qualities. Alloxan, carrageenan, and acetic acid-induced animal models were used. The separated fatty acid showed pain resistance by reducing abdominal writhing in rats with carrageenan-induced paw edoema, with IC50 values of 12.5 g/ml-1.08 g/ml and 10.21 g/ml-1.02 g/ml, along with decreased paw volume [8]. The antidiabetic activity showed a dose-dependent decrease in blood sugar stages, with IC50 values of 6.02 g/ml-0.01 g/ml. A new fatty acid found in R. simplex extraction has been shown in an investigation to have potential applications in the management of diabetes, inflammation, and discomfort [9]. Ruellia simplex, also known as Mexican petunias, has been the subject of hybridization and genetic studies by Rosanna Freyre et al. (2015). Ploidy modification and hybridization were used in development to produce sterile Ruellia cultivars with

a range of flower colors and development patterns [10]. “Mayan White,” “Mayan Pink,” and “Mayan Purple” are the first three sterile hybrids that are currently available for purchase. These hybrids have exceptional growth habits and flowering efficiency. In addition, Ruellia simplex genetics and the anthocyanins that determine bloom color were studied. According to an HPLC analysis, compounds of pelargonidin produced the pink corolla color, while compounds of delphinidin produced the purple corolla color [11]. As a consequence, there are 21 elements that make up Ruellia brittoniana. At 8.51%, β-sitosterol was the predominant sterol. The primary fatty acid (57.25%) in fatty acid methyl esters was determined by GLC testing to be myristic acid. Arachidonic acid, on the other hand, is discovered to have the lowest amount of fatty acids (0.35%) [12]. Additionally, a phytochemical analysis of Ruelllia brittoniana showed an abundance of 2, 2’, 4’, 6’-tetrahydroxy-chalcone, 5, 7, 4’trimethoxy 3-O-Rhamnoflavone, and 5, 2’, 3’trihydroxy 7-O-glucoflavone. Additionally, the present investigation used the stable form of free radical DPPH to determine the antioxidant function of the substance in vitro. Ruelllia brittoniana sample concentration 50 value 4.8 µg/m yielded 4.2% antioxidant activity utilizing vitamin C and positive control [13].

1.3 Major purposeful and therapeutic properties of Ruellia simplex

Ruellia simplex, commonly known as Mexican petunia or wild petunia, is a flowering plant that is often used for ornamental purposes in gardens and landscapes. While it has been traditionally used in some folk medicine practices, it’s important to note that scientific research on the therapeutic properties of Ruellia simplex is limited, and the plant should not be considered a substitute for conventional medical treatments [14]. Here are some general properties associated with Ruellia simplex:

Ornamental Purposes:

The plant is primarily grown for its attractive, trumpet-shaped flowers. It is valued for its ability to add color and visual interest to gardens and landscapes [15].

Traditional Medicine:

In some traditional medicine practices, various parts of Ruellia simplex, such as the leaves and roots, have been used for medicinal purposes. However, there is limited scientific evidence supporting the efficacy and safety of these uses [16].

Anti-Inflammatory Potential:

Some plants in the Ruellia genus are known to contain compounds with potential anti-inflammatory properties. However, specific studies on Ruellia simplex are lacking [17].

Antioxidant Activity:

Plants, in general, can contain antioxidants that may help protect cells from oxidative stress. While there is limited information on the antioxidant properties of Ruellia simplex, some plants in the same family have demonstrated antioxidant activity [18].

Wound Healing (Traditional Use):

In traditional medicine, extracts from Ruellia simplex have been used topically for wound healing. However, scientific studies supporting this claim are needed.

Potential for Other Medicinal Uses:

Traditional uses of Ruellia simplex include treatments for conditions such as diarrhea, dysentery, and respiratory issues. However, these uses are based on traditional knowledge and have not been extensively studied in a scientific context. It's important to approach the use of Ruellia simplex or any plant for medicinal purposes with caution. The efficacy and safety of such uses should be validated through rigorous scientific research. Additionally, it's crucial to consult with healthcare professionals before using any plant-based remedies, especially if you are already on medication or have pre-existing health conditions [19].

1.4 Habitat and heredity of Ruellia simplex

Habitat:

Native Range: Ruellia simplex is native to Mexico but has been introduced and has become naturalized in various other regions [20].

Adaptability: This plant is adaptable to a variety of habitats, including disturbed areas, roadsides, and wetlands. It can thrive in both sunny and partially shaded conditions.

Water Preferences: Mexican petunia is often found in areas with moist to wet soils, and it can even tolerate standing water. This makes it well-suited for wetland habitats [21].

Invasiveness: While it is appreciated as an ornamental plant in gardens, it has the potential to become invasive in some areas, particularly in places with a warm climate. Invasive plants can outcompete native vegetation and disrupt local ecosystems [22].

Heredity:

Botanical Classification: Ruellia simplex belongs to the Acanthaceae family. This family includes a wide variety of flowering plants, many of which are found in tropical and subtropical regions.

Genetic Characteristics: Like all living organisms, Ruellia simplex exhibits genetic variation. The heredity of traits in plants involves the passing of genetic material from one generation to the next. This process includes the transmission of genetic information through seeds.

Reproduction: Mexican petunia reproduces both by seeds and vegetative. The plant produces tuberous roots that can give rise to new shoots, allowing it to spread and form dense stands [23].

1.5 Magnitude of ethno pharmacological cram in drug innovation

Ethno pharmacology plays a significant role in drug innovation by providing valuable insights into traditional medicinal practices and indigenous knowledge of medicinal plants. The magnitude of ethno pharmacological research in drug innovation is substantial, and it contributes to the discovery and development of new therapeutic agents. Here are some key aspects highlighting its importance [24]:

Biodiversity and Traditional Knowledge:

Ethno pharmacological studies often focus on regions with high biodiversity, where indigenous communities have developed a rich knowledge of medicinal plants over centuries. Traditional knowledge about the uses of plants and other natural resources is a valuable source of information for identifying potential therapeutic compounds [25].

Drug Discovery and Development:

Ethno pharmacology provides a starting point for the discovery of bioactive compounds. Many modern drugs have their origins in traditional remedies. By studying the traditional uses of plants, researchers can identify compounds with potential pharmacological activity, which can then be isolated, purified, and further developed into pharmaceuticals [26].

Cultural Relevance and Local Health Practices:

Understanding the cultural context of traditional medicine is crucial in drug development. Local communities often have unique health practices that are tailored to their specific needs and environments. Integrating cultural knowledge into the drug development process ensures that resulting medications are more likely to be accepted and effective in the target populations [27].

