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# A Review on Phytochemicals and Pharmacological Activity of Bharmi







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Keywords: Bacopa monnieri, bacoside-A, phytoconstituents, pharmacological activity

# ABSTRACT

In India, people have been using the herb Bacopa monnieri (BM) for memory enhancement for at least 3000 years. Spiritual groups began using Brahmi to assist pupils improve their recollection for studying old spiritual anthems. Many plants were utilized conventionally as cognitive or neuron tinctures in Indian medical culture. Alkaloids, saponins, herpestine, and brahmine are the main phytoconstituents that are found. The creeping branches that root at the nodes give rise to the 10-30 cm long leaves and flower-bearing stems. The Scrophulariaceae family, which includes the pale blue or pinkish white flowers, are grown near rice fields, freshwater stream banks, and other damp places. In addition, it serves as a diuretic and nervine stimulant for nervous and mental exhaustion, an agent in insanity, epilepsy, hysteria, esthenia, and emotional collapse, an antistress agent, a cardiotonic, an analgesic, a relaxant, a tool for learning, an attention restorer, an enhancer for language and creative thinking, and so on.

# **INTRODUCTION:**

Herbal medicine, also referred to as medicinal plants, which involves the application of medicinally valuable plants as the foundation for traditional treatments.<sup>[1]</sup> Because of their based on plants sources and low risk of side effects, using herbs is becoming more and more common in nations that are both developed and developing.<sup>[2]</sup> *Bacopa monnieri*, frequently called *Herpestis monniera*, *Bacopa monniera*, water hyssop, and "Brahmi," has been utilized as a brain stimulant to improve concentration since the beginning of time. All over India, *B. monnieri* is a perennial creeping plant that thrives in humid, damp, and damp environments.<sup>[3,4]</sup> *Bacopa monnieri* is a small member of the Scrophulariaceae family, creeper herbs. It features a number of branches, brief, elliptical leaves, and tiny, white blossoms with four or five pale purple or white petals.<sup>[5]</sup>

In tropical and subtropical India, the type of plant is an everlasting herb with a short growth season that is often found in damp areas and by streams. It grows best in plains and highlands near running water and damp environments, and is prevalent during the yearly monsoon season.<sup>[6]</sup> A variety of chemical substances such as bacosides A and B , d-mannitol, hersaponin, betulinic acid, stigmasterol, β-sitosterol, brahmine, and herpestine,have demonstrated their presence in the organism.<sup>[7]</sup>

In Ayurveda, the plant is applied for therapy like mental instability, nervousness, and convulsions, antiulcerogenic, cardioprotective, anti-anxiety, and psychological tiredness. In addition, it's frequently used for managing bronchitis, and antinociceptive.<sup>[8–16]</sup>

#### **PLANT PROFILE:**

#### SYNONYMS-

- 1. Bacopa monniera Hayata and Matsum.
- 2. Bramia monnieri (L.) pennell
- 3. Gratiola monnieria L.
- 4. Herpestes monnieria (L.) Kunth
- 5. Herpestis fauriei H.Lev
- 6. Herpestis monniera
- 7. Herpestis monnieria
- 8. Lysimachia monnieri

9. Capraria monnieri (L.)L

10. Moniera cuneifolia Michx.

### BOTANICAL/SCIENTIFIC CLASSIFICATION-

Kingdom	: Plantae
Division	: Tracheophyta
Class	: Magoliopsida
Order	: Lamiales
Family	: Plantaginaceae
Sub Family	: Scrophulariaceae
Genus	: Bacopa
Species	: Bacopa monnieri (L.)

#### **VERNACULAR NAMES OF B. monnieri**

Tamil name	: Neera brahmi	
English name	: Thyme leaved gratiola, water hyssop, Indian pennywort	
Hindi name	: Brahmi	
Kannada name	: Jala brahmi	
Marathi	: Brahmi, Jalabrahmi	
Sanskrit name	: Bramhi, Tikalonika	
Telugu name	: Sambarenu	

#### CHEMICAL CONSTITUENTS<sup>[17]</sup>

**Major:** Alkaloids, saponins, herpestine, and brahmine are the principal constituents. The saponins bacoside A and B, betulic acid. D-mannitol,  $\beta$ -sitosterol, and stigma sterol were isolated. By using acid hydrolysis, three sugars were produced, of which glucose and arabinose are known to be two. Bacoside B also hydrolyzed to glucose and arabinose.

