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Research Article

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In vitro anti-microbial potential of *Bacopa monnieri* and *in silico* OMPX inhibitory activity of its active components

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ABSTRACT

The need for investigating the protective effects of herbal extracts and compounds from natural sources has gained considerable attention among researchers. Current study was aimed at investigating the in vitro anti-microbial activity of methanolic leaf extracts of the medicinal plant Bacopa monnieri (Brahmi)and in silicoOuter Membrane Protein X (OMPX) inhibitory activity of its active components. The antimicrobial potential methanolic extract was tested by antibiotic susceptibility testing (AST) using Kirby Bauer disc diffusion method and compared to that of commercial antibiotics. Three different bacterial strains were used in the study: Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. OMPX was docked against active compounds of Bacopa monnieriand the docked complexes were analysed using PyMol molecular viewer. Bacopaside I gave highest docking score against OMPX followed by bacopaside II, bacopaside A, β-sitosterol, luteolin and apigenin.

Keywords: *Bacopa monnieri*, antimicrobial, OMPX, docking

INTRODUCTION

Antibiotic resistance is the biggest challenge in treating bacterial infections due to multidrug resistant strains.[1] Frequent and inappropriate administration of antibiotics to patients with bacterial infections aids the bacteria to develop resistance to specific antibiotics.[2] Treating such infections becomes difficult when the strain develops resistance to multiple drugs. This has become very common in case of nosocomial infections where most of the bacterial strains are multidrug resistant. Minimizing the frequent use of antibiotics would partly prevent such strains from developing resistance to antibiotics.[3] Hence, the use of natural agents with antimicrobial activity would help bringing about reduction in the usage of antibiotics for mild bacterial infections and wounds.

Bacopa monnieri is a well-known cognitive enhancer studied extensively in models of neurological disorders.[4] It belongs to the Scrophulariaceae family and is found in wet, marshy areas. It is also known to possess anti-inflammatory activity and used to treat conditions such as asthma, bronchitis and rheumatism in traditional Indian and Chinese medicine.[5] Studies have reported that this medicinal herb possesses several beneficial properties such as antioxidant, antidepressant, antiepileptic, anthelminthic, antiparkinsonian and anticholinesterase activities.[6]

Current study is aimed at investigating the *in vitro* antimicrobial effect of the ethanolic and methanolic leaf extracts of *Bacopa monnieri* collected from Vellore district, Tamilnadu, India.

EXPERIMENTAL SECTION

Chemicals: The chemicals used in the experiment were purchased from Sigma Aldrich. The culture media and antibiotic discs used in the study were purchased from HiMedia laboratories.

Plant material: The plant was collected from the local market in Vellore district and the species was identified and confirmed by certified botanists. The leaves of the plant were chosen as the herbal part for the current experiment. Hence, they were collected, dried in the shade and pulverized.

Preparation of ethanolic and methanolic extracts of the leaves of *Bacopa monnieri*:5 g of finely powdered leaves were homogenized in 50 ml of 10% methanol/ethanol separately for 24 h at 25˚C using shaker. The extracts were filtered using Whatman filter paper No. 1. The crude extract obtained was again dissolved in50 ml of 10%ethanol/methanol separately and concentrated at low pressure using a rotary vaporizer. The extract thus obtained was stored at 4°C until further use.

Antimicrobial testing:

Preparation of test organism: Microbes such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were isolated and pure culture of each individual organism was prepared. These organisms were used in the study of antimicrobial activity of ethanolic and methanolic extract of *Bacopa monnieri* by Kirby Bauer disc diffusion method. All the organisms were sub-cultured into nutrient agar media under sterile conditions. Gram's staining and biochemical tests (mannitol motility test, triple sugar iron agar, indole test, citrate utilization test) were performed to confirm the characteristics of organisms.

Antimicrobial assay: Kirby Bauer method was carried out to evaluate the inhibitory effect of extracts against chosen test organisms.[7]About 1-2 colonies of all three organisms were separately inoculated into 1ml of nutrient broth and incubated at 37°C for 16 h. After incubation, organisms were uniformly streaked across the whole area of Muller Hinton agar to form a bacterial lawn using sterile swab. Sterile paper discs (6mm diameter) were impregnated with loopful of extract and placed on the bacterial lawn. This was carried out for both ethanolic and methanolic extracts against each organism. Commercial antibiotic discs such as ampicillin, cefotaxime and ofloxacin were used for the purpose of comparison. Sterile conditions were maintained throughout the experiment. The plates were incubated at 37°Cfor 16 h. The clear zones of inhibition in the plates were measured to estimate antimicrobial activity.

