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In- vitro propagation, antioxidant properties and phytochemical analysis of *Bacopa monnieri* (*Brahmi*)

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Abstract
Bacopa monnieri (Brahmi) a well-known herbal drug. Extensively using as memory enhancer all over the world. It is traditional ayurvedic herb there are several benefits. The leaves of this plant are widely used. The present review discusses the phytochemical constituent, antimicrobial analysis, and antioxidant property of *Bacopa monnieri Linn.* (Brahmi) along with *in-vitro* tissue culture propagation comparative *in vitro* & *in vivo* analysis based on the pharmacological effect. *Bacopa monnieri* treating various disorder like- Alzheimer's, Schizophrenia, Parkinson's disease, Dementia and, skin, hair disease blood pressure so on. These are antidepressant, antianxiety, antioxidant, anti-inflammation, antimicrobial, antifungal properties. Compound have been isolated from *Bacopa monnieri Linn*. Bacoside A & Bacoside B other phytochemical such as alkaloids flavonoids, saponins etc.

Keywords: *Bacopa monnieri*, antidepressant, antianxiety, antioxidant, anti-inflammation, antimicrobial, antifungal properties, bacoside A & bacoside B, etc

Introduction
The plant *Bacopa monnieri L* (BRAHMI) is a perennial creeping aquatic herb with rooting at nodes, branching stems, leaves, flowers are present that has 220 genera and 4500 species (approx.). It has a bitter & sweet taste, and it is known as to import a cooling energy. It is non- aromatic herb. In 1996 a special extract of *Bacopa monnieri* was lunched by the Indian government's Central Drug Research Institute, Lucknow termed CDRI08. It is Ayurvedic traditional medicine plant it has been used by ayurveda in India for almost 3000 years to treat various ailment. Whole part of this plant is medicinally useful, it is enlisted as the most endangered plant due to its over exploitation. Herbal plant products are not only less expensive but also have fewer side effects than synthetic drugs (Saha, *et al.*, 2020; Mohan, *et al.*, 2011) [16, 10].

Distribution
B. monnieri is moist habitat and water edges throughout tropical and subtropical India it grow best near flowing water and wetland area it is commonly grows in marshy area India**,** Nepal, shri Lanka, China, Pakistan, Taiwan, Tropical and southern Africa, Madagascar in Australia, south America. The plant develops all over India particularly in Bengal, Uttarakhand, U.P, Kerala, Tamil Nādu, Karnataka etc (Praveen *et al*, 2016; Rai, *et al.*, 2017;) [14].

Table 1: Synonyms/ Common name/Local Names/ Taxonomical classification: (Rai, *et al.*, 2017) [14]

Synonyms	Common name/Local Names	Taxonomical classification	
Bacopa monniera Hayata & Matsum	Brahmi, water hyssop		
Bramia monnieri (L) Pennell	Neerbrahmi	Domain	Eukaryote
Gratiola monnieria L.	In Bengali- brahmisaka	Kingdom	Plantae
Herpestes monnieria (L)kunth	In Gujrati-baam, jalanevari, kadavi luni	Phylum	Spermatophyta
Herpestis fauriei H.Lev.	In Hindi- jalbuti, jalnim,safed chamani	Sub-phylum	Angiosperms
Herpestris monnieria	In Manipur- brahmi sak	Class	Dicotyledons
Lysimachia monnieria L.	In Oriya-prusni parnni	Order	Personals
Moniera cuneifolia Michx	In Sanskrit- tiktalonika	Family	Scrophulariaceae
Bramia indica	In Tamil- pirami, piramiyam,taray	Genus	*Bacopa*
Bacopa micromonnieria	In Telugu- sambrani aku	Species	*monnieri*

Genetics: The chromosome number reported for *B. monnieri* is 2n = 64 (Samaddar, *et al.*, 2012) [18].

