A DISSERTATION ON

ANTIOXIDANT AND NEUROPROTECTIVE ACTIVITY OF *BERBERIS ARISTATA*

SUBMITTED TO THE DEPARTMENT OF BIOSCIECES,

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT FOR THE

DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY

BY

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UNDER THE SUPERVISION OF

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DECLARATION

I hereby declare that the present work on **"Antioxidant and neuroprotective activity of *Berberis aristata*"** is a record of original work done by me under guidance of Dr. Mohammad Hayatul Islam, Assistant Professor, Department of Biosciences, Integral University, Lucknow during Feb, 2022 to June, 2022. I also declare not part of this thesis has previously been submitted to my Institution or any examining body for acquiring any diploma or degree.

Place: **Khushnuma Fatima**

Date:

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TO WHOM IT MAY CONCERN

This is to certify that Ms. Khushnuma Fatima, a student of M.Sc. Microbiology (II Year, IV semester), Integral University has completed her four months dissertation work entitled **"Antioxidant and neuroprotective activity of *Berberis aristata*"** successfully. She has completed this work from Department of Biosciences, Integral University, under the guidance of **Dr. Mohammad Hayatul Islam.**

The dissertation was a compulsory part of her M.Sc. degree. I wish her good luck and bright future.

(Dr. Mir Snober Shabnam)

Head,

Department of Biosciences,

Integral University, Lucknow

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by Ms. Khushnuma Fatima during the four months training from February – June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by him are genuine and script of the thesis has been written by candidate himself. The thesis entitled is **"Antioxidant and neuroprotective activity of *Berberis aristata*"** Therefore, being forwarded for the acceptance in partial fulfilment of the requirements for the award of the degree of Master of Science in MSc Microbiology, Department of Biosciences, Integral University, Lucknow (U.P.).

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Khushnuma Fatima

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1. INTRODUCTION

Berberis aristata commonly known as “Daruhaldhi and chitra” is spinous shrub native to northern Himalaya region. The plant is widely distributed from Himalayas to Srilanka, Bhutan, and hilly areas of Nepal in Himalaya region. It is found in Himachal Pradesh. It grows at the height of 2000-3000m especially in Kumaon and Chammba region of Himachal Pradesh. It is also found in Nilgiris hills in South India.The shrub *B. aristata* belongs to Berberidaceae family and commonly known as tree turmeric, Daruhaldi (in Hindi), Daruharidra (in Sanskrit), Indian barberry (in English) and Zarishk (in Unani). The genus *Berberis* comprises approximately 450-500 species of deciduous evergreen shrubs and is found in the temperate and sub-tropical regions of Asia, Europe, and America [1]. *B. aristata* is used in ayurvedic medicines from very long time. The plant is used traditionally in inflammation, wound healing, skin disease, menohrrhagia, diarrhea, jaundice and infection of eyes. A very valuable ayurvedic preparation ‘Rashut’ is prepared by this plant. *B.aristata* is a spinous shrub with height ranging from 2 to 3 metres. Wood of the plant is hard and bark is yellow to brown in colour in outer appearance whereas from inside it is of deep yellow colour. Roots of berberis aristate are also yellow in colour [2].

Classification:

Class -Magnoliopsida

Subclass-Magnoliidae

Order- Ranunculales

Family- Berberidaceae

Genus- *Berberis*

Species-*aristata*

Leaves

The leaves are arranged in tufts from five to eight and are about 4.9 cm long and 1.8 cm wide. Leaves are simple, spiny, lanceolate, toothed, leathery in texture, sessile, acuminate, verticillate, intense green on the dorsal surface and light green on the ventral surface and show reticulate pinnate venation.

Flowers

The flowers are yellow in colour, hermaphrodite, actinomorphic and form a racemose inflorescence, with 11-16 flowers per cluster, arranged along a central stem. The calyx whorl of the flower is yellow in colour, polysepalous in condition, 6 in number out of which 3 sepals are large and 3 sepals are small, 4-5mm long caducous calyx Corolla has six petals in total which are yellow in colour. The petals are free (polypetalous), imbricate with 2-3 bracts. The male reproductive structure, androecium, is polyandrous and contains six stamens, 5–6 mm long. The female reproductive structure, the gynoesium, is 4-5 mm long and is composed of a short style and a broad stigma. Ovary is simple, club shaped with 1-12 ovules that are erect and in sub basal arrangement.

