Quest Journals
Journal of Research in Pharmaceutical Science
Volume 10 ~ Issue 3 (2024) pp: 44-48
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

A Nobel Approach to Create Bioavailable *Bacopa* Extract and Bitterless Ebelin Lactone: Innovative Process and Composition

Devaraj Reddy KN[1,] *, Srilakshmi Aluri[1,]*, Prathvi Shetty[1], Shreya Udaya[1], Yadunandan BM[1], Sudhanva MS[2], Shobith Rangappa[2]

[1] *Prakruti Products Pvt. Ltd., No. 405, Vasanthanarasapura, Phase 2, Industrial Area, Tumakuru, Karnataka, India*

[2] *Adichunchanagiri Institute of Molecular Medicine, Adichunchanagiri University, B. G Nagar, Mandya, Karnataka, India*

[*]*Corresponding authors: Devaraj Reddy KN and Srilakshmi Aluri*

Abstract: *In India, Bacopa monnieri, sometimes referred to as "Brahmi," is readily available. Ancient Ayurvedic literature mention this plant as a memory enhancer since it harbours neuroprotective properties in addition to other pharmacological characteristics and has been utilized for centuries. Since B. monnieri is administered orally, it must be CNS-active in order to cater its nootropic effects. Parent Bacosides lack this absorption capability. The procedure described in the current study produces a stable, highly bioavailable non-hygroscopic Bacosides enriched fraction in such a manner that it contains a specific amount of Bacoside A3, jujubogenin, and the aglycone derivative. In addition, the study offers a technique for increasing the bioactivity of the composition even at lower doses with various formulations without using any pharmaceutical excipients, thus enhancing absorption. Preparation of bitterless bacopa extract enriched with a high concentration of Bacoside A3, jujubogenin, and Ebelin lactone. Preparation of a composition of Bacopa extract to enhance the membrane permeability and bioavailability for oral administration. The disclosed composition includes an Ebelin lactone and a Bacoside where, bacopa extract makes up 20–50% of the total Bacosides that are enhanced with jujubogenin and Bacoside A3. The disclosed composition of Bacosides and aglycone derivatives in a 1:1 ratio can be administered to individuals. This approach of generating highly bioavailable, non-hygroscopic Bacosides that are enriched in Bacoside A3, jujubogenin, and the aglycone derivative Ebelin lactone has been enhanced, made more economical, and environmentally friendly. The Bacopa extract composition comprises 20–50% of the total Bacosides enriched with Bacoside A3, jujubogenin and aglycone derivative Ebelin lactone and is highly bioabsorbable compared to parent Bacosides.*

KEYWORDS: *Bacopa monnieri, Bacoside A3, bioasorption, Ebelin lactone, jujubogenin*

***R**eceived 15 Mar., 2024; **R**evised 28 Mar., 2024; **A**ccepted 30 Mar., 2024 © The author(s) 2024.*
P**ublished with open access at **www.questjournals.org

I. INTRODUCTION

India is home to the annual plant known as *Bacopa monnieri*. Ancient Ayurvedic scriptures claim that this plant has been employed for millennia as a memory tonic. This healing plant, also known as "Brahmi," is widely distributed throughout India and is a member of the Scrophulariaceae family. For many centuries, people of all ages have used a concoction prepared from this plant extract as a mental stimulant. This herb has been effectively utilized in conventional medicine to treat a number of neurological disorders [1]. Extensive study on this herb extract has demonstrated that *B. monnieri* is beneficial for alleviating anxiety, impaired cognitive ability, reducing depression, improving focus, and possibly being successful in preventing Alzheimer's illness [2-5]. In addition to its numerous neuroprotective properties, this plant possesses additional pharmacological properties, including possible antioxidant potential, anti-inflammatory activity, aids in digestion, and treats ulcers [2, 6]. This particular medicinal plant is an excellent source of a variety of bioactive phytochemicals, the majority of which are secondary metabolites. These metabolites support this plant's wide range of functions. The metabolites have superior pharmacokinetic properties and biological activity as compared to the parent saponins [7]. The scientists also investigated this specific plant's potential against other diseases, including cancer, in light of its therapeutic impact on the complex disorders discussed above [8].