Table 2: Biological activity of different plant extract of *Bacopa monneri*: (Rai, *et al*, 2017) [14]

Plant part	Extract	Activity observed
1. Whole plant	Methanolic extract	Arthritis, antimicrobial activity show on gram positive and gram-negative bacteria *Bacillus subtilis, Klebsiella, staphylococcus, pseudomonas*, anti-tumour, anti-inflammation
2. Leaves	Bacoside A & B	Neuroprotective, antioxidant, anti-epileptic, protective effect on gastric ulcers,
3. Leaves	Asiatic acid, glutathione	Treat Alzheimer's
4. leaves	Ethanolic acid	Anti- diabetic,

Reproduction

In India, plants of *B. monnieri* bear hundreds of small blue-white flowers throughout the year. The reproductive system was found to be self-compatible with entomophilous behaviour. Pollination with the honeybees *Apies Dorset* and *Apies florae* are regular visitors of the flowers of *B. monnieri* in India. The seeds of *B. monnieri* show a distinct dormancy period and sunlight appears to be an essential requirement for seed germination (Mathur and Kumar, 2001) [9].

***In vitro* propagation**

The multiplication of shoots for the clonal propagation of *B. monnieri* have been reported using pre-existing meristems such as nodes and shoot tips derived from both ex-vitro (45% reports) and *in vitro* grown plants (16% reports). Among the two types of explants used, nodes have been found to be the better choice of explants for *in vitro* shoot multiplication. The rate of propagation was found to depend on the type and concentration of cytokinin used in the culture medium. Although three different types of cytokinin such as BAP, Kn (Kinetin), and TDZ (Thidiazuron), have been used, maximum reports on *in vitro* propagation (57%) involved the use of BA alone or in combination with other hormones like auxin (NAA, IBA), whereas the effect of Kn alone or in combination with other PGR was investigated in only 9% of published reports. Apart from hormones, other additives such as algal extract, organic supplements etc. have been used in 12% of published reports to improve the rate of shoot induction in *B. monnieri* (Vijayakumar, *et al.*, 2010; Gaurav, *et al.*, 2018;) [24, 5].

In vitro phytochemical analysis and antimicrobial activity of crude extract of *Bacopa monneri* using methanol, ethanol, chloroform, and petroleum ether. The phytochemical screening was carried out to know the compounds responsible for these activities. Methanol, ethanol, and chloroform extract was tested against Bacillus amyl liquefactions, Streptococcus pyrogens, Vulgarise, Bacillus megaterium, Aspergillus Niger, Bacillus pugilist, Salmonella typhi, Bacillus subtilis, Micrococcus luteus. The susceptibility of the bacteria to the crude extracts based on zones of growth inhibition varied according to microorganism and extracting solvent. In most of the above-mentioned plants, the methanol extract produced the highest activity. Based on the results obtained, it could be concluded that methanol could be used for extracting antimicrobial compound, Nature has been a supply of medicinal agents since thousands of years and providing various drugs to the modern world. It is estimated that 80% of the global population plant derived medicines to address their health care needs the 250,000 to 500,000 known plant species, very few have been investigated for their pharmacological qualities, and compounds of significant medicinal value may still remain undiscovered in plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy while there are some advantages of using medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature. For these reasons, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multiple drug resistant microbial strains. Herbal medicine is still the main stay of about 75-80% of the whole population, and a major part of traditional therapy involves the use of plant extract and their active constituents. *Bacopa monnieri* (family: Scrophulariaceae) commonly known in both India and Bangladesh as 'Brahmi' is an ancient and renowned medicinal plant with legendary reputation as a memory vitalizer in the traditional system of medicine (Ayurveda) (Gaurav, *et al.*, 2018; Rawat, *et al.*, 2021; Thapa, *et al.*, 2022; Gaurav, *et al.*, 2016; Banerjee and Shrivastava, 2008; Bhardwaj, *et al.*, 2019; Sharma, *et al.*, 2016; Sanyal, *et al.*, 2022) [5, 15, 23, 6, 1, 2, 20, 19].