A **B** **C**

Figure 1. A) Plant B) Leaves and flowers, C) Fruits of *B. aristata*

Fruits

Fruits are globose to ovoid, usually covered with bloom as in plums. Fruits are 7 mm long, 4 mm in diameter, weighing 227 mg, 237 microlitres in volume. Fruit colour is aconite violet. Seeds are 2 to 5 in number, varying in colour from yellow to pink, each weighing 25 mg each.

Stem

Stem has rhytidome with cork consisting of 3 to 45 rectangular and square, yellow coloured, thin-walled cells that are arranged radially. Phloem fibers are arranged tangentially into rows having 1 to 4 rows of cells, the fibers are short, spindle-shaped, thick walled with wide lumen these cells contain the yellowish-brown content. The walls of phloem fibers are lignified. The secondary phloem rays are obliquely arranged having parenchyma cells that are elongated. These rays for the half portion rhytidoma. Secondary xylem consists of xylem vessels, tracheids, xylem fibers and transverse by multi seriate xylem rays. Xylem vessels are numerous ranging from small to medium in size, distributed either in groups or individually throughout the xylem region. They are usually arranged radially, walls are thick and filled with lignin. Xylem rays are straight and the cells are rectangular, some of the cells have brown content [3, 4].

B. aristata, The berberine alkaloid can be found in the roots, rhizomes, and stem bark of the plants. Berberine extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminths, and chlamydia. In China, berberine is an overthe-counter drug for the treatment of bacterial diarrhea. In 1988, the hypoglycemic effect of berberine was firstly reported when berberine was prescribed to treat diarrhea in diabetic patients. Moreover, several clinical and preclinical studies demonstrate ameliorative effect of berberine against several disorders including metabolic, neurological and cardiological problems. This review provides a summary regarding the pharmacokinetic and pharmacodynamic features of berberine, with a focus on the different mechanisms underlying its multispectrum activity.However, numerous literatures had been published by various authors exploring the phytochemical and pharmaceutical aspects along with traditional uses yet there is no much more literature concerning so far the importance of Berberine, which is important constituent of this species

Ayurveda is a traditional system of medicine using a wide range of modalities to create health and well-being. The primary aim of Ayurveda health care is to restore the physical, mental and emotional balance in patients, thereby improving health, preventing disease and treating any current illness. The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are now in great demand in the developing world for primary healthcare not because they are

inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries for primary healthcare2. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phyto-chemically. Therefore, it seems necessary to evaluate the herbs properly. Berberis aristata DC.

(Berberidaceae) is one of the herbs mentioned in all ancient scriptures of Ayurveda, Charaka andSusruta have mentioned it's different properties along with various used for the treatment of numerous illnesses3. The genus Berberisrepresents the around 12 genera and 600 species worldwide and about 77 species have been reported fromIndia4. In Indian Himalayan ecosystem most of the species have reported from Nilgiri hills at an altitude of 1,000–3,000 mASL5. Among the various species of Berberis genus Berberi aristata DC is one of the most important species due to its wide medicinal properties and its occurrence has reported from sub-tropical areas (1800-3000 m ASL) of the mountain state of Uttarakhand and Himachal Pradesh6. It is used invarious crude drug formulations and in different ayurvedic and homeopathic medicines since ancient times.

Potential secondary metabolites/bioactive compounds of *B. aristata*

Different plant extracts, essentials oils and their bioactive secondary metabolites have been extensively used for the treatment and management of distinct metabolic diseases due to their safety and less or no toxicity [5–7]. A recent study demonstrated that *B.aristata* constitutes phytochemicals of different classes such as alkaloids, flavonoids, saponins, steroids, coumarins, glycosides, tri-terpenoids, polyphenols, tannins, reducing sugars, metal ions and, among these, the alkaloids are the most abundant phytoconstituents from *B. aristata* stem [1,8].

