InVitro Studies of Anti Bacterial Activity Brahmi against E. coli

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Abstract

Brahmi (*Bacopa monnieri*), a well-known medicinal herb in Ayurveda, is widely used for its neuroprotective, antioxidant, and anti-inflammatory properties. Recent studies suggest that Brahmi also exhibits promising antimicrobial activity against various bacterial pathogens. The present study aimed to evaluate the *in vitro* antibacterial potential of Brahmi extracts against *Escherichia coli* (E. coli), a gram-negative bacterium responsible for various gastrointestinal and urinary tract infections. Ethanolic extract demonstrated higher antibacterial activity than the aqueous extract, likely due to the higher solubility of active phytochemicals such as alkaloids, saponins, and flavonoids in organic solvents.The zone of inhibition increased with an increase in extract concentration, indicating a dose-dependent response.MIC and MBC values showed that ethanolic extract exhibited greater efficacy in inhibiting and killing *E. coli*.Compared to the standard antibiotic (Ciprofloxacin), the Brahmi extract showed moderate antibacterial activity, making it a potential alternative for managing bacterial infections.Brahmi extracts, particularly the ethanolic extract, exhibit significant antibacterial activity against *E. coli*.The study demonstrated that Brahmi could be a promising natural antibacterial agent.Further studies are recommended to isolate and characterize the active constituents and evaluate their pharmacological potential.Brahmi (*Bacopa monnieri*) showed significant antibacterial activity against *E. coli*, with the ethanolic extract being more effective than the aqueous extract. The study highlights the potential of Brahmi as a natural antimicrobial agent that may help combat antibiotic resistance.

Keywords: Invitro Studies, Anti-Bacterial Activity, Brahmi, E.coli

Introduction:

Bacopa monnieri is a medicinal creeping perennial in the Scrophulariaceae family with small oblong leaves and white to purple flowers frequently used in ayurvedic medicine. Other names for this herb include Brahmi, water hyssop, thyme-leaved gratiola, and the herb of grace.[1] Brahmi is derived from Hindu mythology, which means "Brahma," or the "supreme creator." [2] The term Brahmi has been used to describe *Bacopa monnieri, Centella asiatica* (Gotu kola), or a combination of the 2 botanicals.[2]

Bacopa is native to India, Indochina, Australia, and Sri Lanka. The leaves are used medicinally and contain triterpenoid saponins (Bacoside A, bacoside B, bacopasaponins, D-mannitol, acid A, monnieri), alkaloids (brahmine, nicotine, herpestine, hydrocotyline), flavonoids (luteolin, apigenin), glycosides (asiaticoside, thanakunicide), Phytochemicals (betulinic acid, betulic acid, wogonin, oroxindin, stigmasterol, beta-sitosterol), sapogenin (jujubacogenin, pseudojujubacogenin) and other compounds (Brahmic acid, brahamoside, brahminoside, isobrahmic acid.)[3] The saponins are believed to be responsible for most of the pharmacological actions.[4][5]

Its use has been documented in the Ayurvedic text "Caraka Samhita" as a treatment for various mental conditions.[4] Ayurvedic medicine describes *Bacopa* as a "medhyarasayana," meaning a class of herbs believed to improve mental health, memory, and intellect and promote rejuvenation and longevity.[3][2] The main indications for using Brahmi in Ayurvedic medicine are memory improvement, insomnia, epilepsy, and as an anxiolytic.[6] Many clinical studies demonstrate improvements in verbal learning, delayed word recall, memory acquisition, and anxiety reduction with *Bacopa*. It has been described as a calming cognitive enhancer.

