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Twinkal Revatkar
Department of Pharmacology,
Wadhwani College of
Pharmacy, Dhamangaon
Road, Yavatmal,
Maharashtra, India

Rakesh Bhute
Department of Pharmaceutics,
Bhausaheb Mulak College of
Pharmacy WCL, Umred
Nagpur, Maharashtra, India

Anand Purohit
Department of Pharmaceutics,
Bhausaheb Mulak College of
Pharmacy WCL, Umred
Nagpur, Maharashtra, India

Ashish Lande
Department of Pharmaceutics,
Rajarshi Shahu College of
Pharmacy, Sadak Arjuni,
Maharashtra, India

Amar Jaiswal
Department of Pharmacognosy
and phytochemistry,
Bhausaheb Mulak College of
Pharmacy WCL, Umred
Nagpur, Maharashtra, India

Corresponding Author:
Twinkal Revatkar
Department of Pharmacology,
Wadhwani College of
Pharmacy, Dhamangaon
Road, Yavatmal,
Maharashtra, India

Comparative study of antimitotic activity on *Allium cepa* by using naturally growing *Bacopa monnieri* extract

Twinkal Revatkar, Rakesh Bhute, Anand Purohit, Ashish Lande and Amar Jaiswal

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Abstract

Bacopa monnieri, a prominent herb in the Indian Ayurvedic tradition, is renowned for its health benefits, particularly in enhancing cognitive function and supporting the immune system. This remarkable plant is packed with medicinal properties, including memory enhancement, antioxidant effects, promotion of hair growth, and potential anticancer benefits. In this study, we focused on evaluating the antimitotic activity of extracts from organically cultivated *Bacopa monnieri* using the *Allium cepa* model. We conducted a thorough phytochemical screening of the extract to identify its phytoconstituents. The hydroalcoholic extract exhibited positive results for several compounds, including alkaloids, flavonoids, sterols, saponins, proteins, and glycosides. To assess the antimitotic potential, we examined the root tips of *Allium cepa*, targeting the meristematic cells because their growth rate is comparable to that of cancer cells. We calculated the mitotic index and measured mitotic inhibition, comparing our results to both water and the standard antimitotic drug methotrexate. Notably, the hydroalcoholic extract of *Bacopa monnieri* demonstrated significant effects, highlighting its promising role in antimitotic activity.

Keywords: *Bacopa monnieri*, *Allium cepa*, antimitotic, anticancer, methotrexate.

Introduction

Bacopa monnieri is commonly used in the Indian Ayurvedic system of medicine and is seen as helpful as a health supplement and for strengthening the immune system. This article covers the important phytochemistry, medicinal benefits, and clinical studies on *Bacopa monnieri*. Brahmi, a herb, is used in treating minor and chronic diseases. Brahmi has been used for many years and now India is a key country in Ayurveda, Siddha, and Unani medicine. In India, the plant is sold commercially as Brahmi, a name that comes from Brahma, the legendary creator in Hindu mythology. Brahmi is used for brain healing, improving intelligence, treating epilepsy, healing wounds, fighting cancer, and reducing anxiety^[3]. Taking Brahmi every day for two months can help reduce stress, anxiety, and depression, and also improve patients' energy and mood^[1]. *Bacopa monnieri* grows in southern and eastern India, Australia, Europe, Africa, Asia, North and South America, and Sri Lanka. Brahmi is also known by other names such as hyssop, brahmi, thyme-leafed gracile, herb of grace, and Indian pennywort. Many trials on *Bacopa monnieri* are used to differentiate healthy and unhealthy cases from various diagnostic and symptom groups, but the assessment tools used were not always comparable. *Bacopa monnieri* is a medicinal plant used in Ayurveda to improve memory and brain development. In Brahmi extract, five different compounds are found, namely bacopaside A, bacopaside B, bacopaside C isomer, bacopasaponin C, and Bacopa saponin-I, which were identified using the HPLC method. In preclinical studies, different extracts of *Bacopa monnieri* have shown positive cognitive effects on animals. In animal behavior studies, animals given *Bacopa monnieri* have shown improved motor skills^[30].

