Phytochemical and Pharmacological investigation of methanolic extract of the leaves of Hibiscus subdarifa.

PROJECT WORK

THIS IS A PROJECT REPORT THAT FULFILLS SOME OF THE REQUIREMENTS FOR A BACHELOR OF PHARMACY DEGREE. IT HAS BEEN SUBMITTED TO THE DEPARTMENT OF PHARMACY AT DAFFODIL INTERNATIONAL UNIVERSITY.

SUBMITTED TO

DEPARTMENT OF PHARMACY, FACULTY OF HEALTH & LIFE SCIENCES

DAFFODIL INTERNATIONAL UNIVERSITY

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Date of submission:

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APPROVAL

A project paper investigating the medicinal properties of the leaves of Hibiscuss Sabdariffa has been submitted and accepted by the Department of Pharmacy at Daffodil International University for partial fulfillment of a Bachelor of Pharmacy degree.

BOARD OF EXAMINERS

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DECLARATION

I hereby declare that this project report is done by me under the supervision of **Dr. Sarowar Hossain Professor**, Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for a Bachelor's or any degree award.

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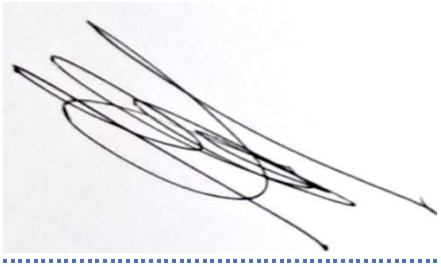

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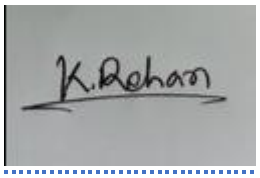

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- **Khandaker Md. Rehan Uddin**

DEDICATED TO MY

BELOVED PARENTS,

RESPECTED TEACHERS,

AND WELL-WISHERS.

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ABSTRACT

Traditional uses for Hibiscus sabdariffa (roselle) include food, herbal drinks, hot and cold beverages, food industry flavoring, and herbal medicine. This study aimed to investigate the phytochemical, thrombolytic, and antioxidant properties of Hibiscus sabdariffa. Phytochemical screening was conducted using various chemical tests to identify the presence of different bioactive compounds. The thrombolytic activity was assessed by measuring the percent inhibition of clot lysis, while the antioxidant activity was determined using the DPPH (2,2- diphenyl- 1-picrylhydrazyl) radical scavenging assay. The results revealed that the plant extract exhibited significant antioxidant and thrombolytic activities compared to the standard compounds. The observed biological activities were correlated with the presence of phytochemicals, suggesting that Hibiscus sabdariffa could be a potential source of natural compounds with therapeutic applications for conditions related to oxidative stress and thrombosis.

CHAPTER ONE:

INTRODUCTION

1.1 GENERAL INTRODUCTION

Natural compounds, derived from various sources, have been instrumental in drug discovery for centuries. Their structural diversity and wide range of biological activities offer inspiration for novel drug designs. Some natural compounds can be used directly as drugs, while others serve as templates for creating new analogs with improved properties. Additionally, they can be utilized as biological probes to study diseases and identify potential drug targets. Despite challenges like complexity, supply, and bioavailability, natural compounds continue to hold great promise in the field of drug discovery, thanks to advancements in technology. [1,2]

1.2 STATUS OF MEDICINAL PLANTS IN BANGLADESH:

Bangladesh, a country with a subtropical climate, has a wealth of natural medicinal plant reserves. Early in the 1980s, domestic forests were a major source of supply for local herbal medicine enterprises, mainly Ayurvedic and Unani, which imported 20% of their needs while producing 80% locally. But since then, the pattern has changed. Currently, just 20% of the demand is met domestically, with a startling 80% coming from imports. The Bangladesh Agricultural Research Institute (BARI) lists a meager 722 medicinal plant species, much fewer than India's 4,000, despite Bangladesh's vast biodiversity. Only about 700 of these are used in traditional medicine in Bangladesh; makers of Ayurvedic and Unani medicines use 255 of these species.[3]

1.3 PLANTS IN TRADITIONAL MEDICINE:

An estimated 70–80% of people worldwide primarily rely on traditional herbal therapy to meet their medical needs. This indicates how commonplace the practice is. In addition, there is a sizable and growing market for herbal medicine. In China's Yunnan province alone, the amount of medicinal plants gathered has increased tenfold in the last 10 years. In India, for example, the market for Ayurvedic medicines is growing at an astounding rate of twenty percent every year.[4][5]

Numerous reasons, including as population increase and the scarcity of Western allopathic medicine in many developing countries, contribute to this rising demand. [6]

Consequently, there is now greater strain on gathering places, like the Indian Himalayan Gori Valley, and the medicinal and aromatic plant (MAP) harvesting season now lasts between two and five months annually.[7][8]

1.4 AN OVERVIEW OF Hibiscus sabdariffa

Hibiscus sabdariffa, commonly known as **roselle**, is a flowering plant belonging to the Malvaceae family. Native to West Africa, it has been cultivated and used for centuries for its medicinal and culinary properties.

Botanical Characteristics:

- **Annual shrub:** It grows as an annual shrub, reaching heights of up to 3 meters.
- **Reddish stems:** The stems are typically reddish in color.
- **Large flowers:** The flowers are large and showy, often with a bright yellow or red center.
- **Edible calyces:** The most valuable part of the plant is the fleshy, red calyx that surrounds the flower.