**Others:** Bacoside A is among the primary chemical elements of *Bacopa monieri*. Bacobitacins A-D, plantioside B, jujubogenin, bacoside B, bacoside A1, bacogenin A2, bacogenin A3, bacogenin A4, bacosides A-C, bacosides 1 and 2, bacopasides 3-5, bacopasides 6-8, bacobitacins A-D, monnieraside 1, monnieraside 3, monnierin, (1-3)- $\beta$ -D-glucopyranosyl-3-O-[ $\beta$ - D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranosyl]- $\beta$ -D-glucopyranosyl

Jujubogenin Nicotine, wogonin, oroxindin, betulinic acid, taraxerone, luteolin, luteolin-7glucoside, luteolin-7-glucuronide, apigenin-7-glucuronide, D-mannitol, 3-formyl-4-hydroxy-2H-pyran, bacosine, bacosterol, bacosterol-3-o- $\beta$ -D-glucopyranoside,  $\beta$ -sitosterol, bacosterol, and an unidentified glycoside <sup>[18].</sup>

# MORPHOLOGICAL DESCRIPTION OF B. MONNIERI<sup>[19, 20]</sup>

Fig 1. Picture of [Brahmi] Bacopa monnieri plant

**Leaf:** 0.6 to 2.5 cm long and 3 to 8 mm wide, obovate-oblong to spathulate in form, entire, lower section dotted with tiny specks, obscuringly 1 to 3 nerved, pale green in color. Smooth, clear, somewhat sessile, vertically and horizontally divided.

**Stem:** The stem is constructed from cylinder-shaped, transparent parts that have noticeable nodes. The stem is occasionally attached to vertically growing branches and ventrally tied to a cluster of challenging, brittle roots. The stem's connecting nodes are reddish in color and lengths ranging from 1 to 1.5 cm and thickness from 3 to 4 mm.

**Flower:** 0.6 to 3cm in length, axillary, nearly regular, solitary, pale blue or pinkish white, with two linear bracteoles; pedicel thin; calyx translucent; deeply five partite; corolla gamopetalous; stamens four; didynamous; anthers two; pistil bicarpellary, syncarpous; ovary two chambered with many ovules; style dilation towards the apex; stigma bound.

**Fruit:** Fresh, purple, 1-3 cm in length. A 5 mm long, globose to ovoid, glabrous capsule with a protracted calyx and pedicel within it.

Seed: Numerous, tiny, elliptical or erratic, less than 1 mm in width.

**Root:** The dried main portions of the root are off-white in color, cylindrical in shape, and are around 5 mm in diameter. They also exhibit longitudinal wrinkles.

#### Microscopic Characters<sup>[18,21-24]</sup>

Leaf: The leaf that passes through the midrib has a very thin elevation on the top side of the middle and an almost cylindrical form. The epidermis is composed of two layers: the top layer has larger cells with striated cuticle in certain areas, and both layers include embedded stomata in addition to sessile-gladular trichomes with multicellular heads. Beneath the outer layer of the midribs is a brief collenchymatous group that exhibits together collateral meristele centered and encircled by a parenchymatous sheath. Prism-shaped and a few cluster crystals of calcium oxalate are lodged in the parenchymatous leaf cells, making up the mesophyll cell of the lamina, which is composed of spongy parenchyma that is crossed by vascular threads.

**Stem:** The stem is nearly spherical in shape, with a wide aerenchymatous cortex spanning the majority of the segment, an odd endodermis around the stellar tissue ring, and a parenchymatous pith in the center that has been covered in a thin layer of nail cuticle and has densely packed epidermal cells. The endodermis layer is conspicuous and encircles the center of the parenchymatous pith in addition to the narrow band of parenchymatous phloem and xylem. The cortex is quite broad and contains starch-embedded chlorenchymatous aerenchyma.

**Root:** The root is unevenly shaped, ranging from cylinder to angular, with a solid core of xylem surrounded by thin phloem in the middle, a parenchymatous cortex with air spaces between it and the xylem, and an outermost piliferous layer. The piliferous layer is replaced by the growth of cork cells; the outermost layer is broad and parenchymatous, with air spaces between simple and the substance starch grains that pass through it; the solid core of xylem is surrounded by a distinct band of phloem called the endodermis, which is composed of fibers, medullary rays, and distinct vessels that make structures outward.