Pharmaceutical activities of *Bacopa monnieri* **(**Singh, 2012; Jain, *et al*., 2017**)** [21, 8]**:**

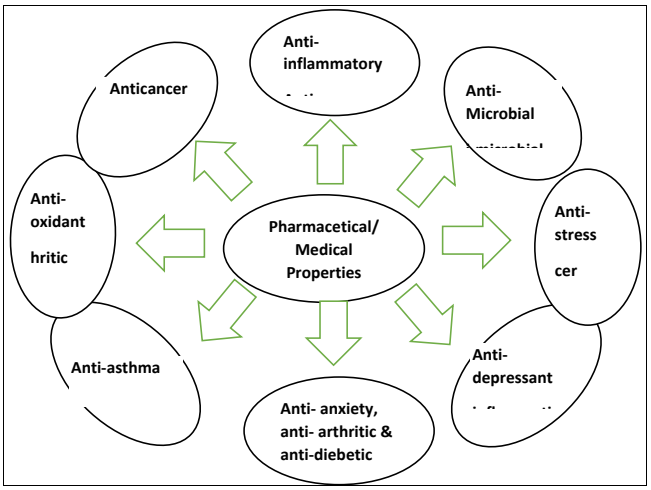


Fig 1: Pharmaceutical activities of *Bacopa monnieri*

Materials and methods

To perform different tests and different media has been prepared. The successful result of any testing depends upon the material and instrument which are used during the performance of the test.

Method of *In Vitro* Propagation (Saini, *et al.*, 2022**)** [17]

Standard tissue culture laboratory should provide facilities for

Refrigerator for keeping stocks solution and chemicals

Temperature controlling facilities

Culture vessels (conical flask petri dishes, beaker)

Take the flask discard by autoclave than wash with tween 80 and water, then place on hot plate and wash with distilled water, then place in oven for sterilization.

Media preparation

Medium used for tissue culture for *in vitro* growth and regeneration the standard MS medium (Murishige and Skoog 1962) [11] contain macronutrient salts, micronutrient salts, vitamins, Fe-EDTA, hormones, sucrose, and agar. The stock solution was kept in dark glass or bottles and store in refrigerator **(**Saini, *et al.*, 2022**)** [17].

Preparation of stock solution for MS medium

Macronutrient

Macronutrient are the component which the plant needs in major or high quantities. The growth and morphogenesis of

the plant tissue in culture is the composition of the culture medium. They provide the six major element nitrogen N, phosphorus P, potassium K, calcium Ca, magnesium Mg, and sulphur S for satisfactory growth and morphogenesis. Potassium is required for cell growth of most plant, sulphur is root nodule formation, phosphorus important has a key role in reactions in ATP in involve, magnesium is required as a chlorophyll molecule. Macromolecules stock for MS medium was prepared at the final concentration of 100x. all the salts were weight individually and dissolved separately in distilled water. Separately dissolved salts were mixed in a conical flask already containing an appropriate amount of distilled water so as to avoid precipitation (Pant, *et al.*, 2022; Binita, *et al*., 2005) [12, 3].

Micronutrient

Micronutrient are needed in only very small quantity the element is sometime called trace elements. The micronutrients are boron, copper, iron, chloride, manganese, nickel, sodium, molybdenum, and zinc. Iron is usually the most critical of all the micronutrient. The element is used as critical salts in culture media, boron involved in cell elongation and nucleic acid metabolism. micronutrient prepared at the 100x, all the salts were weight individually and dissolved separately in distilled water. Separately dissolved salts were mixed in a conical flask already containing an appropriate amount of distilled water so as to avoid precipitation (Pant, *et al.*, 2022; Binita, *et al*., 2005) [12, 3].

Vitamins

Some plants are able to synthesise the essential requirements of vitamins for their growth. Some vitamins are required for normal growth and development of plants plant catalysis in various metabolic process. They are act as limiting factors for cell growth and differentiation when plant cells and tissue are grown *in vitro*. Vitamin most used in the cell and tissue culture media include thiamine, nicotinic acid, and pyridoxine(B6), thiamine is required all cell for growth, vitamin of MS medium was prepared as 100x separately dissolved vitamin were transferred to a volumetric flask and final volume was made with distilled water (Pant, *et al.*, 2022; Binita, *et al*., 2005) [12, 3].