Plant Profile ^[2]

1. **Name:** Brahmi
2. **Synonym:** Nir Brahmi, Indian pennywort, Jalanevari, Jalbrahmi.
3. **Source:** It consist of fresh leaves and stem of plant known as *Bacopa Monniera* Linn.
4. **Family:** Scrophulariaceae
5. **Chemical Constituents:** Brahmi contain Bacoside- A and Bacoside-B.
6. **Genus:** *Bacopa*
7. **Species:** *B. Monniera*
8. **Zoological Name:** *Bacopa monnieri*
9. **Class:** Mangoliopsida
10. **Subclass:** Asteranae

**Fig 1:** *Bacopa Monnieri***Phytoconstituents**

Pharmacologically active compounds found in *Bacopa monnieri* include saponins, steroids, and alkaloids. Early research confirmed that an alkaloid called "brahmin" was isolated from *Bacopa monnieri*. Other alkaloids, such as herpesin and nicotine, were also reported in the same year. Later, D-mannitol, saponins, hersaponin, and potassium salts were isolated. Bacoside A is the main compound responsible for the neuropharmacological and nootropic or anti-amnesic effects of *Bacopa*. Bacoside A is usually found along with Bacoside B; the difference between them is only in optical rotation, which may be an artifact from the separation process. The main chemical components isolated and characterized through various spectroscopic, chemical, and 2D NMR studies by different research groups from alcoholic extracts of medicinal herbs are jujubogenin saponin and pseudojujubogenin saponin as aglycones, along with dammarane type triterpenoid saponins. When Bacoside is broken down with acid, it forms a mixture of aglycones, including bacogenin A1, A2, and A3. Three major types of triterpenoid saponins have been isolated: *Bacopa* saponins A, B, and C, along with *Bacopa* saponin. Pseudojujubogenin was isolated, and a new dammarane-type pseudojujubogenin glycoside, bacosasaponin-D, was identified using chemical transformations and spectroscopic methods. Two new sapogenic glycosides of *Bacopa* were isolated from the glycoside part of the methanol fraction and named Bacoside I and II. Later, three new saponins, called Bacosides III, IV, and V, were isolated. In addition, three new phenylethanoid glycosides, known as monnierasides I to III, were identified from the glycoside part of *Bacopa monnieri* as well as from the known plantainoside-B analogue ^[6, 39, 42].

Table 1: Phytoconstituents of *Bacopa monnieri* ^[5]

Sr, No.	Chemical Constituents	Structure
1.	Bacoside-A	
2.	Bacoside-B	
3.	Jujubogenin	

Mechanism Action of *Bacopa monnieri*

The effects of Brahmi, also known as *Bacopa monnieri*, on the nervous system have been widely researched. This plant contains compounds like saponins and bacosides, which help improve nerve signal transmission. Brahmi supports the repair of damaged nerve cells by enhancing kinase activity, promoting the production of new neurons, and restoring the function of synapses, which are the connections between nerve cells. In animal studies, *Bacopa monnieri* has been found to relax tissues such as the pulmonary artery, aorta, trachea, ileum, and bronchial passages. This effect is achieved by preventing calcium ions from entering the cells. Numerous clinical and research studies have been carried out by various scientists to explore the nootropic, or brain-enhancing, benefits of *Bacopa monnieri* ^[41].

Materials and Methods**Procurement of *Bacopa monnieri* Herb**

The procurement of *Bacopa monnieri* for research work were collected from Saraswati herbal farm at. Umari, tah. pauni, Dist. Bhandara.

Extraction of *Bacopa monnieri* Herb ^[43, 44]

The extracts of *Bacopa monnieri* were made using a traditional maceration process. The dried plant powder of *Bacopa monnieri* was mixed with a combination of ethanol and water. Each mixture was placed separately and left at room temperature for 72 hours, with periodic stirring. Once the maceration was done, the clear liquid on top was poured off, and the mixture was filtered. The filtered liquid was then heated to remove the solvent completely. After the solvent had fully evaporated, the resulting extract was weighed and stored in a sealed glass container for future use.



