***Bacopa monnieri* (Linn.) Pennell - A Possible Plant for Impossible Diseases (A Review)**

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Abstract Since the beginning of humankind, people are using numerous plants to cure diseases and abnormalities. *Bacopa monnieri* has been used by people for a long time. This plant has several medicinal and biochemical properties which would be beneficial to treat diseases. The current review article summarizes the medicinal, presence of the bioactive compound, mechanism of action, therapeutic importance, clinical trials, ethnobotanical usage, and distribution of the plant with respect to India and worldwide. Relevant information and literature on *Bacopa monnieri* were extracted from different sources and their finding were discussed in the manuscript. The number of therapeutic properties of the plant has been seen in the animal model experiment including anti-diarrheal, antioxidant, anti-inflammatory, anti-cancer, anti-fungal, anti-bacterial, anti-convulsant, hepatoprotective, antiulcer, anti-depressant, anti-hyperglycemic and anti-nociceptive activity, immunostimulatory activity, and wound healing activity. This article recapitulates ethnopharmacological uses of *Bacopa monnieri* to explore its therapeutic potentials thereby providing a basis for future research. *Bacopa monnieri* exhibits a comprehensive of pharmacological activities that could be observed by the presence of a vast range of chemical constituents. Manuscript also comprises tissue culture techniques and propagation of *Bacopa monnieri*. The study also provides a descriptive outlook on the uses of *Bacopa monnieri* in wastewater treatment. This review article highlights the endless possibilities present in the plant and also sheds light on research that has not yet been done on this plant and may prove useful if done in the future.

Keywords *Bacopa monnieri*, Medicinal Properties, Future Aspects, Bioactive Compounds, Ethnobotany

1. Introduction

Since the age of human civilization, we are using plants for treating different ailments. Ayurvedic medicine system had been established and practiced in India before 500 BC according to Subhose et al., [1]. From ancient times to the modern age practicing herbal medicine is going on a large scale and is famous throughout the world since it has an almost negative impact on patients and low costs. According to a report by WHO, 80% of the world population depend on the herbal and traditional drugs for primary health care as already discussed by Kumar et al., [2]. *Bacopa monnieri* is also a medicinal plant that has been used for many purposes in the Ayurvedic medicine system. The present study summarizes all aspects of this plant.

Morphology of the plant:

Bacopa monnieri relates to the family of Plantaginaceae. It is a short, succulent, and perennial herb that normally grows on the bank of the river. The leaves are elongated, and 4–6 mm (0.16–0.24 in) thick, oblanceolate. An opposite leaf arrangement exists. Leaves are sessile, petiole less, dorsoventrally flattened alternate and spirally arranged. Leaf lamina is the entire type with an obtuse apex. The blossoms are little, actinomorphic, pedicellate, solitary,

axillary, and white, with five petals. Sepals are five and unequal in size. Four stamens are presented in didynamous within the corolla tube. Length of leaves 4-5mm and its width is about 0.6 to 0.9. Similarly, the length of the petals is around 0.8mm and the length of the pedicle is about 10mm recorded by Vasu [3]. It prefers slightly marshy and wet conditions for cultivation. Stem propagation can be done easily. Each node arises a puff of roots. It starts blooming in the month of March and fruiting would be occurring in the month of May to June. Fig. 1 shows the morphological view of *Bacopa monnieri*.

Scientific classification

Kingdom: Plantae
 Clade: Tracheophytes
 Clade: Angiosperms
 Clade: Eudicots
 Clade: Asterids
 Order: Lamiales
 Family: Plantaginaceae
 Genus: *Bacopa*
 Species: *B. monnieri*

Figure 1. *B. monnieri*

Anatomy of the plant

Gubbannavar et al., [4] studied the anatomy of the plant. The transverse section of the stem revealed the outer epidermis followed by the cortex and endodermis. Two to three layers of hypodermis are made up of parenchymatous cells having chlorophyll and tannin-containing cell. Cortical cells modify and form aerenchyma which covers a two-thirds portion of the section. Tannin-containing cells are present at the connection side between two adjacent cells giving it a Y-like appearance. The endodermis is single-layered. The vascular system is radial. Xylem is present in numbers. Metaxylem is situated toward the pericycle and the protoxylem is situated toward the pith. Centrally located large pith is present.

T.S. section of leaf show outer layer epidermis. Lamina is differentiated into spongy and palisade. Three to five layers of palisade are present below the epidermis. Palisade and spongy cells are made up of chlorenchymatous tissue. The midrib portion consists of a vascular bundle. Crystal of

calcium oxalate found in midrib and lamina. Stomata are diacytic and anisocytic types. Chromosome number of plant is recorded $2n=64$ by Raghavan [5] in 1959.

Distribution in India and Worldwide:

This herb is distributed in the tropical and sub-tropical zone of India. It is native to the Himalayan zone such as Uttarakhand and Northeast. It also grows enormously in Chitrakoot Satna, Madhya Pradesh. It is distributed throughout the Indian subcontinent including Nepal, Bangladesh, and Pakistan. The presence of this herb is also reported in various other continents including Africa, America, and Australia.

Description of *Bacopa monnieri* in Ayurveda

In Ayurveda, this plant is known by the name of “Bramhi” which means knowledge deriving from gods. It is the most important and useful plant in the Indian system of traditional medicine used as a memory enhancer and sharpener. *Bacopa* has been used in various ailments including jaundice, diabetes, cough, leprosy, swelling, diabetes. It is also beneficial for treating skin and blood disorders. From ancient times this plant is used as a memory tonic and booster. The medicinal property of *Bacopa monnieri* has been mentioned in two famous books of Ayurveda i.e. Charak Samhita (2500 B.C) and Sushruta Samhita (2300 B.C) where it has mentioned that Brahmi removes toxic substances from the body along with giving intelligence, anti-inflammatory, strong memory, cooling. Apart from removing phlegm, it is also beneficial in skin-related diseases by purifying the blood P.V., [6]; Rai et al., [7]. Charak Samhita which is an important book on the Indian medicine system has mentioned the medicinal usage of this plant. This plant is used to cure throat infection, constipation, and blood filtering. It has been recommended for treatment of anxiety, lack of concentration, and poor cognition Russo et al, [8].

Memory Enhancer Activity

An animal trial has been performed by Joshi et al., [9] to assess the nootropic activity of *Bacopa monnieri*. Scopolamine (0.4 mg) has been administered to induce amnesia in both young and aged mice. *Bacopa*Rasayana (An ayurvedic formulation of *Bacopa monnieri*) at doses of 100mg and 200mg/kg of body weight administered for eight days. Elevated plus maze and passive avoidance paradigm were applied to evaluate the learning and memory enhancement in mice. Piracetum 200mg per kg of body weight is used as a standard drug. It has been noted BR enhanced memory and learning skills by decreasing acetylcholinesterase activity in the whole brain.

Anti- Inflammatory Activity:

Channa et.al, [10] showed anti-inflammatory activity

action of *Bacopa monnieri* in carrageenan-induced paw edema in mice and rats. In this experiment, researchers used ethanol extract of *Bacopa monnieri* for treating inflammation. Inflammation action is induced by injecting carrageenan (1%) into the right-hand paw. After thirty minutes animals received an ethanolic extract of BM at different concentrations. Anti-inflammatory activity was seen in Aspirin or 10% DMSO. As result, it has been noticed by the author that BM extract cause significant decline (33-95%) of paw edema at a dose of 50mg/kg and 100mg/kg that was more efficient than that caused by aspirin (28-60%) *Bacopa* show anti-inflammatory activity by inhibiting prostaglandin (58-100%).