**Powder Microscopy:** Exhibits portions of the outermost layer of the skin in appear view, encased in sessile-glandular trichomes with 4–8 celled heads and diacytic to anomocytic stomata; these are more common on the bottom, with sinuous anticlinical walls and striated cuticle at times; Calcium oxalate (CO) clump aggregates that are prismatic in shape, crystalline starch grains, oily particles that are distributed or imbedded in the parenchymatous

cells; segments of circular and spiralling veins that have been cut across the board, and diagonally sliced stem portions displaying aerenchymatous cortical cells, papillose marginal cells of the petal, test of the seed in surface view, and transversely cut fragments of cotyledon are displayed.



Figure 2: (a)Vascular bundles, (b)parenchymatous cells, (c) calcium Oxalate crytals, (d) xylem and phloem

### **Phytoconstituents**

### **Chemical structure**

S.NO	STRUCTURE	CHEMICAL NAME
1.	HO HO HO HO HO HO HO HO HO HO HO HO HO H	Bacosde A







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S.No.	Phytoconstituetent	Result
1	Tannins	-ve
2	Saponins	+ve
3	Alkaloids	-ve
4	Carbohydrates	+ve
5	Protein	+ve
6	Sterols	-ve
7	Volatile oil	-ve
8	Flavonoids	+ve
9	Triterpenoids	+ve
10	Glycoside	+ve
11	Fixed Oil	-ve

# Table 1: The plant-derived compounds found in the BM aqueous extract <sup>[18, 25]</sup>

#### +ve – presence, -ve- absence

# **Pharamacology activity**

Ethanol extract (10 mg/kg) improved motor learning in rats. Hersaponin, the active component and the ethanol extract of *Bacopa monnieri* both demonstrated soothing properties. The active component also decreased noradrenaline and 5-hydroxytryptamine concentration in the central nervous system. Ethanol extract and saponin from rats were demonstrated to have antianxiety properties. There additionally been reports of depression therapy. The anti-Helicobacter pylori and anti-gastric ulcer properties of ethanol extract (50 mg/kg) were demonstrated in both normal and diabetic rats. Additional pharmacological results included those that were antifungal properties, analgesic, antihistamine, an antioxidant, epileptic, heart depression, and cardio-tonic.

# Major therapeutic claims

Antileprotic, antiepileptic, antipyretic, antidiabetic, anti-inflammatory, and anxiolytic.<sup>[26]</sup>

# Antiepileptic

24 participants with an assortment of psychiatric illnesses participated in a clinical study that employed the plant's unrefined watery and reduced-fat alcohol extracts. As stated by the study, epileptic individuals who received two daily doses of a crude watery extract of "brahmi" and lower fat alcoholic extract (2-4 mg/kg b.w.) for five months shown increases in their capacity for learn and correct abnormal behavior. It was discovered that the watery form

of "brahmi" was less efficient than the defatted alcoholic extract in decreasing seizures.<sup>[27]</sup> A placebo-controlled study was carried out in epileptic patients to support the sedative and relaxing effects of crude BMEs (4 patients), *Marsilea minuta* (2 patients), and *Acorus calamus* (6 patients), with particular attention to changes in electroencephalography (EEG). Recuperated patients with petit mal epilepsy and cerebral lobe seizures, respectively, using the defatted alcoholic extract of "brahmi." There was a direct relationship between better clinical results and the EEG changes in each of those cases.<sup>[28]</sup>

#### Antidepressant and anti-anxiety qualities

Studies have demonstrated the efficacy of a BME containing 25% bacoside in treating clinical anxiety in mice. An anxiolytic effect comparable to that of the well-known benzodiazepine calming drug lorazepam. It was noted with great caution that the BME enhanced recall as opposed to producing forgetfulness, that is a lorazepam adverse effect.<sup>[29]</sup> The depressed therapeutic potential of BM was evaluated in a prior study. In the forced swim test and learning assistance tests—the two most popular behavior paradigms in animal models of depression—the results showed a robust antipsychotic effect. BME was given once a day for five days at a dose of 20–40 mg/kg in the trial. It was demonstrated to have psychiatric effectiveness in rats comparable to that of the prescription drug imipramine. The same study claimed the participation of serotonin and gamma amino butyric acid (GABA) in the mechanism of action attributed for its antidepressant characteristics along with its soothing potential, according to extensive proof that the indications of anxious conditions and mood disorders overlap.<sup>[30]</sup>