Culinary Uses:

- **Beverages:** The dried calyces are often steeped to make a refreshing and tangy tea, known as "zobo" or "carcade" in various regions.

- **Jams and jellies:** The calyces can also be used to make jams and jellies with a unique flavor.

- **Food coloring:** The vibrant red color of the calyces is used as a natural food coloring agent.

Medicinal Properties:

- **Antihypertensive:** Studies have shown that hibiscus sabdariffa can help lower blood pressure.

- **Antioxidant:** The plant contains antioxidants that may help protect against cell damage.

- **Anti-inflammatory:** It has anti-inflammatory properties that can be beneficial for various health conditions.

- **Diuretic:** Hibiscus sabdariffa can act as a diuretic, helping to increase urine production.[9]

1.5 THE BENEFITS OF NATURAL MEDICINE:

For centuries, people have used natural medicine before modern technology and doctors. Patients and doctors relied on natural techniques and herbal remedies to heal illnesses and injuries, from common colds to life-threatening conditions like cancer, diabetes, and heart disease. These conditions were successfully treated without modern medical equipment.[10]

1.6 PLANT PROFILE:

1.6.1 Scientific name [11]

Scientific name*:* *Hibiscus sabdariffa*

Family: *Malvaceae*

1.6.2 Taxonomic Classification [12]:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malvales

Family: Malvaceae

Subfamily: Malvoideae

Tribe: Hibisceae

Genus: Hibiscus

Species: H. sabdariffa

Binomial name: Hibiscus sabdariffa L.

1.6.3 SYNONYMS:

- *karkadeh* Arabic
- *chin baung* Burmese,
- *luòshénhuā* - Chinese,
- *chukur* , Bengali.
- *amlamadhur* Bengali.
- *Rosella Australia*
- *ìsápá* Africa
- Florida Cranberry America

1.6.4 COMMON VERNACULAR NAMES [13]

- Roselle,
- Jamaica Sorrel,
- Red Sorrel,
- Sorrel,
- Indian Sorrel,
- Asam Susar,

1.6.5 PLANT DESCRIPTION/ MORPHOLOGY [14]:

Hibiscus sabdariffa, commonly known as **roselle**, is a tropical plant with distinctive morphological characteristics. Its unique appearance and nutritional value have made it a subject of interest for researchers.

Key Morphological Features

- **Habit:** Hibiscus sabdariffa is an annual or perennial shrub, typically reaching a height of 2-3 meters.
- **Stem:** The stem is erect, often branching, and can be green, reddish, or purplish in color.
- **Leaves:** Leaves are alternate, simple, and usually lobed with serrated margins. They can vary in color from green to reddish-green.
- **Flowers:** Flowers are large, showy, and often have a bright yellow or red color. They are typically composed of five petals and numerous stamens.
- **Calyx:** The calyx, which is the part most commonly used in culinary and medicinal applications, is often red or purplish and can be fleshy or fibrous.
- **Fruit:** The fruit is a capsule containing numerous seeds.

1.6.6 THE PLANT PART UTILIZED IN THE STUDY [15]

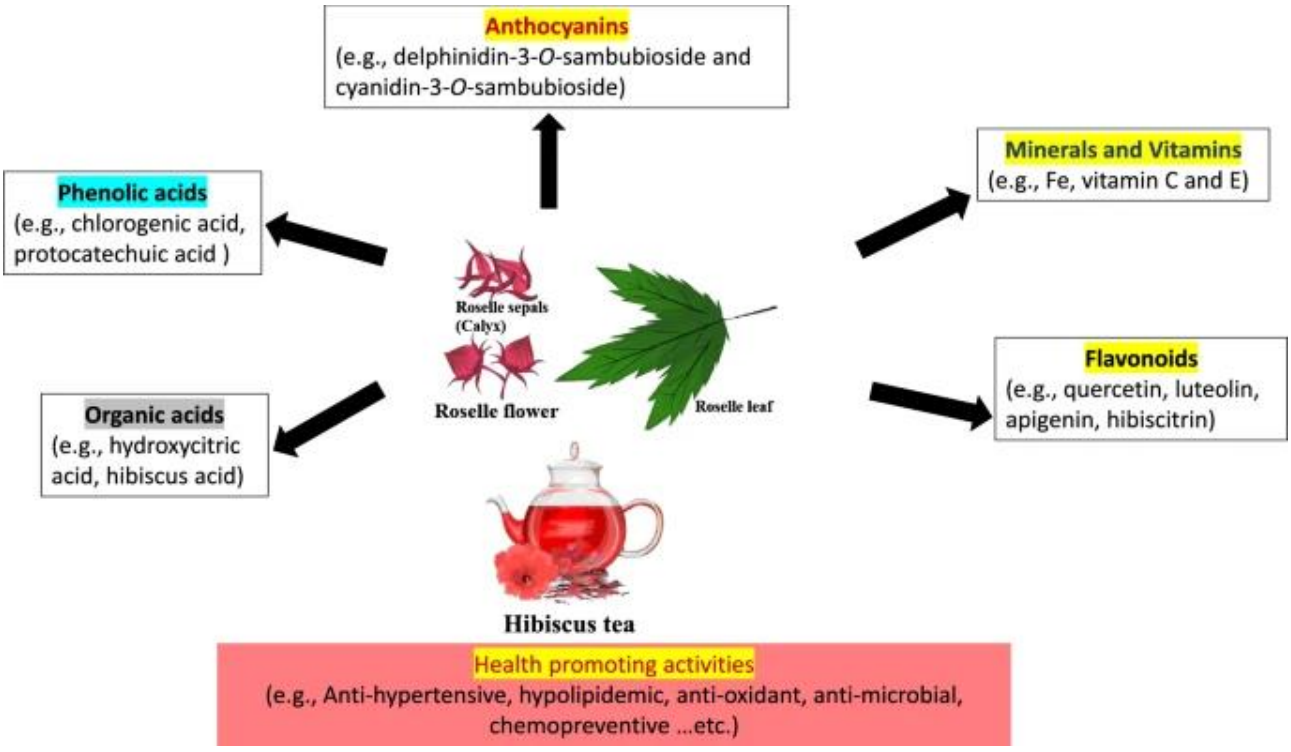

Figure 1: plant parts of Hibiscus subdariffa

My target was to find the therapeutic activities of leaves of Hibiscus sabdariffa.

