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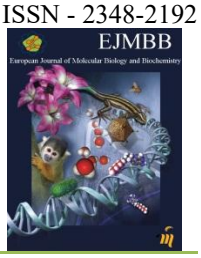

EXPLORING THE ANTIOXIDANT, CYTOTOXIC, AND BACE-1 INHIBITION POTENTIAL OF OCHNA SQUARROSA ROOT BARK: IN VITRO AND IN SILICO INSIGHTS

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ABSTRACT

Alzheimer's disease (AD) is marked by amyloid beta peptide (Aβ) accumulation in high-order association brain regions, and growing evidence suggests that Aβ plays an essential early role in AD pathogenesis. The ethanol extract of the root bark was prepared and used to identify the antioxidant activity by DPPH and nitric oxide scavenging assay. The cytotoxicity of the extract was identified by brine shrimp lethality assay. The molecular docking was performed with the phytoconstituents reported in the root bark of O.squarrosa. The DPPH and nitric oxide assay showed a maximal inhibitory potential of 68.62 % and 73.14 % at 1000 µg/ml. Also, the IC50 of the extract was found to be 307.1 µg/ml (DPPH) and 247.5 µg/ml (nitric oxide). The lethality assay was carried out for 24hrs and 50 % death was observed at 1000 µg/ml. The molecular docking study revealed that the phytoconstituent 3',4'-Dihydroxyfurano [3'',2'': 6,7] flavone showed high docking score with a mechanism-based inhibition of BACE-1 protein. These data suggest that the phytoconstituents may have a BACE-1 inhibition potential and further in-vitro and in-vivo screening is required to confirm the inhibitory potential.

INTRODUCTION

Highlights

- The ethanol extract of the root bark was prepared and used to identify the antioxidant activity by DPPH and nitric oxide scavenging assay.
- The DPPH and nitric oxide assay showed a maximal inhibitory potential of 68.62% and 73.14% at 1000 µg/ml.
- The lethality assay was carried out for 24hrs and 50% death was observed at 1000 µg/ml.
- The molecular docking study revealed that the phytoconstituent 3',4'-Dihydroxyfurano[3",2": 6,7]flavone showed high docking score with a mechanism based inhibition of BACE-1 protein.

Alzheimer's disease (AD) is a neurodegenerative condition that causes cell degeneration in the brain.

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It is the leading cause of dementia, which is defined by a deterioration in thinking and independence in routine daily tasks [1]. AD is thought to be a complex illness, with two primary theories presented as causes: the cholinergic and amyloid hypothesis. The clinical stages of Alzheimer's disease may be divided into two categories: pre-clinical or pre-symptomatic, which can last several years or more, and clinical, which can last many years or more [2,3]. This stage is distinguished by minor memory loss and early degenerative alterations in the cortex and hippocampus, as well as little functional impairment in everyday activities and the lack of clinical signs and symptoms of Alzheimer's disease [4].

Pathologically, Alzheimer's disease (AD) is defined by the deposition of amyloid beta peptide (Aβ) in high-order association brain areas as fibrillar plaques and soluble oligomers. This illness is further distinguished by the presence of intracellular neurofibrillary tangles, neuroinflammation, neuronal malfunction, and death. Increasing evidence shows that Aβ plays an important

early role in AD pathogenesis, and the core tenet of the amyloid (or Aβ cascade) hypothesis is that Aβ aggregates initiate a complicated pathogenic cascade that leads to neurodegeneration [5, 6].

The transmembrane aspartyl protease BACE1 cleaves APP at the β-site. The generation and release of Aβ peptide in the brain is caused by the successive proteolytic cleavage of APP by BACE1 and β-secretase [7]. As a result, amyloidogenic secretases are important therapeutic targets that are now being investigated for AD-modifying intervention. Several studies have found that BACE1 inhibitors have a high potential as a therapy for lowering Aβ brain concentrations and hence halting the course of AD [8, 9].

