Pharmacological study of *Nelumbo nucifera* (leaf)

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Abstract:

Nelumbo nucifera, popularly known as "Lotus," is a member of the Nelumbonaceae family. From ancient times, it has allegedly been used to relieve pain and insomnia. Nowadays, the entire plant is used as a herbal medicine to treat gastritis, diarrhea, sleeplessness, fever, and body heat imbalance. It also has anti-inflammatory, analgesic, and antipyretic properties. *Nelumbo nucifera's* phytochemical activity, antimitotic, antibacterial, and antifungal characteristics are examined in this study using the plant's ethanolic (95%) extract. The *Allium cepa* root tip test was used for the antimitotic study, and the good diffusion method was used to detect antibacterial activity.

Introduction:

The aquatic perennial plant *Nelumbo nucifera*, often known as the lotus or sacred lotus, belongs to the Nelumbonaceae family. (2n = 16). The plant has a 1.5 meter maximum height and a 3 metre maximum horizontal spread. It has enormous, orbicular leaves that range in size from 20 to 90 cm and are non-wettable. Both aerial and floating leaves have glaucous petioles. The aerial leaves are cup-shaped, while the floating leaves are flat. Aerial leaves occasionally have rough, erect, smooth, greenish or greenish-brown, or greenish-brown with tiny brown patches, and petioles. The length of the aerial leaves ranges from 24-33 centimeters, whereas the floating leaves are normally 23–30 cm long. The odor is strange, and the fractures are fibrous. Young leaves are consumed as vegetables and used in traditional medicine. Leprosy weakness, hemoptysis, hemorrhoids, metrorrhagia, skin irritation, hematemesis, epistaxis, and hemorrhoids are among the conditions that are treated with lotus leaves in traditional medicine. Lotus leaves are said to have qualities that include lipolytic, anti-obesity, cardiovascular, and hypocholesterolemic. Several qualities of the leaf extract have been reported to include analgesic, antiobesity, anthelmintic, and hypolipidemic effects. According to reports, the leaf extract contains analgesic, anthelmintic, antiobesity, and hypolipidemic properties According to research, the liquor made from lotus leaves and blossoms has antioxidant characteristics and helps reduce oxidative stress. To treat diarrhea, use the leaf juice. The dried leaf is used to lower blood temperature and boost the spleen's function to stop bleeding during the summer. The leaf extract is used to treat fever and excessive sweating as an astringent, diuretic, and styptic. The leaves are used to treat a variety of conditions, including hemoptysis, metrorrhagia, hematemesis, hyperlipidemia, and epistaxis. They are primarily used to halt bleeding, treat heatstroke, eliminate heat from the body, and cool the blood. The stem is used in Ayurvedic medicine to treat diarrhea, leprosy, vomiting, and nervous exhaustion in addition to being a diuretic and anthelmintic.

Methodology:

Collection and processing of plant sample:

The *Nelumbo nucifera* leaf was grind into a fine powder, and the sample is used in extraction procedure.

Solvent extraction :

200 g of plant material were dissolved in 600 ml of a 100% hydroalcoholic (ethanol) solvent. During 48 hours, this solvent gadget was left at ambient temperature. After 48 hours, solvents were filtered using Whatman filter paper no. 1 in a beaker. The filtrate was dried in a rotary evaporator at a temperature of 55 °C to get the concentrated yield of extracts (Equitron, India). De Mesquita et al. developed a significantly modified version of the accepted solvent extraction procedure. The material was extracted without maceration; it was only dissolved in the solvent. The extract was stored for future research in a dark, frigid environment (4^0C).

Phytochemical analysis :

Using the prescribed methods, tests were run in an aqueous extract solution (ethanol + plant extract) to determine the presence of components. It was done to determine the qualitative presences of alkaloids, flavonoids, terpenoids, saponins, steroids, tannins, phenols, carbohydrates, and cardiac glycosides of amino acids.

(1) Test for flavonoids

(A) Alkaline reagent test:

A yellow colour will be visible after adding 1 ml of 10% NaOH to the plant extract. Add a few drops of diluted HCl, and if the yellow colour vanishes, the test is successful.