Figure 2: Leaves of *Hibiscus sabdariffa* [16]

1.6.7 TRADITIONAL USES:

Traditional Uses

- **Antihypertensive:** Perhaps the most widely recognized traditional use of Hibiscus sabdariffa is for managing high blood pressure.

- **Diuretic:** The plant has been used as a diuretic to promote urination.
- **Anti-inflammatory:** Traditional healers have employed Hibiscus sabdariffa to treat inflammation and associated conditions.
- **Antioxidant:** The plant's antioxidant properties have led to its use in promoting overall health and protecting against cellular damage.
- **Digestive Aid:** Hibiscus sabdariffa has been used to support digestion and relieve digestive discomfort.
- **Cooling Effect:** In some cultures, the plant is believed to have a cooling effect on the body, making it a popular beverage during hot weather.
- **Cooling Effect:** In some cultures, the plant is believed to have a cooling effect on the body, making it a popular beverage during hot weather**.[17]**

CHAPTER TWO:

LITERATURE REVIEW

2.1 Phytochemical and antioxidant activity of Roselle (Hibiscus Sabdariffa L.) petal extracts

The Hibiscus sabdariffa L. roselle is prized for both its medicinal and delicate qualities. The current study intends to investigate the phytochemical screening of Hibiscus sabdariffa for a number of chemicals that are significant to medicine and their quantification. The findings indicated that the petals of the H. sabdariffa contain alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols, and tannins. The concentration of anthocyanins was maximum, whereas the contents of flavonoids and phenols were minimum. Two phenolic acids, sixteen flavonoids, and four anthocyanins were found in the petals of H. sabdariffa by HPLC analysis. The main anthocyanins were cyanidin 3-O-sambubioside and delphinidin 3-O-sambubioside, whereas the main flavonoids were gossypetin, hibiscetin, quercetin, and sabdaretin. The examined extract's antioxidant activity possesses a scavenging capacity of around 97% for DPPH radicals. The H. IC50 values.

Ascorbic acid, the reference control, had a concentration of 0.35 mg/ml, but the sabdariffa extract had a concentration of 0.24 mg/ml. This suggested that anthocyanins, flavonoids, and phenolic acid—the chemicals that make up the majority of H. sabdariffa petals—contribute to their antioxidative properties. Our results validate the use of H. sabdariffa flower extract in folklore remedies by showing it to be a possible natural antioxidant source.**[18]**

2.2 GC-MS Analysis, Antibacterial, and Anticancer Activities of *Hibiscus sabdariffa* L. Methanolic Extract: In Vitro and In Silico Studies

The methanol extract of Hibiscus sabdariffa L. was the most effective against all tested bacteria, with the highest growth inhibition recorded against E. coli. It also showed promising anticancer effects against Caco-2 cells. GC-MS analysis confirmed the presence of bioactive components in the extract, and molecular docking studies suggested potential binding interactions with target proteins. Overall, the results suggest that Hibiscus sabdariffa L. is a promising candidate for further investigation as a natural therapy for infections and cancer.[**19]**

2.3 Antioxidant and Anti-Thrombotic Properties of Selected Plant Extracts of Asia

The study evaluated the antioxidant, anticoagulant, and anti-platelet properties of eight plant species commonly used in Asian food and traditional medicine. The results showed that the plant species varied significantly in their activities. Emblica officinalis exhibited strong antioxidant and anti-platelet properties, while Hizikia fusiforme and Hibiscus sabdariffa demonstrated strong anticoagulant activity. The study suggests that the antioxidant and anti-thrombosis effects of these plants might not be directly related, indicating different active components responsible for each activity. Emblica officinalis, with its combined antioxidant and anti-thrombotic properties, could be a promising functional food ingredient for preventing oxidative stress and thrombosis.**[20]**

2.4 Phytochemical analysis and medicinal uses of Hibiscus sabdariffa

The study investigated the phytochemical composition, nutrient value, and economic importance of Hibiscus sabdariffa, a plant widely used in Nigeria to make a popular beverage called Zobo drink. The analysis revealed that the plant is rich in essential minerals and nutrients like iron, copper, calcium, magnesium, and manganese, crucial for human health. Phytochemical extraction using solvent extraction revealed the presence of alkaloids, tannins, saponins, glycosides, phenols, and flavonoids in the plant extract. Quantitative analysis showed that tannins were the most abundant phytochemical, followed by flavonoids. Hibiscus sabdariffa has medicinal benefits, including lowering hypertension and cholesterol. The study suggests that the plant's protective effects are due to its phytochemical content, vitamins, and minerals. Medicinal and aromatic plants offer therapeutic benefits and are more affordable than synthetic alternatives. Hibiscus sabdariffa, readily available, can provide these benefits.[**21]**