#### Memory enchancer<sup>[31]</sup>

An investigation was conducted into how well a plant may revive the cognitive powers of forty urban Varanasi pupils in the classroom. One group was given one tablespoon (350 mg) of "brahmi" drink three times a day for a period of three months, whereas the other group got the same amount of "simplex" syrup as a placebo. Throughout the formative period, the perceptual-motor capacities of those receiving "brahmi" were enhanced and renovated. In a single-blind, randomized experiment, 110 male students with an average IQ of 100 were given a single month of treatment with a micromedicine (referred to as "suksma") made from a plant. Positive results were found for boosting math performance, straight memorizing

information, and particular language components of cognitive capacity. Studies having a lengthy duration were judged essential.

# Calming and sedating qualities<sup>[32]</sup>

Effects on the central nervous system: In mice and rats, oral doses of the Ayurvedic medication Brahmi Rasayan ranging from 1 to 30 g/kg were studied. A multiparametric check list was employed for the objective of observational mouse screening. Investigations were conducted into the results of the supplied chemical on shock therapy, chemoconvulsions, motor coordination, tail-withdraws reaction time, haloperidol-induced dementia, and persistent rejection behaviour. The test compound exhibited sedative qualities and significantly prolonged the hallucination effects of pentobarbitone.

# Effects on the central nervous system<sup>[33]</sup>

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#### Endocrine effects<sup>[34]</sup>

Oral BME (200 mg/kg) increased thyroid hormone (T4) levels in mice by 41%. It's probable that the material being removed increases T4 synthesis and/or distribution at the secretory level without affecting T4 to T3 conversion since T3 was not boosted. BMEs caused reversible suppression of sperm production and fertility. The drug altered the somniferous tubules in mice and reduced sperm motility, survival, and the amount of male reproductive cells in the testicles and epididymis.

# **Consequences of stopping morphine**<sup>[35]</sup>

The effect of an alcoholic extract of the whole BM (Scrophulariaceae) plant on morphine withdrawal was evaluated using a living guinea-pig ileum. After exposing the samples to morphine Invitro for 4 minutes, naloxone was brought up on induce a strong shrinking. Afterward, various dosages of the alcoholic BM extract (ranging from 100 to 1000  $\mu$ g/ml) were administered. The naloxone-induced tightness decreased 15 minutes before being exposed to morphine in an orderly fashion. The findings imply that BM extraction might be helpful in lowering the symptoms and indications of stopping morphine.

#### Effects of Scavenging free radicals<sup>[36]</sup>

In herbal medicine therapy, BM is used for the medical intervention of impairment of memory, insomnia, epileptic seizures, and mild drowsiness. This work examined the consequences of BM extract of methanol on DNA cleavage caused by H<sub>2</sub>O<sub>2</sub> UV-photolysis and its capacity in order to absorb oxygen species that are reactive. Additionally, this plant extract's ability to lessen the harmful effects of cytotoxicity and DNA harm brought on by the presence of hydrogen peroxide in human non-immobilized fibroblasts is studied. It revealed a dose-dependent capacity of eliminating dangerous free radicals as well as a protective impact towards DNA breakage. These results were corroborated in individual non-immortalized cells by a significant shielding effect against H<sub>2</sub>O<sub>2</sub>-induced damage to DNA and pathogenicity. Several of the known antistress, immunological modulating, cognitive-enhancing, anti-inflammatory, and antiaging benefits of BM on both clinically and experimental volunteers might be clarified by its possible antioxidant compounds. It could also encourage more examination of the other beneficial aspects of BM. Furthermore, because of its anti-oxidant qualities, this ayurvedic drug may be helpful in managing human conditions where free radical generation occurs is a fundamental element, based on the laboratory findings.