It has been seen that when N9 microglial cell lines were treated with extract of *B. monnieri*, it prevented the secretion of pro-inflammatory cytokines (Tumor Necrosis factor- α and (Interleukin-6) IL-6 which are a major cause of inflammation in neuron cells, and ultimately it led to neuronal cell death. Tea fusion extract, alkaloid extract of *Bacopa*, and infusion extract were examined against the N9 microglial cell line in order to determine anti-inflammatory activity if they inhibited the release of the pro-inflammatory cytokines. Result reveals that tea, infusion, bacoside A and alkaloid extract significantly inhibited the release of inflammatory cytokines. The extract also inhibited the secretion of caspase-1 and matrix metalloproteinase-3 (enzymes responsible for inflammation) and caspase-3 in the cell-free assay and cleave the Tau protein major cause of Alzheimer's disease (Nemetchek et al. [11]. The study proved that *Bacopa monnieri* can control inflammation in the CNS.

Bacopa monnieri showed anti-inflammatory action in mice and prevents colchicine-induced dementia as demonstrated by Saini et al., [12]. They induced dementia by a single intracerebroventricular injection of colchicine (15 μ g/5 μ l). A group of colchicine-treated animals was given an extract of *Bacopa monnieri* orally at the dose of 50mg/kg body weight for the next 15 days on daily basis. Colchicine-treated animals with extract of BM showed a significant reduction in escape latency in comparison to only colchicine-treated animals and in the retrieval test, it has been found that BM administered colchicine-treated animals effectively restore memory and rather than control.

Anti-Oxidant Activity:

Kapoor et al., [13] demonstrated antioxidant activity of *Bacopa monnieri* extract in streptozotocin-induced diabetic Wistar albino male rats. In hyperglycemia conditions, free radicals are generated which leads to peroxidative damage to the tissues. This will increase the level of MDA (Malondialdehyde) a well-known compound of lipid peroxidation. The level of MDA has used an Index of oxidative injury which was performed by oxygen free radicals. In this study, the level of MDA has been checked in different parts of the body including the Kidney,

Cerebellum, Cerebrum, and Midbrain. After treating with *Bacopa* extract MDA level in the Midbrain region was significantly reduced from 2.96 ± 0.19 to 1.18 at 125mg/kg body weight. In kidney region extract at a dose of 125mg/kg performed maximum inhibition in MDA level has been seen. It reduced from 8.78 ± 0.54 to 6.20 ± 0.05 . The same thing occurs in Cerebrum and Cerebellum region where MDA concentration has been reduced from 2.30 ± 0.15 , 2.73 ± 0.24 to 1.45 ± 0.02 and 1.82 ± 0.03 at different doses respectively 125mg/kg body weight and 50mg/kg body weight which was better than the standard drug Glibenclamide.

Mathur et al., [14] evaluated the anti-oxidant activity of *Bacopa* extract through DPPH radical scavenging method and found methanolic and aqueous extract show maximum anti-oxidant activity with IC₅₀ value of 46.00 μ g/ml and 43.10 μ g/ml respectively.

Ramachandran et al., [15] demonstrated that an equal combination of extract of *Bacopa monnieri* and *Rosmarinus officinalis* has superior antioxidant potential and anti-lipid peroxidation in human glial (U-87MG) and embryonic mouse hypothalamus cells than either one of them has been used. This combination showed almost similar inhibition of phosphor tau expression as any one of the extracts does alone. This combination of the extract showed a better inhibitory effect on amyloid precursor protein synthesis and higher brain-derived neurotrophic factor production in the hypothalamus than any single extract.

Ramdas et al., [16] performed a DPPH assay to evaluate the antioxidant activity of the plant. The researcher prepared various concentrations (1-100 μ g) of plant leaf protein, mixed it with 1ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2ml of 0.1M acetate buffer which has pH 5.5. BHA and Ascorbic acid were used as control. *Bacopa monnieri* shows the highest scavenging activity of DPPH radical at a dosage of 10 μ g. The author also showed scavenging nitric oxide free radical which is also dose-dependent manner at a dose of 1 to 10 μ g. *Bacopa* leaves protein at concentrated 10 μ g showed similar scavenging property against the ascorbic acid.

Neurodegenerative diseases are the result of harmful action of oxidative stress which causes inflammation and injury in a different region of brains and the main reason of oxidative stress is the production of reactive oxygen species. H₂O₂ is the precursor of deleterious oxygen species which causes serious brain injury by producing ROS. Bhatia et al., [17] addressed the problem and performed an experiment, and examine the neuroprotective role of *Bacopa monnieri* extract (BME) against H₂O₂ persuade oxidative stress using a cellular model neuroblastoma IMR32 cell lines. Protective nature of methanolic ethanolic and water extract examined through MTT assay. Although all extracts had neuroprotective potential but methanolic extract had higher protection than other solvent extracts. UPLC study revealed that the methanolic fraction of *Bacopa monnieri* contains various

polyphenols including quercetin, catechin, umbelliferace and caffeic acid. The levels of oxidative stress markers such as NF200, HSP70, and Mortalin were decreased at the same time. Finding suggests methanolic extract of *Bacopa monnieri* as an impactful drug against neuropathological disorders.

Anti-Cancer Activity:

Cancer is a very fatal disease worldwide impact human health adversely and contributes 10 million deaths in 2020 according to a report published by Ferlay J et al., [18]. Various anti-cancer agents including Cucurbitacins, Bacopaside I, Stigmasterol reported and evaluated in recent studies. Ghosh et al., [19] conducted a study to examine the Anti-tumor property of stigmasterol isolated from *Bacopa monnieri*. Stigmasterol at different doses 5mg/kg of body weight and 10mg/kg of body weight were given to Ehrlich Ascites Carcinoma mice against the standard drug S- fluorouracil at 25mg/kg of body weight. Finding suggests stigmasterol as a potent anti-cancer agent. It not only decreases tumor volume but also diminished packed cell volume, viable cell count. After treatment with *Bacopa* extract life span of EAC tumor-bearing mice is increased. It is believed that stigmasterol control anti-tumor activity of cell by activation of protein phosphatase 2A by ceramide which regulate apoptosis.

Mallick et al., [20] reported that *Bacopa* extract possesses an anticarcinogenic property by conducting an experiment over two different cell lines i.e. MCF-7 and MDA-MB231. He found that the DCM fraction of ethanolic extract shows maximum cytotoxic activity up to 72 hours. The IC₅₀ value DCM extract is 72µg/ml for MCF-7 and 75.0µg/ml for MDA-MB-231 cell lines. DCM fraction of extract show anti-cancer and anti-proliferative activity due to the presence of cucurbitacins and betulinic acid which arrest cell cycle at the G2/M phase

Mallick et al., [21] conducted a study to evaluate the anti-cancer properties of *Bacopa* extract by implementing in vitro and in vivo approaches. For conducting in vitro study different cancer cell lines from the colon (HT29, Coo320, Caco2) Lung (A549), cervix (HeLa SiHa), Breast (MCF-7, MDAMP-231) were selected. For conduction in vivo study, Ehrlich ascites carcinoma tumor having mice have been used. As a result, the dichloromethane fraction of extract showed a great response in both studies. In vivo studies, oral administration of 40mg/kg of body weight rendered prominent reduction of tumor regression parameters such as tumor weight, packed cell volume tumor, volume, and viable tumor count as compared to the untreated mice of the EAC control group. In the case of in vitro study best cytotoxic activity has been recorded with DCM extract (41 to 60 µg/ml at 72 hours).

Smith et al., [22] found in their study that extract of *Bacopa monnieri* (*Bacopa* side II) discourage the growth of colon cancer cell in vitro by persuading cell cycle arrest and apoptosis. It has been observed that *Bacopa* side II

blocked aquaporin I water channel expression in HT-29, SW480, SW620, HCT116 colon cancer cells. Inhibition of HT-29 at 20µM was predominantly mediated by G0/G1 cell cycle arrest and at 30 µM by G2/M arrest and apoptosis. Inhibition of SW480, SW620, and HCT116 at ≥15 µM was mediated by G2/M arrest and apoptosis.