Conservation of Traditional Knowledge:

Ethno pharmacological research helps in documenting and preserving traditional knowledge that might otherwise be lost over time due to cultural changes and globalization. Collaboration with indigenous communities in the drug discovery process also promotes the ethical and responsible use of their traditional knowledge [28].

Bio prospecting and Sustainable Development:

Ethno pharmacology contributes to bio prospecting efforts, where researchers explore natural resources for potential medicinal compounds. Sustainable development practices are often emphasized to ensure that the harvesting of medicinal plants is done in an environmentally friendly and socially responsible manner [29].

Validation of Traditional Remedies:

Ethno pharmacological studies validate the effectiveness of traditional remedies through scientific methods. This helps bridge the gap between traditional and modern medicine. By identifying active compounds and understanding their mechanisms of action, researchers can enhance the credibility of traditional medicinal practices [30].

Global Health Impact:

Many regions with a rich tradition of ethno pharmacology are also areas where access to modern healthcare is limited. Ethno pharmacological research contributes to global health by providing alternative and affordable treatment options. The magnitude of ethno pharmacological research in drug innovation is substantial, as it combines traditional knowledge with modern scientific approaches to identify, validate, and develop new drugs with therapeutic potential. This interdisciplinary approach has the potential to address global health challenges and improve healthcare practices [31].

Chapter two
Purpose of the study

2. Objective of the study

The primary objective of this research is to comprehensively investigate the phytochemical constituents present in the methanol extract of Ruellia simplex, a plant known for its traditional medicinal uses. The study aims to elucidate the chemical composition of the extract through phytochemical screening, with a focus on identifying bioactive compounds that may contribute to its potential therapeutic properties.

Phytochemical Screening:

- To identify and quantify the presence of various phytochemical classes such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds in the methanol extract of Ruellia simplex.
- To assess the abundance of specific phytochemicals that have been correlated with medicinal properties, thereby providing insights into the potential therapeutic value of the plant.

Biological Tests:

To evaluate the biological activities of the methanol extract through a series of in vitro assays.

- **Antioxidant Activity:** Assess the extract's ability to scavenge free radicals and its potential as an antioxidant agent.
- **Antimicrobial Activity:** Investigate the extract's effectiveness against a range of pathogenic microorganisms, shedding light on its possible role as an antimicrobial agent.
- **Anti-inflammatory Activity:** Evaluate the extract's ability to mitigate inflammation, which is crucial for understanding its potential in inflammatory disorders.
- **Cytotoxicity and Anti-cancer Properties:** Explore the impact of the extract on the viability of cancer cells, providing valuable information about its anticancer potential.
- **Enzyme Inhibition Studies:** Investigate the extract's ability to modulate specific enzymes, such as acetylcholinesterase or tyrosinase, which may have implications for neuroprotective or skin-related applications.

Correlation between Phytochemical Composition and Biological Activities:

Establish correlations between the identified phytochemicals and the observed biological activities. This will help in understanding which components may be responsible for the observed effects.

Implications for Medicinal Applications:

To provide a foundation for further research on the therapeutic applications of Ruellia simplex, based on both its chemical composition and biological activities. To contribute to the development of natural and potentially safer alternatives in the fields of medicine and pharmacology. By addressing these objectives, the study aims to contribute valuable insights into the pharmacological potential of Ruellia simplex, laying the groundwork for its further exploration as a source of bioactive compounds with potential medicinal applications.

Chapter three
Methodology

3. Materials & methodology

Phytochemical analysis involves the study of bioactive compounds present in plants. Various methods and apparatus are used for the extraction, isolation, and characterization of these compounds.

3.1 Description of plant extraction is given below:

Plant extraction is a process used to isolate bioactive compounds, such as phytochemicals, from plant material. These compounds can include alkaloids, flavonoids, terpenes, phenolic compounds, and other secondary metabolites with potential medicinal, nutritional, or industrial applications. The extraction process aims to obtain a concentrated extract containing the desired compounds while minimizing the presence of undesirable components.

Here is a general description of the plant extraction process:

Selection of Plant Material:

Choose plant parts rich in the target compounds (leaves, stems, roots, etc.). Ensure the plant material is harvested at the right stage of growth for optimal phytochemical content.

Cleaning and Drying:

Clean the plant material to remove dirt and contaminants. Dry the material to reduce water content, which can affect the extraction efficiency and the stability of the extract.

Size Reduction:

Mill or grind the dried plant material to increase the surface area, facilitating better contact with the extraction solvent.

Choice of Solvent:

Select an appropriate solvent based on the polarity of the target compounds. Common solvents include ethanol, methanol, water, chloroform, and hexane.

Extraction Techniques:

0. Soxhlet Extraction:

- Suitable for extracting lipophilic compounds.

- Involves continuous percolation of a solvent through the plant material.

b. Maceration:

- Simple soaking of plant material in a solvent for an extended period.
- Suitable for extracting a wide range of compounds.

c. Ultrasonic Extraction:

- Uses ultrasonic waves to enhance the penetration of solvent into plant cells.
- Rapid and efficient extraction method.

d. Microwave-Assisted Extraction (MAE):

- Applies microwave energy to accelerate the extraction process.
- Can reduce extraction times compared to traditional methods.

Filtration:

- Separate the liquid extract from the solid plant material using filtration.
- Filtration may be followed by centrifugation to further clarify the extract.

Concentration:

Remove the solvent from the extract using techniques such as rotary evaporation or freeze-drying to obtain a concentrated extract.

Analysis and Quality Control:

- Analyze the extract for the presence of target compounds.
- Perform quality control to ensure the consistency and purity of the extract.

Storage:

Store the final extract in appropriate conditions to maintain stability and prevent degradation.

Further Processing:

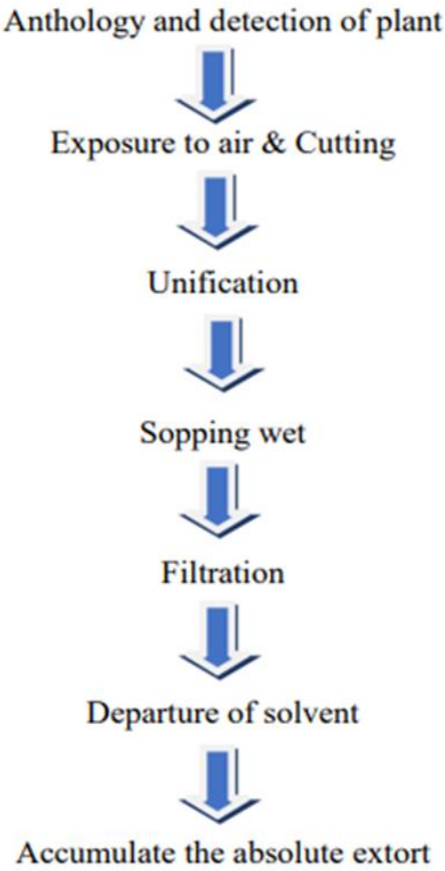
The concentrated extract can be further processed into various forms such as powders, tinctures, or standardized extracts depending on the intended use.

