Chemical and Biological investigation of methanolic extract of Alstonia scholaris

PROJECT REPORT

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

Submitted To

The Department of Pharmacy

Faculty of Allied Health Sciences

Daffodil International University

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APPROVAL

This project paper, **"Chemical and Biological investigation of methanolic extract of *Alstonia scholaris*",** 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 Bachelor programmed and approved as to its style and contents.

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DECLARATION

I hereby declare that this project report **"Chemical and Biological investigation of methanolic extract of *Alstonia scholaris*",** is done by me under the supervision of Dr. Sharifa Sultana Associate Professor and Associate Head Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University. I am declaring that this project is my original work. I also declare that neither this project nor any part therefore has been submitted elsewhere for the award of Bachelor or any degree.

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

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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.

My Parents,

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

My teacher,

The persons who guided me in this process and the committee who kept me on track.

Abstract

Alstonia scholaris, commonly known as the "Saptaparni" or "Blackboard tree," is a medicinal plant widely distributed throughout tropical and subtropical regions. In this study, we conducted a comprehensive chemical and biological investigation of the methanolic extract derived from Alstonia scholaris. The phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds etc. In the biological evaluation, the methanolic extract exhibited significant pharmacological activities, including **antioxidant, analgesic & anti-diarrheal** properties. The extract demonstrated notable antioxidant potential, as evidenced by its ability to scavenge free radicals and inhibit lipid peroxidation. The methanol extract's dose-dependent suppression of abdominal constrictions suggests that the plant may have antinociceptive properties. Overall, our findings provide valuable insights into the chemical composition and pharmacological properties of the methanolic extract of Alstonia scholaris, supporting its traditional use in various medicinal practices and suggesting its potential for further pharmaceutical development.

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Chapter one
Introduction

1. Introduction

***Alstonia scholaris*,** commonly known as the "Saptaparni" or "Devil's Tree," is a significant plant species found predominantly in the tropical regions of Asia, including India, Myanmar, Sri Lanka, and Thailand. This tree holds immense cultural, medicinal, and ecological importance due to its diverse chemical composition and biological properties. The methanolic extract of Alstonia scholaris has garnered considerable attention from researchers due to its potential pharmacological applications. Methanol, being a polar solvent, efficiently extracts a wide range of bioactive compounds from plant materials, making it an ideal solvent for phytochemical investigations [1]. Chemical investigation of the methanolic extract involves the isolation, identification, and characterization of various phytoconstituents present in Alstonia scholaris. These phytoconstituents may include alkaloids, flavonoids, terpenoids, phenolic compounds, and other secondary metabolites. These compounds often exhibit diverse chemical structures and biological activities, contributing to the medicinal properties of the plant. Biological investigation of the methanolic extract focuses on evaluating its pharmacological effects through in vitro and in vivo studies. These investigations explore the extract's potential as an antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, and other therapeutic agents. Understanding the biological activities of the methanolic extract can provide valuable insights into its medicinal potential and facilitate the development of new drugs or therapeutic agents derived from natural sources [2]. In this study, we aim to comprehensively investigate the chemical composition and biological properties of the methanolic extract of Alstonia scholaris. Through a combination of phytochemical analysis and pharmacological assays, we seek to elucidate the potential therapeutic benefits and underlying mechanisms of action associated with this plant extract. Such research endeavors contribute to the growing body of knowledge on natural products and their applications in modern medicine, offering promising avenues for drug discovery and development [3].

Figure 1: *Alstonia scholaris*

1.1 Medicinal plant

Medicinal plants are rich in metabolites that are secondary and are probably a good source of pharmaceuticals. Steroids, alkaloids, glycosides, coumarins, and flavonoids are among these auxiliary metabolites. Many meanings have been put up for the phrase "restorative plant," which refers to plants that have compounds in more of their organs or components that can be employed for various purposes, such as helping achieve specific goals or serving as pioneers in the field of semi-combination chemotherapy [4]. It remedies a few common mishaps with plants including aloe, tulsi, neem, turmeric, and ginger. In many parts of the nation, these are regarded as home remedies. It is a well-known fact that many consumers use tulsi in their daily routines for various activities, pooja, black tea, and medicine preparation [5]. There are about 297 Unani, 204 Ayurvedic, and 77 homeopathic medicine manufacturing companies in Bangladesh, and medicinal plants grown there are frequently employed in both crude and semi-handled forms of medicine in various drug proportion plans. These plants will serve as important raw materials for various state-of-the-art restorative protocols. The pharmaceuticals that these businesses supply from restorative plants have an estimated market value of around Tk. 300 crores. Additionally,

an average amount of medicinal plants are used by tribal people, road vendors, and city of Kobraj to treat various illnesses [6].

1.2 Plants in traditional medicinal

There are about 297 Unani, 204 Ayurvedic, and 77 homeopathic medicine manufacturing companies in Bangladesh, and medicinal plants grown there are frequently employed in both crude and semi-handled forms of medicine in various drug proportion plans [7]. These plants will serve as important raw materials for various state-of-the-art restorative protocols. The pharmaceuticals that these businesses supply from rehabilitative plants have an estimated market value of around Tk. 300 crores. Additionally, an average amount of medicinal plants are used by tribal people, road vendors, and city of Kobraj to treat various illnesses [8].