Anti-convulsant Activity:

Brahmighrita (BG) and Saraswatarishtha (SW) two ayurvedic formulations contain *Bacopa monnieri* as the main ingredient was checked for anticonvulsant activity in Wister albino rats. In this experiment, animals were divided into four groups. The first group serves as blank received only water and feed ad libitum. Group 2nd received phenytoin as standard drug and group 3rd and fourth received Brahmi Ghrita (BG) (0.9ml/kg) and Saraswatarishtha (SW) (0.9ml/kg) orally for eight days in the morning time. Animals received convulsion after 1 hour of drug administration. Convulsion induced by Electro convulsometer. Maximal electroshock seizure was elicited by the application of electric shock (60 Hz AC, 150mA) for 0.2 seconds using the corneal electrode. Symptoms of epileptic attacks like jerking grooming, tail Straub, an extension of the hind limb, and recovery were observed compared with the control and standard group. The result showed Brahmighrita produced a more significant impact in the phase of extension (0.622±0.23 S) and recovery (2.221±0.04) compared to control whereas in jerking and tail straub significant reduction recorded in SG group by Giramkar et al., [23] at the end of the experiment.

Antifungal Activity:

Antifungal activity of plant extract was checked against Fusarium oxysporum, Alternaria species, Rhizocotonia solani, and Sclerotium rolfsii by Jain et al., [24] Different concentrations of plant extract were subjected to antifungal activity. Plant extract at various concentration i.e. 12.5µg/ml, 25µg/ml, 37.5µg/ml 50µg/ml 62.5µg/ml were added to the PDA media after autoclaving. It has been seen that plant extract at doses of 50µg/ml and 62.5µg/ml showed 100% inhibition against Fusarium oxysporum, Sclerotium rolfsii, Alternaria species, and Rhizootonia solani. IC₅₀ value of the plant was recorded as 31.25 g/ml against Fusarium oxysprum. Sclerotium rolfsii showed better antifungal activity exhibiting 90%inhibition at 25µg/ml. IC₅₀ value has been recorded for Sclerotium rolfsii is 6.25µg/ml whereas IC₅₀ for Alternaria was recorded 28.75 g/ml and value of IC₅₀ was recorded as 18.75 g/ml.

Antibacterial Activity:

Antibacterial activity has been evaluated in two parameters. MIC (Minimum inhibitory concentration) and disk diffusion, both methods were used to assess antibacterial properties against 9 different strains of

bacteria which included gram-negative and gram-positive both by Ghosh et al., [25]. Minimum inhibitory concentration (MIC) was evaluated by broth dilution method by dissolving plant extract in DMSO as described previously. Different concentrations of plant extract in DMSO ranging from 25µg/ml to 500µg/ml have been prepared. The Agar disc diffusion method has been used for measuring the zone of inhibition. Different concentrations of 2, 5, 10 mg/ml of extract in DMSO prepared and the disc has dipped in mentioned concentration. Ciprofloxacin (5 µg/ml) has been used as a reference control for antibacterial activity. *Bacopa* extract at 10mg/ml formed maximum zone of inhibition against various bacterial species including *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus polymyxa*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholera*, *Shigella dysenteriae*, *Escherichia coli* *NCTC*.

Antiulcer Activity:

Swiss albino mice of male sex have been used by Karim et al., [26] for evaluating the anti-ulcer property of ethanolic extract of *Bacopa monnieri*. The animals were divided into nine groups each having 6 mice. Group 1st served as normal control only fed 0.5ml/100gm 0.2tween 80. Group 2nd was given the same diet but treated to absolute alcohol (0.5ml/100gm of body weight) Group 3rd was administered standard drug omeprazole (20mg/kg body weight) than alcohol 0.5ml/100gm. Group 4th and Group 5th received aqueous extract of *Bacopa monnieri* at respective doses of 200mg/kg body weight and 400mg/kg body weight. Group 6th and 7th were administered ethanolic extract of plant respective doses of 200mg/kg body weight and 400mg/kg body weight. Group 8th and 9th received carbon tetrachloride extract respective doses of 200mg/kg body weight and 400mg/kg body weight. All the extracts were given with 0.2% tween 80. After one hour of treatment of extract, animals were administered with absolute alcohol to cause a gastric ulcers. Animals were sacrificed after one hour of alcohol treatment. Ulcer index was calculated according to the formula described by Brzozowski et al., [27]. The result show in group 2nd where no extract or drug had been used, ulcer index recorded highest 14.5 ± 0.35 which was significantly reduced by ethanolic extract of *Bacopa monnieri*. At doses of 200mg/kg body weight ulcer index was recorded 3.13 ± 0.38 and protection was recorded at 78%. And in 400mg/kg doses of ethanolic extract of *Bacopa monnieri* maximum protection was recorded (82%) from ulcer score and ulcer index was reduced at 2.75 ± 0.32 .

Antidepressant Activity

Depression is a common mental illness in the world. According to a report of Lancet [28] around 32.2 crore people are suffering from this mental disease. Depression is also the main reason for committing suicide. Lots of

diseases have oriented from this mental disorder including high blood pressure, heart attack, paralytic strokes, and diabetes so it is necessary to evaluate potent antidepressant agents from natural resources. Depression is the second-largest psychiatric problem which affects around 21% population of the world. Almost all people suffer at their different stages of life so it is necessary for us to search for potent anti-depressant agents from natural sources including plants for the betterment of mankind.

A number of studies have been performed that prove *Bacopa monnieri* possess anti-depressant property. The study suggests it is just because of the presence of bacoside I in plant extract of *Bacopa monnieri* Sairam et al., [29]. Anti-depressant activity of *Bacopa monnieri* has been evaluated in Charles-foster (CF) albino rats using behavioral despair test and learned helplessness test. The study reveals that methanolic extract of *Bacopa monnieri* at doses of 20mg/kg body weight and 40mg/kg body weight shows a significant decrease in the period of immobility induced by behavioral despair due to restrictive swimming by the rats. At the same doses, animals show good responses in learned helplessness tests by reducing escape failure. *Bacopa monnieri* show almost equal response standard drug imipramine 15mg/kg of body weight. Antidepressant activity of this plant has been performed in albino mice. *Bacopa monnieri* produced a significant reduction in the duration of immobility at 80 mg/kg dose.

In another study where the same result was obtained conducted Chandrashekar [30] evaluated the anti-depressant activity of *Bacopa monnieri* reported in an animal model of Forced swimming test (FST) and tail suspension test (TST). The researcher reported a significant reduction in the duration of immobility at 80mg/kg of body weight. It is believed that *Bacopa* mediated interaction with serotonergic and noradrenergic nervous system

Anti-Alzheimer Activity of Plant

An animal trial has been performed by Uabundit et al., [31] to see the impact of *Bacopa monnieri* extract on ethylcholine aziridinium induced Alzheimeric male Wister rats. Rats were tested for spatial memory using the Morris water maze test. Histological study was also performed to check the density of neurons and cholinergic neurons. Result reveals that BME improved escape latency time in Morris water maze test. The study suggests *Bacopa monnieri* is an impactful drug against Alzheimer disease.

Anti -Alzheimer's activity of *Bacopa monnieri* in Human Clinical Trail

Calabrese et al., [32] performed a study to evaluate the impact of *Bacopa monnieri* on cognitive performance anxiety and depression during elderly age by using a randomized double-blind placebo-controlled trial. 54

volunteers having a mean age of 73.5 participated in this study out of which 48 remains at the end of the study. These 48 volunteers are divided into two equal groups. One group has received a placebo while the second group received a standard extract of *Bacopa monnieri* at the dose of 300mg/day received till 12 weeks. Different cognitive tests including auditory verbal learning test (AVLT), Stroop Task assessing the ability to ignore irrelevant information and the Divided Attention Task (DAT), and the Wechsler Adult Intelligence Scale (WAIS) letter-digit test of immediate working memory, State-Trait Anxiety Inventory, Center for Epidemiologic Studies Depression scale (CESD)-10 depression scale, and the Profile of Mood States. Vital signs were also monitored. The result shows that *Bacopa* participants had enhanced AVLT delayed word recalls comparison to the placebo group. Stroop result was also significant with the *Bacopa* group improving. CESD-10 depression scores, combined state plus trait anxiety scores, and heart rate decreased over time for the *Bacopa* group but increased for the placebo group. In rest all cases no significant changes have been noted.