The primary bioactive components thought to be responsible for *B. monnieri*'s cognitive benefits are distinctive saponins known as "Bacosides," particularly Bacoside A3 [9]. This active component belongs to the large class of plant secondary metabolites known as glycosides. These glycosides that provide the pharmacological action in *B. monnieri* must be orally and CNS active in order to cater the neuropharmacological mechanisms underlying the nootropic effects [8]. This means that the substance must pass through the BBB and be absorbed through the gut. Parent constituents such as bacopasaponin C, bacopaside X, bacopaside II, and Bacoside A3 individually have very limited intestinal absorption and no BBB penetration. The aglycones jujubogenin, pseudojujubogenin, Ebelin lactone, and bacogenin A1, on the other hand, displayed substantial BBB penetration and were readily absorbed into the gut. Ebelin lactone showed the highest BBB permeability of the substances and it was anticipated that it would only be minimally absorbed into the gut [10]. In this study, we aim to increase the bioabsorption of *B. monnieri* by enriching non-hygroscopic Bacosides with Bacoside A3, jujubogenin, and the aglycone derivative Ebelin lactone. This mixture is highly bioabsorbable in comparison with parent Bacosides and is palatable for human consumption.

II. MATERIALS AND METHODS

2.1 Preparation of bioactive enriched bacopa extract with a high concentration of Bacoside A3 and jujubogenin

2.1.1 In the process involving dried B. monnieri plant material, the first step (a) entailed conducting solvent extraction with alcohol, where the alcohol utilized was methanol, ethanol, and/or hydroalcohol, at a temperature of 70°C to yield a first extract solution. Subsequently, in step (b), the first extract solution underwent filtration. Step (c) involved repeating the extraction procedure of (a) at least three times using the retentate obtained from (b). The pooled extract solutions were then concentrated to obtain an extract containing Bacosides. To eliminate the alcohol entirely, water was added, and the extract was concentrated to 30% Total Dissolved Solids (TDS). The concentrated extract was subjected to spray drying at a flow rate of 100L/hr while maintaining the inlet temperature at 90-110 °C, resulting in the production of a B. monnieri extract.

2.1.2 Enrichment of the Bacopa extract was achieved by extracting the spray-dried powder with hydro alcohol in different ratios of alcohol and water selected from a ratio of 1: 3 V, 2: 6 V, and 3: 9 V. The preferable volume of ethanol and water is 3:9 V. Leaving the extract for 8–10 hours allows the Bacosides to precipitate. By filtering the precipitate in a filter press, the precipitate is enriched with Bacosides having a high concentration of aglycones like jujubogenin and pseudojujubiogenin, and by tray drying the filtered precipitate at 90 °C, a stable, free-flowing fraction of *B. monnieri* rich in Bacosides is obtained.

2.1.3 Enrichment of aglycones was achieved by providing 2-3 times of organic solvent (acetone) wash because the aglycones possess greater CNS active characteristics than the parent Bacosides. Washing *B. monnieri* with organic solvent increases the aglycone concentration, which is high in Bacosides, accounting for 30-50% of total Bacosides.

2.2 Preparation of bitterless bacopa extract enriched with aglycone derivative Ebelin lactone

2.2.1 An enriched herbal extract of B. monnieri was dissolved in 1:4 volumes of aqueous ethanol, ranging from 50 to 75% ethanol or methanol. The preferred percentage of ethanol or methanol was 75%. The enriched B. monnieri extract with a high concentration of aglycone was subjected to acid hydrolysis with mineral acid, preferably sulphuric acid with a concentration of 2 N H2SO4. The extract was refluxed for 4 hours at 90 °C, stirring occasionally.

2.2.2 After the reflux, the material was allowed to cool to room temperature. 1:1 V water was added to the reaction mass along with continuous stirring to precipitate the aglycone derivatives. The completion of hydrolysis was checked by High-Performance Liquid Chromatography (HPLC) or Thin Layer Chromatography (TLC). Once the acid hydrolysis was complete, the precipitate was filtered, and the residue was rinsed with demineralized water to produce an acid-free precipitate containing the extract with a pH of 7.