Plant extraction is a crucial step in the production of herbal medicines, natural products, and functional foods, as it allows for the harnessing of the therapeutic or beneficial properties present in plants. The choice of extraction method depends on the properties of the target compounds and the desired characteristics of the final product.

3.2 Exodus of the solvent:

A rotating evaporator operating at 55 degrees Celsius was used to distribute the ethanol liquid deposit. The solvent (ethanol) is hence far away. It produces an abundance of sticky black color. It was then put in a little pipette and filled to the brim with foil paper for fortification. Not to mention, it was kept frozen until exertion started.

Diagrams of the grounding of extort:



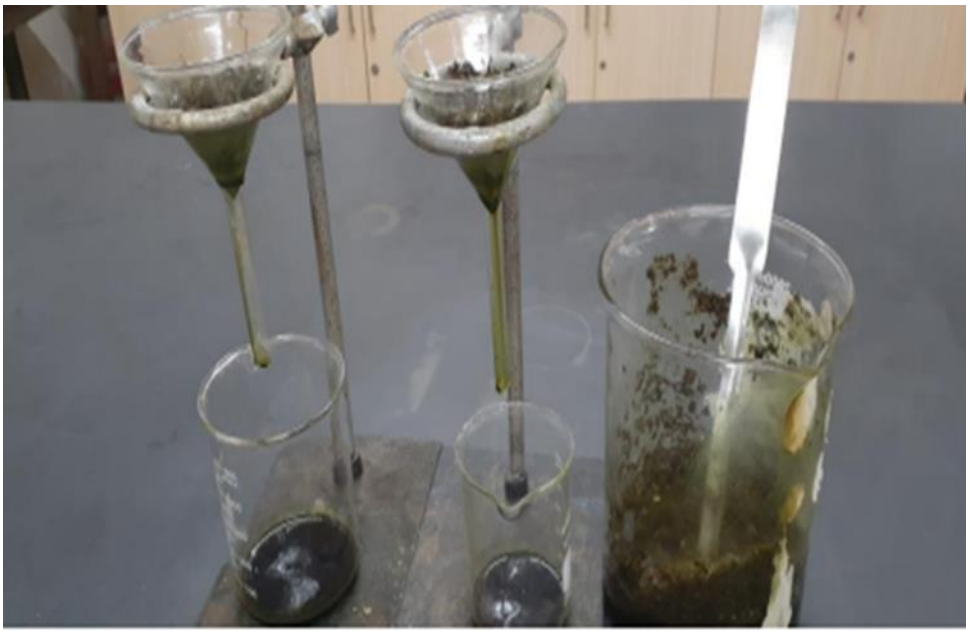


Figure 2: Filtration of extract via funnel with filter paper

Figure 3: Evaporation of extract via Evaporator

3.3 My investigation

In my investigation I have been gadget

- Phytochemical Screening test
- Thrombolytic Activity Assessment
- Anti-diarrheal activity
- Analgesic activity
- Anti-oxidant activity

3.4 Phytochemical Assessment

Phytochemical assessment involves the analysis and study of phytochemicals in plants. Phytochemicals are bioactive compounds that are naturally present in plants and have potential health benefits for humans. These compounds contribute to the plant's defense mechanisms, protection against diseases, and interactions with their environment. Phytochemicals include a wide range of compounds such as alkaloids, flavonoids, terpenoids, saponins, phenolic acids, and more.

3.4.1 Reagents applied for the diverse chemical group analysis

Reagents were used for the different chemical group analysis is given below:

0) **Mayer's reagent:**

5 gm potassium iodide in 20 ml water was combined with 1.36 gm mercuric iodide in 60 ml water to make a solution.

ii) Dragendroff's Reagent:

1.7 gm basic bismuth nitrate and 20 gm tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm potassium iodide and 40 ml water.

iii) Fehling's solution A:

34.64 gm copper sulphate was dissolved in 0.50 ml sulfuric acid and sufficient water to make a 500 ml solution.

iv) Fehling's solution B:

To make 500 milliliters at the time of the exploit, an equal amount of excessive solution, 176 gram of sodium potassium tartarate, and 77 gm of sodium hydroxide were liquefied with sufficient water.

v) Benedicts Reagent:

100 mL Benedicts Reagent made with water the volume was generated by dissolving 1.73 gm cupric sulphate, 1.73 gm sodium citrate, and 10 gm anhydrous sodium carbonate in water.

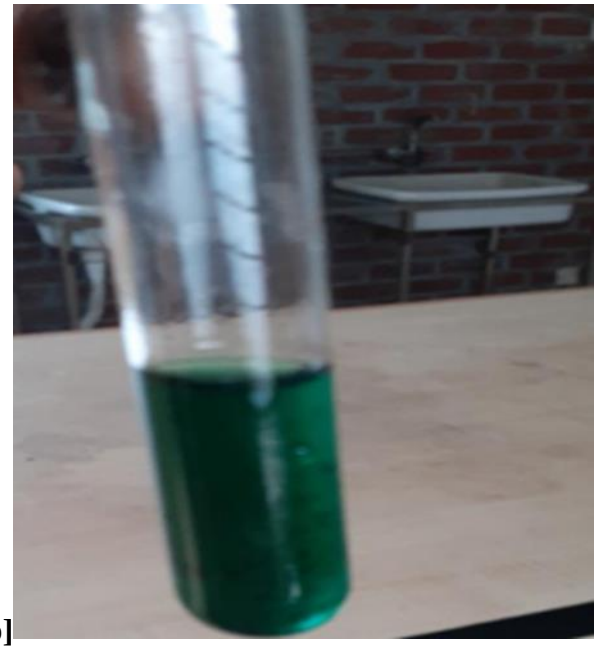
3.4.2 Assessment for alkaloids

Mayer's evaluation

Two milliliters of extort solution and two milliliters of diluted hydrochloric acid were put into a test tube. Then, 1 milliliter of Mayer's reagent was applied. The appearance of a precipitate with a yellow tint indicated the existence of alkaloids.