OBJECTIVES

I. **Solvent based extraction and phytochemical screening of *B. aristata stem*.**

II. **Evaluation of antioxidant potential of *B. aristata* stem extracts.**

III. **Molecular docking analysis of *B. aristata* compounds against acetylcholineesterase enzyme.**

2. REVIEW AND LITERATURE

2.1. Pharmacological effects of *B. aristata* in targeting various diseases

B. aristata is well known for their pharmacological activities such as; anticancer, hepatoprotective, anti-diabetic, anti-inflammatory and neuroprotective activity. various *in vitro* and *in vivo* studies are the agreement of its medicinal propreties.

2.1.1 Antioxidant effects

Till date, various *in-vitro* methods have been used for the determination of antioxidant properties of different medicinal plants and their secondary metabolites *i.e*., DPPH, FRAP, OH radical and ABTS assay along with other *in-vivo* biomarkers of oxidative homeostasis *i.e*., CAT, SOD, GST, GPx, and PON-1 [9-14].Gaccheand Dhole assessed the antioxidant potential of ethanolic extract of *B. aristata* and reported that it exerts potent *in-vitro* DPPH free radical scavenging activity with an IC_{50} value of 150 µg/ml [15]. Further, they also analyzed the hydroxyl radical scavenging activity of *B. aristata* ethanolic extract and demonstrated that it does not show hydroxyl radical quenching activity [15].

Recently, berberine has been shown to exhibit potent antioxidant activities and suppressed the mitochondrial ROS generation in spiral ganglion cells via targeting sNMDAR1/Nox3 pathway [16]. .In addition, berberine significantly alleviated the oxidative stress in the lenses of diabetic rats via modulation of SOD, CAT, and GPx activities and the level of thiobarbituric acid reactive substances (TBARS) [17]. Berberine also protected against lead (Pb)-mediated oxidative imbalance and hepatotoxicity in rats by increasing SOD, CAT, and GPx activities along with GSH content, a non-enzymatic antioxidant. These beneficial effects of berberine against Pb-induced oxidative stress, hepatic necrosis and inflammatory response were comparable to the effects of reference standard silymarin [18].

2.1.2. Anti-inflammatory effects

Administration of *B. aristata* extract markedly suppressed the protein expression of inflammatory markers i.e., interleukin-1β (IL-1 β), IL-6, TNF-R1 and cyclooxygenase-1 (COX-1) that are reckoned to trigger the inflammatory cascades, whereas, the expression of anti-inflammatory IL-10 was stimulated in peritoneal macrophages [19].

Further, *B. aristata* extract supplementation resulted in down-regulation of IL-1 β), IL-6, TNF-R1, NF-κB and vascular endothelial growth factor (VEGF) expression in an adjuvant-induced arthritis (AIA) model. In addition, the HO-1/Nrf-2 ratio was also improved and the protein level of matrix metalloproteinases (MMP)-3 and 9, known to degrade the extra cellular matrix, was also suppressed after *B. aristata* extract treatment [19].

Similarly, the treatment with berbamine also regulated the MAPK (JNK and ERK1/2) pathways and reduced the activation of NF-κB in LPS-stimulated macrophages [20]. Moreover, incubation with berbamine evidently combated the LPS or CH_3COOH-induced IL-1β and lL-6 mRNA expression and p65 and STAT3 phosphorylation in RAW264.7macrophage cells and protected C57BL/6J mice against alcoholic liver disease [21]. Two sitosterol's namely β-sitosteryl-3β-glucopyranoside-6′-O-palmitate and 3-O-(6′-O-linoleoyl-β-D-glucosyl)-β-sitosterol isolated from *Berberis spp*. exhibited strong anti-inflammatory effects via inhibiting *in-vitro* activity of COX-1 and COX-2. In conclusion, *B. aristata* and its secondary metabolitesexhibit promising anti-inflammatory effects and may be further evaluated for their therapeutic potential against various inflammatory diseases including atherosclerosis, diabetes and cancer.