(B) **Lead Acetate test:**

0.5 ml of plant extract should be added together with a few drops of 10% lead acetate solution. If yellow colour is seen, the test is successful.

(2) Test for terpenoids

(A) Salkowski test:

Combine 0.2 ml of chloroform and 0.5 ml of plant extract. If reddish brown colour is seen after adding 0.3 ml of concentrated H2SO4, the test is positive.

(3) Test for Amino Acids

(A) Biuret test:

0.2 ml of plant extract should be treated with a few drops of 2% copper sulphate solution. Add 1 ml of ethanol and then a lot of potassium hydroxide pellets. When the extract layer turns pink, amino acids are present.

(4) Test for Cardiac Glycosides

(A) Killer Killani Test:

If a brown ring forms at the interface and a greenish ring spreads gradually throughout the thin layer after adding 0.5 ml of plant extract, 0.08 ml glacial acetic acid, and 1-2 drops of Fecl3, the test is positive.

(5) Test for Saponins

(A) Foam test:

Combine a tiny amount of ethanol with 0.5 ml of plant extract. After adding a little amount of D/W, shake the graduated cylinder for 15 minutes. If stable foam is seen, the test was successful.

(6) Test for Steroids

(A) Libermann- Burchared test:

Each 0.5 ml of plant extract was combined with 2 ml of acetic anhydride and then 2 ml of H2SO4. if the violet colour did not turn blue or green, this is a sign of the presence of steroids.

(7) Test for Tannins

(A) Ferric chloride test:

Add 0.5 ml of plant extract along with a few drops of 1% Fecl3; tannins are indicated by a vivid green or black tint.

(8) Test for phenols :

(A) Ferric chloride test:

0.5ml of plant extract and 3–4 drops of 5% Fecl3 solution should be added. Blueish hue formation suggests the presence of phenols.

(9) Test for Carbohydrate

(A) Molisch's test:

In a test tube, mix 0.5 ml of plant extract with 2-3 drops of 1% alcohol-napthol solution. When the violet rings form at the junction, there are carbohydrates present.

(10) Alkaloid test:

(A) **Dragendroff's reagent** (Potassium-bismuth-iodide solution):
Empty beaker, add 0.5g of bismuth nitrate. Concentrated hydrochloric acid, 10 ml, is added. In another beaker, add 4g of potassium iodide, a little water, and stir until all of the KI is dissolved. Take note of the development of a dark orange solution.

(B) **Mayer's reagent** (Potassium-mercuric-iodide solution): If a cream-colored precipitation forms after dissolving a solution of mercuric chloride (1.36g) and potassium iodide (5.00g) in 100ml of water, the test is affirmative.

(C) **Wagner's reagent** (iodine-potassium-iodide solution): Dissolve 100ml of water with 2g of iodine and 6g of potassium-iodide.

(D) **Hager's reagent** (Saturated solution of picric acid): 1g of picric acid and plant extract should be dissolved in 100ml of water.

Antimitotic assay:

Using an enhanced technique created by Fiskesjo, the antimitotic behaviour of the sample in *Allium* cepa roots and *Allium cepa* bulbs was assessed (1985). This was grown for 96 hours in the dark with daily water changes. Bulbs with uniform root growth were employed for greater activity. The produced and diluted plant extract was then applied to the fully formed and consistently growing roots of *Allium cepa* bulbs. 9 samples of onion bulbs were picked. Three sets were created as a result.

water	With drug (colchicine) 0.1 mg/

Figure 1.1 The *Allium cepa* bulbs with water and drug

Figure 1.2 The *Allium cepa* bulbs with plant sample

Both the dilution solution and the blank were made of water. Because colchicine has a strong and precise antimitotic activity, it was utilized as the standard. The last sample used was a

plant extract. For 24 hours in the dark and at room temperature, roots were allowed to germinate under these concentrations. Following incubation, the root tips were dyed with acetocarmine dye to assess the anticipated activity of the corresponding solutions, and were then examined under a 10x light microscope. Following the count, a mitotic index was performed on the corresponding sample that had been developed under the influence of the corresponding solution. To check for the mitotic index, following formula was used:-

$$mitotic\ index = \frac{number\ of\ dividing\ cells}{number\ of\ total\ cells} \times 100$$

Statistical Analysis:

The data was previously collected and expressed as the mean over three replicates with standard deviation. Graph Pad Prism software was utilised for the study of the p value. ANOVA was used to distinguish between the standard, sample, and blank for the plant extract of *Nelumbo nucifera*. To be deemed statistically significant, the p value for the ethanolic extract of *Nelumbo nucifera* leaf extracts must be lower than 0.05.