Goswami et al., [33] conducted a clinical trial on Alzheimer's disease patients to evaluate the Anti-Alzheimer properties of *Bacopa monnieri*. (Standard extract of *Bacopa monnieri*). Total 39 patients having a mean age of 65.23 years were given plant extract at doses of 300mg twice a daily till 180 days. Before starting this trial mini-mental state examination scales (MMSES) were recorded for all patients. MMSES includes an orientation of time, place and person, attention, and in their language component in terms of reading learning writing, and comprehension. The patient involved in this study shows significant improvement in the MMSES parameter at the end of the trial.

A randomized double-blind parallel phase 2b study has been performed by Prabhakar et al., [34] to find out the efficacy of *Bacopa monnieri* against the standard drug Donepezil. 48 patients were participated in the study which received 300mg of *Bacopa monnieri* extract and 10mg donepezil. Results were analyzed through Alzheimer's disease assessment scale cognitive subscale (ADAS-cog) and postgraduate institute (PGI) memory scale. No significant differences have been recorded between *Bacopa monnieri* and donepezil which show *Bacopa* as a potent anti-Alzheimer agent.

McPhee et al., [35] performed a randomized, double blind, placebo controlled trail in 28 healthy older adults. Participants have to complete cognitive training (CT) 3 hours weekly for 12 weeks. Cognitive tasks, life satisfaction, memory complaints and mood were assessed and blood analyzed for serum brain derived neurotrophic factor (BDNF) before and after 12 weeks of intervention. *Bacopa monnieri* has higher mean accuracy in image discrimination task and menan accuracy is higher in spatial working memory task than placebo group. Although neuroimaging outcomes of white matter and grey matters conflicts the behavioral results.

Hepatoprotective Activity

The hepatoprotective activity of *Bacopa monnieri* has been examined in paracetamol-induced liver damage in Wistar albino rats. In this study, animals were categorized into four groups. The first group received 5ml/kg normal saline. The second group received the same dose. Except for the 1st group, all received 500mg/kg paracetamol for seven days. Group third received 300mg/kg of ethanolic extract of BM whereas group fourth received standard drug silymarin 25mg/kg. After sacrificing different assays including serum glutamate oxaloacetate (SGOT), Serum glutamate pyruvate transaminase (SGPT) alkaline phosphatase (ALP) Bilirubin (Direct and Total) Cholesterol (total and HDL) have been performed to analyze the activity. Levels of SGOT, SGPT, ALP, Bilirubin, and cholesterol are used as index to evaluate liver damage. It has been recorded that ALP, SGOT, SGPT, Bilirubin and Cholesterol significantly reduced comparison to control along with significantly enhancement in the level of GSH, SOD, CAT and HDL cholesterol by Ghosh et al., [36] during this experiment:

Another study on hepatoprotective action of *Bacopa monnieri* has been conducted by Menon et al., [37] on Nitrobenzene induced liver damage in rats. *Bacopa* extract at a dose of 200mg/kg of body weight given orally to induced liver damaged mice. Increased serum marker enzymes Aspartate, Transaminase, and Phosphatase were restored towards normalization significantly by the extract. Level of SOD CAT and GPx were significantly increased after treatment with *Bacopa monnieri* extract in liver injured experimental rats.

Immunostimulatory effects of the medicinal plant

The immune stimulatory effect of *Bacopa monnieri* has been evaluated by Yamada et al., [38] in four-week-old male Sprague Dawley rats. In this study, animals were divided into four groups of ten rats. Animals received 10g of *Bacopa* per kg of rat feed, 10g of Echinacea per kg of rat feed, and 10g of Withania per kg of rat feed. Bodyweight and food intake was recorded in two days interval. After four weeks, animals were sacrificed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia. The spleen lymphocytes isolated from each dietary group were cultured in 100ml FBS/RPMI1640 medium. Cellular concentration maintained to 2×10^6 cell/ml in 24 well microtiter plates. Cells were incubated at 37 °C for 48 hours in the absence and presence of 10µg/ml, Concanavalin A, and lipopolysaccharides. The concentration of IgA IgG and IgM was measured by ELISA. Results revealed that the production of IgA, IgG were significantly higher in the serum of rat-fed *Bacopa* compared to Echinacea and Withania and also much higher than control. These herbs also stimulate the production of antibodies in the presence of LPS or ConA. *Bacopa* produced higher concentration of various antibodies

including IgA, IgG, IgM, IgE in comparison to *Echinacea*, *Withania*.

Antihyperglycemic Activity

The antihyperglycemic activity was examined in streptozotocin-induced diabetic Albino Wister male rats by Taznin et al., [39]. Diabetic-induced mice were categorized into seven groups. Each group has received particular doses respectively 50mg/kg, 125mg/kg, 250mg/kg. Standard drug glibenclamide is given to one group @0.6mg/kg BW/day. The level of glucose was recorded on 1st day after diabetic induction and the last day of extract treatment. A significant increase of glucose in blood level has been recorded after streptozotocin induction i.e. 250mg/dl which was reduced by *Bacopa* extract at 125mg/kg BW. A maximum 49.21% of reduction of blood glucose level has been seen after the treatment of diabetic rats whereas standard drug glibenclamide shows a 53.77% reduction in blood glucose level. The present study evaluated *Bacopa monnieri* extract as a potent antidiabetic agent.

Antinociceptive Activity

The analgesic activity has been evaluated by Siraj et al., [40] using the model of acetic acid writhing in swiss albino mice against the standard drug Diclofenac sodium at a dose of 25mg/kg of body weight. At different doses i.e. 250mg/kg of body weight and 500mg/kg of body weight of crude extract were given to acetic acid-induced mice. The result shows 36% writhing inhibition caused by 250mg/kg of extract whereas 500mg/kg (body weight) of extract inhibited 61.33% writhing in mice.

The antinociceptive activity has been checked by Taznin et al., [39] in Swiss albino mice. The acetic acid-induced constriction method was used for evaluating antinociceptive activity. Animals were divided into six groups. Animals of all groups were given 1% acetic acid, 10ml per kg body weight through intraperitoneal injection for inducing abdominal pain. 1st the group which served as control, received vehicle only (1% tween 80 in water, 10mg per kg body weight) Group 2nd was administered standard antinociceptive drug aspirin @200mg/kg body weight rest all groups (group 3rd to 6th) received extract @ 50, 100, 200, 400 mg/kg of body weight. The number of constrictions induced by gastric pain in mice was then counted for the next 10 minutes. *Bacopa* Extract at 400mg concentration inhibit 53.4% abdominal constriction which has significantly higher than Aspirin group which inhibit 40.0% abdominal concentration at 200mg/kg of body weight.