Dragendroff's examination

Two milliliters of extort solution and two milliliters of diluted hydrochloric acid were put into a test tube. Dragendroff's reagent (1 mL) was then added. An orange-brown precipitated was observed, indicating the possible involvement of alkaloids.

[a]  **[b]** 

A=Mayer’s analysis

B= Dragendroff’s analysis of extract

3.4.3 Assessment for Flavonoids

The presence of flavonoids is shown by the red color, which was obtained by adding a couple of droplets of strong hydrochloric acid to a tiny amount of an alcoholic solution of the plant component.

Figure 4: Flavonoids analysis of extract

3.4.4 Assessment for Saponins

After diluting 1 milliliter of the aqueous solution with 20 milliliters of distilled water, it was agitated in a glutted container for fifteen minutes. A foam layer just one centimeter thick indicates the presence of saponins.

Figure 5: Saponins analysis of extract

3.4.5 Assessment for Tannins

10ml of purified water will be used to absorb 5g of extraction. The mixture will then be filtrated, and the resultant filtrate will be treated with ferric chloride reagents. The formation of a blue-black, green, or blue-green precipitation suggests the presence of tannins.

Figure 6: Tannins analysis of extract

3.4.6 Assessment for Glycosides

One milliliter of water was mixed with a small amount of alcohol extract, and then a small drop of watery sodium hydroxide was applied. The presence of glycosides was indicated by the measurement of yellow color. A small amount of an extract made of alcohol has been heated in Fehling liquid. The presence of glycosides was determined by measuring the red brick precipitated. A further part of the material was heated with a few droplets of diluted sulfuric acid after it had been mixed in water and alcohol. Boil the Fehling solution after neutralizing it with sodium hydroxide water. As a sign glycoside, the red brick precipitated was evaluated.

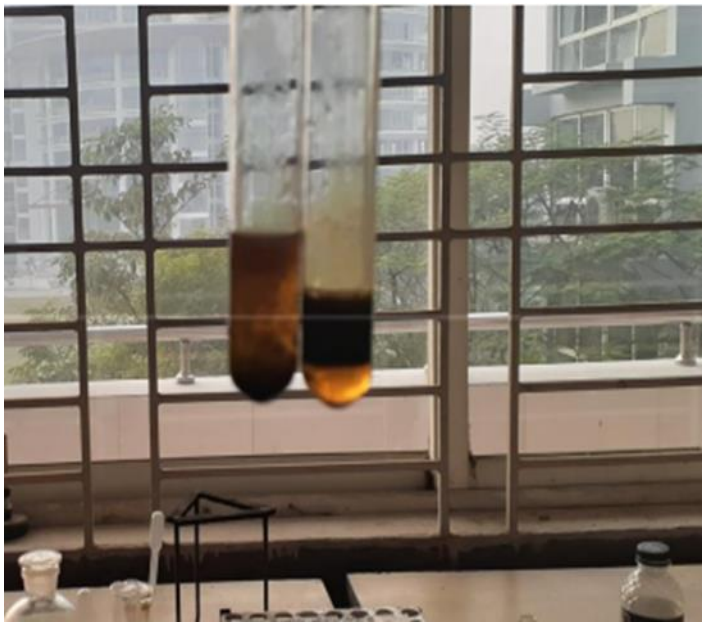


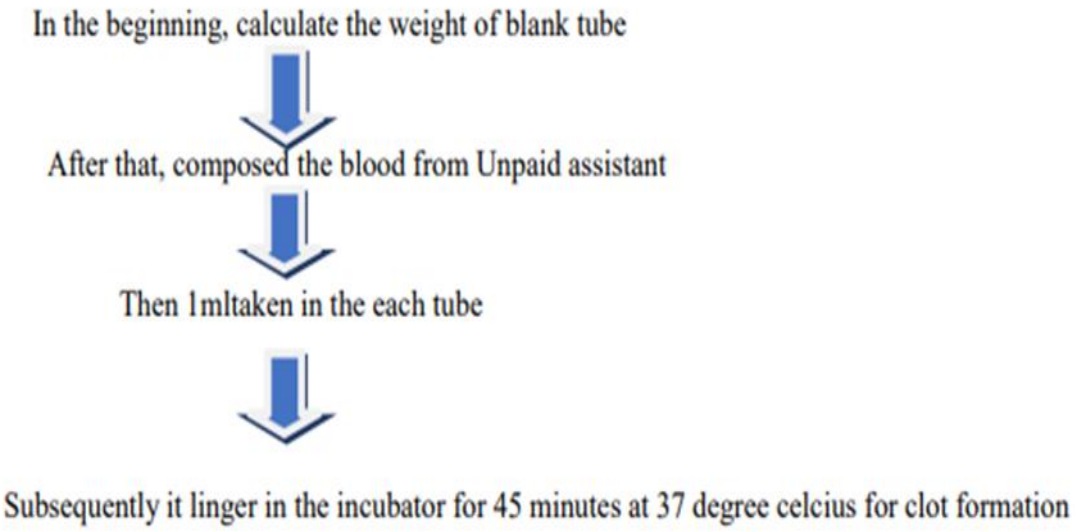
Figure 7: Glycosides analysis of extract

3.5 Assessment of Thrombolytic activity

Machinery and Reagent

- Three Tube.
- Syringe filter.
- Distilled water.
- Blood from volunteer
- Extort solution.
- Streptokinase (STD).
- Incubator.
- Electronic balance

Procedure



1st clot weight= 1st clot with tube weight – blank tube weight

Currently, set on the standard 100 l explicitly30000IU, controlled 100 l distilled water and extort 100 l i.e. 1000 g/ l

Yet againit linger in the incubator for 90 minutes at 37 degree celcius for clot lysis formation .