Taxonomical classification

Kingdom	**Plantae**
Kingdom:	**Plantae**
***Clade*:**	**Tracheophytes**
***Clade*:**	**Angiosperms**
***Clade*:**	**Eudicots**
***Clade*:**	**Asterids**
Order:	**Gentianales**

1.2 Chemical Composition of *Alstonia scholaris*

Alstonia scholaris, commonly known as the devil's tree or dita bark, is a species of evergreen tree native to the Indian subcontinent, Southeast Asia, and Australia. Its chemical composition includes several alkaloids, which are the primary active compounds responsible for its pharmacological properties [9]. Some of the alkaloids found in Alstonia scholaris include:

1. **Alstonine**: This alkaloid is one of the major constituents of Alstonia scholaris. It has been studied for its potential pharmacological effects, including its antimalarial and antiarrhythmic properties.

2. **Echitamine**: Another important alkaloid found in Alstonia scholaris, echitamine has been investigated for its potential anticancer and antimalarial activities.
3. **Picrinine**: This alkaloid is known for its bitter taste and has been reported to possess antimicrobial and antimalarial properties [10].
4. **Vincoside**: Vincoside is a well-known alkaloid found in various plant species, including Alstonia scholaris. It has been studied for its potential neuroprotective effects and ability to improve cognitive function.
5. **Reserpine**: Reserpine is a well-known alkaloid with antipsychotic and antihypertensive properties. It is found in various plant species, including Alstonia scholaris.

These alkaloids, along with other minor constituents, contribute to the medicinal properties of Alstonia scholaris, making it a subject of interest in traditional medicine and pharmacological research. However, it's important to note that while these compounds have demonstrated various pharmacological activities in studies, further research is needed to fully understand their potential therapeutic applications and mechanisms of action [11].

1.3 Therapeutic use and pharmacological activity of *Alstonia scholaris*

Alstonia scholaris, commonly known as the "Devil's Tree" or "Saptaparni" in Ayurvedic medicine, has been traditionally used for various therapeutic purposes. Here are some of its known therapeutic uses and pharmacological activities:

Anti-inflammatory Activity: *Alstonia scholaris* has demonstrated anti-inflammatory properties in various studies. Its extracts have been shown to inhibit the production of inflammatory mediators, making it potentially useful in the treatment of inflammatory conditions [12].

Antioxidant Activity: The plant contains compounds with antioxidant properties, which can help in scavenging free radicals and reducing oxidative stress. This property makes it beneficial in preventing oxidative damage to cells and tissues.

Antimicrobial Activity: Extracts from *Alstonia scholaris* have exhibited antimicrobial activity against various bacteria and fungi. This property suggests its potential use in treating microbial infections [13].

Antidiarrheal Activity: In traditional medicine, *Alstonia scholaris* has been used to treat diarrhea. Its extracts have shown antidiarrheal activity in experimental studies, possibly through modulation of intestinal motility and secretion.

Anti-diabetic Activity: Some studies have suggested that *Alstonia scholaris* extracts may have hypoglycemic properties, which could be beneficial in managing diabetes. It may help in reducing blood glucose levels by various mechanisms [14].

Antimalarial Activity: Certain compounds isolated from *Alstonia scholaris* have exhibited antimalarial activity in laboratory tests. This property suggests its potential in the development of new antimalarial drugs.

Antiulcer Activity: Alstonia scholaris extracts have demonstrated antiulcer activity in animal studies. It may help in protecting the gastric mucosa and reducing ulcer formation.

Analgesic Activity: The plant has been traditionally used for pain relief. Some studies have reported its analgesic properties, indicating its potential in alleviating pain [15].

Immunomodulatory Activity: *Alstonia scholaris* extracts have shown immunomodulatory effects in experimental studies. It may help in enhancing the immune response, which could be beneficial in various immune-related disorders.

Anticancer Activity: Preliminary research suggests that certain compounds present in *Alstonia scholaris* may have anticancer properties. Further studies are needed to explore its potential in cancer therapy [16].

It's important to note that while Alstonia scholaris shows promise in various therapeutic applications, further research, including clinical trials, is necessary to validate its effectiveness and safety for medical use. Additionally, it's essential to consult a healthcare professional before using any herbal remedies for therapeutic purposes.