Growth hormone

They are not nutrient, but they influence growth and development. They play vital role in stem elongation, apical dormancy, they generally classified into the following group, auxin, cytokinin, gibberellins, and abscisic acid. Auxins to cytokinin determine the type and organogenesis in plant cell cultures, auxin have been used for cell division and root differentiation, the auxin commonly used in tissue culture are indole 3 acetic acid, IBA, NAA. Auxins are usually dissolved in either ethanol, or dilute NaOH, cytokinin are incorporated mainly for cell division and differentiation of adventitious shoots from callus and organ, commonly used cytokinin are BAP, Kinetin, Zeatin, gibberellin are less commonly used in plant tissue culture, they stimulate elongation of internodes, ethylene is an unusual it is produced by ageing. 0.01 gm BAP and 1ml HCL pour it into test tube than mixed with vortex mixer than add 9ml distilled water that so on. Were prepared separately as stock solution and were used according to the requirement of the medium (Pant, *et al.*, 2022; Binita, *et al*., 2005) [12, 3].

Amino acid

Amino acid as the organic source of reduced nitrogen the presence of inorganic nitrogen in the medium is generally sufficient to ensure protection against any possible nitrogen deficiency supplementation with amino acid may not be required. It required amino acid for optimal growth are usually synthesized by most plants. Amino acid used for enhancement of cell growth in culture media amino acid mixture such as casein hydrolysate, L -glutamine and adenine are frequently used as source of organic nitrogen in culture media (Pant, *et al.*, 2022; Binita, *et al*., 2005) [12, 3].

Carbon and energy source

In plant cell culture media, besides the sucrose, it provides energy, fructose considering that glucose is utilized by the cells in the beginning following by fructose. It was frequently demonstrated that autoclaved sucrose was better for growth than filter sterilized sucrose., most plant tissue culture are unable to photosynthesis because of the absence of chlorophyll it is add to the culture most commonly used carbon source is sucrose (Pant, *et al.*, 2022; Binita, *et al*., 2005) [12, 3].

Solidifying agent agar

Solidifying agent most common use gelling agent such as agar, agarose gel a polysaccharide obtain from seaweed is universal use as a gelling agent for preparing semi-solid plant tissue culture media. Agar has several advantages mixed with water, it is easily melted in temp, it gets do not react with constituent of media and not digested by plant enzymes (Pant, *et al.*, 2022; Binita, *et al*., 2005) [12, 3].

Table 3: Preparation of culture medium

S.NO	Stock solution	Concentration in ml/L or gm/L	
		1000ml	**500ml**
1	Major salt	1000ml was dissolved in 20 ml	500ml was dissolved in 10ml
2	Minor salt	1000ml was dissolved in 2ml	500ml was dissolved in 1ml
3	$CaCl_2.2H_2O$	1000 ml was dissolved in 10ml	500 ml was dissolved in 5ml
4	Vitamin	1000ml was dissolved in 10ml	500ml was dissolved in 5ml
5	Fe EDTA	1000ml was dissolved in 0.08gm	500ml was dissolved in 0.040gm
6	Sucrose	1000ml was dissolved in 30gm	500ml was dissolved in 15 gm added fresh
7	Hormone	BAP 200micro lit and kinetin 50micro lit	BAP 100 micro lit or 1.0mg and kinetin 50 micro lit
8	Agar	1000ml was dissolved in 8gm	500ml was dissolved in 4gm
9	pH	5.8	5.8

Total volume will be 500ml, take already sterilized conical flask washed by (DH_2O) distilled water, added 200ml distilled water in 500ml size conical flask with measuring cylinder. add 10ml major salt, then added 1ml minor salt, 5ml $CaCl_2$ solution added in flask, then add 5ml vitamins, 0.040 EDTA added, then added 15 gm sucrose, then place on hot plate for dissolved process, then added 100ml distilled water, added BAP and kinetin hormone 100micro lit and 50 micro lit, than check pH=5.8, than added 4gm agar, solution put on hot plate for dissolving, than added 200ml distilled water and place in autoclave with cotton plug for sterilization process