2.1.3. Antidiabetic effects

In this regard, *B. aristata* has been reported to positively affect glycemic control, significantly decreasing HbA1c, basal insulin, homeostatic model assessment of insulin resistance[22].A randomized and placebo control clinical trial on 102 patients observed that treatment with *B. aristata* extract in combination with *Silybum marianum*improves the glycemic control in diabetic dyslipidemic subjects via increasing the insulin C-peptide [23].Another clinical trial with same combination showed the protective effects on T1DM as well as reduced the insulin doses required before meals [24].The level of fasting blood glucose (FBG), PPG, HbA1c, TC, TGRLs and LDL-C were also reduced after the administration of *B. aristata/S. marianum* extracts [24]. Another interventional study concluded that *B. aristata* increases the level of insulin through its antioxidant mechanisms and ability to repair damaged pancreatic β-cells in T1DM and T2DM cases [25].

2.1.4. Anticancer effects

A recent study showed that the treatment with methanolic extract of berberis exerted significant anticancer effects in human osteosarcoma cells (HOS) via induction of ROS generation, enhanced apoptosis, nuclear fragmentation, autophagy, and caspase-3 activity, as well as diminished cell viability (either alone or in combination) [26].

Another study with stem plus bark extract of *B. aristata* evident its anti-tumorigenesis potential in Ehrlich ascites carcinoma (EAC)-bearing mice and it also increased the survival rate and declined the body weight due to the decreased tumor cell proliferation after receiving intraperitoneal injection of 100 mg/Kg B.W. and 6.5 mg/Kg B.W. of aqueous and ethanolic extracts, respectively [27]. Apart from the anticancer effects of *B. aristata* extracts, its bioactive ingredients like berberine, oxyberberine, berbamine and palmatine also showed remarkable antitumorigenic and antiproliferative efficacy in different *in-vitro* and animal model studies. In this context, berberine has been extensively investigated for its beneficial effects against the management of different cancers [28-30].

2.1.5. Antimicrobial effects

Extract of *B. aristata* showed potent bactericidal effects against a serious of microbes with maximum antibacterial efficacy against *Klebsiella pneumoniae* having a zone of inhibition (ZOI) of 25 mm, however, *K. pneumoniae* and some other bacteria showed a marked resistance against *B. aristata* extract. The same extract exerted strong antimicrobial effects against *Candida albicans* (ZOI: around 23 mm) [31]. Berberine, a major constituent of *B.aristata*, showed potent inhibitory effects against various methicillin-resistant *Staphylococcus aureus* (MRSA)strains with minimum inhibitory concentrations (MICs) between 32 and 128 µg/mL and extended the antibacterial efficacy of commercially available beta-lactam antibiotics*i.e.*, ampicillin and oxacillin [32,33].

3. MATERIALS AND METHODS

3.1 Chemicals

Chemicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), ascorbic acid, and dimethyl sulfoxide (DMSO), were purchased from Himedia Laboratories, Mumbai, India. Methanol (MeOH) was obtained from Merck, India. All chemicals were of analytical grade.

3.2 Plant material and its extracts preparation

Berberis aristata stem was obtained locally from Botanical garden, NBRI, Lucknow. The formal identifcation of the plant material was carried out in NBRI, Lucknow. Plant stem was shed dried and made in coarse powder, avoiding sun dried due to the signature modification of the biochemicals. The dried powder (25 g) of the plants was extracted using nonpolar, partially polar, and polar solvents successively with the required amount of each solvent (n-hexane, ethyl acetate, dicholoromethane, methanol and aqueous) and water solvents in Soxhlet apparatus until it turned colour less. The solvent was removed, filtered, and dried at room temperature, and residues were scratched out and stored at −20◦C for future use [34].

3.3 Phytochemical Screening

Qualitative chemical tests were carried out to identify the phytochemicals present in various extracts of *B. aristata* using standard procedure [34].