After that, lysis is alienated from the tube

Over again calculate the 2nd clot with tube weight

Afterward, 2nd clot weight= 2nd clot with tube weight – blank tube weight

In addition to weight of lysis clot = 1st clot weight – 2nd clot weight

To conclude,

% of lysis = (wt of lysis clot/1st clot weight) × 100

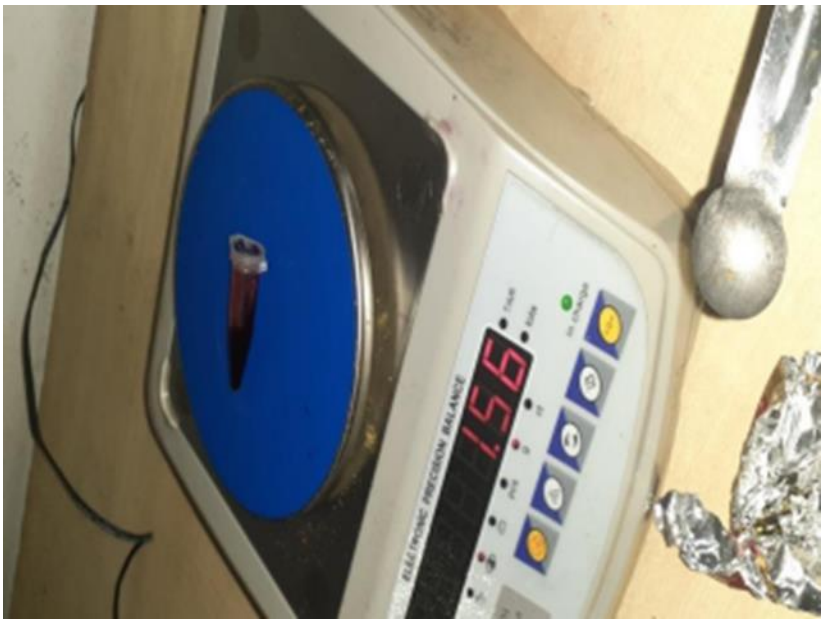


Figure 8: Weight of tube+ clot via Balance machine

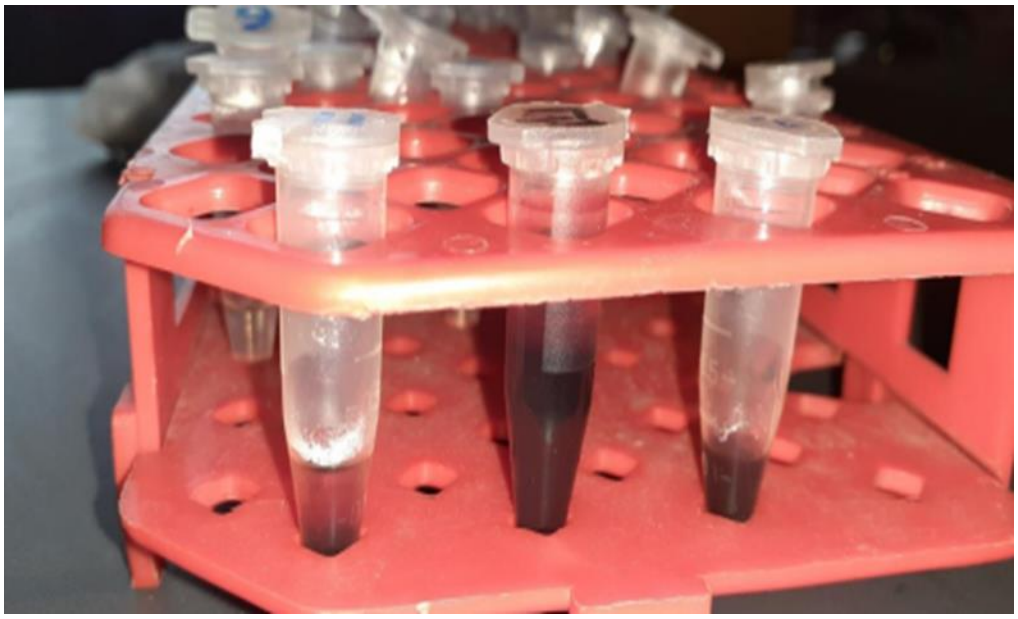


Figure 9: Tube with clot

3.6 Assessment of Anthelmintic effect

Collection of Ruellia Simplex

Medium Preparation

Preparation of Drug Solutions

Evaluation of Anthelmintic Activity

3.7 Assessment of Antioxidant

Reagents:

Ethanol.

2. 0.004% DPPH (1, 1 – diphenyl – 2 – picrylhydrazyl – hydrate).

Ascorbic acid

Methanol

Chloroform

Carbon tetra chloride

Machinery

Beakers

Test tubes and stands

Volumetric flasks

Pipettes

Electric balance

Spatula

Foil paper

Sonicator

Funnel

Tissue paper

Marker

UV spectrophotometer

Progression

FirstlyTo make 6 different types of concentration (1, 5, 10, 50, 100 and 500 µg/ml),6 volumetric flasks was taken

Then,rapped test tubes and volumetric flasks via foil paper.

Serial dilution of extract is completed in 6 volumetric flakst henmanifest them correspondingly.

3 ml of 0.004% DPPH solutionand1ml of sample from each concentration is taken via pipette in 6 test tubes correspondingly

Afterward solution of each test tube was kept in dark place for 30 minutes with raping of foil paper

3ml 0.004% DPPH & 1ml methanol is taken in another test test tube to prepare blank solution.

Subsequently by the help of UV Spectroscopy absorbance was taken.

% of inhibition is measured by using amethod: These formula is given below:

$$\% \text{ Inhibition: } \frac{\text{Blank absorbance} - \text{Solution}}{\text{Blank absorbance}} \times 100$$