Wound Healing Activity

The wound healing property of ethanolic extract of *Bacopa monnieri* has been evaluated by using Wister Albino rat of the male sex by Ghosh et al., [41]. The

excision wound model that has been explained by Shirwaikar et al., [42] was used in this experiment. The hair of the back skin was removed by using hair remover cream. A circular wound having a diameter of 10mm has been formed under mild ether anesthesia. Animals were divided into three groups. 1st group served as negative control which received simple ointment i.p. whereas group 2nd received ointment having plant extract (10% w/w) in simple ointment and the third group received nitrofurazone (0.2w/v) as a standard drug in ointment considered as a positive control. The entire test samples were given daily till the 12th day. On the 12th of the wounding post-day wound healing activity has been checked for all samples. 47.42% of wound healing activity recorded for negative control (maybe due to the immunity of animals) 67.08% wound healing activity recorded for BM extracts and 80.99% wound healing property are shown by standard drug nitrofurazone. The level of Hydroxyproline and DNA synthesis has been also checked in wound tissues. As rapid DNA synthesis has been required in wound tissues to overcome the damage. Hydroxyproline is a major component of protein collagen describe by Szpaz, [43]. Collagen is required for blood clotting. It has been found that after treatment of *Bacopa* extract and nitrofurazone content of Hydroxyproline in wound tissue has been increased and reached 112.80 ± 2.01 mg/g, 117 ± 1.41 mg/g respectively whereas it is only recorded in 71.11 ± 1.85 . DNA content also triggered in wound tissue has been recorded in 6.44 ± 0.31 in the negative control, 9.97 ± 0.40 recorded in *Bacopa* extract and 11.2 ± 0.58 has been noted in the standard drug.

Adaptogenic Activity

Anti adaptogenic activity of *Bacopa monnieri* has been checked by Rai et al., [44] using male Sprague-Dawley rats weighing 180-200 g. Stress was induced in mice by keeping them in a hemicylindrical tube having a diameter of 4.5 cm and 12 cm long for 150 minutes in case of acute stress (AS). For inducing chronic stress (CS) in animals same procedure is performed twice a day. Animals are divided into control non-stress group, AS group, and CS group and extract treating group for both AS and CS. Animals (AS and CS group) were fed with plant extract at different doses of 40mg/kg and 80mg/kg 9prior to the stress regimen for seven days. Animals were sacrificed at the end of the experiment. The blood plasma was used for evaluation of different parameters including glucose, triglyceride, alanine aminotransferase, (ALT) aspartate aminotransferase (AST), and creatinine kinase by using an autoanalyzer. Their high concentration caused stress conditions in animal beings which should be lowered by extract if they possess adaptogenic properties. The result showed AS exposed mice significantly increase the blood plasma level of glucose which was significantly decreased in AS group. In the CS group, no significant change has been recorded whereas a significant increase in plasma

level glucose has been recorded in the extract-treated CS group. In the case of plasma level triglyceride, it has decreased in the CS group but no significant change has been observed in drug-treated groups. In plasma ALT level no significant change has been reported in the CS group but it has significantly decreased in AS group pretreated with *Bacopa* extract at 80mg/kg of body weight. In AS and CS groups plasma level AST has been significantly raised but it has been significantly declined in the drug-treated AS and CS groups. In the case of plasma level of CK activity, It has significantly risen in AS and CS groups which have been lowered by a drug-treated group of AS and CS at 40mg/kg of body weight and 80mg/kg of body weight.

Anti-diarrheal Activity

Siraj et al., [40] evaluated the anti-diarrheal property of *Bacopa monnieri* using a model of castor oil-induced diarrhea in mice. Diarrhea symptoms showing mice were categorized into three groups. One group is administered only 1% tween 80 at a dose of 10ml/kg of body weight serves as a control. The second group received standard drug Loperamide at a dose of 50mg/kg of body weight treated as a positive control. The third and last group received a methanolic extract of a plant at a dose of 500mg/kg of body weight. The result was analyzed by stool count per hour. It has been found at the end of the experiment that *Bacopa monnieri* extracts at doses of 500mg/kg having a latent period of 1.16 ± 0.16 against standard drugs having a latent period of 2.28 ± 0.20 whereas normal control group shows a latent period of 0.75 ± 0.06 . The latent period is the time gap of two diarrheal episodes which has significantly raised in *Bacopa* extract. Mean of feces are also recorded in this study which has significantly decreased in *Bacopa* extract at doses of 500mg/kg which reduced from 7.6 ± 1.43 to 3.6 ± 0.98 whereas the standard group show feces mean with the value of 2.4 ± 0.51 . *Bacopa* possesses numbers of phytochemicals out of which tannin has to be supposed for the presence of anti-diarrheal activity in the plant extract.

Anti-helicobacter Activity

Anti-helicobacter pylori activity of *Bacopa monnieri* extract was evaluated in vitro condition against the bismuth subcitrate which is known Anti-pyloric agent by Goel et al., [45]. At the concentration of 1000µg/ml of *Bacopa monnieri* extract showed 75% inhibition against H. pylori which is equal to Bismuth subcitrate. The study demonstrates that *Bacopa monnieri* extract increases the amount of prostaglandin E (PGE) and prostacyclin (PGI₂). *Bacopa monnieri* extract at 100µg/ml increased 40.8% PGE and PGI₂. Both prostaglandins are special types of lipid which play an important role in protection of gastric mucosa.

Anti-Toxic Effect

Shahid et al., [46] performed a study and evaluated the

beneficial effect of *Bacopa monnieri* extract on opioid-induced toxicity. Toxicity in rats was induced by administration of 20mg/kg for 14 days and 21 days for street heroin. Methanolic extract of *Bacopa monnieri* extract was given to rats two hours before the opioid treatment. It has been noted that morphine and street heroin cause elevation of serum alanine aminotransferase, aspartate, aminotransferase, and creatinine. The three compounds also cause toxicity in the liver and kidney. Rats treated with *Bacopa* extract prevent the elevation in ALT, AST, and creatinine in comparison to standard drug Ascorbic acid.

Anti-hypertensive & Anti-allergic Activity

Very few studies have been conducted till now which show limited cardiovascular action of *Bacopa monnieri*. When *Bacopa* extract at the dose of 20-60mg/kg of body weight is given intravenous to the anesthetized rats, it decreases systolic and diastolic pressure without affecting heart rate in the study of Kamkaew et al., [47] Brahmi reduces blood pressure partly via releasing nitric oxide from the endothelium, with additional actions on vascular smooth muscle Ca²⁺ homeostasis. Onsa-ard et al., [48] performed a clinical trial to see the anti-hypertensive activity of *Bacopa monnieri* against L-NAME administered Male Wister rats. Captopril was used as a standard drug in this experiment. Systolic blood pressure which attains elevation of 94.7 ± 7.5 mmHg (week 0, n = 7) to 166.6 ± 3.5 mmHg can be reduced to 129.9 ± 6.8 mmHg after the treatment of *Bacopa* extract whereas in the case of standard drug captopril from 166.4 ± 7.2 mmHg (week 4) to 140.4 ± 5.8 mmHg (week 8, P < 0.01, n = 6-7) but had no effect on normotensives. It is to be believed Brahmi extract elicited endothelial independent vasorelaxation, suggesting that it acts directly on the vascular smooth muscle cells.

A study has been conducted by Samiulla et al., [49] showed that methanolic extract of *Bacopa* leaves has a potent mast cell stabilization effect comparable to disodium cromoglycate. This result proves that *Bacopa* has anti-allergic properties.

Anti-Parkinsonian effect

Anti- Parkinsonian effect of *Bacopa monnieri* was evaluated by Jadiya et al., [50]. It has been seen that in the case of neurodegenerative Parkinson's disease, the accumulation of protein alpha-synuclein ultimately leads to the death of dopaminergic neurons. In this study researcher used *Caenorhabditis elegans*; a transgenic model expressing different strains of human alpha-synuclein [NLS901 (Punc 54: alpha synuclein YFP+ VNC-119 and pharmacological model expressing green fluorescent protein (GFP).

Babita Singh et al., [51] also checked neuroprotective role of *Bacopa monnieri*. In their study, they found *Bacopa* extract reduced the reactive oxygen species [ROS], decreased the pro inflammatory cytokines, and decreased

the level of alpha synuclein in male Wister Albino rats. Study outcomes suggest *Bacopa* control inflammation in brain region and thus it can consider as novel therapeutics against Parkinson's disease.