2.5 Phytochemical Screening and Antibacterial Activities Of *HIBISCUS SABDARIFFA* L. Leaf Extracts

The study evaluated the phytochemical properties and antibacterial activity of Hibiscus sabdariffa L (rosselle) leaf extracts. Phytochemical analysis revealed the presence of tannins, flavonoids, saponins, and steroids in the methanolic extract. Antibacterial testing against Salmonella typhi, Escherichia coli, and Staphylococus aureus showed that the extract exhibited the strongest activity against Staphylococus aureus. Further studies are necessary to assess the toxicity of the extract.**[22]**

2.6 Evaluation of Co-administration of Roselle Water Extract (*Hibiscus sabdariffa* L.) and Aspirin for Antiplatelet Therapy in Male Sprague-Dawley Rats

The study investigated the potential interaction between roselle water extract and aspirin in rats. Both substances have similar functions in maintaining cardiovascular health. The study found that co-administration of roselle water extract and aspirin did not significantly affect bleeding time, survival rate, or the number of microthrombus, indicating that roselle water extract does not interfere with aspirin's pharmacodynamics. **[23]**

CHAPTER THREE:

PURPOSE OF THE STUDY

3.1 EXPLORING THE THERAPEUTIC POTENTIAL OF HIBISCUS SABDARIFFA: A PATHWAY TO NEW DRUG DEVELOPMENT IN BANGLADESH

To fully realize the therapeutic potential of Hibiscus sabdariffa in Bangladesh, several key areas of research and development need to be prioritized:

1. **Phytochemical analysis:** A comprehensive analysis of the bioactive compounds present in Hibiscus sabdariffa grown in Bangladesh is essential to understand its unique properties.
2. **Pharmacological studies:** In-depth pharmacological studies are needed to elucidate the mechanisms of action of these compounds and their potential therapeutic benefits.
3. **Clinical trials:** Well-designed clinical trials are necessary to evaluate the safety and efficacy of Hibiscus sabdariffa-derived products in human subjects.
4. **Drug formulation and delivery:** Research on the development of effective drug formulations and delivery systems is crucial for maximizing the bioavailability and therapeutic potential of the plant's bioactive compounds**.[24]**

CHAPTER FOUR:

MATERIAL & METHODS

4.1 PLANT COLLECTION:

The Hibiscus sabdariffa leaves were collected from the local area. It was collected during the day in June 2024. During collection of the leaves any chance of adulteration, hydrolysis, or chemical degradation was avoided.

4.2 PREPARATION OF PLANT FOR THE TEST:

Collect the Sample

The experimental leaves were cleaned by removing any unwanted material and plant part

Plant leaves were cleaned with fresh water

Then dried under the sun for five days and due to weather conditions, it shifted to a microwave dryer for 3 days.

When well-derided crush the plant leaves into fine powders by blender.

250 g of leaf powder were kept in a container. Then move it through the Soxhlet extraction machine for three days.

Then the sample is extracted by 400 ml methanol. Then filter it with a Vacuum (2 times) and filter paper.

Then we got the plant extract solution.

Methanol was vaporized at a temperature between 65 and 670 degrees Celsius and a rotation rate between 34 and 38 revolutions per minute. The material that had evaporated was moved to a 50 ml beaker. Once the pure extract was collected, the beakers were covered with aluminum foil and the extract was placed immediately under the fan. The residual solvent in the extract was then evaporated, and the leftover water was then partially removed using a cold hair dryer.

4.3 Preparation of plant sample

During their freshest state, the leaves were harvested. In order to guarantee that any remaining moisture was gone and the leaves were crispy, the leaves were sun-dried for two hours each day for two days following about five days of shed drying. Until needed, the finely powdered leaves are stored in an airtight container and kept somewhere dry, cool, and dark using a grinder.

4.4 Extraction

The dried leaves were extracted using methanol for twelve days. The jar containing the leaves and methanol was sealed to prevent air from entering and was shaken regularly to improve the extraction process.

4.5 Filtration

After the extraction process, sterile cotton and Whatman filter paper were soaked in the required solvent and put inside a funnel to filter the plant extract. A glass jar held the collected filtrate.

4.6 Evaporation

The liquid extract was evaporated using a rotating evaporator. The methanol was vaporized at a temperature of 65-670 degrees Celsius and a rotation rate of 34-38 revolutions per minute. The evaporated material was collected in a beaker and the residual solvent was evaporated using a cold hair dryer.

4.7 Chemical Group test

Testing of several chemical groups found in the extract that serves as a representation of the initial phytochemical research. The chemical group test is carried out in the manner described below:

4.7.1 Test for Alkaloids

➢ **Mayer's test:**

Two milliliters of the extract solution and 0.2 milliliters of diluted hydrochloric acid were put into a test tube. Potassium mercuric iodide, or Mayer's reagent, was then added in a milliliter. The presence of alkaloids is indicated by the formation of a yellow precipitate.

➢ **Dragendroff's Test**

Two milliliters of the extract solution and 0.2 milliliters of diluted hydrochloric acid were put into a test tube. Next, 1 milliliter of the Potassium Bismuth Iodide Solution, or Dragendroff's reagent, was added. Alkaloids are present when an orange-brown precipitate forms.

➢ **Wagner's Test:**

1 ml of Wagner's reagent (iodine potassium iodide) and 2 ml of the extract solution.There are alkaloids present when brown or reddish-colored precipitation forms.