% Inhibition: Blank absorbance – Solution

3.8 Assessment of Anti-diarrheal activity

Reagent & machinery

Electronic balance

Extort solution

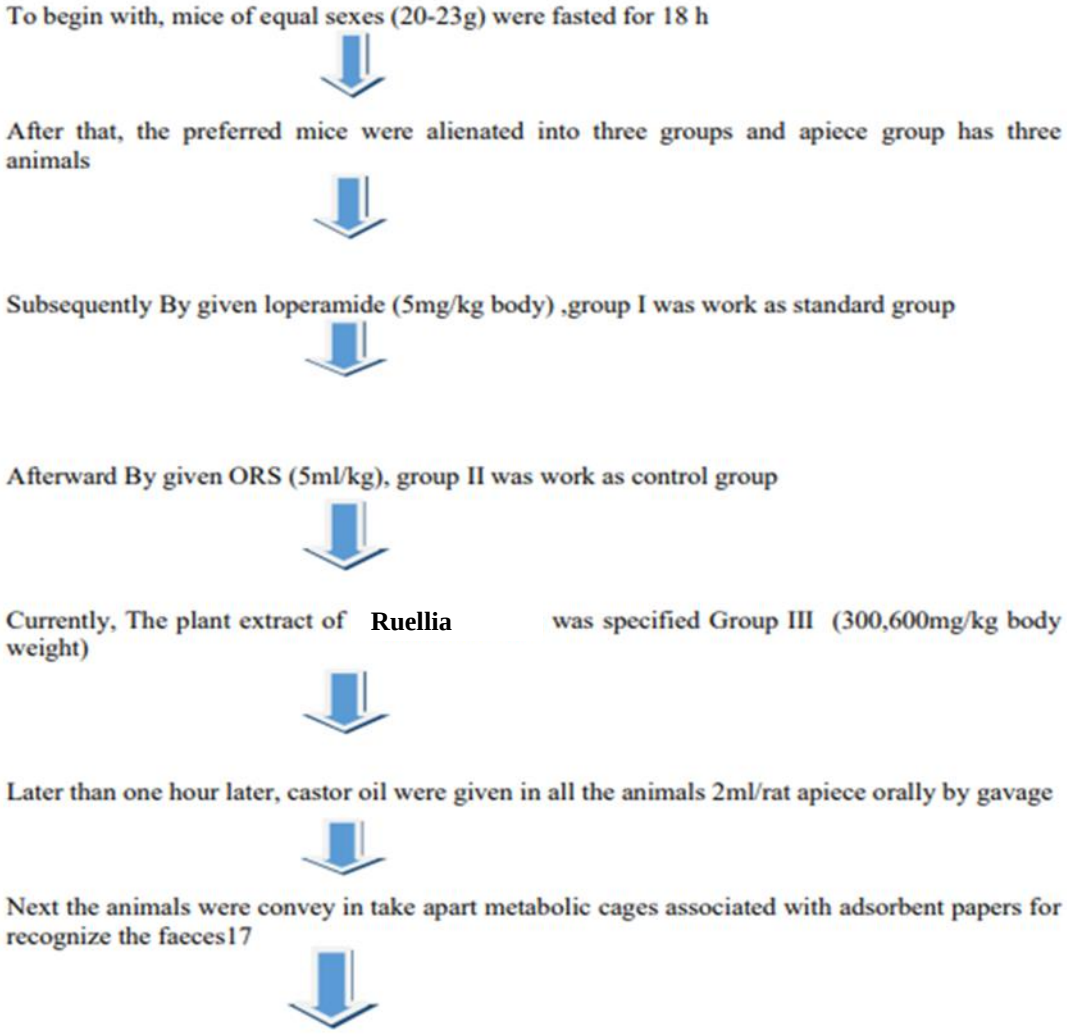
Castrol oil

Loperamide (Opsonin Pharmaceuticals Ltd)

Distilled water

ORS (NaCl 0.9%)

Needle



Followed by the sum of faeces (equally diarrheal and non-diarrheal) excluded were evaluate with the control group.

The totality score of diarrheal faeces for the control group was measured as 100%. The consequences were articulated as a percentage of mortification of diarrhea.

$$\text{Fraction (\%) inhibition of defecation} = \left(\frac{A - B}{A} \right) \times 100$$

Here, A = Mean no. of defecation by control and

B = Mean no. of defecation by drug/extract

Chapter four
Results and Discussion

4.1 Result and Discussion of phytochemical evaluation

Phytochemical constitution	Results
Alkaloids	existence
Saponins	existence
Flavonoids	existence
Glycoside	nonexistence
Tannin	existence
Carbohydrate	existence

Table 1: Result of phytochemical evaluation

The table that follows displays the corresponding dried extract amount, physical characteristics, and qualitative chemical analysis of the several Ruellia Simplex extracts:

4.2 Consequence of Thrombolytic effect

Sample	Blank Tube Weight Gm (A)	1st clot+ Tube Weight gm (B)	1st clot Weight gm (B-A)	2nd clot+ Tube Weight gm ©	2nd clot Weight gm (C-A)	Weight of lysis clot gm (1st clot – 2nd clot)	% of Lysis
Control (Distil water)	0.80	1.82	1.02	1.67	0.87	0.15	14%
Extract	0.71	1.89	1.18	1.07	0.36	0.82	69%
Standard (Streptokinase)	0.81	1.70	0.89	0.97	0.16	0.73	74%

Table 2: Result of Thrombolytic effect

% of lysis = ((weight of lysis clot)*(100))/(1st clot of weight)

Discussion

Distilled water was used as a negative control and showed a very small amount of clot lysis (14%). A statistical analysis revealed that the mean variance in clot lysis percentage among the positive and negative controls was quite middling. Ruellia Simplex methanol extract showed adequate thrombolytic effectiveness (69%), according to this investigation.