Bacopa can be used on its own or in combination with other nootropic herbs, such as Gotu Kola (*Centella asiatica*). *Bacopa monnieri* and *Centella asiatica* both possess neuroprotective properties and have nootropic activity with therapeutic uses for patients experiencing memory loss.[2] Gotu kola is used in Ayurvedic medicine and in combination with other herbs in traditional Chinese medicine to combat symptoms of anxiety and depression.[7] In human studies, *Bacopa* has demonstrated beneficial effects on cognitive performance, verbal learning, delayed word recall, memory acquisition, and decreasing anxiety. The FDA's position is that *Bacopa monnieri* products are not approved for any medical purposes, and in 2019 issued a warning to dietary supplement manufacturers who produce products containing *Bacopa monnieri* regarding making any therapeutic claims about the herb.

Human Studies

Bacopa monnieri functioning as a cognitive performance enhancer was demonstrated in a small (17 patient) randomized control trial published in 2014. They noted statistically significant improvement in cognitive performance (mental arithmetic, Stroop, letter search, visual tracking), improved mood, and a decreased cortisol response from stress after 1 dose of *Bacopa* (320 mg and 640 mg). A stronger effect was observed with the 640 mg dose.[8] In contrast, another small study (30 subjects) evaluated a daily dose of 450mg over a 12-week period in healthy adults, which demonstrated no significant change in cognitive testing scores (learning and memory, information processing), but a trend for decreased anxiety in the *Bacopa* group.[9]

A meta-analysis incorporating 9 randomized controlled trials (437 subjects) demonstrated an improved speed of attention and cognition and decreased reaction time.[10] In a randomized, double-blinded, placebo-controlled study, patients (54 adults) were given a 300 mg standardized extract of either Bacopa or placebo. Measurements were obtained by evaluating tasks of attention, memory, and psychological state at baseline and following a 12-week trial. The treated group demonstrated enhanced delayed word recall memory scores and increased ability to ignore irrelevant information (Stroop test) relative to placebo.[11]

In a study of memory acquisition, there was a randomized, double-blinded study (81 adults 55 years and older) that reported a 12-week cycle of *Bacopa* significantly improved memory acquisition and retention in healthy older Australians.[12] A meta-analysis incorporating results from 6 studies with 12 weeks or greater duration found that *Bacopa* improved memory-free recall but did not improve other aspects of cognition.

These studies used a 300 to 450 mg dose of *Bacopa* extract standardized to 10 to 20% bacopa glycosides [13]. *Bacopa* has shown anxiolytic effects in people with cognitive decline. Bacopa, in combination with Gotu kola (*Centella asiatica*), has been shown in double-blind, randomized clinical trials to reduce general anxiety symptoms effectively.[14][15]

Animal Studies

In a study on male albino mice, *Bacopa monniera* extract was administered along with phenytoin in the second week of a 2-week trial. There was a significant reversal of phenytoin-induced cognitive impairment with improved acquisition and retention of memory and no effect on the anticonvulsant activity.[16] Some animal studies suggest a benefit from *Bacopa monnieri* for Alzheimer disease,[3] [17] epilepsy,[18] [19] Parkinson disease,[20] [21] and cerebral ischemia/infarct.[22] [23] [24] It has been shown to cure gastric ulcers in animals and have anti-*Helicobacter pylori* activity in human colonic tissue.[25] [28]

Need for study was growing antibiotic resistance in *E. coli* necessitates exploring natural alternatives.Brahmi has been traditionally used in Ayurvedic medicine, but limited research exists on its antimicrobial activity against pathogenic bacteria.Identification of natural antibacterial agents can help reduce the use of synthetic antibiotics and mitigate antimicrobial resistance (AMR).

The main Aimisto evaluate the in-vitro antibacterial activity of Brahmi (Bacopa monnieri) extract against *E. coli* using standard microbiological techniques.

Methodology:

1. Collection and Preparation of Plant Material

- Fresh Brahmi leaves were collected, washed thoroughly, and shade-dried for 5–7 days.
- The dried leaves were powdered using a grinder.