Fig 2: Extraction process of *Bacopa monnieri*

Preliminary Phytochemical Screening [44, 45, 46]

A) Test for Alkaloid

- **Mayer's test:** Two drops of Mayer's reagent were added to 2ml of filtrate by the side of the test tube. A white creamy precipitate was formed, which showed a positive result.
- **Hager's test:** 1 ml of Hager's reagent was added to 2ml of filtrate. A noticeable yellow precipitate appeared, indicating the presence of alkaloids.
- **Wagner's test:** Few drops of Wagner's reagent were added to 2ml of filtrate by the side of the test tube. A reddish brown precipitate formed, confirming the presence of alkaloids.

B) Test for glycosides

- **Saponin glycosides (Foam test):** About 50mg of extract was dissolved in successive solvents and made up to 20ml. The suspension was shaken for 15 minutes. A 2cm layer of foam formed and was left to settle for 10 minutes. The appearance of foam indicated the presence of saponins.
- **Anthraquinone glycosides (Borntrager's test):** 2ml of extract were mixed with sulfuric acid. The solution was filtered next. The same amount of chloroform was then added to the filtrate. After shaking, the organic solvent was separated. Finally, the same volume of ammonia solution was added. No bright pink, red, or violet color appeared in the upper layer, showing that anthraquinones were not present.

C) Test for flavonoids

Sodium hydroxide test: 2ml of the extract were taken, and increasing amounts of sodium hydroxide were added. A color change was observed, which disappeared when dilute hydrochloric acid was added. The disappearance of color showed the presence of flavonoids.

D) Test for Steroids

Salkowaski test: 2 ml of the extract were placed in a test tube with two milliliters of chloroform. Then, 2ml of concentrated sulfuric acid were added from the side of the test tube. The mixture was shaken for a few minutes. The appearance of a red color in the chloroform layer and a greenish-yellow color in the acid layer indicated the presence of steroids.

E) Test for proteins

Millon's test: About 2ml of the sample extract were mixed with 5ml of Millon's reagent. A white precipitate formed. After warming the mixture, the precipitate turned into a brick-red color, indicating the presence of proteins.

F) Test for carbohydrates

Molisch's test: 2 ml of the extract were placed in a test tube. 2 drops of alcoholic alpha-naphthol solution were added, and the mixture was shaken well. Then, 1 ml of concentrated sulfuric acid was added carefully along the sides of the test tube. A violet ring formed at the junction, indicating the presence of carbohydrates.

Physiochemical Evaluation [47, 48]

A) Loss on Drying

Loss on drying refers to the decrease in mass, expressed as a percentage by weight. This test helps to measure both the water and other volatile substances present in the crude drug. Moisture is naturally present in crude drugs and needs to be removed as much as possible. A careful amount of about 2 grams of *Bacopa monnieri* (L) powder was placed in a glass petri dish. The dish was left uncovered in a vacuum oven and dried at a temperature ranging from 100 to 105 degrees Celsius for 2 hrs until the weight stopped changing. After drying, the dish was cooled in a desiccator to room temperature, then weighed again and the result was recorded. The percentage loss on drying was calculated using the provided formula.

$$\text{Percentage (\%)} \text{ loss on drying} = (W2 - W3 / W2 - W1) \times 100$$

Where,

W1 = Weight of empty petri dish

W2 = Weight of empty petri dish with sample

W3 = Weight of empty petri dish with dried sample

B) Ash Value

Determination of Ash value of *Bacopa monnieri* (L)

1. Total ash

2 grams of dry, coarsely powdered plant material from *Bacopa monnieri* were placed in a crucible that had been previously heated. The sample was gradually heated to a temperature between 500 and 600 degrees Celsius until it turned white. After heating, the crucible was cooled in a desiccator and then weighed again. The percentage of total ash was calculated based on this weight.

$$\text{Percentage (\%)} \text{ Total ash content} = (W2 - W3 / W2 - W1) \times 100$$

Where,

W1 = Weight of empty crucible

W2 = Weight of crucible with sample

W3 = Weight of crucible with dried sample

2. Acid-Insoluble Ash

It is the residue left after boiling the total ash with dilute hydrochloric acid and then burning the remaining insoluble material. 25ml of dilute HCl was added to the crucible containing the total ash and boiled gently for five minutes. The insoluble material was collected on ashless filter paper, washed with hot water, and then the filter paper was burned, cooled in a desiccator, and weighed. The percentage of acid-insoluble ash was calculated based on this weight.