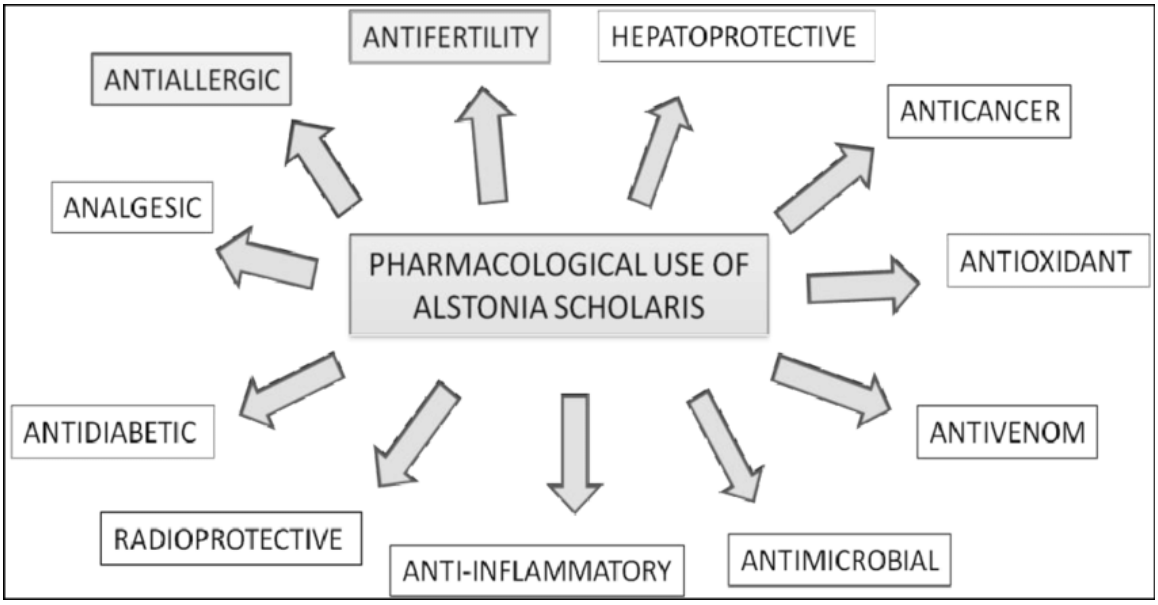

Figure 2: Medicinal Properties of *A. scholaris*

1.4 Anticancer effect of *Alstonia scholaris*

Alstonia scholaris, commonly known as the "Saptaparni" tree or "Devil's tree," is a species of tree native to the Indian subcontinent, Southeast Asia, and Australia. It has been traditionally used in Ayurvedic and traditional medicine systems for various ailments due to its pharmacological properties. However, there is limited scientific evidence specifically regarding its anticancer effects [17]. Some studies have investigated the potential anticancer properties of compounds derived from Alstonia scholaris. For instance, research published in the journal "Bioorganic & Medicinal Chemistry Letters" in 2017 evaluated the cytotoxicity of compounds isolated from the stem bark of Alstonia scholaris against several human cancer cell lines, including lung cancer (A549), colon cancer (HCT116), breast cancer (MCF-7), and prostate cancer (PC-3) cells [18]. The study found that certain compounds exhibited significant cytotoxic activity against these cancer cell lines, suggesting their potential as anticancer agents. However, further research is needed to understand the mechanisms of action and potential efficacy in vivo. Additionally, other studies have explored the pharmacological properties of Alstonia scholaris extracts, which may have implications for cancer treatment. For example, research published in the "Journal of Ethnopharmacology" in 2015 investigated the antioxidant and cytotoxic activities of Alstonia scholaris leaf extracts [19]. While the study did not specifically focus

on cancer cells, it demonstrated significant antioxidant activity, which could potentially contribute to its overall health benefits, including cancer prevention. Overall, while there is some preliminary evidence suggesting the potential anticancer effects of compounds derived from Alstonia scholaris, more comprehensive research, including preclinical and clinical studies, is needed to confirm its efficacy and safety for cancer treatment. Additionally, it's essential to consult with healthcare professionals before using any herbal remedies, including those derived from Alstonia scholaris, especially for cancer management [20].

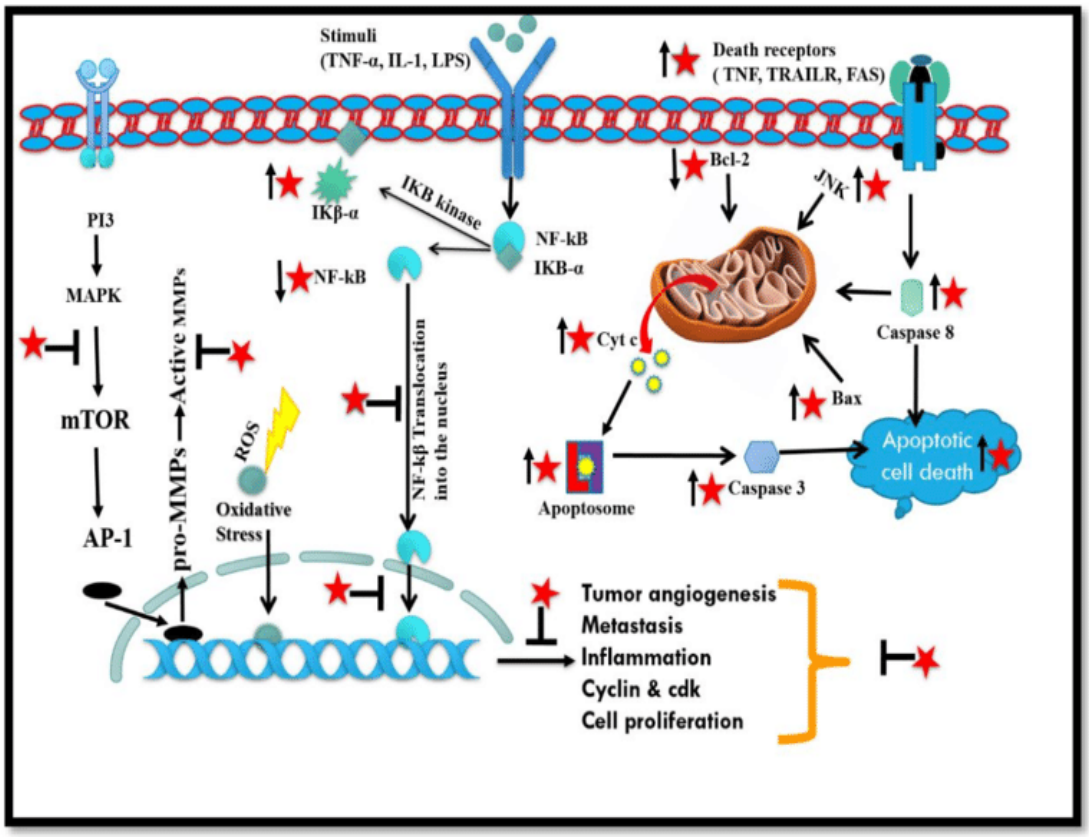

Figure 3: Anticancer effect of *Alstonia scholaris*

1.5 Introduction of in vivo Antioxidant test

In vivo antioxidant testing is a crucial aspect of evaluating the efficacy and safety of potential therapeutic agents, particularly those aimed at combating oxidative stress-related disorders. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants,

leading to cellular damage and dysfunction. In vivo studies involve experiments conducted within living organisms, typically animals, to assess the antioxidant activity of test substances in a physiological context [14].