Antibacterial Assay:

Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, and *shighella flexneri* were the four bacterial strains against which the plant sample was tested for antibacterial efficacy. The media, along with pipettes, petri dishes, and metal borers, were sterilised in an autoclave for 15 minutes at 121 ℃ at 15 psi pressure. Under aseptic conditions, the media was added to Petri dishes. The four bacterial strains were obtained from the biotechnology department at Atmiya University in India. Bacterial surface colony formation was accomplished using nutrient agar. As part of the agar well diffusion procedure, bacterial strains were disseminated on solidified agar media and 7 mm wells were drilled into the agar media using a sterile metallic borer. The agar well diffusion method was developed by Murray et al. and then updated by Olurinola. The petri dishes were incubated for 24 hours at 37 degrees Celsius with antibiotic-containing control wells serving as a supporting control. To determine antibacterial activity, the diameter of the zones of inhibition after 24 hours was measured and compared to the control zone of inhibition. For antibacterial activity against Rifampicin test discs, plant extracts were examined. addition of different concentration (10mg, 25mg, 50mg, 100mg,) of plant extracts was executed and kept in incubator for 24hrs to observe the desire results

Result :

Phytochemical analysis :

The plant extract underwent phytochemical analysis, which revealed the presence of secondary metabolites as flavonoids, terpenoids, carbohydrates, and alkaloids as listed in Table 1.

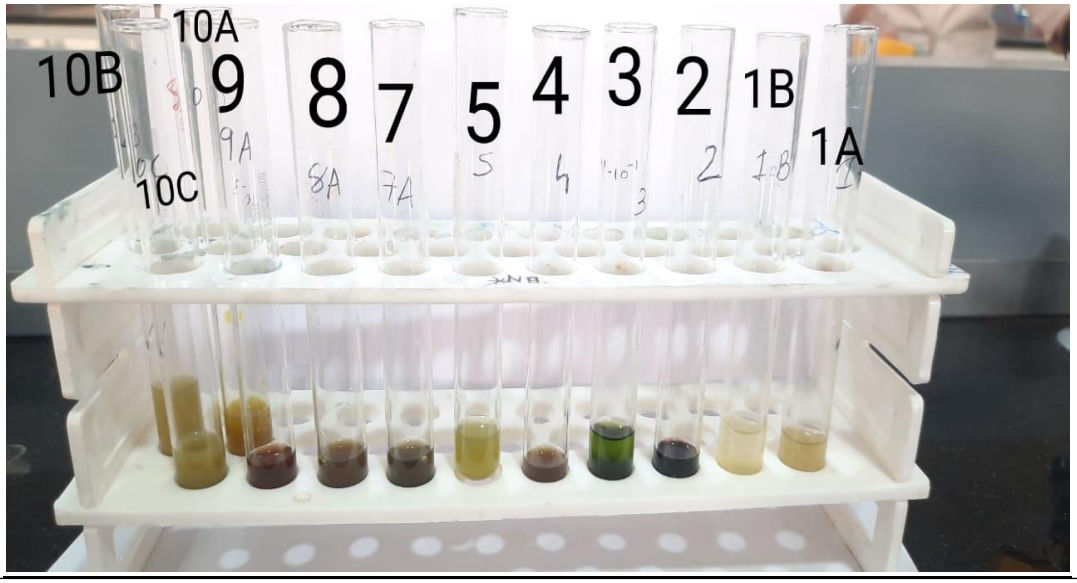


Figure 2 Test result of phytochemical analysis (Test tube 1A- Alkaline test , 1B- Lead-acetate test , 2 - Salkowski test ,*3*- Foam test ,*4*- Libermann-burchared test , 5- Ferric chloride test, 6 - Ferric chloride test , 7- Molisch's test ,8- Biuret test ,9- Killer killani test ,10A-Dragendroff's test , 10B- Mayer's test , 10C - Wagner's test