It has been proposed that creatine kinase (CK) as well as its outshoots are effective markers for the assessment of neurodegenerative and cardiac conditions. The evolution of cardiovascular and cerebrovascular diseases is connected to the application of tobacco products. Consequently, the present work reports on the CK isoenzyme profiles in mice that were ingested nicotine from cigarettes and explains how bacoside counteracts the damage brought on by prolonged smoking. Adult smoking cigarettes was introduced to the male Albino rats and administered bacoside A, the plant's active ingredient BM, for a duration of

twelve weeks. The brain, heart, and serum were tested for CK activity, and electrophoresis was utilized to distinguish the isoenzymes of CK in blood plasma from one another. Not only the rats who were exposed to cigarette smoke showed significant increases in all three isoforms in serum, but they also showed significant increases in circulating CK activity and a commensurate decrease in cardiac and cerebral activity. Bacoside A use stopped these smoking-related alterations. Smoking is thought to induce lipid peroxidation (LPO), which is caused by free radicals and damages brain and cardiovascular system cells by increasing membrane permeability. As a result, CK is released into the circulation of blood. Bacoside's antilipid peroxidative and free radical elimination characteristics may account for its favorable impact on the membrane's structural as well as operational integrity, preventing the passage of CK from the relevant tissues.<sup>[37]</sup>

#### Anticonvulsant

Bacosides, or the unrefined plant extract of BM, showed evidence of having anticonvulsive qualities. It provides neuroprotective effects against memory loss associated with pilocarpine-induced seizures additionally glutamate-mediated toxicity during seizures. The seizure efficacy of the ethanol-based extract of BM was assessed using an assortment of convulsive models, including strychnine-injected rats, maximal electroshock, pentylenetetrazol, hypoxic stress-induced convulsions in mice, and lithium-pilocarpine-induced status epilepticus. An oral dosage of 50–55 mg/kg of the ethanol containing extract of BM was administered to rats and mice, two to four hours prior to the equivalent convulsive stimuli. The ethanol-based leaf extract showed significant anticonvulsant effectiveness in all models tested, with a mechanism of action similar to that of benzodiazepines (GABA agonist). <sup>[27,29]</sup>

#### Adaptogenic qualities and antioxidant qualities

Bacosides, or BME for short, have demonstrated antioxidant and antistress properties.<sup>[32]</sup> A previous study implies that the GABAergic system may modulate the effects of BM on the nervous system in the center.<sup>[33]</sup> Based on results from animal investigations, bacosides have demonstrated advantageous antioxidant qualities in the striatum, frontal brain, and hippocampus areas.<sup>[38]</sup> Study conducted on animals has indicated that BMEs alter the way that certain enzymes implicated in the generation and removal of reactive oxygen species inside the brain. It was hypothesised that the adaptogenic properties of BM, which showed promise in stress in a study involving rats, may aid in the treatment of pressure-related

diseases.<sup>[39]</sup> In all areas of the brain, BME was found to increase cytochrome P450 (CYP450) enzymes furthermore to increasing expression of heat-shock protein 70 (Hsp70). Stress was discovered to cause a rise in Hsp70 levels in the brain. Conversely, Hsp70 was seen to be in lower concentration in the group that received 20–40 mg/kg/daily pretreatment for one week prior to receiving stress. Following exposure to pressure alone and with both doses of BME, An increase in the activity rate of the CYP 450-dependent enzymes 7-pentoxyresorufin-odealkkylase and 7-ethoxyresorufin-o-deethylase was seen throughout each brain regions; however, the degree of stimulation was less pronounced with greater quantities of the same substance. thereby was suggested that whatever lack of harsh circumstances, the BM prepared the brain for stress by storing these beneficial molecules, and that using this medicinal plant may lessen our susceptibility to anxiety. To support the hypothesis that this stimulation is a normal reaction to the tension, more research is necessary. Superoxide dismutase (SOD) levels were likewise elevated within the mind of the BME-pretreated groups. The study suggests that BME may regulate the enzymes CYP 450, SOD, and Hsp70, which may help the body's nervous system prepare to operate under stressful conditions.<sup>[40]</sup>

#### Promotion of hair growth<sup>[41-43]</sup>

Hair medicine produced using an ethyl alcohol extracts of *Emblica officinalis*, BM, and *Cyperus rotundus*, either in combination or individually. The scalp oil was prepared with each of these three herbs separately, at varying amounts, and in a consistent ratio, starting with coconut oil as the base. Different amounts of the produced oil were sprayed topically onto the hairless epidermis of Albino rats to assess its physical, chemical, and characteristics related to hair development. The primary irritation of the skin test, the body hair thickness test, and the rate of hair development test were performed using healthy Albino rats and compared to a standard minoxidil 2 % *ethanolic* liquid. The formula of the hair-growth oil was shown to work better than any other product tested; it showed a prolongation of the anagen phase as well as an increase in follicular size.