Plant marerial

1. Preparation and sterilization of explant

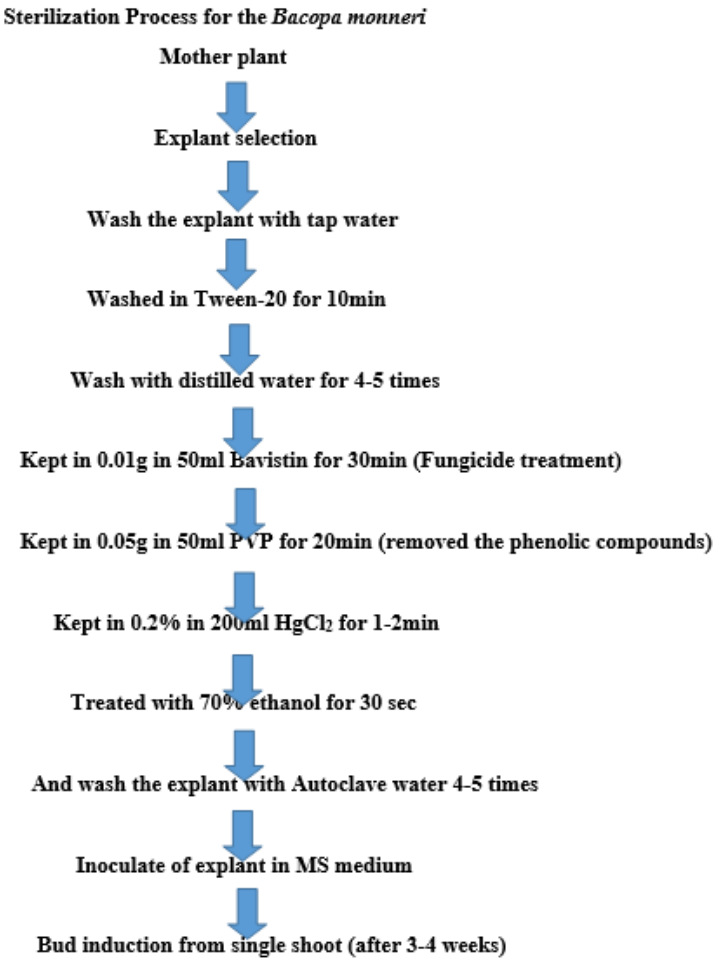
- First cutting of explant used of shoot culture
- Explant were cut into appropriate size and washed under running tap water 5-6min to remove the dust particles adhering to the surface
- Explant were washed with liquid detergent tween 20, 8-9 drops /50 ml by gentle agitation for 10 min
- Explant were washed under running tab water to removed detergent
- explant washed were treated with fungicide (Bavistin 0.01g in 50 ml) for 30 min
- than washed were (PVP 0.05g in 50 ml) for 20min.
- the explants so washed were surface sterilization under laminar air flow hood with ($HgCl_2$ 0.2% in 200 ml) for 1-2 min and treated with 70% ethanol for 30 sec.
- the explant was again washed with sterilized distilled water at least 4 to 5 times to remove the trace of the sterilant.
- prior to inoculation proximal and distal parts of nodal explant were trimmed slightly to removed dead tissue**.**

Inoculation process

- After surface sterilization each explant was inoculated to MS medium with different concentration of hormone
- Transfer of explants to culture tubes containing suitable medium were carried out inside laminar air flow chamber under aseptic condition.
- The chamber was earlier sterilization with absolute alcohol and UV radiation, the instrument used for inoculation were autoclave at 121c for 20 min and sterilized with 100%alcohol.
- Hands were also swapped with 100% alcohol before carrying out operation order to ensure aseptic condition.
- Care was taken not to dip explant completely in the medium and tips of forceps should not touch the agar medium.
- The culture tubes were sealed immediately**.**

Culture condition and sterilization

The culture is incubated in the growth chamber or tissue culture room. Maintain of constant environmental condition it was continuous exposure of explant to light for more than three days all culture were growth under 16 hr light and 8 hr dark period. Temp of the room was maintained between 23-24^{0}c with 60-70% relative humidity, and maintain hygienic condition. To reduce contamination the desirable condition



Leaf extract preparation by distilled water and methanol of antimicrobial, phytochemical analysis process

Preparation of leaf extracts

Fresh healthy leaves of *Bacopa monnieri* were collected from SGRR University Pathri Bagh campus, Dehradun, leaves of plant were washed under running tap water to removed dust. than wash with distilled water and dry at room temperature in shades for 15 days. The dry leaves are taken into fine powder using mixer grinder and weighting of plant powder than store in airtight bottles. About 5gm of dry leaves powder of *Bacopa monnieri* was taken separately in flask and 50 ml distilled water and 50ml methanol were added and soaked separately. Both flask ware labelled as distilled water(W) and methanol. Then these mixtures were properly dissolved and shaking in rotatory shakers for 2day. kept undisturbed under dark place. Again, the solution was properly mixed, extract was passed through Whitman filter paper after filtration sample were collected and evaporated using water bath at 40-45 ^{0}c. when solvent will evaporate at last when extract will remain in beaker it will be store in airtight well or bottle with the help of spatula. sample were store in refrigerator at 4^{0}c.