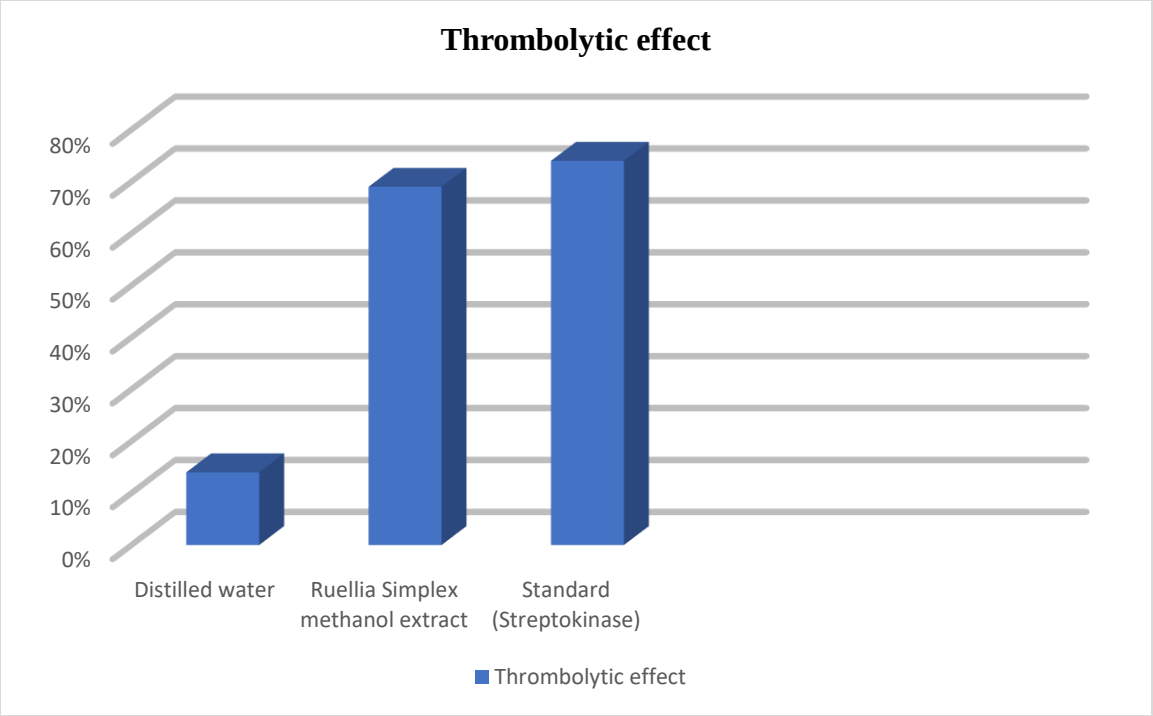


Figure 10: Thrombolytic effect

4.3 Anthelmintic Activity of Methanol extract of Ruellia Simplex

Group	Concentration (mg/mL)	Paralyzed time (min)	Death time (min)
Control	N/A	N/A	N/A
Mebendazole	25 mg/mL	15 min	50 min
Methanol extract of Ruellia Simplex	100 mg/mL	12 min	33 min
	50 mg/mL	15 min	60 min
	25 mg/mL	31 min	82 min
	12.5 mg/mL	45 min	95 min

Table 3: Anthelmintic Activity of Methanol extract of Ruellia Simplex

4.4 Results of Anti-oxidants test

***Methanol extract of Ruellia Simplex**

Concentration (µg/mL)	% of inhibition	IC50 (µg/mL)
500	76.90%	**20.487**
250	73.34%	
125	67.82%	
62.5	58.78%	
31.25	53.42%	

Table 4: DPPH free radical inhibition activity extract (methanol extract of Ruellia Simplex)

***IC50 for standard (Ascorbic acid)**

Concentration (µg/mL)	% of inhibition	IC50 (µg/mL)
500	92.48%	**9.072**
250	85.79%	
125	78.57%	
62.5	69.33%	
31.25	63.74%	

Table 5: DPPH free radical inhibition activity standard (Ascorbic acid)

Discussion: The methanolic extract of Ruellia Simplex and standard ascorbic acid were concentrated on free radical scavenging activity (% of inhibition IC50) using the Brand Williams et al. (1995) technique. Standard ascorbic acid's DPPH inhibitory effect displays the IC50 (9.072 µg/ml) as the positive control. The Methanol extract of Ruellia Simplex has a notable DPPH inhibitory effect, with an IC50 of 20.487 µg/ml when compared to standard.

4.5 Result of anti-diarrheal evaluation

Result:

Table:Test materials used in the evaluation of anti-diarrheal activity.

Code no	Test sample	Group	Identification	Dose (mg/kg)*
CTL	1% Tween-80 in normal saline	I	Control group	0.1 ml/10g of body wt.
STD	Loperamide	II	Standard group	50
MESF 200	Methanolic extract of branch of ruelia simplex	IIIa	Test sample	200
MESF 400	Methanolic extract of branch of Ruelia simplex	IIIb	Test sample	400

Table:Total number of diarrheal faces stool given by each mouse.

Code no	Mice no	No. of diarrheal feces				Total of diarrheal feces	Average
		1 hour	2 hour	3 hour	4 hour	Total feces	
CTL	1	1	6	5	3	16	16.33
	2	3	3	1	6	13	
	3	7	5	6	1	19	
STD	1	0	0	0	0	0	0.00
	2	0	0	0	0	0	
	3	0	0	0	0	0	
MESF 200	1	1	0	6	3	9	8.78
	2	0	3	2	3	8	
	3	1	1	3	3	8	
MESF 400	1	3	2	2	0	7	9.32
	2	6	0	1	1	8	
	3	4	3	2	4	13	

Table:Effects of methanolic extract of branch on castor oil (1ml/mice)induced diarrhea in mice.

Group	t-test value	%Reduction of diarrhea	Degree of freedom	P value	Statistical Significance
II	9.2601	100.0	4	0.0008	Extremely statistically significant
MESF 1	4.4567	50.98	4	0.0114	statistically significant
MESF 2	3.592	44.78	4	0.0229	statistically significant

Table 6: Result of anti-diarrheal evaluation

Chapter five
Conclusion

5.1 Conclusion

To sum up, the investigation into the biological testing and phytochemical screening of Ruellia simplex methanol extract has yielded important new information about the plant's possible therapeutic uses. A thorough phytochemical examination demonstrated the existence of a wide range of bioactive chemicals, indicating the plant's abundance of supplemental metabolite. In addition, the methanol extract's high bioactivity has been shown by the biological tests that were carried out, whether they were centered on antioxidant, antibacterial, or other pertinent activities. The biochemical properties of the Ruellia simplex methanol extract were then assessed using a battery of in vitro experiments and in-vivo anti-diarrheal, in-vitro thrombolytic activity, in vitro antioxidant & in vivo anthelminthic activity. Ruellia Simplex methanol extract showed adequate thrombolytic effectiveness (69%), according to this investigation. Standard ascorbic acid's DPPH inhibitory effect displays the IC50 (9.072 µg/ml) as the positive control. The Methanol extract of Ruellia Simplex has a notable DPPH inhibitory effect, with an IC50 of 20.487 µg/ml when compared to standard. All things considered, the study advances our understanding of plant-based medicine and supports more research on Ruellia simplex as a possible source of bioactive chemicals with a range of medicinal uses.

Chapter six
Reference

6 Reference

[1] Villarruel GR, Langley GE, Abedi GR, Anderson LJ. (2009) Respiratory syncytial virus activity-United States, July 2008 December 2009, MMWR Morbidity and Mortality Weekly Report, 59, 230-233.

[2] Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. (2005) Respiratory syncytial virus infection in elderly and high-risk adults. The New England Journal of Medicine, 352, 1749-1759.

[3] Cingoz O. (2009) Motavizumab. MAbs, A new assay system for evaluation of developmental immunotoxicity of chemical compounds using respiratory syncytial virus infection to offspring mice. Environmental Toxicology and Pharmacology,25, 69-67.

[4] Gill MA, Welliver RC. (2009) Motavizumab for the prevention of respiratory syncytial virus infection in infants. Expert Opinion on Biological Therapy, 9, 1335-1345.

[5] Pelaez A, Lyon GM, Force SD, Ramirez AM, Neujahr DC, Foster M, Naik PM, Gal AA, Mitchell PO, Lawrence EC. (2009) Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. Transplantation infection, 28, 67-71.

[6] Weisman LE. (2009) Respiratory syncytial virus (RSV) prevention and treatment: past, present, and future. Cardiovascular & Hematological Agents in Medicinal Chemistry, 7, 223-233.