***In silico* docking:**

Protein: The three-dimensional structure of OmpX was obtained from Protein Data Bank (http://www.rcsb.org) (Figure 1). The protein was prepared for molecular docking using PyMol molecular viewer.

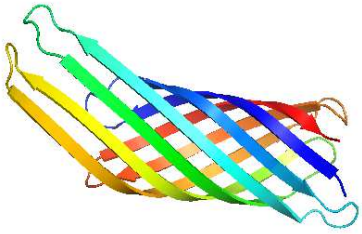

Figure 1: 3-D Structure of OmpX

Ligands: The structures of ligands were obtained in SMILES format from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and submitted on CORINA molecular networks (https://www.molecular-networks.com/online_demos/corina_demo) for generation of three-dimensional structures. Figure 2 shows the two-dimensional structures of the ligands used in molecular docking experiments.

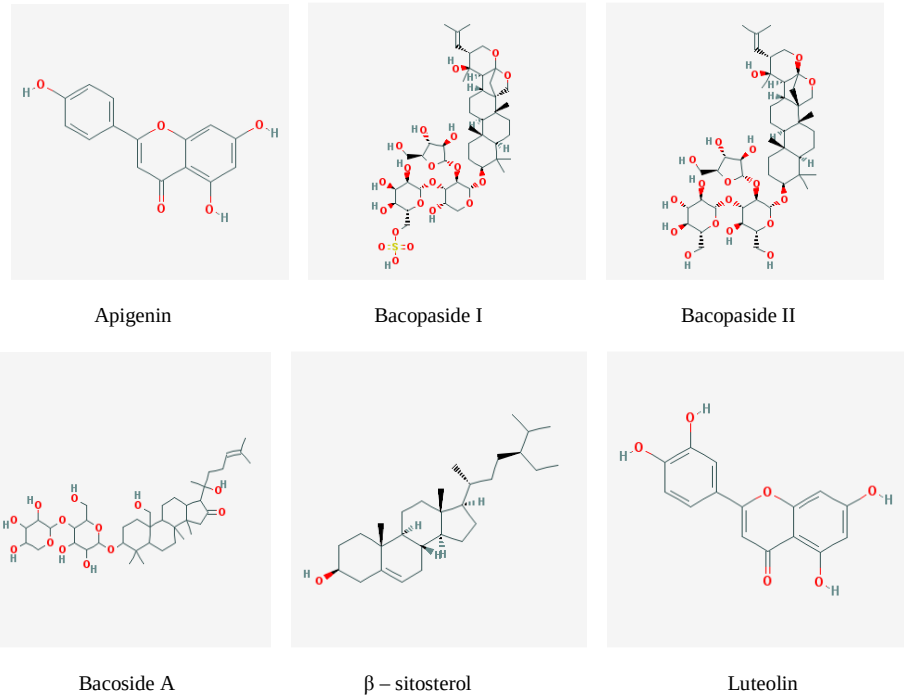

Figure 2: 2D structures of ligands

Table 1.Ligands used in docking experiments

	Ligand	PubChem CID	Chemical Name	Molecular Formula	Molecular Weight g/mol
1	Apigenin	5280443	5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	$C_{15}H_{10}O_5$	270.2369
2	Bacopaside I	71312546	Pseudojujubogenin 3-O-[|A-L-arabinofuranosyl-(1 inverted exclamation marku2)-[6-O-sulfo-|A-D-glucopyranosyl-(1 inverted exclamation marku3)]-|A-L-arabinopyranoside]	$C_{46}H_{74}O_{20}S$	979.13276
3	Bacopaside II	9876264		$C_{47}H_{76}O_{18}$	929.09554
4	Bacoside A	53398644		$C_{41}H_{68}O_{13}$	768.97082
5	β – sitosterol	222284	22,23-Dihydrostigmasterol	$C_{29}H_{50}O$	414.7067
6	Luteolin	5280445	3',4',5,7-Tetrahydroxyflavone	$C_{15}H_{10}O_6$	286.2363

Analysis of Docked Complexes: The binding interactions between OmpX protein and each ligand were analyzed using PyMol molecular viewer (http://www.pymol.org/).The interacting residues of the protein, interacting atoms of the ligands were labeled and hydrogen bond length were labeled using Pymol.