No.	***Phyto constitute***	***Name of test***	***Plant-1***
1	***Flavonoids***	Alkaline test	+ +
		Lead-acetate test	+ +
2	***Terpenoids***	Salkowski test	+ +
3	***Saponins***	Foam test	+ +
4	***Steroids***	Libermann-burchared test	--
5	***Tannins***	Ferric chloride test	+ +
6	***Phenol***	Ferric chloride test	+
7	***Carbohydrates***	Molisch's test	+
8	***Amino acid***	Biuret test	+
9	***Cardic glycoside***	Killer killani test	--
10	***alkaloids***	Dragendroff's test	--
		Mayer's test	+
		Wagner's test	+ +

Table 1. Phytochemical constituents present in *Nelumbo nucifera where* + slightly present, ++ moderately present, +++ highly present, − absent,

Nelumbo nucifera contains alkaloids, which made up the majority of the earliest anticancer medications discovered. Several of these alkaloids were also employed to make potent chemotherapeutic medications, including CPT, a well-known topoisomerase-I inhibitor. An alkaloid called vinblastine interacts with the protein tubulin and affects how spindle fibres are arranged. Other secondary metabolites present in it include flavonoids, terpenoids, and sugars.

Antimitotic Assay:

After using the acetocarmine dye to stain the root tip cells, they were examined under a light microscope. Fig. 3 depicts the root tips in microscopic detail

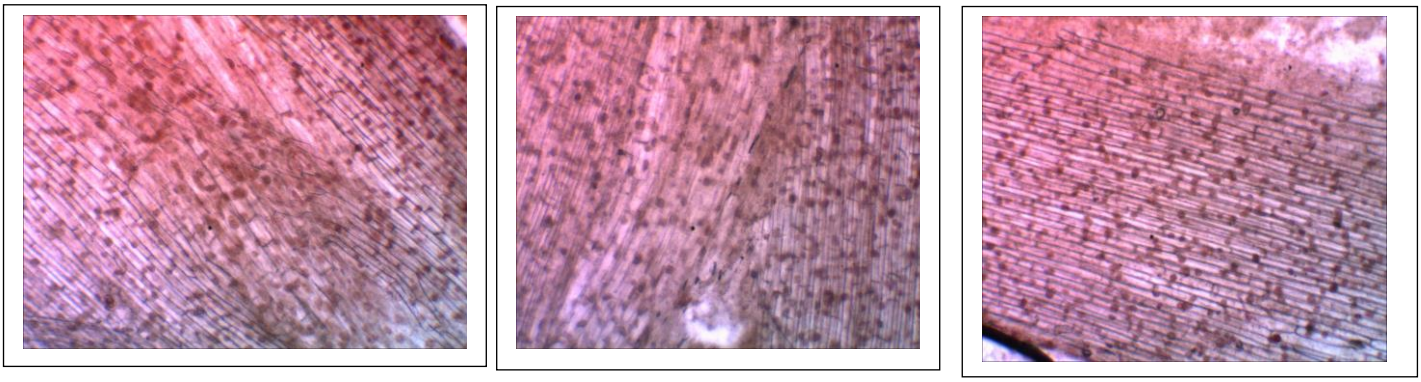


Figure 3.1 Observation of water-treated meristematic cells in *Allium cepa* under a microscope at a 10X magnification.

