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Short Communication

Exploration of Therapeutic Potential of *Bacopa monnieri* Using *in silico* Approach and Optimization of Culture Conditions for Bacoside Production

Anuja Koul[1], Munish Sharma[2], Shagun Sharma[1], Jyotsna Sharma[1], Vijeshwar Verma[1], Sharada Mallubhotla[1*]

[1]School of Biotechnology, Shri Mata Vaishno Devi University, Kakryal, Katra, Jammu and Kashmir–182320, India;[2]Department of Plant Sciences, Central University of Himachal Pradesh, Shahpur, Himachal Pradesh–176206, India

ABSTRACT

Bacopa monnieri plant extract has been used as a nerve tonic in ancient Indian folklore since ages and bacosides are the main key component behind neuro-potential of the herb. Accumulated β-amyloid protein causes the hyperphosphorylation of tau protein which in turn is the cause of tangled nerve projections in Alzheimer's brain. However, the interaction of bacosides with amyloid protein has not been studied so far. Computational approach including docking analysis showed hydrogen bonding of bacoside molecule with important amino acid residues present in amyloid protein which proved the potential of bacoside A_3 in targeting Alzheimer's. In addition to this, *in vitro* culture conditions have demonstrated their critical influence on secondary plant metabolism. Kinetic studies conducted in our experiment showed that cell suspension cultures of bacopa placed under shaking light conditions produce more bacoside content as compared to other conditions. Besides these properties, bacopa plants have also been known to improve the chemical characteristics of saline and clayey soils thus, enhancing the growth of agriculturally important crops. In this work, we attempted the docking of the bacoside A_3 molecule to find the binding mechanism with the suitable target which will be helpful in understanding the molecular mechanism involved in the development of Alzheimer's disease. Up-scaling of memory enhancer bacoside molecule has also been attempted under *in vitro* conditions that should deliver maximum benefits with minimal tissue loss during harvestation.

Keywords: Alzheimer's; Bacoside A_3; Cell cultures; *In vitro*; Secondary metabolism

INTRODUCTION

B. monnieri commonly known as Brahmi is a perennial succulent herb present in marshy areas of Indian subcontinent and is a well-known nootropic herb [1]. Bacoside A_3 is mainly responsible for the therapeutic potential of herb [2]. Plant growth media augmented with different growth regulators and *in vitro* culture techniques have been reported to impact potentially on *in vitro* regeneration and secondary metabolite production [3-6]. Alzheimer's disease (AD) affects millions of people both in developing and developed countries [7]. Immuno-reactivity and β-amyloid protein is the cause behind hyper-phosphorylation of tau protein which in turn causes neuritic tangle, fibrillar and plaque in AD brain [8]. Studies have shown that the *B. monnieri* plant extract has a neuroprotective effect on β-amyloid induced cell and can attenuate Tau protein [9-11]. Certain soil conditions limit agricultural production mainly in arid and semi-arid regions. In those conditions, the use of halophytes has been reported to be an alternative to resolve salinization issues. Bacopa is one such important plant where association with fungus can increase soil productivity. Therefore, the present study attempts to adopt an *in silico* approach (molecular docking) to study the complex interaction between the β-amyloid receptor and bacoside molecule. Docking method is known to be an important approach that is helpful in understanding the affinity of an active compound in relation to its biological target [12]. The present study also aimed to increase bacoside production by evaluation of the influence of different culture conditions on cell cultures of *B. monnieri* raised as suspension cultures.

DOCKING

Docking interaction between bacoside and β-amyloid protein has been attempted and evaluated. The crystal structure of the β-amyloid protein (PDB ID: 1AMB; www.pdb.org) was pre-processed by deleting all water molecules and inserting hydrogen atoms [13]. SDF format of Bacoside A_3 (Compound ID: 44152167) and Cucurmin ligand (Compound ID: 969516/ for comparison) were converted to .pdb format using Open Babel [14] and geometrical optimizations using Hartree-Fock (HF) method by ArgusLab 4.0.1 software. Then we performed rigid docking simulation using Argus Lab 4.0.1 [15,16]. The ligand was docked onto the entire protein surface. "Dock" was opted as the calculation type, "rigid" for the ligand, binding site box size was "26 × 26 × 26 angstroms" and A Score was used as the scoring function. Visualization was carried out using Pymol (PYMOL, Schrödinger) [17]. The interaction

Correspondence to: Sharada Mallubhotla, School of Biotechnology, Shri Mata Vaishno Devi University, Kakryal, Katra, Jammu and Kashmir–182320, India, E-mail: sharda.p@smvdu.ac.in

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sites and binding module of β-amyloid consisted of residues VAL 18, PHE 19 and GLU22 which played crucial part in interaction (Figures 1 and 2). The used β-amyloid protein structure was chosen of length 28 amino acids and consisted of a single helical chain.