Ochnasquarrosa, sometimes known as sunari belongs to the Ochnaceae family. It is a little shrub with a thin, dark brown stem that can grow up to 50 cm tall [10]. It is recognised as an important component in traditional medicinal systems for the treatment of a wide range of maladies, including constipation, ulcers, wounds, and cancer. This plant's root bark is used as a digestive tonic [11]. Boiling the leaves of *O.squarrosa* is used to treat lumbago and ulcers. Its root decoction is used to treat menstrual difficulties and asthma.

The purpose of this work was to determine the BACE-1 inhibitory potential of *O.squarrosa* root bark using molecular docking studies. In addition, the brine shrimp lethality experiment was used to determine antioxidant activity and toxicity.

METHODS AND MATERIALS

Collection and Preparation of extract

The root bark of *O.squarossa*was collected, washed under running water and then shade dried. They were powdered and extracted using ethanol. The extract was then kept in incubator shaker for 48 hours, filtered using whattman filter paper (No.1) and evaporated to remove the excess solvent. The extract were store in an airtight container and kept for further use.

Molecular Docking

Ligand Preparation

3',4'-Dihydroxyfurano [3'',2'': 6,7] f lavone, 3'-methoxyfurano [3'',2'': 6,7] flavone, 5-Methoxyfurano [3'',2'': 6,7] flavone, 5-O-Methylsquarrosin, 5,7,8-Trimethoxy-3',4'-(methylenedioxy)isoflavone, Lophirone A, Lophirone H, Lophirone L, Calodenone and Chrysophanol. The ligands were then prepared for docking using the Ligprep tool in the suite. The OPLS4 field force was chosen because it has been demonstrated to be more accurate than the previous field for protein-ligand docking [12]. Following ligand pre-processing, the final ligands for docking were chosen based on state penalty ratings.

Protein Preparation

The Crystal Structure of Human BACE-1 in Complex with CNP520 (6EQM) was obtained from the RSCB website and processed for protein preparation [13]. PRIME was first used to add all hydrogen, zero-order bonds, disuphide groups, missing atoms, and chains. The protein was optimized and finally, the protein was minimized, integrated into the workspace of the suite [14].

Receptor Grid generation

The docking grid was created in the active site of the protein. The protein already contains a CNP520 inhibitor linked to its active site. This pocket was picked, and a docking grid was created [15].

Docking

The previously built receptor grid was selected, and the prepared ligands were selected from the workspace. The standard precision technique was used, and the docking position was limited to a 0.10 tolerance. After that, the docking method was started, and the findings were saved with 5 pose per ligand-protein interaction.

Brine shrimp lethality assay

The artificial saltwater was made by dissolving 27g of sodium chloride in 1 litre of distilled water in a separating funnel and aerating it. The aeration supply produced 75% of the dissolved oxygen measured with the dissolved oxygen metre. The frozen shrimp eggs (a few grammes) were weighed and combined with the artificial seawater. The arrangement was left alone for 24 hours. The extract was serially diluted at 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml, and 1000 µg/ml concentrations. The Petri dishes were cleaned and filled with seawater. 10 nauplii in each Petri plate were exposed to the five various concentrations during 24 hours, and the number of nauplii was counted after that time. As a control, artificial seawater was employed. After 24 hours of exposure to the concentration, death was found. The movement of the nauplii was carefully examined and documented under the microscope [16].

The percentage of death was calculated by using the formula,

$$\% \text{ Death} = \frac{\text{no of dead nauplii}}{\text{No of dead + no of live nauplii}} \times 100$$

Antioxidant activity:

DPPH assay:

3 ml of A DPPH solution (200µM) was added to the 2 ml of extracts (10-1000 µg/ml), and the reaction was allowed to proceed in the dark for around 20 minutes. Ascorbic acid was utilized as a reference. At 517nm, the absorbance was measured and the % inhibition was measured [17].