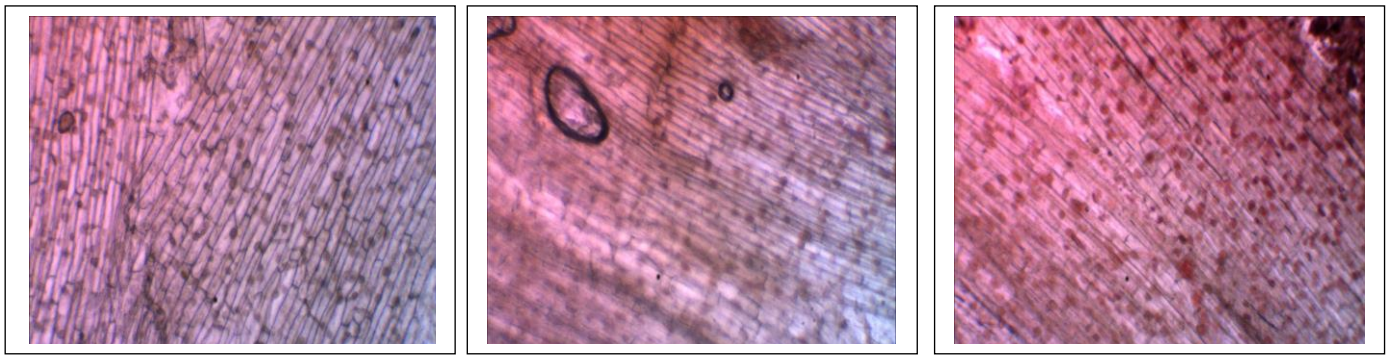


Figure 3.2 Observation of colchicine-treated meristematic cells in *Allium cepa* under a microscope at a 10X magnification.

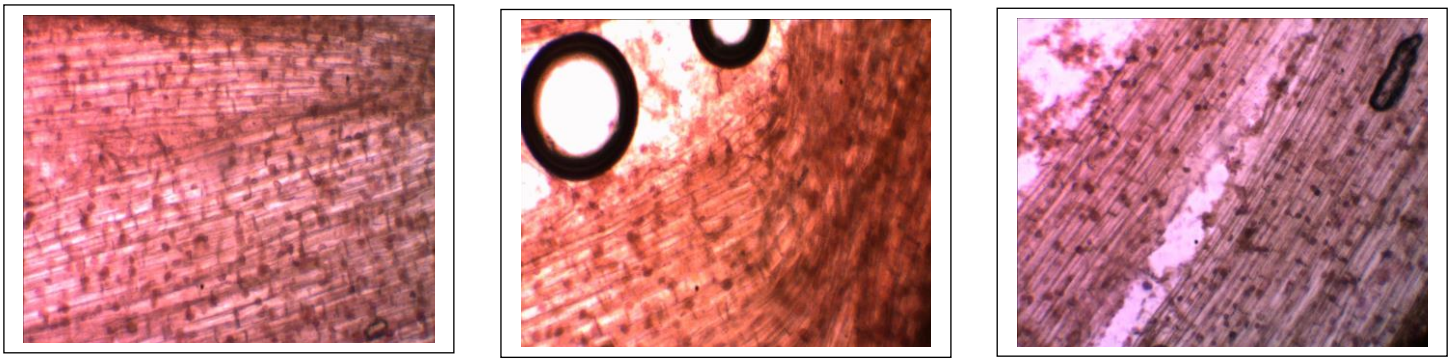


Figure 3.2 Observation of plant extract-treated meristematic cells in *Allium cepa* under a microscope at a 10X magnification.

	Sample 1			**Sample 2**			**Sample 3**		
	dividing cells	Non Dividing Cells	Total Cells	deviding cells	Non Dividing Cells	Total Cells	deviding cells	Non Dividing Cells	Total Cells
Water	296	117	413	275	162	437	160	169	329
Colchicine	60	210	270	98	222	320	104	271	375
Plant Extract	112	190	302	130	268	398	169	233	402

Table 2.1 Number of cells visible under a microscope in the root tips of *Allium cepa.*

Table 2.2 shows the impact on Nelumbo nucifera mitotic index of *Allium cepa* root tip cells.

	Water	**Colchicine**	**Plant**
Sample 1	71.67%	22.22%	37.08%
Sample 2	62.92%	36.29%	32.66%
Sample 3	48.92%	27.73 %	42.03%

Table 2.2 Compares the mitotic index for water, colchicine, and plant extract.