3. Water Soluble Ash Values

The total ash was boiled for five minutes with 25 ml of water. The soluble portion was collected in a crucible,

burned, and weighed. The percentage of water-soluble ash was then calculated.

C) Swelling Index

Take 1 gram of the sample and place it in a 25 ml stoppered measuring cylinder. Fill the cylinder up to the 20 ml mark with water. Gently shake the cylinder occasionally over a period of 24 hours and let it stand. Measure the volume taken up by the swollen sample. The original sample should occupy a volume of at least 10 ml.

$$\text{Swelling index} = (V_2 - V_1)/V_1 \times 100$$

Where,

V₁ = Initial volume

V₂ = Final Volume

D) Foaming Index

Many medicinal plants contain saponins, which can create a lot of foam when their water-based decoction is shaken. The ability of a plant material's water extract to produce foam is measured using a foaming index.

Recommended Procedure: Take about 1 gram of the plant material and grind it into a coarse powder with a mesh size of 10, 22, 44, or 85. Weigh it accurately and put it into a 500 ml conical flask that already has 100 ml of boiling water. Let it boil gently for 30 minutes. Allow it to cool, then filter the liquid into a 100 ml volumetric flask and add water through the filter to bring the volume up to 100 ml. Pour this decoction into 10 stoppered test tubes, adding successive amounts of 1 ml, 2 ml, 3 ml, and so on, up to 10 ml. Make sure each tube has exactly 10 ml of liquid by adding water as needed. Stopper each tube and shake them in a lengthwise motion for 15 minutes. Then measure the height of the foam formed in each tube. The results are evaluated as follows: If the foam height in every tube is less than 1 cm, the foaming index is less than 100. If any tube shows a foam height of 1 cm, the volume of plant material decoction in that tube (a) is used for determining the index. If this tube is

the first or second in the series, prepare an intermediate dilution in the same way to get a more accurate result. If the foam height in all tubes is more than 1 cm, the foaming index is over 1000. In this case, repeat the test using a new series of dilutions to get a more precise result. Calculate the foaming index using the following formula: $-1000/a$

Where,

a = the volume in ml of decoction used for preparing the dilution in the tube where foaming to height to a height cm is observed.

Antimitotic Assay

A method that was adjusted by Fiskesjo (1985) was used to assess the antimitotic effect using the root of *Allium cepa*⁽³⁰⁾. *Allium cepa* bulbs weighing 40 ± 10 grams were germinated in water for 72 hours at room temperature in the dark. Only bulbs that developed uniform roots were selected for the study (Fig. 6). These onion roots were then placed in beakers containing water, methotrexate (0.1 mg/mL), and plant extract (10 mg/mL) for 24 hours. Water was used for dilution, and methotrexate served as the standard for comparison. The plant extract, along with the control (water) and the standard (methotrexate), were tested separately. After 24 hours, the root tips were stained with acetocarmine and examined

under a microscope at 1800X magnification. The number of dividing and non-dividing cells was counted. The mitotic index was calculated using the following formula:

$$\text{Mitotic index (MI)} = \frac{\text{No of cell in mitosis}}{\text{Total number of cells}} \times 100$$

$$\% \text{ Mitotic inhibition} = \frac{\text{Mitotic index in Control} - \text{Mitotic index in test}}{\text{Mitotic index in control}} \times 100$$

Results

Phytochemical Screening

Table 2. Phytochemical screening of aqueous and ethanolic extracts of *Bacopa monnieri*

Sr. No.	Secondary metabolites	Phytochemical tests	Aqueous	Ethanol
1.	Test For Alkaloids	Mayer's Test Wagner's Test Hager's Test	+	+
2.	Test for Glycosides Saponin glycosides Anthraquinone glycosides	Foam Test Borntrager's Test	+	+
3.	Test For steroids	Salcowaski Test	+	+
4.	Test For Flavonoids	Sodium Hydroxide Test	+	+
5.	Test for Tannins	Dilute Nitric Acid Test	+	+
6.	Test For Proteins	Millon's Reagent Test	+	+



Fig 3: Phytochemical screening of aqueous and ethanolic extracts of *Bacopa monnieri*