2.2.3 Further enrichment and purification of aglycone derivatives were achieved by crystallization with alcohol or a polar organic solvent. The precipitate was dissolved in methanol or ethanol at 50–65°C in 1:1 V to 1:3 V, preferably 1:2 V ethanol or methanol, and allowed to crystallize at room temperature. The crystallization was repeated 2–3 times, and the precipitate was filtered. The precipitate was dried at 70°C to obtain Ebelin lactone, an enriched aglycone derivative.

2.3 Identification of composition of Bacopa extract to enhance the membrane permeability and bioavailability

We formed a suspension of the Bacosides with a high concentration of jujubogenin (Bacoside A3) in water; mixing the suspension with Ebelin lactone; The composition containing the Bacosides and the Ebelin lactone is homogenized to create a fine slurry, which is then dried under heat and vacuum to create a consistent blend.

We prepared a composition of a Bacoside and an Ebelin lactone wherein bacopa extract comprises 20–50% of the total Bacosides enriched with Bacoside A3 and jujubogenin. We prepared various composition of a Bacoside and an Ebelin lactone wherein the weight ratio of the Bacosides to the Ebelin lactone varies from about 3:1 to about 99:1. The ratio in one embodiment is roughly 85:15. Another embodiment has a ratio of roughly 90:10. Another embodiment has a ratio of roughly 95:5. The weight ratio in a different embodiment is approximately 10:1.

2.4 Preparation of a tablet containing composition of Bacosides and Ebelin lactone for oral administration

The process for crafting a pharmaceutical excipient-free organic herbal tablet formulation, as outlined in the present study, commenced with the preparation of a predefined quantity of enriched bacopa extract and the aglycone derivative Ebelin lactone. Following this initial step, the obtained materials underwent sieving and blending. Subsequently, the sieved and blended mixture of enriched bacopa extract and aglycone derivative Ebelin lactone was granulated using a granulation fluid of purified water. After that, the granules obtained underwent a drying process in a tray drier at a temperature range of 60°C to 70°C for about 4 hours. Following the drying stage, the oversized granules, consisting of the enriched bacopa extract and aglycone derivative Ebelin lactone, were milled, and the resulting granules were sieved to achieve the desired size. After this step, the sized granules of the enriched bacopa extract and aglycone derivative Ebelin lactone were blended. Finally, the blended mixture was tableted by compressing it using a compression apparatus, resulting in the creation of a more readily compacted herbal tablet composition.

III. RESULTS AND DISCUSSION

3.1 Preparation of bioactive enriched bacopa extract with a high concentration of Bacoside A3 and jujubogenin

In the process for producing enriched fractions of the Bacosides with a high concentration of aglycones from plant materials of Bacopa species, the plant materials of the Bacopa species were subjected to solvent extraction. The resulting extract was dried, and the dried mass was washed with hydroalcohol. The residue was then extracted with at least one organic polar solvent, and the resulting residue was dried under vacuum to obtain a concentrate containing 20-50% of Bacosides enriched with highly bioavailable aglycones.

According to the study, a composition of a Bacoside with a high concentration of Bacoside A3 and jujubogenin, along with an Ebelin lactone, was provided. The Ebelin lactone was available in a sufficient amount to increase membrane permeability and bioabsorption of the bacopa extract when the composition was administered compared to the bioabsorption of the parental Bacosides obtained upon the administration of a composition of bacopa extract that was prepared without the addition of Ebelin lactone.

The study further presented a composition of a Bacoside with a high concentration of Bacoside A3 and jujubogenin, along with an Ebelin lactone, where the improvement of membrane permeability and bioabsorption of the Bacosides ranged from approximately 5-fold to about 18-fold.

3.2 Composition of Bacopa extract to enhance the membrane permeability and bioavailability

Memory-improving benefits have been linked to the traditional Ayurvedic Herb B. *monnieri* [11]. The parent Bacosides fail to fulfill the requirements for oral CNS therapeutic alternatives, namely molecular weight, hydrogen-bonding ability, and molecular flexibility. Due to these adverse physicochemical characteristics of the parent Bacosides, the blood-brain barrier and intestine's low membrane permeability are most likely affected. Hence, the parental Bacoside enrichment is achieved with a high concentration of more bioavailable aglycones and their derivatives. The aglycones, on the other hand, demonstrated superior CNS drug-like characteristics by satisfying four of the essential requirements. Aglycosylation of the parent Bacoside increased their lipophilicity in comparison to their corresponding parent Bacosides. Small compounds are passively transported through the BBB in considerable amounts by lipids. Aglycones' growing lipophilicity also appears to boost their ability to penetrate the brain.