Antifertility Activity

Antifertility activity of plant extract was evaluated in mice (Parkes Strain) by Akanksha et al., [52]. The study reveals that *Bacopa* extract at a dose of 250mg/kg of body weight cause antifertility in mice by making reduction in mobility, viability morphology of spermatozoa in cauda epididymidis. *Bacopa* extract caused alternation in seminiferous tubules including intraepithelial vacuolation, loosening of germinal epithelium, and exfoliation of germ cells. The study also reveals that extract of *Bacopa monnieri* caused a significant reduction in the height of germinal epithelium and diameter of the seminiferous tubules compared to control.

Anti-aging Activity

Saha et al., [53] conducted a study for evaluation of the anti-aging activity of *Bacopa monnieri*. They found that *Bacopa* diminished the Benzo[a] pyrene-induced apoptosis and senescence in human astrocytes. In previous study it has already reported that Benzo[a]pyrene is a neurotoxic agent, responsible for impaired neuronal development, induced apoptosis which is the main cause of aging. *Bacopa monnieri* also prevents cell cycle arrest induced by Benzo[a]pyrene and protects the cell from reactive oxygen species. The formation of ROS also triggers by B[a]P by reducing the damaged mitochondria.

Anti-emetic Properties

Ullah et al., [54] conducted a study to see the anti-emetic properties of *Bacopa monnieri*. Both metanolic and butanolic fractions of *Bacopa monnieri* had been tested for anti-emetic property in chemotherapy induced emesis in *Suncus murinus*. Cisplatin (30mg/kg) was injected to the animal for induction of emesis. Different concentration of Butanolic extract (5-20mg/kg) and methanolic extract (10-40mg/kg) were evaluated for anti-emetic screening. The result showed butanolic extract antagonized vomiting response 59.4% and methanolic extract counter it by 71%. It suggests methanolic extract as a good source of the anti-emetic drug against chemotherapy-induced emetic condition.

Side Effect & Cytotoxic Activity

Bacopa monnieri has been using in India for a long time. Till now none of the side effects has been recorded. Joshua Allan et al., [55] conducted a study to evaluate the safety

and efficacy of BacoMind which is an enriched phytochemical composition of *Bacopa monnieri*. *Bacopa monnieri* recorded median lethal dose after administration of 2400mg/kg of bodyweight in Sprague Dawley rats whereas no sign of toxicity or significant changes with respect to neurological examination, food consumption, body weight gain, hematological and blood biochemistry parameters had been observed in case of subchronic toxicity. Again acute and chronic toxicity of *Bacopa monnieri* has been checked in Sprague Dawley rats by Sireeratawong et al., [56]. Female rats were administered a single dose of 5000mg/kg of body weight. Rats were monitored for 14 days. No abnormalities in behavior and health were recorded in this period. In chronic toxicity, both male and female rats were administered different doses of extract (30, 60, 300, or 1,500 mg/kg) for 270 days. During these days health and behavior of animals were observed. At the end of the experimental animals were sacrificed. Body and organ weight were measured. Different parameters including hematology, blood clinical chemistry, and microanatomy have been examined. No significant differences have been reported between experimental and control group rats which established *Bacopa monnieri* extract as a safe drug. Cytotoxic activity has been evaluated by Siraj et al., [40] by using brine shrimp lethality bioassay. The extract shows 50% (L50) mortality at 26.30µg/ml and 90% (L90) mortality at 141.25µg/ml.

Pharmacognostic & Physiochemical Properties

Phompittayarat et al., [57] performed a study to see the seasonal impact of saponin content in *Bacopa monnieri* and recorded the highest saponin quantity in the rainy season while the highest weight yield was recorded in summer. A high quantity of saponin [1.91±0.48 w/w] was detected at the shoot of Brahmi. The physiochemical property includes solubility of extract in different solvents, loss on drying, total Ash value, Acid insoluble ash, water-soluble ash. Extractive value of *Bacopa monnieri* in methanol recorded 10.1% followed by ethanol 8.6%, water 7.6%, chloroform 2.0%, Acetone 1.5%, Dichloroethane 0.6%, and petroleum ether 0.5%. Total ash value recorded 13.5%, insoluble ash value 5.5%, Water soluble ash value 2.5%, and loss on drying recorded 1.5% by Pawar et al., [58].

Pharmacognostic Characterization of *Bacopa monnieri* has been done by Chaurasia et al., [59]. They did powder analysis which showed the presence of cuticle, raphide calcium late crystal, and starch in the plant. However, stone cells are absent. Fluorescence analysis was also performed by the researcher which was cited below.

Table 1. Fluorescence study conducted by Chaurasia et al., [59]

Sr.No.	Sample	FTC	Visible Light	DAPI
1	Plant Powder	White	Yellow	White
2	Plant Powder + H ₂ O	Light green fluorescence	Dark brown	White
3	Powder with H ₂ SO ₄	Light green fluorescence	Dark brown	Brown
4	Powder with NH ₃	Light green fluorescence	Dark green	White Fluorescence
5	Powder with C ₂ H ₅ OH	Light green fluorescence	Brown	White
6	Plant Powder +KOH	Light green fluorescence	Greyish Brown	Red
7	Plant Powder with NaOH	Dark green fluorescence	Brick red	Brick Red
8	Powder +Iodine	Light green fluorescence	White	White

Phrompittayarat et al., [60] compared various extraction methods and found that *Bacopa monnieri* gave the highest yield of *Bacopa* saponin if it is soaked in 95% methanol for 3 days after maceration. Content of *Bacopa* saponin evaluated through HPLC.

Silpa et al., [61] reported that drying of fresh herbage (*Bacopa monnieri*) at 50°C of 12 h in cabinet drier retained the highest Bacoside content.

Phytochemical and Bioactive Compound

Phytochemical study reveals that plant possess numbers of phytoconstituents in it. It contains proteins (10.54 ± 1.71) mg/gm carbohydrate (150.63 ± 6.61) mg/gm., phenols (3.71 ± 0.23) mg/gm., 2.03 ± 0.11 mg/gm, flavonoid (1.12 ± 0.02) mg/gm. Numbers of bioactive compounds were isolated and identified from the plant extract. It also contains saponins, cardiac glycosides, steroids, tannins, phlobetanin and terpenoid.

Sivaramakrishna et al., [62] isolated two triterpenoids glucosides along with 10 known saponins. These triterpenoids glucosides are 3-O-[beta-D-glucopyranosyl-(1-->3)-beta-D-glucopyranosyl] jujubogenin (1) and 3-O-[beta-D-glucopyranosyl-(1-->3)-beta-D-glucopyranosyl] pseudojujubogenin (2). Their structures were elucidated by NMR spectroscopy and chemical correlation.

Bhandari et al., [63] isolated a new sterol glycoside, bacosterol-3-O-beta-D- glucopyranoside along with *Bacopa* saponin-C, *Bacopa* side-I, *Bacopa* side-II, bacosterol bacosine and luteolin-7-O- beta- glucopyranoside from *Bacopa monnieri*. Identification of structure has been done by using IR, 1D, 2D NMR (HMQC, HMBC, COSY) HR-ESI, QTOP MS and EI mass spectral techniques.

Estimation of twelve *Bacopa* saponin in *Bacopa monnieri* extract had performed by Murthy et al., [64] through developing a new method based on reversed-phase high-performance liquid chromatography.

Bhandari et al., [65] isolated cucurbitacin (A-E) from the

aerial part of *Bacopa monnieri*. Structures were identified by using 1D, 2D NMR, ESI-QTOF-MS/MS.