Nitric oxide assay:

At physiological pH, an aqueous solution of sodium nitro prusside spontaneously creates nitric oxide (NO), which combines with oxygen to produce nitrate ions and was colorimetrically detected. 3ml of a reaction mixture containing sodium nitro prusside, 10mM in phosphate buffered saline (PBS), and different extract concentrations (10-1000 µg/ml) was incubated at 37°C for 4 hours. The control without the test substance was kept in the same way. Following the incubation period, 0.5ml of Griess reagent was applied. At 546nm, the absorbance was measured. Ascorbic acid was utilized as a reference [18, 19].

RESULTS AND DISCUSSION

Molecular docking

The ligands were docked to the BACE-1 protein and the obtained poses were analyzed (table 1). It was found that 3',4'-Dihydroxyfurano[3'',2'': 6,7]flavone showed highest docking score of -7.708 Kcal/mol. Only few ligands were able to interact with the active site while rest of the compounds did not show any interaction. The 3',4'-Dihydroxyfurano[3'',2'': 6,7]flavone showed interaction with ASP32 and a water molecule (Figure 1). The CNP520 present in the active site of the protein was taken as a standard. It was found that the ligand showed similar binding pattern as the CNP520 and expressed a mechanism based inhibition as reported previously [20].

Brine shrimp lethality assay

The brine shrimp (Artemia salina) is a very sensitive indicator species utilised in early cytotoxicity studies of plant extracts and other chemical substances. The nauplii need 24 hours to hatch from the eggs and become alive, drawn to light. The experiment was then continued with artificial seawater utilised for hatching. Ten nauplii were taken from the separating funnel and placed in five different concentrations in a Petri plate. After 24 hours, certain nauplii were discovered to be dead at a certain concentration, but all nauplii in the control were alive. According to Table-2, as the concentration of extract increases, so does the mortality of the nauplii.

DPPH assay

The DPPH radical scavenging activity of the extracts was measured and compared to that of the reference molecule ascorbic acid. The ethanol extract demonstrated a dose-dependent inhibition with a maximal inhibition of 68.62 % at 1000 µg/ml a **Figure 2**. The decrease in DPPH must be attributed to either free hydrogen atom transfer or electron transfer. This research suggests that phytoconstituents are a good source of antioxidants. Also the IC_{50} of the ethanol extract was found to be 307.1 µg/ml and that of ascorbic acid was found to be 60.92 µg/ml.

Nitric oxide assay

The nitric oxide inhibition was measuring for the plant extract and compared to that of the ascorbic acid. The assay showed that the ethanol extract showed a maximum inhibition of 73.14 % at 1000 µg/ml with a dose-dependent inhibition. The IC_{50} of the ethanol extract was found to be 247.5 µg/ml **Figure 3**, also ascorbic acid IC_{50} was 63.41 µg/ml. The results suggest that the Phytoconstituent can act as superoxide anion scavenger and exhibit therapeutic anti-oxidant activity.

Table 1: Docking scores of Ligands against 6EQM.

S.No	Ligands	Docking score	Glide score	Glide e-model score
1.	3',4'-Dihydroxyfurano [3'',2'': 6,7] flavone	-7.708	-7.709	-37.068
2.	5-Methoxyfurano [3'', 2'': 6, 7] flavone	-6.636	-6.636	-34.729
3.	5,7,8-Trimethoxy-3',4'-(methylenedioxy)isoflavone	-4.719	-4.719	-27.312
4.	5-O-Methylsquarrosin	-4.704	-4.704	-28.717
5.	Chrysophanol	-4.448	-4.448	-15.582
6.	Lophirone H	-4.381	-4.426	-30.740

Table 2: Brine shrimp lethality assay

Sl.No	Concentration (µg/ml)	Control	No of live Naupli	% Death
1	0.1	10	9	10
2	1	10	8	20
3	10	10	8	20
4	100	10	7	30
5	1000	10	5	50

Figure 1: Interaction of 3',4'-Dihydroxyfurano[3'',2'': 6,7]flavone with 6EQM.