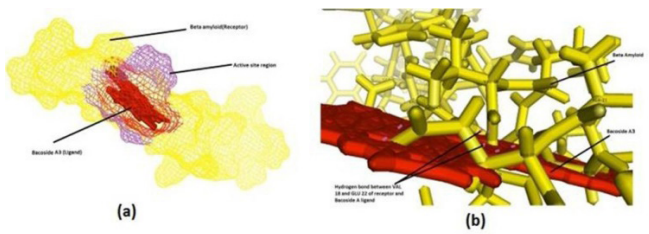

Figure 1(a): Overview of the docking process (b) Stick view of the entire process.

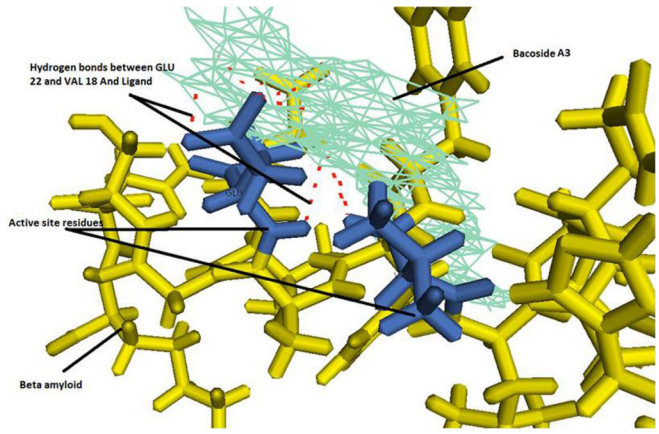

Figure 2: Representation of hydrogen bonds between valine18 and Glutamic acid 22 and the ligand Bacoside A_3.

IN VITRO CULTURE STUDIES

In addition to the docking studies, we also attempted to evaluate the influence of *in vitro* culture conditions (light, dark, static and shaking conditions) on bacoside production. *B. monnieri* plant material (as runners) was collected from CSIR-Indian Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India and habituated at Herbal Garden, Shri Mata Vaishno Devi University, Katra (Figure 3). The effect of various physical parameters on bacoside production in suspension cultures raised from *in vitro* friable callus [18] on Gamborg's (B5) media fortified with 2,4 D (1.0 mg/l) was analyzed. For static and light conditions, cultures were placed on clean tissue culture racks fitted with cool white florescent light (1500-3000 lux), photoperiodic timer of 16/8 hrs day/night regime and ambient temperature of 25 ± 2°C. For dark and shaking parameters, the cultures were placed in an incubator shaker at 80 rpm with same ambient temperature. The suspension cultures were harvested at regular intervals of 3, 6, 9, 15, 30, 45 days for further calculation of growth index, bacoside extraction and HPLC quantification analysis [18].

STATISTICAL CALCULATION

The data collected from different treatments for 10 replicates were analyzed using statistical tool ANOVA (analysis of variance) using SPSS version 17 (SPSS Inc., Chicago, USA). The intention of using this tool was to assess the differences in means (for 10 replicates) and major differences between means were analyzed at $P \leq 0.05$.

Bacoside A_3 had XLogP3 value of 2.8 and had 10 rotatable bonds. Bacoside A_3 has an A score -8.599 kcal/mol and Curcumin has -13.68 kcal/mol along a poor H-bonding in comparison to Bacoside A_3. Reports also demonstrated that 20 Curcuminoid compounds showed an A score between -12.03 to -15.58 kcal/mol with β-amyloid protein. This data further corresponds to our results that Bacoside A_3 could be a more potent inhibitor than Cucurmin. Further analysis of binding interaction reveals that four hydrogen bonds are formed between receptor and ligand. Two bonds are formed with valine at position 18 and two with glutamic acid at position 22. Since glutamic acid is reported to be engaged in neural transmission, interaction with it can modify its functionality rendering it to be inhibited by the ligand [19-25]. Therefore bacoside A_3 can be considered an effective inhibitor for β-amyloid. Earlier also, interaction of bacoside A/A_3 with tryptophan hydroxylase at glutamate residue regulated the activity of serotonin biosynthesis thus enhancing memory [20]. The present study interaction results create more avenues to be exploited in future for better understanding of these molecules [25-32].