#### Antibacterial activity

BM's antibacterial efficacy was evaluated using petroleum-based ether, methanol, alcohol, and chloroform towards many bacterial strains. A screening based on plants was carried out to ascertain the chemicals responsible for these activities. Methanol, ethanol, and extracts from chloroform were tested against an assortment of bacteria, including *Aspergillus niger* 

(MTCC 281), *Bacillus pumilus*, *Salmonella typhi*, *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus amyloliquefaciens* (MTCC 1270), *Streptococcus pyogens* (MTCC 1923), and *Vulgarica*. The microbes reaction to the fundamental extraction varied based on the microorganism and extracting solvent, as shown by zones that experienced inhibition. For the majority of the species mentioned above, the methanol extract generated the most level of potency. Given the findings obtained, it is plausible to conclude ethanol may be used for the extraction of antimicrobial substances utilizing leaflets.<sup>[44]</sup>

#### **Gastrointestinal effects:**

Numerous studies on people, animals, and In vitro have looked into how BME affects the gut. Acute spasmolytic impact on colonic muscle smoothness has been revealed In vitro through the decrease in calcium influx across cell membrane channels. This feature suggests that BME could be advantageous for intestinal spasm-producing illnesses like irritable bowel syndrome. The outcomes demonstrated that smooth muscles were directly impacted by the extract.<sup>[41,45]</sup> Furthermore, BME (10-700 mcg/mL) was proven to be directly associated to the influx of calcium ions by lowering the calcium chloride-induced effects noticed in the rabbits' blood vessels and jejunum. Despite this, since the drug under investigation had no impact on contractions induced by noradrenalin or caffeine, the researchers concluded that it had no discernable effect on the intracellular mobilization calcium. Considering study's findings, a suggestion is proposed that the decrease in calcium influx is what primarily causes BME's spasmolytic effect on smooth muscles. This idea is applicable to calcium channels found in cell membranes that are triggered by electrical impulses additionally receptor impulses. Depending on In vitro and experimental animal research, reports of BM's antiulcerogenic action as well as its preventative and curative properties on ulceration of the stomach have been made.<sup>[46,47]</sup>

The preventative and therapeutic effects of a BME standardized for bacoside A were evaluated in five types of stomach ulcers in rats.<sup>[48]</sup> When given for 10 days at a dosage of 20 mg/kg, BME significantly enhanced the mucosal barrier's function, decreased mucosal exfoliation, and healed entering lesions brought on by acetic acid. Furthermore, the extract avoided lesions caused by stress, as demonstrated by a noteworthy decrease in LPO in the animal's gastrointestinal mucosa. The impact was believed to be explained by the antioxidant properties the capability of BM to keep the levels of SOD and catalase in equilibrium. Additionally, a new In vitro research shown its unique antibacterial efficaciousness against *H*.

*pylori*, a bacterium connected to chronic ulcers in the digestive system. Prostaglandins that are thought to help safeguard the interior of the stomach, prostacyclin accumulated in the liquid extract when it was cultivated with H. pylori and human mucosal cells of the colon.

### Membrane stabilizing activity:

The stabilizing membrane's properties of botanical materials was investigated using heatinduced and hypotonicity-induced hemolysis assays.<sup>[49]</sup>

#### The thrombolytic action:

The sample was exposed to thrombolytic testing according to standard protocol. Positive and negative thrombolytic controls were streptokinase, SK (30,000 I.U.), and water from pure sources, respectively.<sup>[50]</sup>

# Antioxidant potential:

The ability of antioxidant was evaluated using the DPPH test method. In conclusion, DPPH solution (20  $\mu$ g/ml) in methanol was mixed with the test specimens of *B. monnieri* that had been submerged in alcohol. The degree of sensitivity was 517 nm in measurement after 20 minutes of incubation. In this case, ascorbic acid (AA) and tert-butyl-1-hydroxytoluene (BHT) were employed as comparative standards.<sup>[51]</sup>

# **Cytotoxic activity:**

The samples that were utilized to assess the cytotoxic activity among the substances under test were brine shrimps, or *Artemia salina* Leach.<sup>[52]</sup> Dimethyl sulfoxide accustomed to produce the test sample to the desired amount (DMSO). Plant-based extracts were put into vials with nauplii submerged in artificial seawater. After a day, the chambers were inspected, and the quantity of viable nauplii was computed. It was determined what percentage of the nauplii were fatal for the various samples.

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