4.7.2 Test for Flavonoids

1ml of crude extract was added with a few drops of strong hydrochloric acid. The presence of flavonoids was indicated by the immediate production of red color.

4.7.3 Test for Saponins:

A 20 ml distilled water solution was diluted with 1 ml of extract solution. After that, give it a good shaking for 15 minutes, during which time clear foam will form, signifying the presence of saponin.

4.7.4 Test for Phenols:

Add 3 or 4 drops of ferric chloride solution to a 2 ml extract solution. The development of a bluish black hue signifies the existence of phenols.

4.7.5 Test for Glycosides:

i. 1ml of water was mixed with a few quantity of an methanolic extract made from either fresh or dried plant material. Next, a few drops of sodium hydroxide aqueous were added. The presence of glycosides was thought to be indicated by a yellow color.

ii. Fehling's solution was heated with a tiny quantity of an alcoholic extract of the plant material, water, and alcohol. The precipitates that was brick-red was thought to be a sign that glycosides were present.

iii. To investigate the appearance of glycosides, a portion of the extract was solubilize in water and alcohol, heated, and then neutralized with sodium hydroxide solution after a few drops of diluted sulfuric acid were added.

4.7.6 Test for Gum:

Molish reagent and sulfuric acid were then added to the 5 ml extract of solution. A violet ring forms at the intersection of two liquids, signifying the presence of gum.

4.7.7 Test for Steroids:

In a test tube, 1 ml of the aqueous solution was dissolved in 2 ml of chloroform. A lower layer was formed by carefully applying concentrated sulfuric acid to the test tube wall. The existence of a steroid ring—that is, the aglycone part of the glycoside—was indicated by a reddish-brown tint at the interface.**[25]**

4.8 Thrombolytic Activity Test:

Together with the coagulation factors, thrombocytes, commonly known as platelets, are a component of blood whose job it is to react to bleeding from blood vessel damage by clumping together and starting a blood clot. The development of a blood clot, or thrombus, inside a blood vessel is referred to as thrombosis. It stops blood from passing through the circulatory system in a regular manner. Venous thromboembolism is the term used to describe a blood clot that originates in the veins. Pulmonary embolisms and deep vein thrombosis may result from this. Atheros-thrombosis is the term for a clot that develops in the arteries and can cause a heart attack or stroke.

Working procedure and dose calculation of Thrombolytic activity

Standard:

Streptokinase 1500000 IU/5ml

Dose: 30000 IU in 100 µl

That means, 1500000 IU streptokinase dissolved in 5 ml distilled water

= 100 µl distilled water contains 30000 IU streptokinase

Extract Dose:

1000 µg in100 µl

Extract Concentration:

Stock solution=100mg/10ml

That means, 100 mg extract dissolved in 10 ml distilled water

Or, 100000 µg extract dissolved in 10000 µl distilled water

1000 µg extract dissolved in 100 µl distilled water = 100µl distilled water i.e. 1000µg/100 µl

Procedure:

1. At first, measured the blank tube weight
2. Then, Collected the blood from the volunteer and put in 1 ml blood in each tube.
3. And stands for 45 minutes for incubation at 37^{o}C temperature for clot formation
4. Then, Separate serum from blood and weight 1st clot with tube
5. 1st clot weight = 1stclot with tube weight – blank tube weight

6. Then, put on the dose of standard **100 µl i.e. 30000 IU**, control **100 µl distilled water** and extract **100 µl i.e. 1000 µg/100 µl**
7. And again, stands for 90 minutes incubation at 37°C temperature for clot lysis
8. Separate the lysis blood fluid from the tube.
9. And measured the 2nd clot with tube weight
10. Then, 2nd clot weight = 2nd clot with tube weight – blank tube weight
11. And weight of Lysis clot = 1st clot weight – 2nd clot weight
12. Finally,

$$\% \text{ of lysis} = \frac{\text{wt of Lysis clot}}{1^{st} \text{ clot weight}} \times 100$$

[26]

4.9 Anti- Oxidant Activity:

A serial dilution of the plant extract was performed using a stock solution that was made in methanol (10 mg/ml). Initially, five volumetric flasks are used to create five distinct concentration types: 10, 25, 50, 100, and 500 µg/ml. Foil paper is used to cover volumetric flasks and test tubes. A serial dilution of the extract is carried out and labeled in five volumetric flasks.

Using a pipette, 2 ml of each concentration's sample and 2 ml of the 0.004% DPPH solution are added to 5 test tubes, respectively. After that, the solution is placed in a dark area for half an hour while each test tube is wrapped in foil. 2 ml of 0.004% DPPH and 2 ml of methanol are taken and put in another test tube to make the blank solution. Then absorbance was taken by UV Spectroscopy. The percentage of inhibition was calculated by using following formula [27,28,29]

$$\%\text{inhibition} = \frac{\text{Blank absorbance} - \text{Solution absorbance}}{\text{Blank absorbance}} \times 100$$

Working Procedure for Anti-oxidant Test

Stock Solution Concentration:

10 mg/ml

Measured 2.5 ml stock solution:

1ml contain 10 mg

2.5ml contain (10X25) mg =25 mg

Dilution of Stock solution:

Dilution Factors = Old Concentration/ New Concentration

Then, **Dilution Factors** = New volume/Old volume

Serial Dilution:

Serial Dilution of Concentration **500µg, 100µg, 50µg, 25µg, 10µg**

500µg = 1ml stock solution + 19ml solvent (methanol)

100µg= 1ml 500µg concentration solution + 4ml solvent (methanol)

50µg= 5 ml 500µg concentration solution + 5 ml solvent (methanol)

25µg= 5 ml 50µg concentration solution + 5 ml solvent (methanol)

10µg= 4 ml 50µg concentration solution + 6 ml solvent (methanol)

DPPH solution:

DPPH Stock solution contain 0.004% DPPH

That means,

100 ml solvent (methanol) contain 0.004gm DPPH

For STD solution, Ascorbic Acid:

25 mg in 2.5 ml methanol solution.