Statistical Analysis:

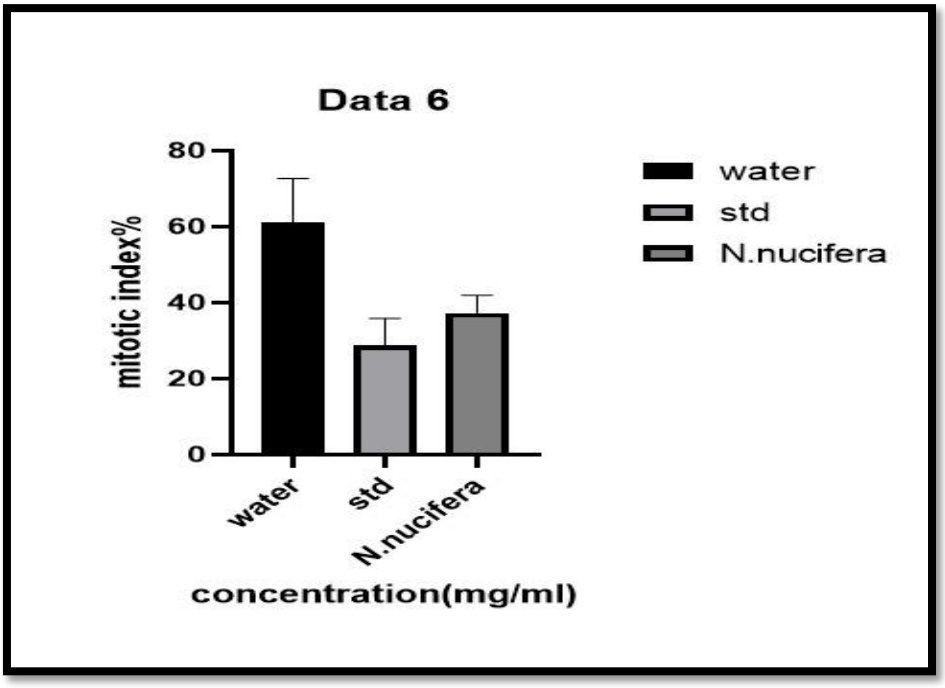


Fig4. Comparison of the mitotic index (MI) in water, colchicine, and plant extracts

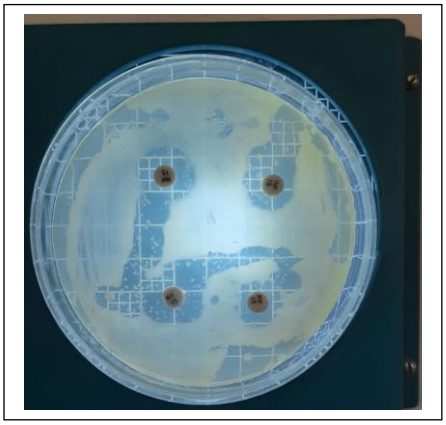
ANOVA summary	
F	12.17
P value	0.0077
P value summary	**
Significant diff. among means (P < 0.05)'	Yes
R squared	0.8022

Table 2.3 The comparative statistical table for the p value

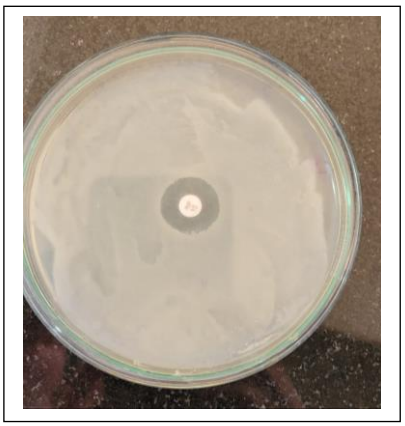
The statistical analysis was carried out using GraphPad Prism. A comparison between the mitotic index of *Allium cepa* root tips treated with water, colchicine, and plant extract was shown on the graph. In contrast to water, *Nelumbo nucifera* extract significantly inhibited mitosis by having antimitotic activity. Colchicine's antimitotic properties (0.1 mg/mL) were utilised as a benchmark. The *Nelumbo nucifera* p value was taken into account. The autonomous classes appear to differ significantly based on the p value (0.05). The experiment's results were statistically significant because the p-value for the study was 0.0077. The sample exhibited high antimitotic activity as a result, suggesting that it could be utilised as a potent antimitotic agent.