RESULTS AND DISCUSSION

Table 2.Antimicrobial effect of methanolic and ethanolic extracts of *Bacopa monnieri*

Organism	ME (200 µg/ml)	EE (200 µg/ml)	A (mm)	CTX (mm)	OF (mm)
Eschericia coli	12	13	28	23	30
Pseudomonas aeruginosa	10	12	23	27	22
Staphylococcus aureus	11	13	24	25	28

Note: ME- methanolic extract; EE- Ethanolic extract; A- Ampicilin; CTX- Cefotaxime; OF- Ofloxacin

Antimicrobial testing: It was found that the ethanolic extract possesses better antimicrobial activity as compared to the methanolic extract. The results obtained were compared with that of commercial antibiotic discs. Our study confirmed previously reported data showing enhanced inhibitory activity of ethanolic extrct of *Bacopa monnieri* in comparison with other extracts such as ethyl acetate, diethyl ether, benzene, dichloromethane and aqueous extracts.[8,9]

***In silico* docking:** The outer membrane protein (OmpX) of *E.coli* was docked with the chosen ligands which showed the patterns of interaction between the ligands and the protein. Table 3 shows the results of the *in silico* docking experiments andFigures 4-9 show the docked complexes. The number of hydrogen bond interactions between Bacopaside I and OmpX protein were seven. The OmpX-luteolin complex showed five hydrogen bond interactions. Three hydrogen bonds each were found in the OmpX-bacopaside II and the OmpX-bacoside A complexes. The number of interactions between β- sitosterol and OmpX are two.

Bacopaside I is a pseudo jujubogenin glycoside isolated from *Bacopa monnieri*.[10] It has been proven to possess significant neuroprotective effect in ischemic brain injury in rats and antidepressant-like effect in mice.[11]

Table 3. Docking scores, area and atomic contact energy of the docked complexes

Protein (IQJ8)	Ligand	Score	ACE	Area
OmpX	Apigenin	4072	-206.25	477.00
	Bacopaside I	6944	-219.89	959.70
	Bacopaside II	6734	-349.88	1061.20
	Bacoside A	6178	-284.12	835.70
	β–Sitosterol	4850	-163.74	584.00
	Luteolin	4104	-220.92	470.40

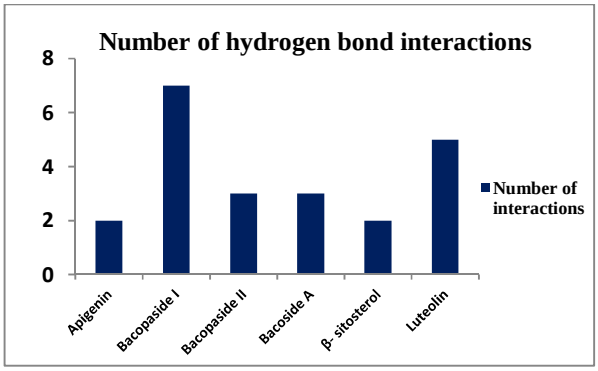

Figure 3: Graphical representation of number of interactions found in docked complexes

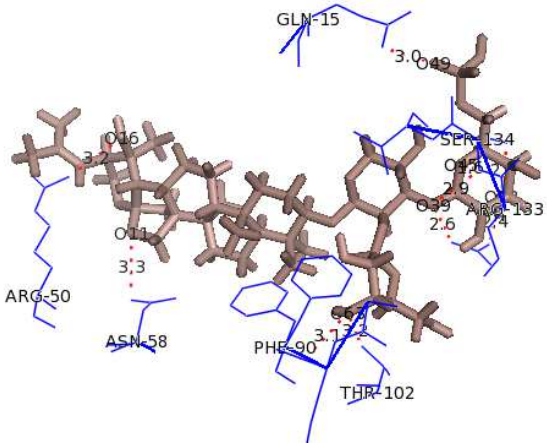

Figure 4: 7 hydrogen bond interactions between bacopaside I and ARG-50, ASN-58, PHE-90, THR-102, ARG 133, SER-134 and GLN-15 amino acids of OmpX

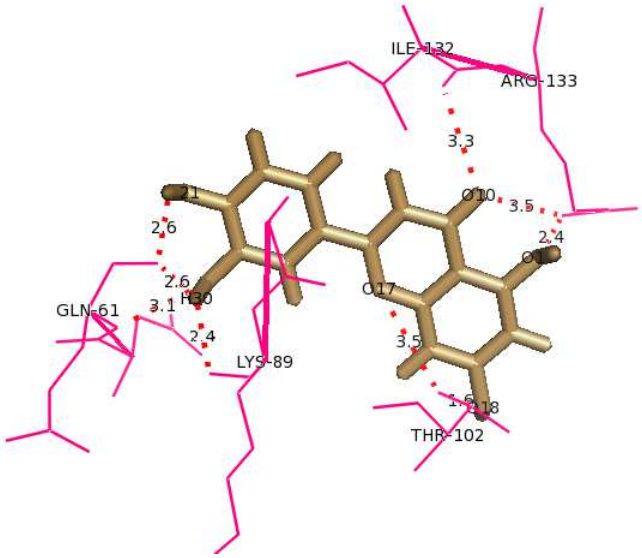

Figure 5: 5 hydrogen bond interactions between luteolin and GLN-61, LYS-89, THR-102, ILE-132, ARG-133 amino acids of OmpX