Serial Dilution of Concentration **500µg, 100µg, 50µg, 25µg, 10µg**

500µg = .5 ml stock solution + 9.5 ml solvent (methanol)

100µg= 2 ml 500µg concentration solution + 8 ml solvent (methanol)

50µg= 5 ml 500µg concentration solution + 5 ml solvent (methanol)

25µg= 5 ml 50µg concentration solution + 5 ml solvent (methanol)

10µg= 4 ml 50µg concentration solution + 6 ml solvent (methanol)

Working Procedure:

1. At first, prepare the stock solution of extract in concentration 10mg/ml.
2. Then, dilution this concentration of 500µg, 100µg, 50µg, 25µg, 10µg concentration of serial dilution
3. Prepared 0.004% DPPH solution in solvent (methanol)
4. Next, DPPH solution and extract solution/standard solution are added in varying concentrations (1:3), meaning that 1 ml of extract solution and 3 ml of DPPH solution are added.
5. 3 ml of DPPH solution is added to 1 ml of solvent (methanol) to create the blank solution.
6. After that, spend forty-five minutes in a dark area.
7. Lastly, the absorbance at 517 nm was determined for the blank solution and various extract solution/standard solution concentrations.

Measurement of % of inhibition:

For Extract:

$$\% \text{ of inhibition} = \frac{\text{Blank solution absorbance} - \text{Extract solution absorbance}}{\text{Blank solution absorbance}} \times 100$$

For STD:

$$\% \text{ of inhibition} = \frac{\text{Blank solution absorbance} - \text{STD solution absorbance}}{\text{Blank solution absorbance}} \times 100$$

Then put in the data of Microsoft excel and X-excess of Concentration and Y-excess of % inhibition.

CHAPTER FIVE:

RESULT AND DISCUSSION

5.1: Results of phytochemical screening

Phytochemical constituents	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Phenols	+
Glycosides	+
Gum	+
Steroids	+

Table 1: Results of phytochemical screening

Positive sign indicates the presence of compounds, so it can be ensured that the plant contain alkaloids, flavonoids, saponins, phenols, glycosides, gum, steroids.

Discussion:

While a positive test indicates the presence of a compound, it is important to consider the sensitivity and specificity of the test, the quantity of the compound, the interactions with other compounds, and the potential safety and toxicity. Further research is necessary to fully understand the properties and potential applications of these phytochemicals.

5.2 Result of Thrombolytic Activity:

Serial	Tube weight (g)	1st clot weight with tube (g)	Weight of 1st clot (g)	2nd clot with tube (g)	Weight of 2nd clot (g)	% of lysis
Blank	.672	1.712	1.4	1.532	.86	17.31
Standard	.71	1.76	1.5	1.004	.294	72
Sample (250 µg/ml)	.754	1.38	.626	1.18	.426	31.95
Sample (500 µg/ml)	.756	1.56	.794	1.22	.464	41.56
Sample (1000 µg/ml)	.795	1.79	.995	1.14	.345	65.32

Table: 2

The results show that the plant extract inhibited clot lysis in a concentration-dependent manner. The blank sample had the highest percentage of lysis (17.31%), while the sample with the highest concentration (1000 µg/ml) had the lowest percentage of lysis (65.32%). This suggests that the plant extract may be effective in preventing clot formation.

Discussion:

A higher percentage of lysis indicates that more of the clot was dissolved, while a lower percentage of lysis indicates that less of the clot was dissolved.

Overall, the results suggest that the plant extract may be a potential anti-thrombotic agent. However, further research is needed to confirm these findings and to determine the safety and efficacy of the extract for clinical use.

5.3 Anti-Oxidant Activity Test:

For STD (Ascorbic acid):

Concentration (µg)	**Absorbance (nm)**	**% of inhibition**
500	0.049	83.77
100	0.055	81.79
50	0.061	79.80
25	0.062	79.47
10	0.069	77.15