Antibacterial Analysis:

Several zones of inhibition were found when *Nelumbo nucifera's* antibacterial effectiveness was tested in vitro against *Staphylococcus aureus, Bacillus subtilis, shighella flexneri, and Salmonella typhi*. Our findings indicate that neither the extracts nor the four bacterial samples were inhibited by them, therefore neither the extract from the plant nor the bacterial samples are antibacterial. Our study led us to the conclusion that all three medications had an inhibitory effect, which the Zone Of Inhibition confirmed.



Bacillus subtilis

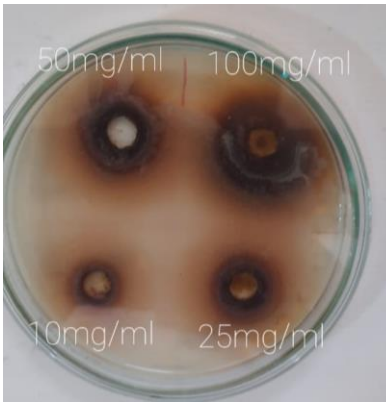


Staphylococcus aureus

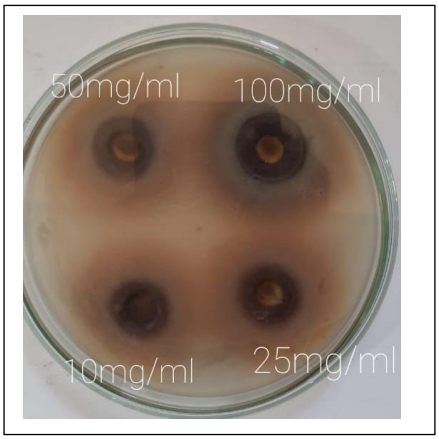
Salmonella typhi

Shighella flexneri

Fig 4.1 Well diffusion assay of the standard antibiotics (Rifampicin) showing zone of inhibition.

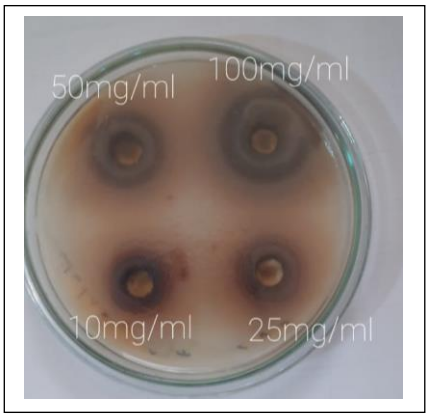


Shighella flexneri



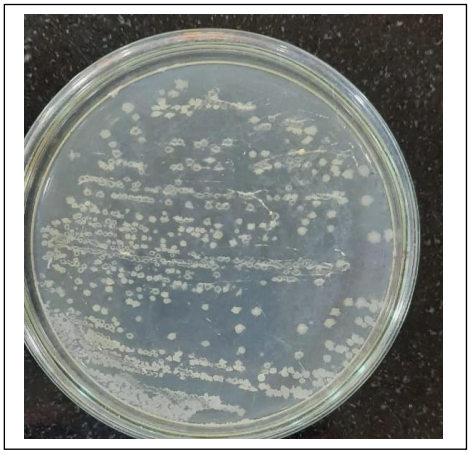
Bacillus subtilis

Staphylococcus aureus



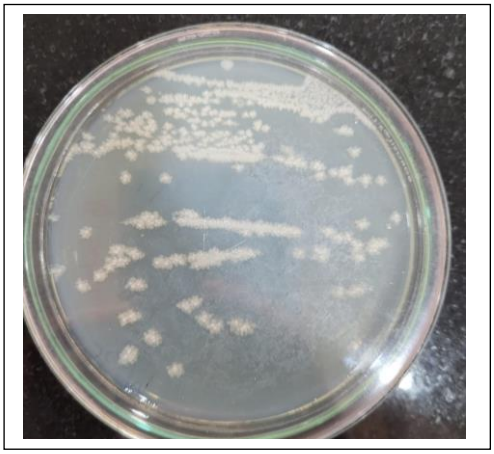
Salmonella typhi

Fig 4.2 N-agar plate showing zone of inhibition in the plates having *Staphylococcus aureus, Bacillus subtilis, Shighella flexneri* and *Salmonella typhi*