Shefin et al., [66] demonstrate that *Bacopa* possess number of phytochemicals. Jeysari et al., [67] listed these phytochemicals in his study. According to the study *Bacopa* possess 52 phytoconstituents including Nicotine, D- Mannitol, Bacoside A, *Bacopa* saponin A, *Bacopa* saponin B, *Bacopa* saponin C, *Bacopa* saponin D, *Bacopa* saponin E, *Bacopa* saponin F, *Bacopa* saponin G, *Bacopa* side I (Fig. 2.7), *Bacopa* side II (Fig. 2.8), *Bacopa* side III, *Bacopa* side IV, *Bacopa* side VIII, *Bacopa* side XII, Plantainoside B, Betulinic acid, Cucurbitacin A, Cucurbitacin B, Cucurbitacin C, Cucurbitacin D, Cucurbitacin E, Steraic Acid, Rosavin, 3-4 Dimethoxycinnamic acid, Ascorbic acid, Aciatic acid, Brahmic acid, Wogonin, Oroxidin, Laliolide, Stigmasterol, beta-sitosterol, Bacosterol, Bacosine, Heptacosane, Octacosane, Nanocosane, Triacontane, Hentriacontane, Dotriacontane, Apigenin, Quercetin, Ursolic acid, Luteolin, Asiaticoside, *Bacopa* side VI and *Bacopa* side VII, *Bacopa* side A3 (Fig. 2.1).

Jain, Paras; et al., [23] showed that total 37 compound are present in this plant including Phytol (Fig. 2.6), Tridecane, Octadec 9- enoic acid, (Fig. 2.2) N-hexadecanoic acid, Stigmasterol (Fig. 2.3), Vitamin E (Fig. 2.10), Icosanoic acid (Fig. 2.5), cis-9- hexadecenal acid (Fig. 2.4), Dodecane, phenol, 2 methoxy-4-(2 propenyl), 2- Nonenal, 2-pentyl, 2,6,10-rimethyl, 14-ethylene-14-pentadecene, Hexaecanoic acid, Methyl ester, Octadecanoic acid (Fig. 2.9), Ergost-5-en-ol, (3-beta-24R)-, Hahnfett, Hneicosane Cis-10-Nonadecenoic acid.

Figure 2.1. Bacoside A3

Figure 2.2. Octadec 9- enoic acid

Figure 2.8. Bacoside-II

Figure 2.3. Stigmasterol

Figure 2.9. Octadecanoic acid

Figure 2.10. Vitamin E

Figure 2.4. Cis-9- hexadecenal acid

Figure 2.5. Icosanoic acid

Figure 2.6. Phytol

Figure 2.7. Bacoside-I

Fig 2.1 to 2.10 Various Compound reported in *Bacopa monnieri*

Tissue Culture Technique

Shrivastava and Rajani [68] found the best result in shoot induction of leafy explant if culture medium is supplemented with 2um benzyladenine and gelled with 0.2% gelrite.

Micropropagation of *Bacopa monnieri* has been performed by Mohapatra et al., [69] by using MS and B5 media supplemented with BAP and NAA using leaf and shoot explant. *Bacopa* shows the best response in MS medium which is supplemented with 2mg/l with BAP.

Another study which reports micropropagation of *Bacopa monnieri* was done by Vijay Kumar et al., [70] stated about suitable hormonal concentration and soil type. According to their research for root initiation MS medium should be enriched with IAA 0.5mg/l and TDZ 0.06mg/l whereas for shoot induction MS-medium was supplemented with 1mg/l BA+ 0.4mg/l KIN + 0.4mg/l NAA for better induction and growth of shoot. For callus growth, MS medium will be supplemented with 0.5mg/l and 0.5mg/l of NAA. They found clay soil is the most appropriate soil for cultivation of *Bacopa monnieri* followed by red soil.

Ali et al; [71] suggested better hormonal configuration for somatic embryogenesis and in vitro regeneration of *Bacopa monnieri*. According to them highest calli

formation from the leaf explant were recorded on NAA (2.5mg/liter) showed the highest regeneration i.e. 94.22 followed by 2, 4-D mg/liter showed 71.4% calli formation. In internode explant it was recorded highest in 2, 4-D showed 65.25% regeneration. The maximum somatic embryogenesis callus, calli induction, and formation were observed on 2, 4-D KIN (2.0+1.5mg/liter) amended solid medium.

Sujipuli et al., [72] performed an experiment with *Bacopa monnieri* for enhancing biomass and bacoside production. Explant of the diploid progenitor of *Bacopa monnieri* treated with different doses of colchicine. Result revealed that (0.05%) colchicine induce tetraploidy which resultant highest Bacoside A3 (4.276 ± 0.019 mg/g dry weight) and Bacoside e-content (5.040 ± 0.070 mg/g dry weight) obtained from tetraploid species.

Sharma et al., [73] added methyl jasmonate in MS medium and found 4.4mg bacoside A/gram dry weight which is 1.8 fold increased from control.

Panda et al [74] reported that if fly ash was added to the prepared garden soil for *Bacopa monnieri* by up to 25%, there was no negative effect on the photosynthetic activity of *Bacopa monnieri*, chlorophyll content, as well as an increase in plant biomass tolerance and oil content. In this way, we can not only get rid of the problem of fly ash but also increase the oil content and biomass.

A study was performed by Lala S., [75] to see the impact of copper Nanoparticle on the production of a secondary metabolite of *Bacopa monnieri*. Secondary metabolites were checked through the spectrophotometric method. At the concentration of 5mg/l CuNP can be used to enhance the concentration of secondary metabolites.

Use of various elicitors such as salicylic acid and jasmonic acid to increase biomass and production of bacoside in *Bacopa monnieri* studied by Anuja Kaul et al [76]. Increased biomass and bacoside production were evaluated over a period of 3, 6, 9, 15 days. The study revealed that Salicylic acid enhances biomass and bacoside production (6.58mg/g of dry weight) maximum between the period of 6 to 9 days.

Use of Microbe for Enhancing Secondary Metabolite Production

Microbes perform a crucial role in the growth and development of the plant body. It has been found that microbial interactivity with plant also provides the defiance biotic and abiotic stress. Banarjee and Modi [77] used a hot extract of *Aulosira fertilissima* (cyanobacterium) added in different proportions to MS as a liquid culture media for the invitro propagation of *Bacopa monnieri* and found maximum numbers of shoot were induced from axillary node after maintaining proportion 40:60 of MS

media and *Aulosira* extract. After induction maximum shoot multiplication found on adding Kn (1mg/L).

Gupta et al., [78] studied the effect and interaction of various rhizospheric bacteria *Bacillus megaterium*, *glomus intraradices*, *Trichoderma harzianum* and their combination on *Meloidogyne incognita* infected *Bacopa monnieri* and found that *Meloidogyne incognita* infection not only significantly decreased upto 2.75 fold but also bacoside content significantly increased 1.40 fold during this period. Estimation of bacoside content done by Fourier transform near infrared.

Prasad et al., [79] demonstrated that when *Bacopa* is grown with root endophyte *Piriformospora indica*, the bacoside content, antioxidant activity of plant, growth rate and nuclear hypertrophy of plant is increased as compared to the control.

In another study conducted by Singh et al., [80] *Bacopa monnieri* has exposed to different microbes bio-inoculants *Pseudomonas montelli*, *Cedecea davisae*, *Cronobacter dublinensis* and *P.aeruginosa*. There was a significant enhancement in the biomass and secondary metabolite content of *Bacopa monnieri* which were prone to these microbes compared to control.

Gupta et al., [81] conducted a study to see the impact of *chitiophilus* sp. MTN22 and *Streptomyces* sp. MTN14 singly as well as in combination modulated the biosynthetic pathway of bacoside A and systemic defense mechanism *Meloidogyne incognita* in *Bacopa monnieri*. The result showed that the expression of bacoside biosynthetic pathway genes (3-Hydroxy-3-methylglutaryl coenzyme A reductase, mevalonate diphosphate decarboxylase, and squalene synthase) in plants treated with these microbes was increased in the presence as well as in the absence of *Meloidogyne incognita*. Total 1.5 fold of bacoside production has increased in plant treated with *Chitiophilus* sp. MTN22 and *Streptomyces* sp. MTN14. Plant resistances against *M. incognita* also increase via enhancement in chlorophyll a, defense enzymes and phenolic compounds like gallic acid, syringic acid, ferulic acid and cinnamic acid.