Table: 3

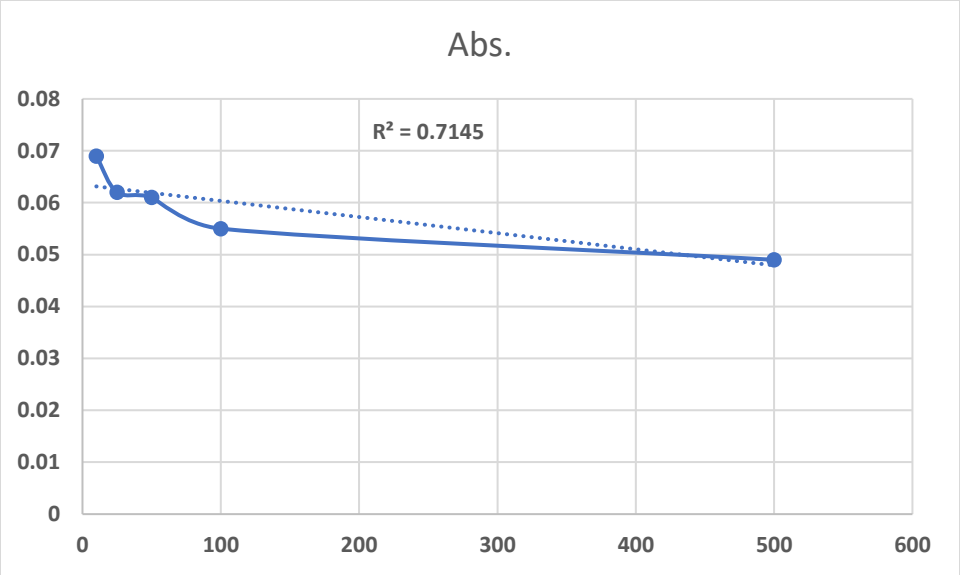

Figure 3: STD - Ascorbic Acid

The results show a concentration-dependent increase in antioxidant activity. As the concentration of the sample increases, the percentage of inhibition of the DPPH radical also increases. This suggests that the sample is a potent antioxidant, and that its effectiveness is directly related to its concentration.

Discussion:

The data shows that as the concentration of the sample increases, the absorbance of the DPPH radical decreases. This suggests that the sample is effectively scavenging the DPPH radical, which is a measure of its antioxidant activity. The percentage of inhibition also increases with increasing concentration, further supporting the conclusion that the sample is a potent antioxidant.

Overall, the results suggest that the sample is a promising antioxidant. However, further research is needed to determine its exact mechanism of action and to assess its safety and efficacy for clinical use.

For sample *(Hibiscus subdariffa L.)*

Concentration (µg)	Absorbance (nm)	% of inhibition
500	0.069	77.15
100	0.076	74.83
50	0.079	73.84
25	0.105	65.23
10	0.108	64.23

Table: 4

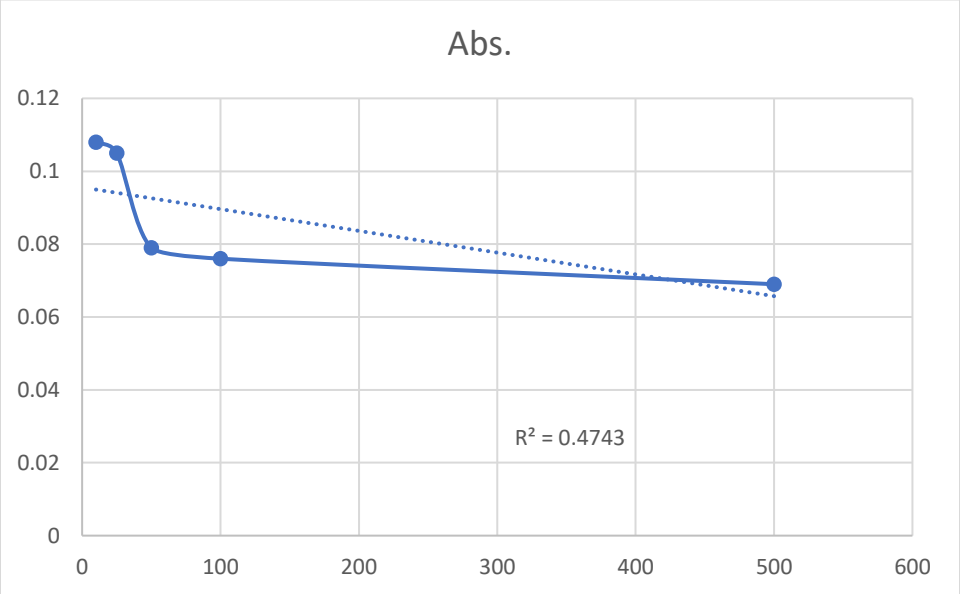

Figure: 4 Sample: *Hibiscus sabdariffa L*

The results show a concentration-dependent increase in antioxidant activity in Hibiscus sabdariffa leaves. As the concentration of the leaf extract increases, the percentage of inhibition of the DPPH radical also increases. This suggests that the extract is a potent antioxidant, and that its effectiveness is directly related to its concentration.

Discussion:

The data shows that as the concentration of the leaf extract increases, the absorbance of the DPPH radical decreases. This suggests that the extract is effectively scavenging the DPPH radical, which is a measure of its antioxidant activity. The percentage of inhibition also increases with increasing concentration, further supporting the conclusion that the leaf extract is a potent antioxidant.

Overall, the results suggest that Hibiscus sabdariffa leaves have significant antioxidant properties. This could be due to the presence of various phytochemicals, such as polyphenols and flavonoids, which have been shown to have antioxidant activity.

CHAPTER SIX:

CONCLUSION

6.1 Conclusion:

Based on the findings of this study, Hibiscus sabdariffa leaves demonstrate promising potential as a source of natural compounds with therapeutic applications. The phytochemical screening revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins, phenols, glycosides, gum, and steroids. These compounds have been associated with various pharmacological properties, such as anti-inflammatory, antioxidant, and thrombolytic activities. The biological activity assays further support the potential of Hibiscus sabdariffa. The plant extract exhibited significant thrombolytic activity, suggesting its potential for preventing or treating thrombotic disorders. Additionally, the extract demonstrated potent antioxidant activity, indicating its ability to combat oxidative stress and protect against related diseases.

However, further research is necessary to fully elucidate the specific mechanisms of action of the bioactive compounds and to evaluate their safety and efficacy in vivo. Isolating and characterizing individual compounds may provide valuable insights into their pharmacological properties and potential applications.

Overall, the results of this study suggest that Hibiscus sabdariffa leaves may be a promising source of natural compounds for the development of novel therapeutic agents. Future research efforts should focus on exploring the potential benefits of this plant in various therapeutic areas.