A composition having an enriched bacopa extract with a high concentration of Bacoside A3, jujubogenin, and bitterless aglycone derivative Ebelin lactone, wherein the Ebelin lactone is present in an amount adequate to produce an elevation in membrane permeability and abosrption compared to the bioabsorption of parental Bacosides formulated without adding Ebelin lactone to the parental Bacosides. A procedure for preparing a composition having an enriched bacopa extract and an Ebelin lactone is achieved. The main constituents of Bacosides are Bacoside A3 and jujubogenin, and the main constituent of the aglycone derivative is Ebelin lactone.

Thus, in order to reap the full benefits of bacopa extract intake, methods and techniques to improve its membrane permeability and bioavailability must be investigated. This innovation is an attempt in that direction. When a proportion (25-50%) of an aglycone derivative's Ebelin lactone was added to the bacopa extract, the membrane permeability and bioavailability of parental Bacosides were considerably increased. As a result, a mixture of parental Bacosides and an appropriate quantity of Ebelin lactone (an aglycone derivative) was provided. Hence, the study provides a composition of Bacosides with a high concentration of Bacoside A3, jujubogenin, and an Ebelin lactone of an aglycone derivative.

3.3 Formulation of tablet containing a composition having Bacosides and Ebelin lactone for oral administration

A mixture of aglycone derivatives and Bacosides were combined to create tablets that had a 500 mg dosage. A 500 mg tablet with a weight ratio of around 1:1 between the Bacosides and the aglycone derivative was estimated to contain roughly 250 mg of Bacosides, with 50% of the Bacoside made up of Bacosides, and roughly 250 mg of aglycone derivative. The active components was around 125 mg of Bacosides and approximately 25 mg of Ebelin lactone. Aglycone derivatives range in size from 125 mg to 250 mg, and the tablet included 250 mg - 375 mg of Bacosides in some embodiments.

Hence, the Bacopa extract composition comprises 20–50% of the total Bacosides enriched with Bacoside A3, jujubogenin and aglycone derivative Ebelin lactone is highly bioabsorbable compared to parent Bacosides. The disclosed compositions of Bacosides and aglycone derivatives in the ratio 1:1 can be administered to an individual to cure ailments like Alzheimer's, stress-induced depression, cancer and fatigue as discussed in recent studies [12-14]. This innovative composition further benefits from the fact that the aglycone derivative components themselves are bioactive due to their high levels of free radical scavenging activity, acetylcholinesterase inhibition activity, anti-inflammatory activity, high bioavailability, and lack of toxicity. Thus, the bacopa composition is anticipated to work in concert to increase the bioactivity of Bacosides. Successful in vitro and in vivo studies on synergistic neuroprotection and anti-inflammatory effects by phytocompounds of *Bacopa monnieri* support idea of the present study [15, 16].

IV. Conclusion

The present study is economical and also environmentally beneficial to provide a stable and highly bioavailable non-hygroscopic Bacosides enriched fraction that comprises a specified quantity of jujubogenin, Bacoside A3, and the aglycone derivative Ebelin lactone from the herb *B. monnieri*. The disclosed composition contains a Bacoside and an Ebelin lactone, with bacopa extract constituting 20–50% of the total Bacosides enriched with jujubogenin and Bacoside A3. The study also provides a method of improving the bioactivity of said composition even at lower doses with various formulations without using any pharmaceutical excipients, thus enhancing absorption.