1.6 Introduction of in vivo Antidiarrhoeal test

An in vivo antidiarrheal test refers to a method used in pharmacological research to evaluate the effectiveness of compounds or substances in treating or preventing diarrhea in living organisms, typically animals. This test is crucial in drug development to identify potential candidates for the treatment of diarrhea, a common gastrointestinal disorder characterized by frequent bowel movements and loose or watery stools. The in vivo antidiarrheal test involves administering the test compound to experimental animals and then inducing diarrhea through various means, such as administration of a diarrheal agent or a laxative, or exposure to stressors [15]. These models are chosen based on factors such as their sensitivity to diarrheal agents, ease of handling, and ethical considerations. The effectiveness of the test compound in reducing or alleviating diarrhea is assessed by comparing the parameters measured in treated animals with those in control groups that receive either a placebo or a reference drug with known antidiarrheal properties. Various endpoints may be measured, including stool consistency, fecal output, intestinal transit time, and electrolyte balance. In vivo antidiarrheal tests provide valuable insights into the potential efficacy, safety, and mechanisms of action of candidate compounds, helping researchers identify promising drug candidates for further development [16].

1.7 Introduction of in vivo Analgesic test

An in vivo analgesic test refers to a type of experiment conducted in living organisms (usually laboratory animals) to assess the efficacy of potential analgesic compounds or treatments in alleviating pain. These tests are crucial in the development and evaluation of new pain-relieving drugs and therapies. The procedure typically involves inducing pain in the animals using various methods such as thermal (heat or cold), mechanical (pressure or pinching), chemical (injection of irritants), or inflammatory (administration of inflammatory agents) stimuli. The animals' responses to these pain-inducing stimuli are then measured using behavioral or physiological endpoints [18].

Chapter two
Purpose of the study

2. Objective of the study

Investigating the phytochemical makeup and possible biological activities of the methanol extract made from leaf of *Alstonia scholaris* is the aim of this work. The following is an outline of the investigation's precise objectives:

- The primary objective of this study is to investigate the chemical composition of the methanolic extract of *Alstonia scholaris*.
- Another key purpose is to assess the biological activities of the identified compounds.
- This study aims to validate the traditional claims regarding the therapeutic efficacy of the plant by scientifically investigating its chemical and biological properties.
- By elucidating the chemical composition and pharmacological activities of the methanolic extract, this study may contribute to the discovery and development of novel drugs or therapeutic agents.
- Additionally, the findings of this study may have implications in the nutraceutical or herbal medicine industry.

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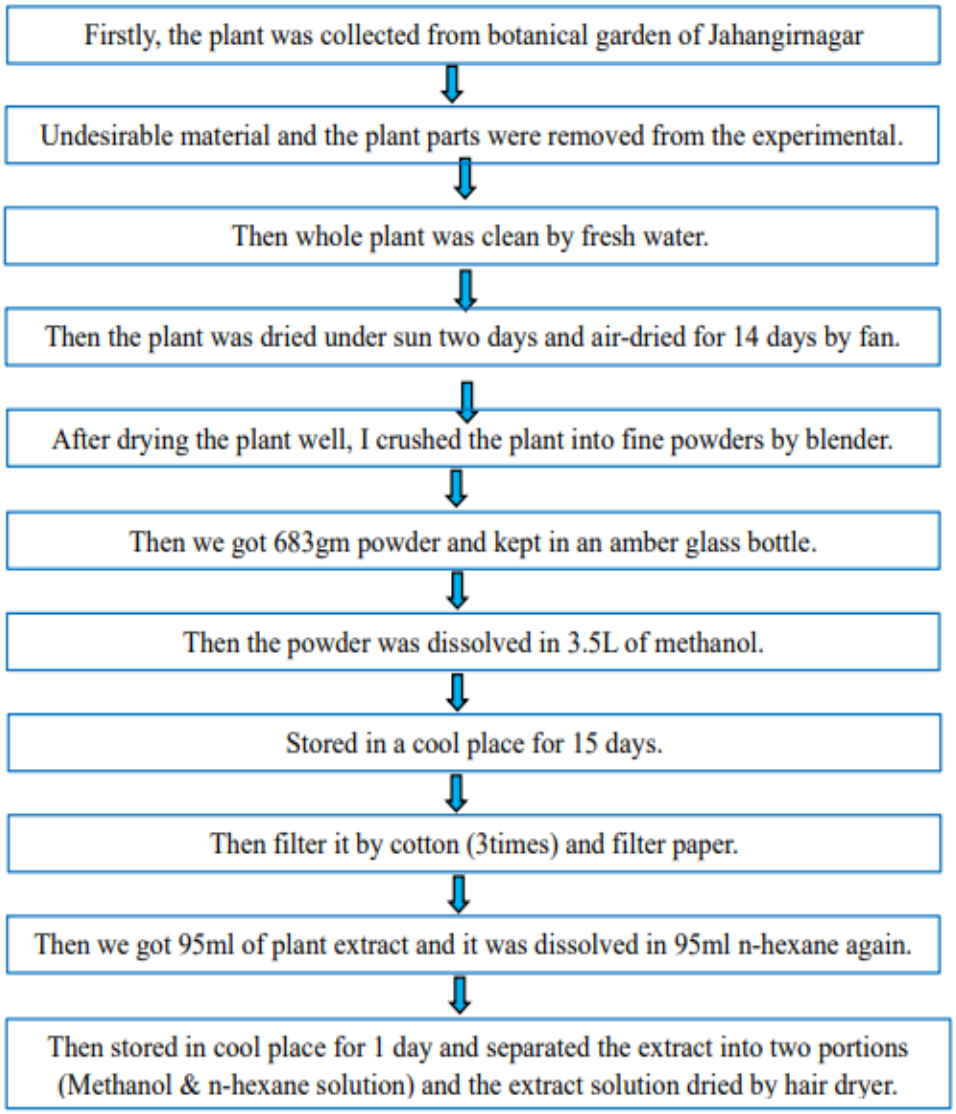
Chapter three
Materials & Methodology

3. Materials & methodology

Analyzing phytochemicals entails studying the bioactive substances found in plants. These chemicals are extracted, isolated, and characterized using a variety of techniques and equipment.