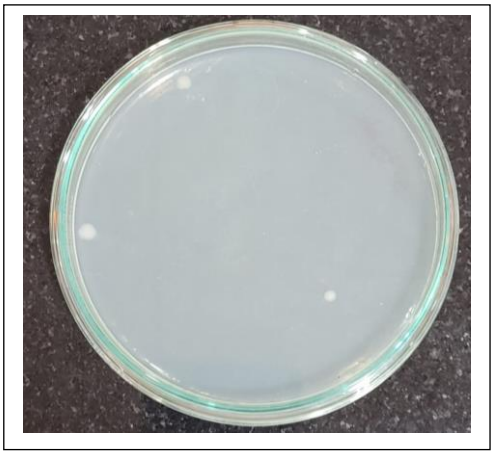


Shighella flexneri

Staphylococcus aureus



Bacillus subtilis



Salmonella typhi

Fig 4.3 Positive control plates

Discussion:

There are numerous reports on *Nelumbo nucifera* plant leaves, but no studies have been done on the plant's antimitotic properties. *Nelumbo nucifera's* antimitotic activity was discovered during a preliminary test, and this suggests that the plant may be further researched for use in the treatment of cancer. Using the proper extraction method and solvent, the active plant components must be separated from the plant portion in the initial step of phytochemical study.

I) Ethanol Extraction:

Extraction is a crucial first stage in the study of healthy plants since it is crucial to separate the designated chemical elements from the plant materials for every isolation and

characterisation. The fundamental procedure includes pre-washing, freeze-drying or drying plant components, and grinding to obtain an even sample.

II) Rotary Evaporator:

A rotary evaporator was used to successfully evaporate the leftover solvent in our sample. Each plant species' powdered material was steeped in 300 mL of ethanol for 48 hours, thoroughly shaken twice daily, and then filtered. The filtrates were then evaporated in a rotary evaporator with reduced heat, producing a sticky residue. The extracts were kept in sterile glass vials and kept at room temperature until they were analyzed.

III) Phytochemical Analysis:

Plants either create phytochemicals through primary or secondary metabolism. They often perform a biochemical task in the plant host, helping the plant grow or protect itself from infections, pests, or competitors. Identification is challenging because plant extracts can contain a combination of substances or phytochemicals with varying polarity. The phytochemicals in plant extracts can be better understood through phytochemical study on a number of therapeutic plants. The plants analysed also contained various levels of alkaloids, tannins, saponins, phenols, flavonoids, and terpenoids. The foam test, killer killani test, Biuret test, Liebmann burchard test, ferric chloride test, and other tests are just a few of the assays that are available for each phytochemical. Each test's measurements and meanings are listed above. Each plant has distinct groupings of phytochemicals for a range of uses, and there are numerous forms and classifications of phytochemicals.

IV) Antimitotic Assay:

Cell division can be interfered with by antimitotic substances at some stage in the cell cycle. As a result, bark extracts are toxic to cells that are actively dividing, either by preventing the formation of spindles in the M-phase or by interfering with the synthesis of deoxyribonucleic acid in the S-phase. The low mitotic index indicates that plant extracts have an immediate genotoxic effect on live cells.

V) Antibacterial Assay

An indisputable test that identifies the precise action of a plant extract against a range of antibiotics as well as two gram positive and two gram negative bacteria is the antimicrobial assay. To develop surface colony development for bacteria culture, agar medium was used. Due to phytochemicals produced by the secondary metabolism of the plant, several plants are employed for their antibacterial qualities. Four microorganism strains, *Staphylococcus aureus ,Bacillus subtilis ,Shighella flexneri* and *Salmonella typhi.*

Conclusion:

In this research, the antimitotic and antibacterial behaviour of *Nelumbo nucifera* was examined, and it was found that it possesses both of these traits. These procedures made it clear that Nelumbo nucifera had considerable amounts of bioactive substances and biochemical components, indicating that it might be employed as a source for antibacterial and cancer treatments. To completely understand the distinctive impact of the bioactive molecule, more investigation is required.

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