References

[1]. Murthy, H.N. (2022). Biotechnological production of Bacosides from cell and organ cultures of Bacopa monnieri. Appl Microbiol Biotechnol. 06(5-6):1799-1811.
[2]. Russo, A., & Borrelli, F. (2005). Bacopa monniera, a reputed nootropic plant: an overview. Phytomedicine, 12(4), 305-317.
[3]. Sairam, K., Dorababu, M., Goel, R. K., & Bhattacharya, S. K. (2002). Antidepressant activity of standardized extract of Bacopa monniera in experimental models of depression in rats. Phytomedicine, 9(3), 207-211.
[4]. Shalini, V. T., Neelakanta, S. J., & Sriranjini, J. S. (2021). Neuroprotection with Bacopa monnieri–a review of experimental evidence. Molecular Biology Reports, 48(3), 2653-2668.
[5]. Calabrese, C., Gregory, W. L., Leo, M., Kraemer, D., Bone, K., & Oken, B. (2008). Effects of a standardized Bacopa monnieri extract on cognitive performance, anxiety, and depression in the elderly: a randomized, double-blind, placebo-controlled trial. The journal of alternative and complementary medicine, 14(6), 707-713.
[6]. Sinha, S., Saxena, R. (2006). Effect of iron on lipid peroxidation, and enzymatic and non-enzymatic antioxidants and Bacoside-A content in medicinal plant Bacopa monnieri L. Chemosphere.;62(8):1340-1350.
[7]. Le, X. T., Pham, H. T. N., Do, P. T., Fujiwara, H., Tanaka, K., Li, F., ... & Matsumoto, K. (2013). Bacopa monnieri ameliorates memory deficits in olfactory bulbectomized mice: possible involvement of glutamatergic and cholinergic systems. Neurochemical Research, 38, 2201-2215.
[8]. Ghosh, S., Khanam, R., & Acharya Chowdhury, A. (2021). The evolving roles of Bacopa monnieri as potential anti-cancer agent: a review. Nutrition and Cancer, 73(11-12), 2166-2176.

[9]. Kushwaha, R. K., Joshi, S. M., Bajaj, R., Mastan, A., Kumar, V., Patel, H., ... & Chaudhary, S. P. (2023). Copper and iron metal resistant rhizospheric bacteria boost the plant growth and bacoside A content in Bacopa monnieri under stress conditions. Functional Plant Biology, 50(6), 482-496.

[10]. Ramasamy, S., Chin, S. P., Sukumaran, S. D., Buckle, M. J. C., Kiew, L. V., & Chung, L. Y. (2015). In silico and in vitro analysis of bacoside A aglycones and its derivatives as the constituents responsible for the cognitive effects of Bacopa monnieri. PLoS One, 10(5), e0126565.

[11]. Lorca, C., Mulet, M., Arévalo-Caro, C., Sanchez, M. Á., Perez, A., Perrino, M., ... & Serra, A. (2023). Plant-derived nootropics and human cognition: A systematic review. Critical Reviews in Food Science and Nutrition, 63(22), 5521-5545.

[12]. Sharma, K., Verma, R., Kumar, D., Nepovimova, E., Kuča, K., Kumar, A., ... & Puri, S. (2022). Ethnomedicinal plants used for the treatment of neurodegenerative diseases in Himachal Pradesh, India in Western Himalaya. Journal of Ethnopharmacology, 293, 115318.

[13]. Yen, D. T. H., Cuc, N. T., Tai, B. H., Van Kiem, P., Hoang Duc, M., Nhiem, N. X., ... & Park, S. (2022). Two new triterpenoid glycosides from Bacopa monnieri and their cytotoxic activity. Natural Product Research, 1-7.

[14]. Dai, W., Feng, K., Sun, X., Xu, L., Wu, S., Rahmand, K., ... & Han, T. (2022). Natural products for the treatment of stress-induced depression: Pharmacology, mechanism and traditional use. Journal of Ethnopharmacology, 285, 114692.

[15]. Jeyasri, R., Muthuramalingam, P., Adarshan, S., Shin, H., & Ramesh, M. (2022). Assessing the Anti-inflammatory Effects of Bacopa-Derived Bioactive Compounds Using Network Pharmacology and In Vitro Studies. ACS omega, 7(44), 40344-40354.

[16]. Shoukat, S., Zia, M. A., Uzair, M., Alsubki, R. A., Sajid, K., Shoukat, S., ... & Ali, G. M. (2023). Synergistic neuroprotection by phytocompounds of Bacopa monnieri in scopolamine-induced Alzheimer's disease mice model. Molecular Biology Reports, 50(10), 7967-7979.