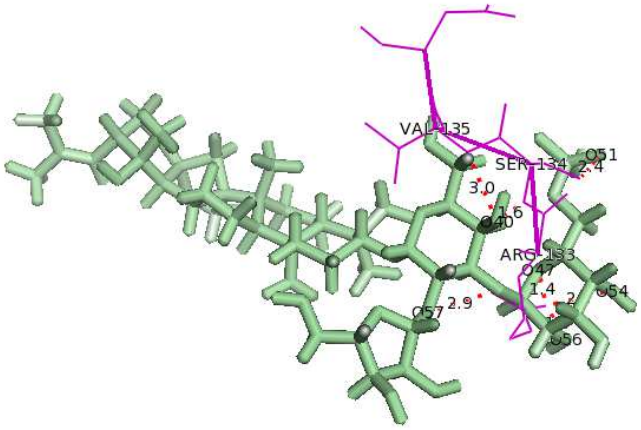

Figure 6: 3 hydrogen bond interactions between bacopaside II and VAL-135, SER-134 and ARG-133 amino acids of OmpX

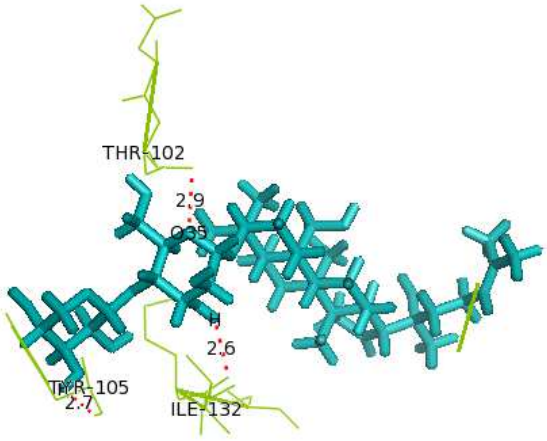

Figure 7: 3 hydrogen bond interactions between bacoside A and THR-102, ILE-132 and THR-105 amino acids of OmpX

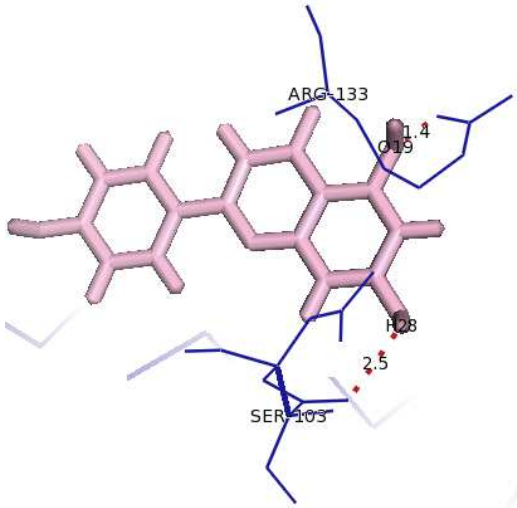

Figure 8: 2 hydrogen bond interactions between apigenin and ARG-133 and SER-103 amino acids of OmpX

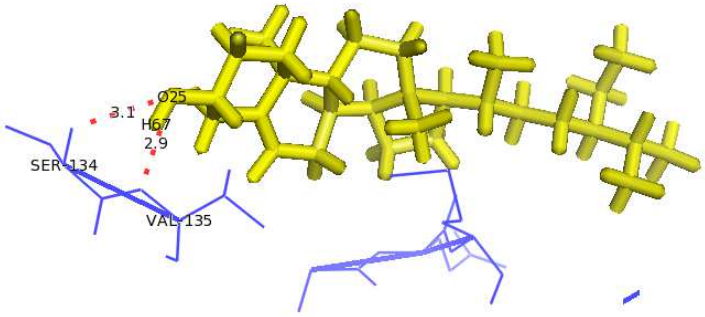

Figure 9: 2 Hydrogen bond interactions between β- sitosterol and SER-134 and VAL 135 amino acids of OmpX

CONCLUSION

Present study shows that ethanolic leaf extract of *Bacopa monnieri* shows significant antimicrobial activity. It is also evident from the molecular docking analysis that *Bacopa monnieri* could be used in the development of effective inhibitors against OmpXand thereby its virulence. The results of *in silico* experiments clearly indicate that among the chosen ligands Bacopaside I was found to have more binding interactions with the target protein OmpX and it could be a potent inhibitor of the same. However, further studies are required in exploring the mechanisms of OmpX inhibition by BacopasideI.

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