It was found that cultures placed under shaking light condition produced fine suspension calli with higher biomass and higher amounts of bacoside content (Figure 4a). The kinetic study analysis showed that there is an initial increase in biomass and bacoside content in bacopa cultures between 3rd to 9th day which is maximum between 6th-9th day period and gradually decreased by 45th day (Table 1 and Figure 4b).

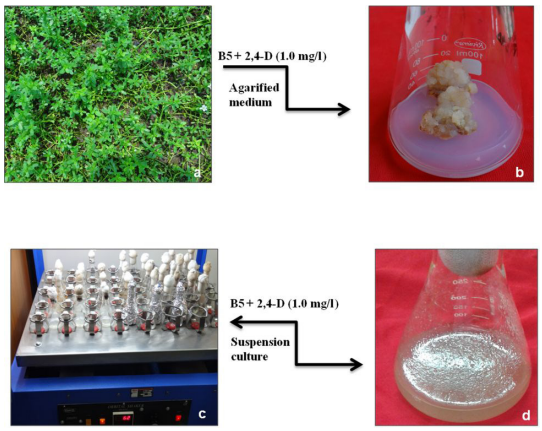

Figure 3: Initiation and scaling up of Suspension calli cultures of *B. monnieri* (a) Field grown bacopa plants; (b) Agarified friable callus culture raised on Gamborg's (B5) medium supplemented with 2,4-D (1.0 mg L-1); (c) Suspension cultures on rotary shaker at 80 rpm; (d) Suspension calli culture.

Table 1: Optimization of culture conditions for suspension cultures and production of bacoside A_3 content.

S. No.	Culture Conditions	Response	Bacoside A_3 content (mg/g)*
1.	Control (without PGR)	Suspended suspension initial culture	1.89 ± 0.03
2.	Male	Male	Male
3.	Male	Male	Male
4.	Male	Male	Male
5.	Male	Male	Male

*Values represent mean ± SE of 10 replicates in each culture condition. SE is the Standared Error calculated in SPSS 17 where $P \leq 0.05$

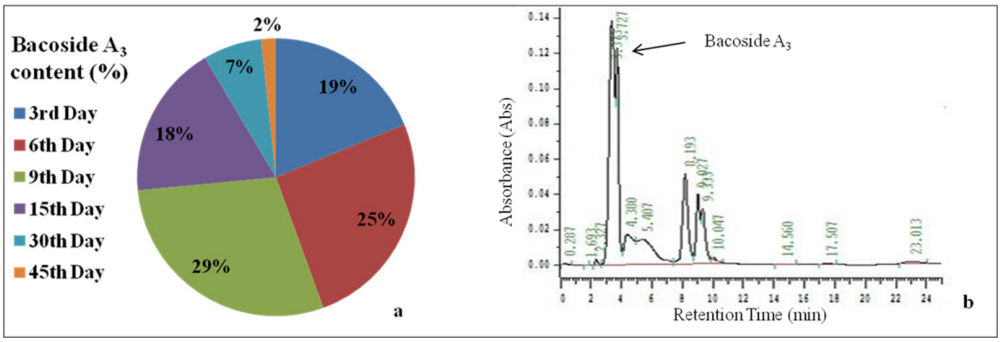

Figure 4: Bacoside A_3 content at different time interval in suspension cultures under shaking light conditions; (b) HPLC chromatogram at 9th day of culture during kinetic study.

CONCLUSION

B. monnieri, an elite medicinal plant has been used since ages for its potential memory enhancing abilities. Also previously reported studies demonstrated bacopa plants association with fungus enhances the chemical properties of soil conditions thus making it more fertile and productive. Keeping in mind the potential of bacopa, studies should be directed to evaluate further this plant for conservation, enhancing metabolites, and its use in various human diseases. The potential application and use of suspension cultures over organ plant cultures for bacoside production is also a step forward towards plant conservation. Thus the present study helped in understanding the inhibitory mode of bacoside towards Alzheimer's tau protein along a method to enhance biomass and bacoside content in bacopa cultures.

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