3.1 Description of plant extraction is given below:

Unprocessed medicine is extracted from the plant and animal and used without modifying its chemical composition. I organized raw concentration in several steps. Here's a general description of the process:



3.2 Extraction:

A glass jar with a plastic top was thoroughly cleaned before being given a methanol rinse. Subsequently, 3500 milliliters of methanol were poured to the jar containing 683 grams of the dry powder, before the surface of the sample was sufficiently coated, reaching a height of one inch above the jar. The jar was appropriately airtight, with a plastic cover that was coated with aluminum foil. The procedure described above was done for twelve days. To enhance the extraction procedure, the jar was shook nearly twice a day.

3.2.1 Filtration

After the process of extraction, sterile cotton and filter paper were used to filter the plant's extract. Both items were subsequently immersed in the required solvents and put within a funnel. The filtered material was stored in a glass jar.

Figure 4: Filtration of extract via funnel with filter paper

3.2.3 Evaporation

The liquid extraction was evaporated using an evaporator that rotates. At a temperature of 65 to 670 degrees Celsius and a spinning speed of 34 to 38 rotations per minute, methanol was evaporated. The liquefied material was transferred to a 50 milliliter beaker. After the pure material was gathered, the resultant solution was put just beneath the fan in beakers wrapped with aluminum foil. A cold hair drier was then used to completely eliminate the leftover water after the rest of the component in the resulting extract had begun to evaporate.

Figure 5: Evaporation of extract via Evaporator

3.3 My investigation

In my investigation I have been gadget

- Phytochemical Screening test
- Anti-diarrheal activity
- In vivo analgesic activity
- Anti-oxidant activity

3.4 Phytochemical Assessment

Plant phytochemicals are analyzed and studied as part of the phytochemical evaluation procedure. Phytochemicals are naturally produced bioactive substances found in plants that may be beneficial to human health. These substances support the plant's defense systems, disease prevention, and relationships with their surroundings. A vast variety of substances,

including alkaloids, flavonoids, terpenoids, saponins, phenolic acids, and more, are referred to as phytochemicals.

3.4.1 Reagents applied for the diverse chemical group analysis

3.4.1 Assessment for alkaloids

Mayer's evaluation

Two milliliters of extort solution and 0.2 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

3.4.2 Assessment for Flavonoids

The presence of flavonoids is shown by the red color, which was obtained by adding 2 mL of droplets of strong hydrochloric acid to a 5 mL amount of an alcoholic solution of the plant component.

Figure 6: Flavonoids analysis of extract

3.4.3 Assessment for Saponins

After diluting 5 milliliter of the aqueous solution with 5 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 7: Saponins analysis of extract

3.4.4 Assessment for Tannins

10 ml of purified water will be used to absorb 5g of extraction (Equivalent 2 mL). The mixture will then be filtrated, and the resultant filtrate will be treated with 2-3 drops of ferric chloride reagents. The formation of a blue-black, green, or blue-green precipitation suggests the presence of tannins.

Figure 8: Tannins analysis of extract

3.4.5 Assessment for Glycosides

0.2 mL of concentrated HCL was added after 2 mL of the sample was initially taken. The evaluation of yellow color indicated the existence of glycosides. Fehling liquid contains a tiny amount of a heated alcohol extract. The amount of red brick that precipitated was measured to ascertain the presence of glycosides. After the substance was combined with alcohol and water, a little portion was heated with a few drops of diluted sulfuric acid. After neutralizing Fehling solution with sodium hydroxide water, bring it to a boil. The red brick precipitated was assessed as a glycoside indication.

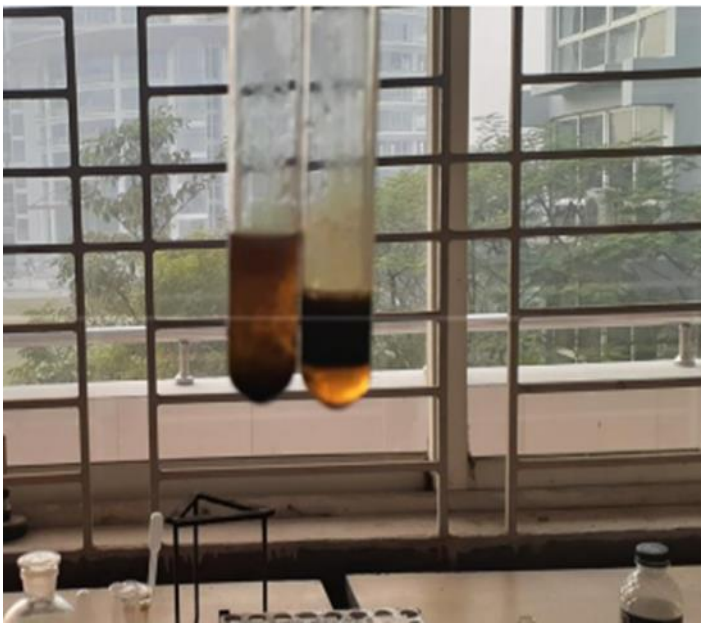


Figure 9: Glycosides analysis of extract

3.4.6 Test for Phenol

A 2-3 drops neutral ferric chloride solution was diluted with 2 milliliters of the extract; the dark blue coloring indicated the presence of phenolic chemicals.