Anti-microbial analysis

Chemical used for analysis of antimicrobial activity- Nutrient agar media, Nutrient broth,

1. Preparation of Nutrient agar media (NAM)

After mixing the chemical show in the table below, the nutrient broth was autoclaved at 15 lbs.

Table 4: Component of nutrient agar media (NAM)

Composition	Amount for 1000ml	Amount of 100ml
Beef extract / yeast extract	3gm	0.3gm
Peptone	5gm	0.5gm
NaCl	5gm	0.5gm
Agar powder	15gm	1.5gm
Distilled water	1000ml	100ml
pH	7.0	7.0

1. Preparation of medium nutrient broth

After mixing the chemical shown in the table below, the nutrient broth was autoclave at 15lbs and 121-degree c for 30min.

Table 5: Component of medium nutrient broth

Composition	Amount of 1000ml	Amount of 100ml
Beef extract	3gm	0.3gm
Peptone	5gm	0.5gm
NaCl	5gm	0.5gm
Distilled water	1000ml	100ml
pH	7.0	7.0

2. Preparation of bacterial inoculum

Prepared nutrient broth is transferred into sterile test tubes, each test tube containing 10ml of broth. All work is done in laminar air flow for maintaining sterilized condition. Different bacterial culture like (Pseudomonas and E. coli) was taken from the already fresh bacterial culture plates by using sterilized inoculation loop and inoculation into the separate test tubes. Inoculation test tube were inoculation at 37^{0}c in B.O.D incubator for 24hr.

3. Preparation of disc

Disc are prepared from the Whatman filter paper which is prepared by the help of punching machine and autoclave for sterilization.

4. Preparation of different concentration of extracted sample

10micro lit extract + 90 micro lit distilled water =100 micro lit

20micro lit extract +80 micro lit distilled water =100 micro lit

30micro lit extract +70micro lit distilled water = 100 micro lit

5. Preparation of control

Weight 2mg streptomycin and dissolved in 2ml of distilled water.

Weight 2mg tetracycline and dissolved in 2ml of distilled water.

6. Procedure for performing the disc diffusion test

- In laminar air flow UV is on for 10 min afterward we can take arranged media and the UV light was switched off at 15 min before work inside the cabinet.
- Take sterilized Petri plate and marking than NAM poured in Petri plate.
- Subsequent to pouring media is permitted to cool and set.
- Take 100ml bacteria culture stock in pipette and poured it on hardened NAM and is spread by the sterilized spreader.
- Whatman disc were prepared than disc deep in one control and three different concentrations of sample were loaded on the Petri plate.
- Plates were than incubated 37^{0}c for overnight
- The zone of inhibition was observed and recorded against control.

7. Procedure for performing the agar well diffusion test

- First made the nutrient agar plate
- Approx. 100ml of inoculums was poured on the plates by micropipette.
- Culture was spared by a sterilized glass spreader.
- 4well in each plate were prepared by using the tip of micropipette.
- A control and 3 different concentrations of sample were loaded in the wells.
- Plates were then incubated 37^{0}c for overnight.
- The zone of inhibition was observed and recorded against control.

Phytochemical analysis (Tanveer, *et al*., 2010) [22]

The presence of phytochemical in Bacopa monnieri leaf removes utilizing various solvent were resolved subjectively. Standard method was adhered to decide the presence of Alkaloids, carbohydrate, saponins, steroids, glycosides, tannins, terpenoids, flavonoids, phenols, amino acid**.**

Alkaloids (Mayer test)

1ml of extract was taken and placed into a test tube. Then 1ml potassium mercuric iodide solution was added and shaken. Emergence of whitish or cream precipitate implies the presence of alkaloids.