Macroscopic Characteristic

Table 3. Macroscopic characteristic of naturally growing *Brahmi*

Features	Naturally growing <i>Brahmi</i>
Colour	Greenish brown
Odour	Characteristics
Taste	Bitter
Touch	Rough
Shape	Leaves–sessile, succulent, opposite Obovate – oblong

Microscopic Evaluation ^[14]

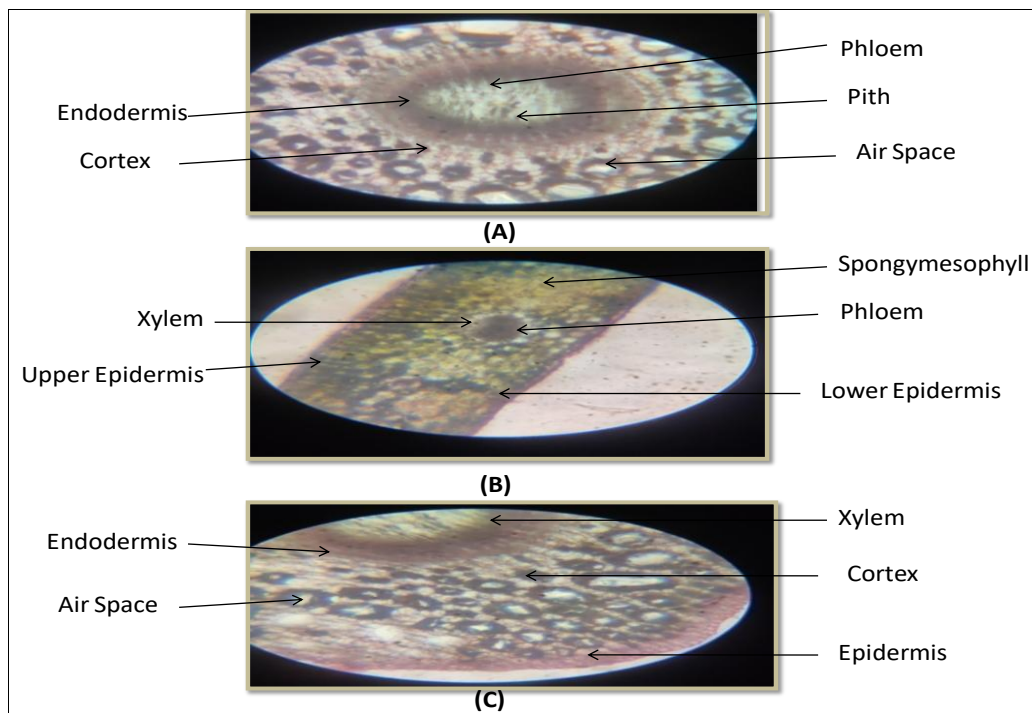


Fig 4: Representative transverse sections of leaf (a), stomata on upper epidermis (b) and (C) stem of *Brahmi* using safranin O staining (Comparative Microscopic images of *Bacopa monnieri*)

Physiochemical Evaluation



Fig 5: physiochemical evaluation of *Bacopa monnieri*

Table 4: Physiochemical analysis of *Bacopa monnieri*

Sr.no	Physiochemical Parameters	Result
1.	Loss On Drying (LOD)	8.5%
2.	Total Ash	8.82%
3.	Swelling Index	No change
4.	Foaming Index	333.33