3.4.7 Test for Steroid

One milliliter of concentrated H2SO4 was added to two milliliters of extract in a test tube. Bright yellow or red coloration.

3.4.8 Test for gums

Sulfuric acid was added after the resultant extract and Molish reagent were combined in five milliliters. A reddish-violet ring formed when two liquids were combined, indicating the existence of gums and carbs.

3.4.9 Test for Reducing Sugar (Benedict's test)

After mixing Benedict's reagent with one milliliter of the analyte sample, place the mixture in a boiling water bath and heat it for three to five minutes. The development of a brick-red-colored cuprous oxide precipitate indicates the presence of reducing sugars in the analyte.

3.4.10 Test for Carbohydrates

2 ml of concentrated H2SO4 plus 2-3 drops of Molisch's reagent and 2 ml of extract. The existence of carbohydrates was indicated by a red or purple ring.

3.5 Assessment of Anti-diarrheal activity

Reagent & machinery

Serial No:	Name
1	Electronic balance
2	Extort solution
3	Castrol oil
4	Loperamide (Opsonin Pharmaceuticals Ltd)
5	Distilled water
6	1% tween 80 in normal saline
7	Needle

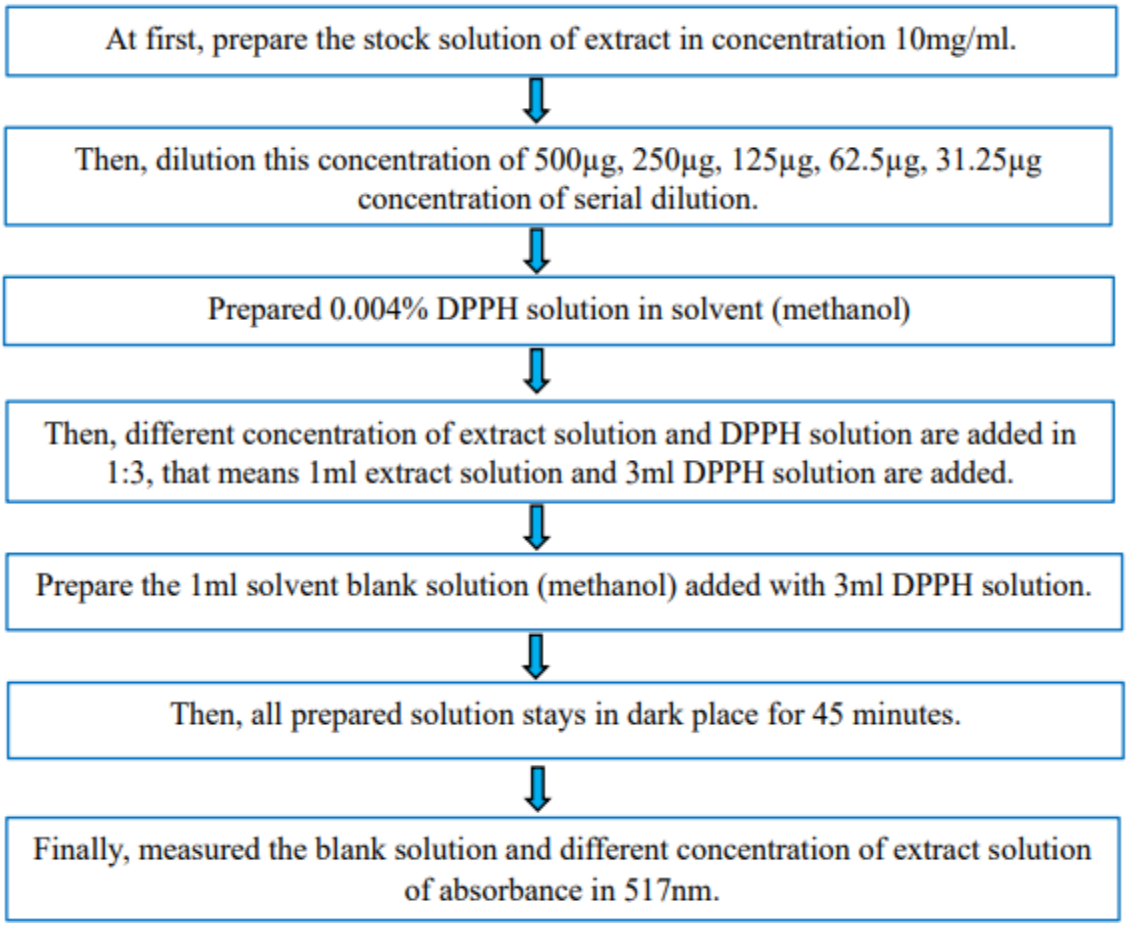
3.5.1 Procedure

With a small adjustment, the methodology outlined by Shoba and Thomas (2001) was used for this investigation. Three mice each were used to create the test, positive control, and control groups for the animal. The control group was given 10 mL/kg of vehicle (1% tween 80 in normal saline) orally. Loperamide was given orally to the positive control group at a dose of 50 mg/kg. 200 mg/kg body weight of a methanolic extract of ziziphus jujuba leaves was given to the test group. Every animal was housed in a separate cage, with a fresh floor covering added every hour. Following the aforementioned therapy, castor oil was given orally to each mouse to produce diarrhea.

3.6 Antioxidant Test

The importance of oxidizing to the organism and to food is well understood. Cells require oxidative metabolism in order to survive. One unintended consequence of this dependence is the production of free radicals, including reactive oxygen species, which cause oxidative changes. Additional data suggests that these species participate in a variety of common in vivo regulatory systems.