Carbohydrate (Fehling's test)
1ml of extract was taken and placed into a test tube. 1ml of Fehling's A and 1ml of Fehling's B solution were added in a test tube and heated in the water bath for 10min. formation of red precipitated colour the presence of carbohydrate.

Saponins
About 1ml of extract was boiled in 1ml of distilled water in a water bath and filtered. The filtrate was mixed with distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drop of olive oil and shaken vigorously, then observed for the formation of emulsion.

Steroids
1ml of extract was treated with chloroform, and then to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red colour indicates the presence of steroids.

Glycosides
Glycosides are compounds which upon hydrolysis give rise to one or more sugar and a compound which is not a sugar, the extract was hydrolysed with HCL solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitated indicates the presence of glycosides.

Tannins
2ml of extract was taken in a test tube and heated on water bath, chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Terpenoids (Salkowski's test)
Small quantity of the extract, 2ml of chloroform was added, 3ml of concentrated sulphuric acid was carefully added along the side of the test tube to form a layer. A reddish-brown colour present indicates the presence of terpenoids.

Flavonoids
Extract of about 500ml was dissolved NaOH and HCL was added. A yellow solution that turns colourless indicates the presence of flavonoids

Phenols
2-3 ml of extract few drop of dilute iodine solution were added. Observed of transient red colour indicates presence of phenolic compounds.

Amino acid
1ml of the extract was treated with few drops of ninhydrin reagent. Appearance of purple colour shows the presence of amino acid.

Result and discussion
The explants (apical meristem & nodal section) of *Bacopa monnieri* were cultured on MS basal medium supplemented with different concentrations of BAP (0.5-3.0 mg/L), Kinetin (1.0 mg/L). The length was measured on a weekly basis for the first 1–2 weeks to see how they developed. After 5-6 days of inoculation, shoots began to appear, and the greatest results (optimum conc. for maximum shoot formation in the shortest period) were obtained with the BAP conc. 1.5 mg/L. Other concentrations (1.0 mg/L Kinetin) exhibited initiation after some time, however the responses were less effective. Apical meristem & nodal section of *Bacopa monnieri* were then inoculated on MS basal media supplemented with BAP (1.5 mg/L)

Table 1a: Effect of various hormone concentrations on shoot regeneration

S.NO	Medium + Growth hormones mg/l	%age of shoot induction	No. of shoots per culture	Average shoot length in cm.
1	MS+0.5 BAP	71%	1-2	1-2
2	MS+1.0 BAP	77%	1-4	2
3	MS+1.5 BAP	79%	1-5	2.6
4	MS+2.0 BAP	59%	1-2	1-2
5	MS+0.5 KN	30%	1	1-2
6	MS+1.0 KN	55%	1-2	1-2
7	MS+3.0 BAP+1.0 KN	35%	1	1
8	MS+1.5 BAP+1.5 NAA	85%	1-4	2-3

Subculture
After 15 days, the healthy shoot buds were removed from the initiation media and transferred to MS multiplication medium (MS1, MS2, MS3, MS4) supplemented with different hormone concentrations (MS1 (1.0 mg/L BAP), MS2 (2.0 mg/L BAP), MS3 (3.0 mg/L BAP + 1.0 mg Kinetin), MS4 (1.5 mg/L BAP + 1.5 mg/L NAA). The MS3 media had the most shoot regeneration.

Table 1b: Effect of growth regulators on shoot induction in *Bacopa monnieri*.1. MS1 (1.0 mg/L BAP); 2. MS2 (2.0 mg/L BAP); 3. MS3 (3.0 mg/L BAP+1.0 mg/L Kinetin); 4. MS4 (1.5 mg/LBAP+1.5 mg/L NAA)

Multiplication Media	No. of explants Inoculated	No. of shoots proliferation / explants	No. of contamination	Survival rate
MS1 media	10	4	-	40%
MS2 media	10	4	2	40%
MS3 media	10	6	-	60%
MS4 media	10	5	1	50%