Antimitotic Activity

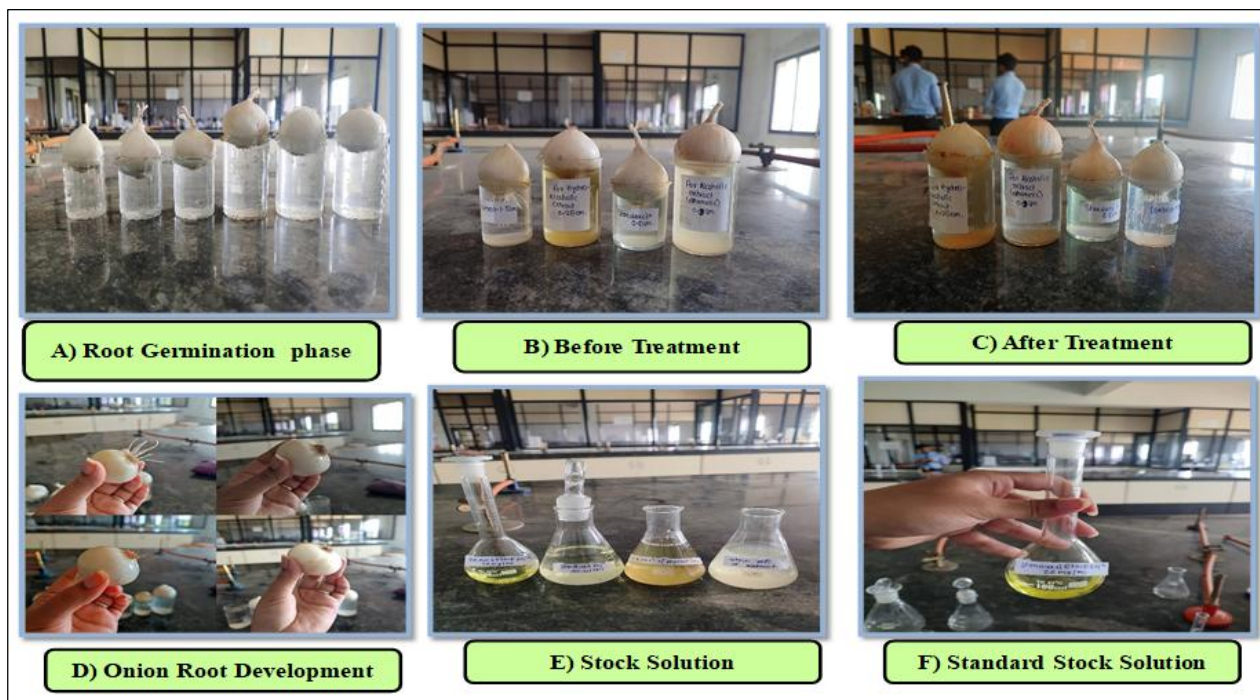


Fig 6: Representation of onion cepa model (A) root germination phase (B) before treatment (C) after treatment (D) onion root development (E) prepration of stock solution (F) Standard stock solution (methotrexate).

Table 5: *Allium cepa* root length attained after incubation with water, methotrexate, *Bacopa monnieri* extract.

Sr.No	Sample	Before Germination	Before Treatment	After Treatment
1.	Water (control)	00cm	1.5cm	4.5cm
2.	Methothrexate (standard)	00cm	0.7cm	0.7cm
3.	Alcoholic Extract	00cm	1cm	1.1cm
4.	Hydro-Alcoholic Extract	00cm	0.9cm	1.3cm