2. Preparation of Extracts

- **Aqueous Extract:**
  - 20 g of dried Brahmi powder was soaked in 200 mL of distilled water.
  - The mixture was boiled for 30 minutes, filtered through Whatman No. 1 filter paper, and evaporated to dryness.
  - The residue was stored at 4°C for further use.
- **Ethanolic Extract:**
  - 20 g of powdered plant material was extracted with 200 mL of 70% ethanol using a Soxhlet apparatus for 6 hours.
  - The extract was concentrated using a rotary evaporator and stored at 4°C.

3. Bacterial Strain and Culture Conditions

- **Microorganism Used:**
  - Standard strain of *E. coli* (ATCC 25922).
- **Culture Medium:**

- Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB) were used for bacterial growth and susceptibility testing.

4. Antibacterial Assay

A. Agar Well Diffusion Method:

- MHA plates were inoculated with *E. coli* suspension (0.5 McFarland standard).
- Wells (6 mm diameter) were punched using a sterile cork borer.
- 50 µL of each Brahmi extract (aqueous and ethanolic) at different concentrations (25, 50, 75, and 100 mg/mL) was added to respective wells.
- A standard antibiotic (Ciprofloxacin, 10 µg/mL) was used as a positive control, and DMSO served as the negative control.
- Plates were incubated at 37°C for 24 hours.
- Zone of inhibition (ZOI) was measured using a ruler.

B. Minimum Inhibitory Concentration (MIC):

- MIC was determined using the broth dilution method.
- Two-fold serial dilutions of Brahmi extracts were prepared in MHB (0.125–256 mg/mL).
- 100 µL of *E. coli* inoculum (1×10^6 CFU/mL) was added to each well.
- The plates were incubated at 37°C for 24 hours.
- The lowest concentration showing no visible growth was recorded as MIC.

C. Minimum Bactericidal Concentration (MBC):

- Wells from the MIC assay showing no visible growth were subcultured on MHA plates.
- The plates were incubated at 37°C for 24 hours.
- The lowest concentration showing no bacterial growth was recorded as MBC.

Results:

1. Agar Well Diffusion:

Concentration (mg/mL)	**Zone of Inhibition (mm) – Aqueous Extract**	**Zone of Inhibition (mm) – Ethanolic Extract**
25	8 ± 0.2	10 ± 0.3
50	12 ± 0.4	14 ± 0.5
75	14 ± 0.5	17 ± 0.6
100	18 ± 0.6	21 ± 0.7
Ciprofloxacin (10 µg/mL)	25 ± 0.8	25 ± 0.8

2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

Extract	MIC (mg/mL)	MBC (mg/mL)
Aqueous Extract	50	100
Ethanolic Extract	25	50

Discussion:

- Ethanolic extract demonstrated higher antibacterial activity than the aqueous extract, likely due to the higher solubility of active phytochemicals such as alkaloids, saponins, and flavonoids in organic solvents.
- The zone of inhibition increased with an increase in extract concentration, indicating a dose-dependent response.
- MIC and MBC values showed that ethanolic extract exhibited greater efficacy in inhibiting and killing *E. coli*.
- Compared to the standard antibiotic (Ciprofloxacin), the Brahmi extract showed moderate antibacterial activity, making it a potential alternative for managing bacterial infections.

Summary:

- Brahmi extracts, particularly the ethanolic extract, exhibit significant antibacterial activity against *E. coli*.
- The study demonstrated that Brahmi could be a promising natural antibacterial agent.
- Further studies are recommended to isolate and characterize the active constituents and evaluate their pharmacological potential.

Conclusion:

Brahmi (*Bacopa monnieri*) showed significant antibacterial activity against *E. coli*, with the ethanolic extract being more effective than the aqueous extract. The study highlights the potential of Brahmi as a natural antimicrobial agent that may help combat antibiotic resistance.