Quantitative Estimation of Secondary Metabolite

Bacopa possesses various secondary metabolites which show a diverse range of therapeutic action. Various researchers quantified total phenolic, alkaloid, tannin, and saponin content. Production of secondary metabolites in the plant was regulated by a number of factors including soil and environmental conditions. Thus, the amount of secondary metabolite may vary in the same plant which was growing in different regions. In this review, the author summarizes various studies to find out the suitable place for the cultivation of *Bacopa monnieri*.

Table 2. Quantitative phytochemical presence in various studies in *Bacopa monnieri* growing in various part of Asia subcontinent

References	Evaluated Phytochemical								Plant Collected from
	Total Phenolic Content	Total Alkaloid Content	Total Flavonoid Content	Total Tannin Content	Total Saponin Content	Total Sugar Content	Total Anthocyanin Content	Total Chlorophyll Content	
Jain et al., [23]	24.75 mg/g	110mg/g	29.666mg/g	12.5mg/g	1.5mg/g		1.436 CGE		Ranchi
Ghosh et al., [35]	47.7ug/mg of extract								Salipur, Orissa
Hossain et al., [82]				105.90mg/g		150.63mg/g			Karamjal, Sundarban Khulna, Bangladesh
Volluri et al., [83]	27.76±1.87m g/gm GAE								Visakhapatnam, Andhra Pradesh, India
Nandi Chakraborty, Swati et al., [84]	46.24 mg/g (chloroform solvent)		38.54mg/g (methanol solvent)						East Kolkata (Wetland area) Bengal India

Table 3. Ethnobotanical uses of *Bacopa monnieri*

Name of Ailments	Method of drug preparation	Area where ethno medicinal practices of plants is going on	References
Epilepsy, Bronchial and Diarrheal	Leaves juice of plant is given to the patient for the treatment of epilepsy, bronchial and diarrheal ailments	Punjab Provinces, Pakistan	Shah et al., [86]
Memory enhancement	Plant juice is given orally to enhance and sharpen memory	Mayurbhanj District, Orissa	Rout et al., [87]
Heaache, Hairfall	Whole plant juice is given orally to treat headache and hairfall.	Rajbari District, Bangladesh	Mukti, M., & Rahmatullah, M. [88]
Blisters	A fresh root decoction is used to treat snakebite in Rajasthan. Dry leaves powder with quantity of 5 g mixed with crushed 2 or 3 black pepper is given in a single dose for the treatment of bone fracture	Rajasthan, India	Verma, M. [89]
Snake Bite	Fresh root decoction used for treatment of snake-bite, scorpion stings	Rajasthan, India	Verma, M. [89]
Hoarseness of voice	<i>Bacopa</i> powder fried with ghee given for the enhancement of memory	Rajasthan, India	Verma, M. [89]
Memory enhancement	<i>Bacopa</i> powder fried with ghee mix with Pushkar mul (Sauseria lappa's root) given for enhancement of memory	Rajasthan, India	Verma, M. [89]
Bone fracture	Leaf powder about 5 g with 2 or 3 black pepper are given in a single dose for the treatment of bone fracture.	Rajasthan, India	Verma, M. [89]
Swelling of legs (Animal)	Leaf paste applied externally on the affected area of the body 3 times daily in the legs of animals for one week	Rajasthan, India	Verma, M. [89]
Asthma	Fresh decoction of leaves and stem filtered in a proper way. Taken twice in a day for 5 to 10 days until disease has been persisted.	Rajasthan, India	Verma, M. [89].
Hair Fall	Plant juice is applied externally on hair to treat disease	Rupandehi district, Nepal	Singh, et al., [90]
Snake Bite	Plant Juice mixed with castor oil is applied externally to treat snake bite. Leaf powder decoction mixed with hot cow's milk is given to the affected person.	Salem district, Tamilnadu, India	Upasani et al., [91], Alagesaboopathi C., [92]
Malaria	Paste of plant is given orally to treat the malarial fever.	Haripur, Abbottabad and Mansehra of Khyber Pakhtunkhwa (KPK) province of Pakistan	Shah et al., [93]
Snake Bite	Leaf powder decoction is used as antidote for Cobra bite.	Kurnool district, India	Basha S Khaleel et al., [94]

2. Ethnobotanical and Ethnopharmacological Study

Pandey et al., [85] describe a various familiar method of using plants for treating health issues in India. According to him there are four common ways to adopt plant parts as medicine, which are taking fresh juice from plant parts, making a decoction of a plant parts, taking plant powder with milk or clarified butter, and decoction of plant parts taken with honey or jaggery. In the case of Brahmi, we reported the same application of taking drugs. Various ethnobotanical surveys revealed several unknown and hidden medicinal properties of *Bacopa monnieri* which are listed in table 3.

3. Application of *Bacopa monnieri* for Treatment of Wastewater

Jauhari et al., [95] used *Bacopa monnieri* for the treatment of heavy metal contaminated wastewater. It has

been observed soil-grown *Bacopa monnieri* remove 64 Cr% and 83% Cd from the wastewater whereas in vitro cultured plants reduce the concentration of Cr 67% and 93% Cd from wastewater. The study recommends that *Bacopa monnieri* can be used for the removal of heavy metal from wastewater.

Nagarajan [96] showed that *Bacopa* removes the enteric infection from the wastewater. The study also showed that *Bacopa* can easily be flourished in wastewater coming out from the toilet and sewage. It can remove coliform bacteria up to 70% which was a potent source of enteric infection.

Abbasi et al [97] tested the phytoremedial activity of *Bacopa monnieri* using their own SHEFROL® ("sheet flow root level") bioreactor. They found in their test that by using *Bacopa monnieri* in the SHEFROL® bioreactor, suspended solids in sewage water were reduced by 90%, chemical oxygen demand was reduced by 76-77%, biological oxygen demand by 80%, Nitrogen 65%, Phosphorus 55%, Zinc 42%, Copper 42%, and Nickel 41%, can only be reduced through hydraulic retention of six hours. Study outcomes established that *Bacopa* can be used

for the treatment of sewage (grey water).

Shanmugam et al., [98] tested the phytoremediation role of *Bacopa monnieri* for degradation of textiles azo dyes and found that hydroponically and invitro cultivated *Bacopa* successfully decolorized azo dyes at 40mg/l concentration of different azo dyes.

Gupta et al., [99] conducted a study to see the impact of Cd ion on the growth and content of Bacoside A and *Bacopa* side I of *Bacopa monnieri*. Various growth parameters including total protein, Chlorophyll content, biomasses were evaluated after the exposure of Cd to the plant. No harmful impact of Cd was recorded till 10µM and content of *Bacopa* side I and Bacoside A gradually increased but when Cd concentration increased up to 50 and 100µM it affect plant adversely as chlorophyll content, protein and biomass decrease although content of *Bacopa* side I and Bacoside I is still higher than control group.

4. Future Prospective

Bacopa monnieri is used since ancient times for enhancing memory. Patel et al., [100] listed 22 herbal formation based products which has *Bacopa* as a main ingredient. So the use of *Bacopa* is still rising in herbal product as a memory tonic and health supplement. The ethnobotanical surveys revealed lots of its medicinal properties. The ethnobotanical studies conducted revealed that this plant was a great source of anti-venomous drugs. The juice of this plant can also be used to prevent hair fall. To date, lots of ethnobotanical studies reveal that this plant has anti-sickling, anti-paralytic and anti-venomous properties but experimentally it remains unexplored. Varieties of phytochemicals and bioactive properties present in this plant are still not tested against several diseases, leaving a huge scope in this field.

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