[7] Yasukawa K. Sun Y, Kitanaka S, Tomizawa N, Miura M, Motohashi S. (2008) Inhibitory effect of the rhizomes of Alpinia officinarum on TPA-induced inflammation and tumor promotion in two-stage carcinogenesis in mouse skin. Journal of Natural Medicines, 62, 374-378.

[8] Kurokawa M, Ochiai H, Nagasaka K, Neki M, Xu H, Kadota S, Sutardjo S, Matsumoto T, Namba T, Shiraki K. (1993) Antiviral traditional medicines against herpes simplex virus (HSV-1), poliovirus, and measles virus in vitro and their therapeutic efficacies for HSV-1 infection in mice. Antiviral Research, 22, 175-188.

[9] Sun Y, Tabata K, Matsubara H, Kitanaka S, Suzuki T, Yasukawa K. (2008) New cytotoxic diarylheptanoids from the rhizomes of Alpinia officinarum. Planta Medica, 74, 427-431.

[10] Lee HJ, Kim JS, Ryu J-H. (2006) Suppression of inducible nitric oxide synthase expression by diarylheptanoids from Alpinia officinarum. Planta Medica, 72, 68-71.

[11] Kiuchi F, Shibuya M, Sankawa U. (1982) Inhibitors of prostaglandin biosynthesis from Alpinia officinarum. Chemical & Pharmaceutical Bulletin, 30, 2279–2282.

[12] Kiuchi F, Iwakami S, Shibuya M, Hanaoka F, Sankawa U. (1992) Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids. Chemical & Pharmaceutical Bulletin, 40, 387-391.

[13] Yadav PN, Liu Z, Rafi MM. (2003) A diarylheptanoid from Lesser Galangal (Alpinia officinarum) inhibits proinflammatory mediators via inhibition of mitogen-activated protein kinase, p44/42, and transcription factor nuclear factor-κB. The Journal of Pharmacology and Experimental Therapeutics, 305, 925-931.

[14] Sawamura R, Sun Y, Yasukawa K, Shimizu T, Watanabe W, Kurokawa M. (2010) Antiviral activities of diarylheptanoids against influenza virus in vitro. Journal of Natural Medicines, 64, 117-120.

[15] Sawamura R, Shimizu T, Sun Y, Yasukawa K, Miura M, Toriyama M, Motohashi S, Watanabe W, Konno K, Kurokawa M. (2010) Anti-influenza virus activity in vitro and in vivo of diarylheptanoids isolated from Alpinia officinarum. Antiviral Chemistry and Chemotherapy, 21, 33-41.

[16] Kawana F, Shigeta S, Hosoya M, Suzuki H, De Clercq E. (1978) Inhibitory effects of antiviral compounds on respiratory syncytial virus replication in vitro. Antimicrobial Agents and Chemotherapy, 31, 1225-1230.

[17] Xie YY, Zhao XD, Jiang LP, Liu HL, Wang LJ, Fang P, Shen KL, Xie ZD, Wu YP, Yang XQ. (2006) Inhibition of respiratory syncytial virus in cultured cells by nucleocapsid gene targeted deoxyribozyme (DNAzyme). Antiviral Research, 71, 31-41.

[18] An N, Xu L-Z, Zou Z-M, Yang S-L. (2006) Diarylheptanoids from Alpinia officinarum. Journal of Asian Natural Products Research, 8, 637–641.

[19] Shin D, Kinoshita K, Koyama K, Takahashi K. (2002) Antiemetic principles of Alpinia officinarum. Journal of Natural Products, 65, 1315–1318.

[20] Sun, Y, Matsubara H, Kitanaka S, Yasukawa K. (2008) Diarylheptanoids from the rhizomes of Alpinia officinarum. Helvetica Chimica Acta, 91, 118-123.

[21] Watanabe W, Shimizu T, Hino A, Kurokawa M. (2008) A new assay system for evaluation of developmental immunotoxicity of chemical compounds using respiratory syncytial virus infection to offspring mice. Environmental Toxicology and Pharmacology,25, 69-67.

[22] Watanabe W, Konno K, Ijichi K, Inoue H, Yokota T, Shigeta S. (1994) MTT colorimetric assay system for the screening of antiorthomyxo- and anti-paramyxoviral agents. Journal of Virological Methods, 48, 257-265.

[23] Amiruzzaman N. 1990. Post-harvest handlilng and processing of fruits and vegetables. In: Kitchen Gardening and Homestead Productive Activities.CIRDAP Action Research Series No. 11. p. 22.

[24] Anonymous. 1979. Extension bulletin No. 22, Indian Institute of Horticultural Research, Bnglalore Jackfruit. In: Fruits of India Tropical and Subtropical. T.K. Bose (eds).Naya Prokash, 206 Bidhan Sarani, Calcutta-700 006, India. PP. 487.

[25] Ashwah. EI, Abd-EI-Baki N M, Samahy EI and Fedul M G.1980. Effect of storage on the characteristics of the concentrated orange and lime beverages.Agricultural Research Review 58(3). 275-288 (1980 pub. 1982). Hort.Res. Inst. Agric. Res. Centre. min. of . Agric. Cairo. Egypt.

[26] Bhatnagar H C and H Subramanyam. 1973. Fruits of India tropical and subtropical. T.K. Bose (eds). Naya Prokash, 206 Bidhan Sarani, Calcutta-700 006, India. PP. 70-71.

[27] BBS. 2006. Statistical Yearbook of Bangladesh. Bangladesh Bureau of Statistics, Ministry of Planning, Dhaka, Bangladesh.PP.158-159.

[28] Carlos J R .1992. Bangladesh. Off season production of horticulture. Proc.of the Six National Horticulture Convention and Symposium. Bangladesh Society of Horticultural Science, Dept. of Hort., Bangladesh Agricultural University, Mymensingh..

[29] Chaudhry T M and Farooque M A R. 1969. Fruits of India Tropical and Subtropical. T.K. Bose (eds). Naya Prokash, 206 Bidhan Sarani, Calcutta-700 006, India. PP. 72.

[30] Ewaidah E H .1972. Studies on commercially canned juices product locally in Soudia Arabia,III. Physicochemical organoleptic and microbiological assessment. Food chemistry. 44(2);103-111.

[31] Frazier W C.1958. Food Microbiology. 2nd Eds. McGraw-Hill Book Co. New York.pp. 199. Hossain A K M A.1989. Manual on Mango Cultivation in Bangladesh. Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, p.64.