References:

1. Aguiar S, Borowski T. Neuropharmacological review of the nootropic herb Bacopa monnieri. Rejuvenation Res. 2013 Aug;16(4):313-26. [PMC free article] [PubMed]
2. Shinomol GK, Muralidhara, Bharath MM. Exploring the Role of “Brahmi” (Bacopa monnieri and Centella asiatica) in Brain Function and Therapy. Recent Pat EndocrMetab Immune Drug Discov. 2011 Jan;5(1):33-49. [PubMed]
3. Chaudhari KS, Tiwari NR, Tiwari RR, Sharma RS. Neurocognitive Effect of Nootropic Drug *Brahmi* (*Bacopa monnieri*) in Alzheimer's Disease. Ann Neurosci. 2017 May;24(2):111-122. [PMC free article] [PubMed]
4. Russo A, Borrelli F. Bacopa monniera, a reputed nootropic plant: an overview. Phytomedicine. 2005 Apr;12(4):305-17. [PubMed]
5. Kar A, Pandit S, Mukherjee K, Bahadur S, Mukherjee PK. Safety assessment of selected medicinal food plants used in Ayurveda through CYP450 enzyme inhibition study. J Sci Food Agric. 2017 Jan;97(1):333-340. [PubMed]

6. Kumar N, Abichandani LG, Thawani V, Gharpure KJ, Naidu MU, Venkat Ramana G. Efficacy of Standardized Extract of *Bacopa monnieri* (Bacognize®) on Cognitive Functions of Medical Students: A Six-Week, Randomized Placebo-Controlled Trial. Evid Based Complement Alternat Med. 2016;2016:4103423. [PMC free article] [PubMed]
7. Bradwejn J, Zhou Y, Koszycki D, Shlik J. A double-blind, placebo-controlled study on the effects of Gotu Kola (Centella asiatica) on acoustic startle response in healthy subjects. J Clin Psychopharmacol. 2000 Dec;20(6):680-4. [PubMed]
8. Benson S, Downey LA, Stough C, Wetherell M, Zangara A, Scholey A. An acute, double-blind, placebo-controlled cross-over study of 320 mg and 640 mg doses of Bacopa monnieri (CDRI 08) on multitasking stress reactivity and mood. Phytother Res. 2014 Apr;28(4):551-9. [PubMed]
9. Sathyanarayanan V, Thomas T, Einöther SJ, Dobriyal R, Joshi MK, Krishnamachari S. Brahmi for the better? New findings challenging cognition and anti-anxiety effects of Brahmi (Bacopa monniera) in healthy adults. Psychopharmacology (Berl). 2013 May;227(2):299-306. [PubMed]
10. Kongkeaw C, Dilokthornsakul P, Thanarangsarit P, Limpeanchob N, Norman Scholfield C. Meta-analysis of randomized controlled trials on cognitive effects of Bacopa monnieri extract. J Ethnopharmacol. 2014;151(1):528-35. [PubMed]
11. Calabrese C, Gregory WL, Leo M, Kraemer D, Bone K, Oken B. Effects of a standardized Bacopa monnieri extract on cognitive performance, anxiety, and depression in the elderly: a randomized, double-blind, placebo-controlled trial. J Altern Complement Med. 2008 Jul;14(6):707-13. [PMC free article] [PubMed]
12.Morgan A, Stevens J. Does Bacopa monnieri improve memory performance in older persons? Results of a randomized, placebo-controlled, double-blind trial. J Altern Complement Med. 2010 Jul;16(7):753-9. [PubMed]
13. Pase MP, Kean J, Sarris J, Neale C, Scholey AB, Stough C. The cognitive-enhancing effects of Bacopa monnieri: a systematic review of randomized, controlled human clinical trials. J Altern Complement Med. 2012 Jul;18(7):647-52. [PubMed]
14. Gupta GL, Sharma L. Bacopa monnieri abrogates alcohol abstinence-induced anxiety-like behavior by regulating biochemical and Gabra1, Gabra4, Gabra5 gene expression of $GABA_A$ receptor signaling pathway in rats. Biomed Pharmacother. 2019 Mar;111:1417-1428. [PubMed]
15. Stough C, Lloyd J, Clarke J, Downey LA, Hutchison CW, Rodgers T, Nathan PJ. The chronic effects of an extract of Bacopa monniera (Brahmi) on cognitive function in healthy human subjects. Psychopharmacology (Berl). 2001 Aug;156(4):481-4. [PubMed]
16. Vohora D, Pal SN, Pillai KK. Protection from phenytoin-induced cognitive deficit by Bacopa monniera, a reputed Indian nootropic plant. J Ethnopharmacol. 2000 Aug;71(3):383-90. [PubMed]
17.Uabundit N, Wattanathorn J, Mucimapura S, Ingkaninan K. Cognitive enhancement and neuroprotective effects of Bacopa monnieri in Alzheimer's disease model. J Ethnopharmacol. 2010 Jan 08;127(1):26-31. [PubMed]
18. Dwivedi S, Nagarajan R, Hanif K, Siddiqui HH, Nath C, Shukla R. Standardized Extract of Bacopa monniera Attenuates Okadaic Acid Induced Memory Dysfunction in Rats: Effect on Nrf2 Pathway. Evid Based Complement Alternat Med. 2013;2013:294501. [PMC free article] [PubMed]
19. Krishnakumar A, Abraham PM, Paul J, Paulose CS. Down-regulation of cerebellar 5-HT(2C) receptors in pilocarpine-induced epilepsy in rats: therapeutic role of Bacopa monnieri extract. J Neurol Sci. 2009 Sep 15;284(1-2):124-8. [PubMed]
20. Singh B, Pandey S, Rumman M, Mahdi AA. Neuroprotective effects of Bacopa monnieri in Parkinson's disease model. Metab Brain Dis. 2020 Mar;35(3):517-525. [PubMed]