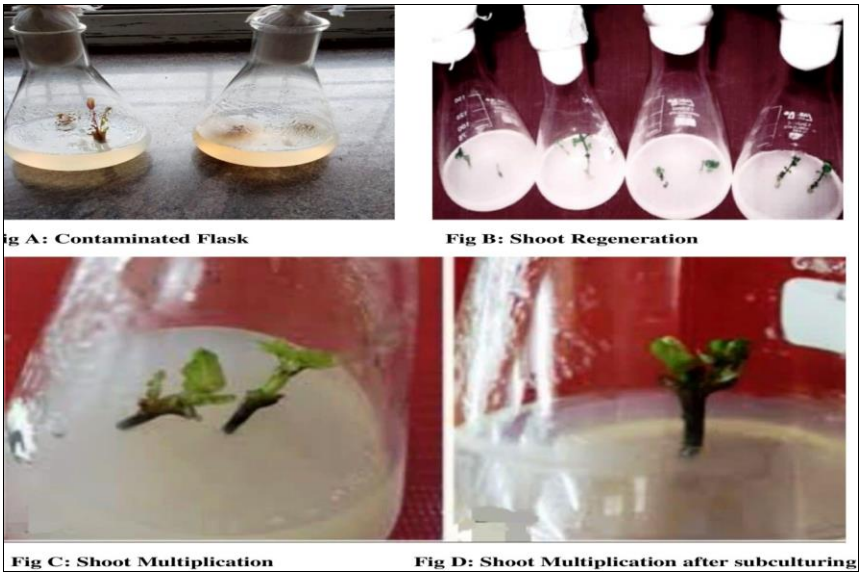


Fig 2: *In vitro* shoot regeneration

Phytochemical analysis result

Phytochemical analysis of extract of *Bacopa monneri* showed the presence of most constituent. The phytochemical analysis of all the two solvent extract of leaves of *Bacopa monnieri* the methanol and distilled water extract showed the presence of saponins, steroids tannins etc. The medical value of the plant can be correlated due to the presence of various bioactive constituents revealed the presence of alkaloids, tannins, carbohydrates, phenols etc. All the results can be understood on the basis of following symbols which are used in the table (-), indicates negative results (+), indicates the positive results

Table 2: Phytochemical analysis result

S.NO	Phytochemical test	Leaf extract prepared from solvents	
		Methanol	Distilled water
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Saponins	+	+
4	Steroids	+	+
5	Glycosides	+	+
6	Tannins	+	+
7	Terpenoids	+	-
8	Flavonoids	+	+
9	Phenols	+	+
10	Amino acid	_	+

Antimicrobial activity results

Antimicrobial activity test given against the following bacteria- *E. coli*, *Pseudomona*, bacterial growth indicate antimicrobial activity against

Methanol extract

The following results are obtained by disc diffusion method after screening of methanol extract.

Table 3: Zone of inhibition when sample methanol extract.

Test organism	Zone of inhibition (mm)				
	Control		Sample		
			Disc 1	Disc 2	Disc3
E. coli	Tetracycline	2.9	1	1.6	2
Pseudomonas	Streptomycin	3	1.9	2	2.3

Distilled water extract

The following result are obtained by disc diffusion method after screening of distilled water extract.

Table 4: Zone of Inhibition when sample distilled water extract.

Test Organism	Zone of Inhibition (mm)				
	Control		Sample		
			Disc 1	Disc 2	Disc3
E. coli	Tetracycline	2.9	1.3	1.8	2
Pseudomonas	Streptomycin	2	1.5	1.9	1.6

Conclusion

B. monnieri showed various potential action the present review around the recently studied data in regard to the therapeutic profile and phytochemical constituent of the *B. z*, anti-cancer, microbial infection, skin and hair disease an improve immune. Biomedical research on *B. monnieri* is still at a roadblock novel compound with their encoding properties, active targets, biological processes, and interactions have opened the research floodgates with the integration of Ayurveda to the modern medicine era. This study also hypothesizes that Bacopa compounds and their combination with other substances as is recommended by the Ayurvedic and modern medicine system may result in synergistic effects and need to be studied further. The ethical implications of drugs which enhance cognition are vital but should be appropriately mitigated with social and ethical considerations as field researchers enter the brave, advancing world of neural enhancement. The explant best shoot induction was occurred in shoot tip and responded in almost, *in-vitro* regeneration from shoot tip is another alternative step for clonal propagation. Hence in the presence study an approach has been made to standardize an efficient protocol for *in- vitro* propagation of highly commercial, *B. monneri* using shoot explants.

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