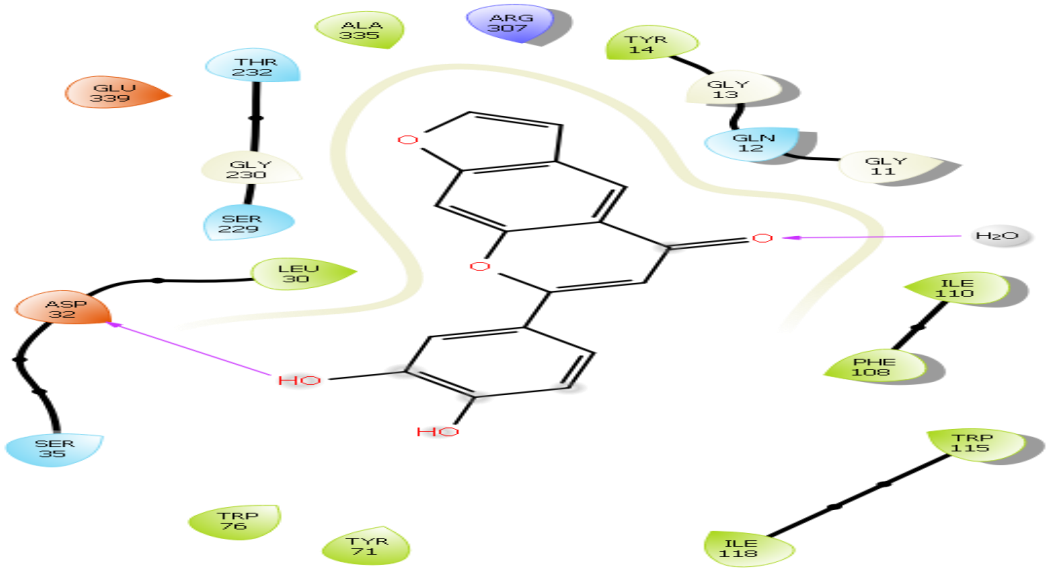


Figure 2: IC50¬of ethanol extract of O.squarrosa – DPPH assay

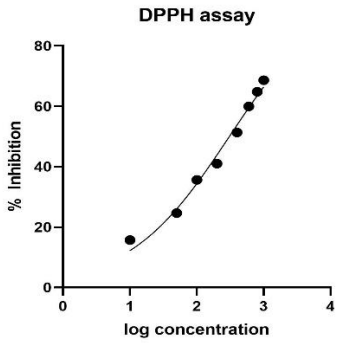
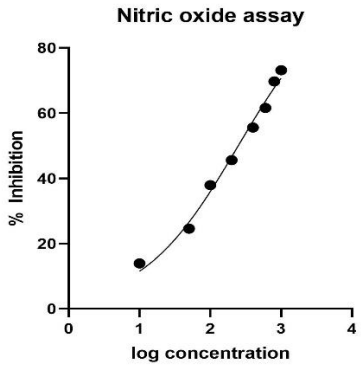


Figure 3: IC50¬of ethanol extract of O.squarrosa –nitric oxide assay



CONCLUSION

Although the actual cause of Alzheimer's disease (AD) is unknown, investigations over the last 30 years have demonstrated that aberrant synthesis or buildup of -amyloid peptides (A) is likely to be a dominating early event in AD clinical progression.Chemical inhibition of BACE1 has been proven in animal research and human trials to diminish A. While BACE1 inhibitors are now being tried in clinical trials to treat Alzheimer's disease patients, it is critical to determine if BACE1 inhibition has a substantial influence on cognitive functioning in Alzheimer's patients. In this study the rootbark of O.squarrosa was subjected to antioxidant and brine shrimp lethality assay. The results analyzed showed a good antioxidant and less lethal effect at 1000 µg/ml. Similarly the docking studies on BACE-1 inhibition were performed based on the phytoconstituents reported early in the root bark of O.squarrosa. The 3',4'-Dihydroxyfurano[3'',2'': 6,7]flavone showed a mechanism based inhibition of BACE-1 through docking studies. These phytoconstituents need to be further studies in-vivo to identify the complete BACE-1 inhibition potential.

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