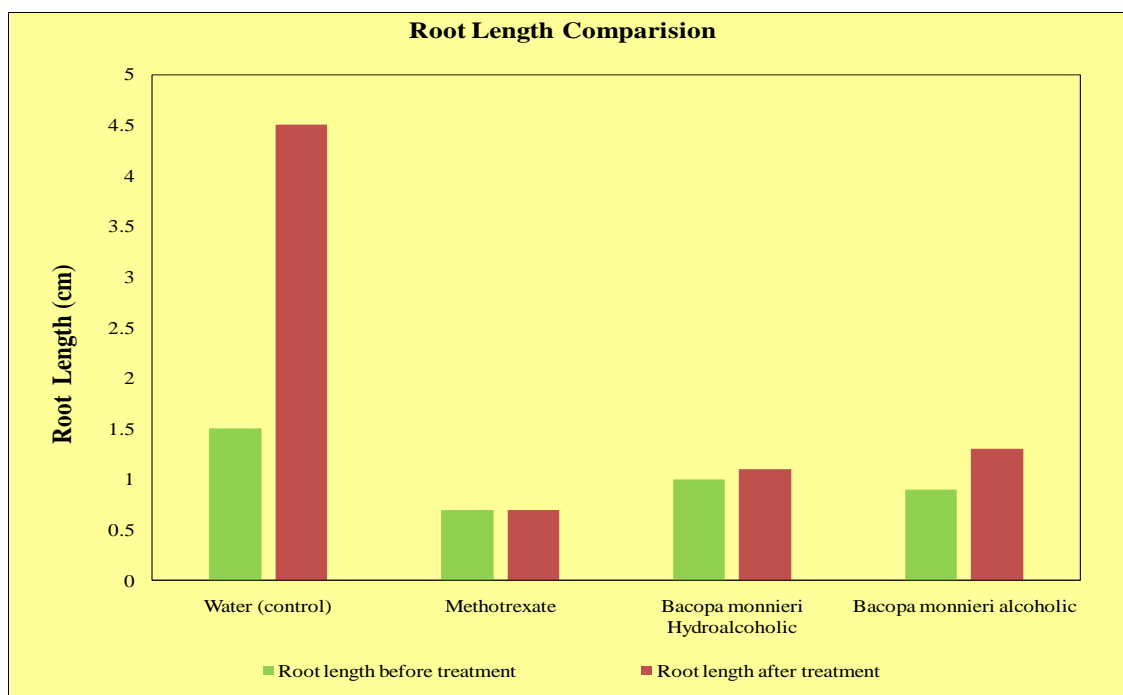


Fig 7: Graphical representation of *Allium cepa* model

Antimitotic assay: After staining, the root tip cells were examined under a light microscope using acetocarmine stain. A microscopic image of the stained root tip cells at 1800X magnification is shown in Figure 8. The effect of B.

monnieri extract on the mitotic index (MI) of *Allium cepa* root tip cells is presented in Table 6. The graph compares the mitotic index of root tips treated with water, methotrexate, and the plant extract. The extract from B.

monnieri showed significant antimitotic activity, as it reduced the rate of mitosis compared to the water-treated group. Methotrexate at a concentration of 0.1 mg/mL was used as a standard and exhibited the highest antimitotic

activity. Therefore, the *B. monnieri* extract demonstrated significant antimitotic properties, suggesting its potential as a strong antimitotic agent (Fig. 9 and 10).

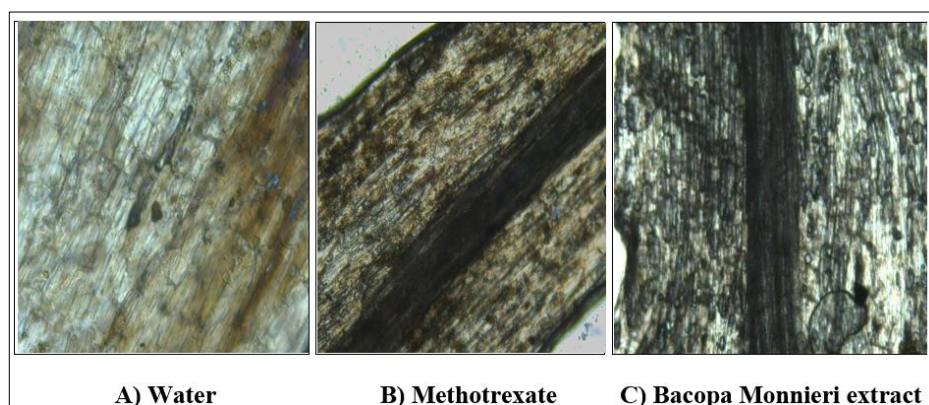


Fig 8: Mitosis study on the roots Meristematic cells(1800X)

Table 6: Cell count in *allium cepa* meristematic root tip section

Sr. no.	Sample	Total no. of cells	No of cells in Mitosis	Mitotic Index	% Mitotic Inhibition
01.	Water	76	53	69.73	0.00
02.	Methotrexate	32	10	31.25	55.18
03.	<i>B. Monnieri</i>	60	27	45.00	35.46