CHAPTER SEVEN:

REFERENCES

1. Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. *et al.* Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov* 20, 200–216 (2021). https://doi.org/10.1038/s41573-020-00114-z

2. David J. Newman and Gordon M. Cragg *Journal of Natural Products* **2007** *70* (3), 461-477.DOI: 10.1021/np068054v

3. Vishwakarma, A.P., Vishwe, A., Sahu, P. and Chaurasiya, A. (2013) Magical Remedies of Terminalia arjuna (ROXB.). International Journal of Pharmaceutical Archive, 2, 189-201.

4. Xiao, P. (1988) A Pictorial Encyclopaedia of Chinese Medicine. v1-10. Commercial Press, Hong Kong, 88-90.
5. Li, L. (2000) Opportunity and Challenge of Traditional Chinese Medicine in Face of the Entrance to WTO (World Trade Organization). Chinese Journal of Information on Traditional Chinese Medicine, 7, 7-8.
6. Uniyal, S. K., Awasthi, A. & Rawat, G. S. (2002). Current status and distribution of commercially exploited medicinal and aromatic plants in upper Gori valley, Kumaon Himalaya, Uttaranchal. Current Science 82, 1246-1252.
7. GJ Chand AV Babu U Spandana A review on meracle tree: Moringa oleiferaJ Pharmacogn Phytochem

8. W Salama A Salem E Yousef Development of Innovative Beverage Based on Milk Permeate Fortified With Dried Leaves of Moringa Oleifera Arab Univ J Agric Sci

9. Oboh, A., & Oyagbemi, E. O. (2016). Hibiscus sabdariffa (Roselle): A review of its phytochemical constituents and pharmacological properties. International Journal of Food Science and Technology, 51(1), 22-32.
10. https://www.stringerchiropractic.com/index.php/wellness-news/news/102-benefits- of-natural-medicine

11. Flora of Pakistan: Volume 111, pages 139-140
12. **NCBI Taxonomy:** https://www.ncbi.nlm.nih.gov/taxonomy
13. https://www.nparks.gov.sg/florafaunaweb/flora/2/0/2096
14. https://www.researchgate.net/publication/269278965_MORPHOLOGICAL_EVALUATION_OF_SOME_ROSELLE_Hibiscus_sabdariffa_L_GERMPLASM_FROM_KADUNA_STATE_NIGERIA#fullTextFileContent
15. Salem, M.A., Zayed, A., Beshay, M.E. *et al. Hibiscus sabdariffa* L.: phytoconstituents, nutritive, and pharmacological applications. *ADV TRADIT MED (ADTM)* **22**, 497–507 (2022). https://doi.org/10.1007/s13596-020-00542-7
16. https://www.gardensall.com/roselle-plant/
17. Yusuf, Daudu. (2014). MORPHOLOGICAL EVALUATION OF SOME ROSELLE (Hibiscus sabdariffa L.) GERMPLASM FROM KADUNA STATE, NIGERIA
18. Obouayeba AP, Djyh NB, Diabate S, Djaman AJ, N'guessan JD, Kone M, Kouakou TH. Phytochemical and antioxidant activity of roselle (Hibiscus sabdariffa L.) petal extracts.
19. Sehim AE, Amin BH, Yosri M, Salama HM, Alkhalifah DH, Alwaili MA, Abd Elghaffar RY. GC-MS Analysis, Antibacterial, and Anticancer Activities of Hibiscus sabdariffa L. Methanolic Extract: In Vitro and In Silico Studies. Microorganisms. 2023 Jun 16;11(6):1601.
20. Rao TP, Htay HH, Yasuda NK, Sugino H, Ohkubo TH, Okamoto T, Suzuki K. Antioxidant and anti-thrombotic properties of selected plant extracts of Asia. Austin J Nutr Metab. 2014;1(1):6.
21. Okereke CN, Iroka FC, Chukwuma MO. Phytochemical analysis and medicinal uses of Hibiscus sabdariffa.
22. Adamu H, Ngwu RO. Phytochemical screening and antibacterial activities of Hibiscus sabdariffa L. leaf extracts. Nigerian Journal of Chemical Research. 2015 Dec 17;20:46-52.
23. Saputri FC. Evaluation of Co-administration of Roselle Water Extract (Hibiscus sabdariffa L.) and Aspirin for Antiplatelet Therapy in Male Sprague-Dawley Rats. Pharmacognosy Journal. 2021;13(2).
24. Oboh, A., & Oyagbemi, E. O. (2016). Hibiscus sabdariffa (Roselle): A review of its phytochemical constituents and pharmacological properties. International Journal of Food Science and Technology, 51(1), 22-32.

25. Kumar D, Kumar S, Sahu M, Kumar A. Phytochemical screening and anti-oxidant activity of Neolamarckia cadamba and cymbopogon citrates from durg district of Chhattisgarh, India. Saudi Journal of Biomedical Research. 2020;5(12):343-8.
26. Ramjan A, Hossain M, Runa JF, Md H, Mahmodul I. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna journal of phytomedicine. 2014 Nov;4(6):430.
27. CRC Handbook of Free Radicals and Antioxidants, 1989; 1: 209-221.
28. Antioxidant Vitamins Benefits Not Yet Proved (editorial) NEJM, 1994; 230(15): 1080 – 1081.
29. Antioxidants and Physical Performance, Critical Reviews in Food Science and Nutrition, 1995; 35(1&2): 131-141.

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