3.6.1 Working Procedure of Antioxidant Test



Measurement of % of inhibition:

$$\%\ of\ Inhibition = Blank\ Absorbance - Extract\ Solution\ Absorbance\ Blank\ Absorbance \times 100$$

3.7 In vivo analgesic activity

Initially, the acetic acid-induced writhing methodology was used to assess the analgesic effectiveness of the *Alstonia scholaris* ethanolic extract in rats. A total of twenty-four animals were split into four groups, with each group containing six rats.

Group I: Treated with vehicle (1% Tween 80 in water, 10 ml/kg body weight p.o.)

Group II: Received Indomethacin (10mg/kg) body weight (p.o.)

Group III: Treated with 500 mg/kg body weight (p.o.) of the extract

Group IV: Treated with 1000 mg/kg body weight (p.o.) of the extract.

The starting medication, indomethacin, was given orally 15 minutes prior to the injection of acetic acid, while the test specimens and vehicle were given orally 30 minutes before intraperitoneal delivery of 0.7% v/v acetic acid. The mice were watched for a specific type of body spasm known as "writhing" for the next ten minutes after a five-minute break. The animal did occasionally completed its writhing; this incomplete writhing was referred to as half-writhing. Two half-writhings were therefore interpreted as a single full squirming. Each treated group's amount of writhing was contrasted to that of a control group. Rats receiving treatment will wriggle less when exposed to samples with analgesic action.

Chapter four
Results and Discussion

4.1 Result and Discussion of phytochemical evaluation

Serial No.:	Phytochemical constitution		Result
1	Alkaloids	Mayer's reagents	Positive
		Dragendroff's	Positive
2	Glycoside		Negative
3	Phenol		Positive
4	Tannin		Positive
5	Flavonoid		Positive
6	Saponins		Negative
7	Gum		Positive
8	Steroids		Positive
9	Reducing sugar		Positive
10	Carbohydrate		Positive

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 *Alstonia scholaris* extracts:

4.2 Result of anti-diarrheal evaluation

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 weight
STD	Loperamide	II	Standard group	50
MESF 200	Methanolic extract of branch of *Alstonia scholaris* extracts	IIIa	Test sample	200
MESF 400	Methanolic extract of branch of *Alstonia scholaris* extracts	IIIb	Test sample	400

Table 2: Test material used in the evaluation of anti- diarrheal activity

Code no	Mice no	No of diarrheal faces				Total of diarrheal faces	Average
		1 hour	**2 hour**	**3 hour**	**4 hour**		
CTL	1	3	3	1	0	7	5.67
	2	0	2	1	0	3	
	3	2	2	0	3	7	
STD	1	0	2	0	1	3	2.34
	2	0	2	0	0	2	
	3	0	1	1	0	2	
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 3: Total number of diarrheal faces stool given by each mouse

Group	t-test value	% reduction of diarrhea	Degree of freedom	P value	Statistical significance
II	8.5667	98.50	4	0.0007	Extremely significance
MESF1	4.1256	48.67	4	0.0112	Statistically significance
MESF2	3.2343	42.45	4	0.0223	Statistically significance

Table 4: Result of anti-diarrheal evaluation

4.3 Result of Antioxidant evaluation

IC50 for n-hexane extract of *Alstonia scholaris:*

Concentration (µg/ml)	% of Inhibition	IC50 (µg/ml)
500	92.06%	**5.641**
250	92.49%	
125	91.47%	
62.5	55.9%	
31.25	44.15%	

Table 5: IC50 for n-hexane extract of *Alstonia scholaris*

Y=19.103ln(x)-17.02

50=19.103ln(x)-17.02 (Where Y= 50)

ln(x)= 1.73

X=5.641

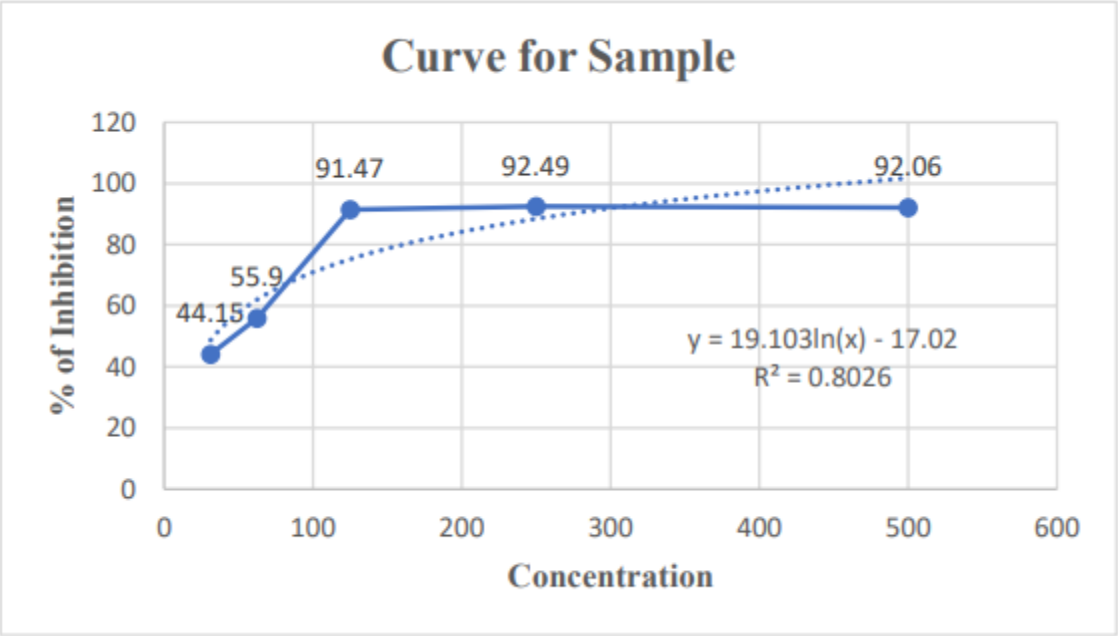


Figure 10: Graphical representation of % of Inhibition and concentration for Sample