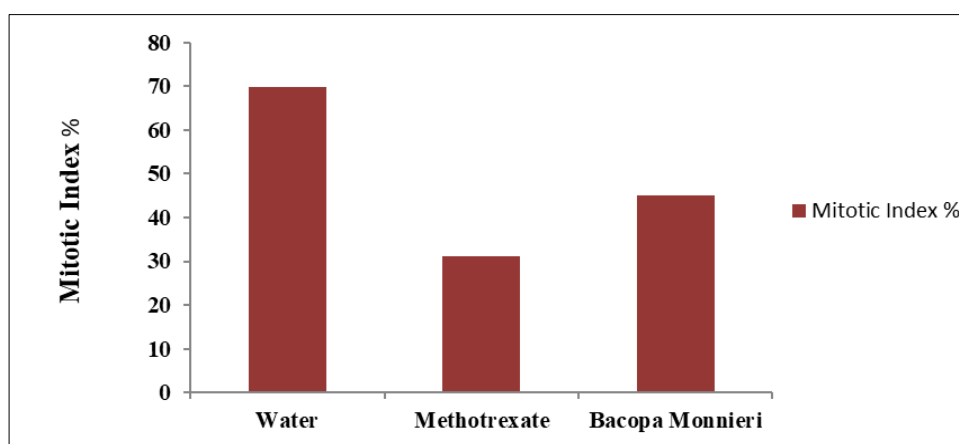


Fig 9: Comparison between % mitotic index of *Bacopa monnieri* extract, Methotrexate and Water.

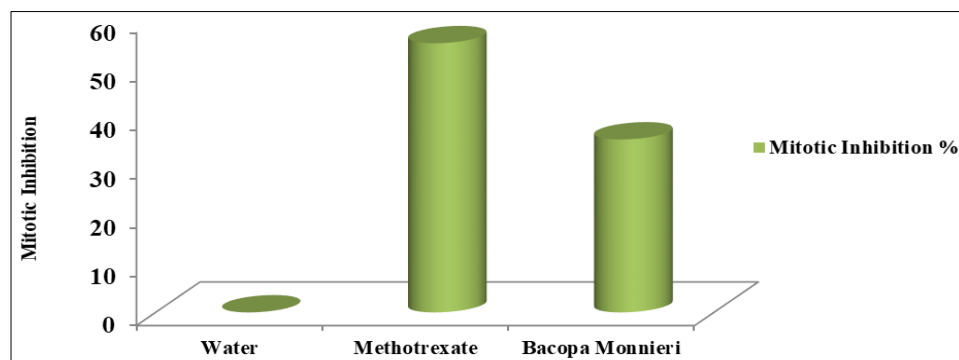


Fig 10: Comparison between % mitotic inhibition of *Bacopa monnieri* extract, Methotrexate and Water.

Discussion

Bacopa monnieri is a plant known for its high medicinal value, including the ability to enhance memory, act as an antioxidant, promote hair growth, and have anticancer properties. This study focuses on evaluating the antimitotic activity of *Bacopa monnieri* extract. A phytochemical screening was done to identify the phytoconstituents present

in the plant. The hydroalcoholic extract showed positive results for alkaloids, flavonoids, sterols, saponins, proteins, and glycosides. The antimitotic activity was tested on the root tip meristematic cells of onion (*Allium cepa*) because these cells grow at a rate similar to cancer cells. In this study, the mitotic index and mitotic inhibition were calculated and compared with water and the standard

antimitotic drug methotrexate. The hydroalcoholic extract of *Bacopa monnieri* showed a significant effect. Pharmacognostical evaluation of *Bacopa monnieri* is an important initial step in standardizing the plant and provides valuable information on its macroscopic, microscopic, and physical characteristics. Macroscopic examination of *Bacopa monnieri* gives detailed information about its morphological features such as size, shape, color, taste, and odor. Microscopic evaluation provides knowledge about the cellular arrangement in the tissue. The transverse section (TS) of the leaves of *Bacopa monnieri* showed layers like the epidermis, cortex, vascular bundle, xylem, and phloem. Physicochemical parameters like loss on drying (LOD), ash value, foam index, and swelling index were also determined. The moisture content of *Bacopa monnieri* was found to be less than 8.5%, and controlling moisture content is very important. The swelling index of the plant material indicates its therapeutic or pharmaceutical value, which may be due to the presence of gums, mucilage, pectin, and hemicellulose. According to proximate analysis, *Bacopa monnieri* does not show a swelling index. The ash value is also useful for determining the quality and purity of crude drugs.

Summary and Conclusion

The preliminary phytochemical screening revealed that *Bacopa monnieri* Extract is responsible for antimitotic activity. In phytochemical screening of *Bacopa monnieri* Extract give positive test for alkaloid, glycoside, tannins, flavonoids, saponins etc. In present work, attempt was made to study for antimitotic activity of *Bacopa monnieri* Extract from the result clearly proved Extract of *Bacopa monnieri* showed promising antimitotic activity which shows significant effect. In this research work show the future prospect in further new research.

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