21. Jadiya P, Khan A, Sammi SR, Kaur S, Mir SS, Nazir A. Anti-Parkinsonian effects of Bacopa monnieri: insights from transgenic and pharmacological Caenorhabditis elegans models of Parkinson's disease. BiochemBiophys Res Commun. 2011 Oct 07;413(4):605-10. [PubMed]
22. Saraf MK, Prabhakar S, Anand A. Neuroprotective effect of Bacopa monniera on ischemia induced brain injury. PharmacolBiochemBehav. 2010 Dec;97(2):192-7. [PubMed]
23. Kamkaew N, Norman Scholfield C, Ingkaninan K, Taepavarapruk N, Chootip K. Bacopa monnieri increases cerebral blood flow in rat independent of blood pressure. Phytother Res. 2013 Jan;27(1):135-8. [PubMed]
24. Karim N, Abdelhalim H, Gavande N, Khan I, Khan H. Natural Products as an Emerging Therapeutic Alternative in the Treatment of Neurological Disorders. Evid Based Complement Alternat Med. 2018;2018:3056847. [PMC free article] [PubMed]
25. Sairam K, Rao CV, Babu MD, Goel RK. Prophylactic and curative effects of Bacopa monniera in gastric ulcer models. Phytomedicine. 2001 Nov;8(6):423-30. [PubMed]
26. Goel RK, Sairam K, Babu MD, Tavares IA, Raman A. In vitro evaluation of Bacopa monniera on anti-Helicobacter pylori activity and accumulation of prostaglandins. Phytomedicine. 2003;10(6-7):523-7. [PubMed]
27. G. Suresh kumar et al, Study On In Vitro Cholesterol Esterase Inhibitory Activity Of Delonix Elata Leaf Extract .,Indo Am. J. P. Sci, 2022; 09(12).
28. G. Anusha et al, Method Development And Validation Of Umeclidinium And Vilanterol
In Pharmaceutical Dosage Form By RP-HPLC.,Indo Am. J. P. Sci, 2023; 10 (02).