IC50 for ascorbic acid (standard):

Concentration (µg/ml)	% of Inhibition	IC50 (µg/ml)
500	99.86%	**7.316**
250	98.79%	
125	78.57%	
62.5	62.33%	
31.25	52.74%	

Table 6: IC50 for ascorbic acid (standard)

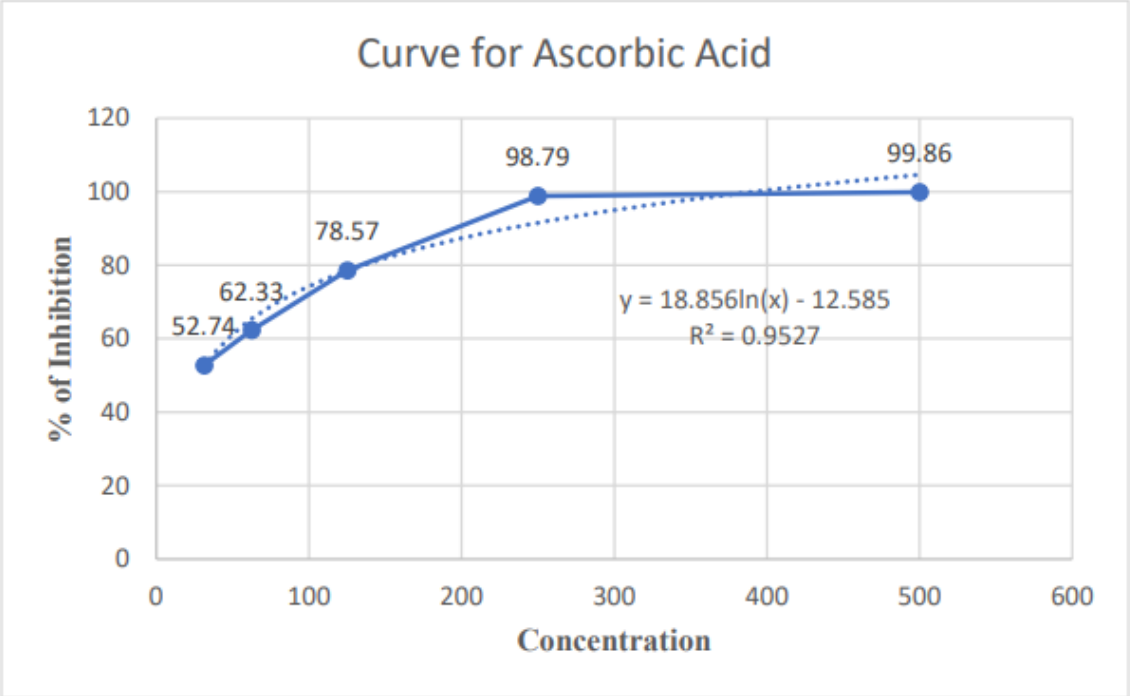


Figure 11: Graphical representation of % of Inhibition and concentration for Ascorbic Acid Test

Y = 18.856ln(x) - 12.585

50= 18.856ln(x) - 12.585 (Where Y= 50)

ln(x)= 1.99

X= 7.316

Discussion on the antioxidant test

The IC50 value of the antioxidant activity was determined using the DPPH method. The statistically significant testing results for the antioxidant activity of ascorbic acid and the *Alstonia scholaris* extract are shown in the aforementioned chart. In conclusion, we found that the solvent employed during the extraction process may have an effect on the substance composition in the extract. This study shows that the methanol extract of *Alstonia scholaris* has antioxidant action.

4.4 Results of in vivo analgesic activity

Analysis group	No of writhing			% Of inhibition
Standard drug	M1	M2	M3	59.27%
	7	6	9	
Alstonia scholaris 500mg	11	9	8	48.16%
Alstonia scholaris 1000mg	5	7	6	70.5%

Table 7: Results of in vivo analgesic activity

Discussion

Alstonia scholaris methanol extract decisively ($p < 0.05$ and $p < 0.01$) decreased the amount of writhing motions caused by intraperitoneal infusion of acetic acid solution in the acetic acid induced writhing test. The methanol extract's dose-dependent suppression (Table 7) of abdominal constrictions suggests that the plant may have antinociceptive properties. At a dosage of 1000 mg/kg body weight, the exerted suppression of writhing was greater than that of the common non-narcotic analgesic medication, indomethacin.

Chapter five
Conclusion

5.1 Conclusion

In conclusion, the chemical and biological investigation of the methanolic extract of ***Alstonia scholaris*** demonstrates its potential as a valuable source of bioactive compounds with significant medicinal properties. The investigations conducted in this study showed that the methanol extract of ***Alstonia scholaris*** included organic components that may be involved in pharmacological activity, including carbohydrates, alkaloids, glycosides, tannins, and diterpenes. Furthermore, the biological assays conducted indicate promising pharmacological activities, such as antioxidant, analgesic, and anti-diarrheal properties. These findings suggest that ***Alstonia scholaris*** holds great promise for further exploration in drug discovery and development. However, additional studies are warranted to elucidate the mechanisms of action and evaluate its safety and efficacy for potential therapeutic applications. Overall, this research contributes to our understanding of the medicinal value of ***Alstonia scholaris*** and highlights its potential as a source of novel pharmaceutical agents.

Chapter six
Reference

6. Reference

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