3.4 DPPH Radical Scavenging activity

The DPPH radical scavenging capacity of the plant was determined by the method of Brand-Williams *et al. (*1995). Briefly the free radical scavenging activity based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. DPPH molecule determines with the occurrence of a purple colour. DPPH solution (132mM) was prepared in methanol in a dark reagent bottle. 100µl of the Iridin (dissolved in 5% CH_3OH) and reference standard drug, ascorbic acid (dissolved in water) (Concentration ranging from 0 to 1000µg/ml) was added to 2ml of DPPH solution and the reaction mixture was incubated for 15 minutes at 27ºC in a water bath and absorbance was measured at 517 nm. The reduced form of DPPH was generated, accompanied by the disappearance of the violet colour. Ascorbic acid was used as a

reference standard. Percent (%) scavenging of DPPH free radical was measured using the following equation [9].

% DPPH scavenging= (Abs. of Control-Abs. of sample)/Abs. of control x100

Further, IC50 [9].

3.5 Superoxide radical scavenging assay

This activity was measured by the reduction of NBT according to a previously reported method [5]. The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to a purple formazan. The 1 ml reaction mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations (0–20 µg/ml) of sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated.

3.6. *In silico* pharmacokinetic analysis of active metabolites of *B. aristata* and molecular docking analysis against Acetylcholineesterase (AChE)

3.6.1. *Software required*

Cygwin, a data storage c:\program was downloaded from www.cygwin.comb, MGL (Molecular Graphics Laboratory) tools AutoDock4.2 downloaded from www.scripps.edu, Discovery studio (DS) visualize 3.0 downloaded from www.accelerys.com, Accelrys draw download from www.accelrys.com and Python 2.5 downloaded simultaneously during cygwin download.

3.6.2. *Prepration of ligands*

B. aristata compounds were collected from available literature. Chemical properties and mol files of the compounds were retrieved from the NCBI–PubChem Compound database (http//pubchem.ncbi.nlm.nih.gov/).

3.6.3. *Pharmacokinetic Analyses*

ADME/Tox properties

The ADME/Tox properties (Absorption, Distribution, Metabolism, Excretion/ Toxicology) of all compounds were calculated using the online server PreADMET http://preadmet.bmdrc.org/). This server calculates pharmacokinetic properties as: Human Intestinal Absorption (HIA), cell permeability Caco-2 in vitro (Pcaco-2), cell permeability of Maden Darby Canine Kidney (PMDCK), skin permeability (PSkin), Plasma Protein Binding (PPB) and the penetration of the blood brain barrier (C_{Brain}/C_{Blood}), and toxicological properties such as: mutagenicity and carcinogenicity (Yashmita et al., 2000).

Drug likeness calculations

Drug scans were carried out to determine whether the plant metabolites fulfil the drug-likeness conditions. Lipinski's filters using Molinspiration (http://www.molinspiration.com/) were applied for examining drug likeness attributes as including quantity of hydrogen acceptors (should not be more than 10), quantity of hydrogen donors (should not be more than 5), molecular weight (mass should be more than 500 daltons) and partition coefficient log P (should not be less than 5). The smiles format of each of the compound was uploaded for the analysis (Singh et al., 2017; Molinspiration, 2016).

3.6.4. *Preparation of Protein*

The protein Beta amyloid and Tau (PDB ID:4M0E) from RCSB PDB (http://www.rcsb.org/pdb/home/home.do). The protein was subsequently cleaned by removing the bound complex molecule, the non-essential water molecules and all the heteroatoms. Finally, hydrogen atoms were merged to the receptor molecule in AutoDock.

3.6.5. Molecular Docking study

The docking was done with the help of AutoDock Tool 4.2.6 in order to find a suitable binding conformation of the target (AChE) and ligands (compounds of *B. aristata*). The analysis of Binding conformation of the target-ligand complex was done and they were ranked according to the scoring function of the free energy of binding and inhibition constant. Four coordinate files are created ligand.pdbqt, receptor.pdbqt, grid.gpf and dock.dpf. The "Lamarckian genetic algorithm" was applied to determine the binding

affinity of the complex. The torsion of the ligand was set random. With the assistance of docking polar hydrogen atoms, atomic solvation parameters, Kollman charges and fragmental volume were allocated to the protein. The grid spacing was 0.375Å between the two connecting grid points. Every grid point in x, y and z-axis was set to 90 x 90 x 90Å. While for docking test, 10 runs with a population size 150 and maximum number of evaluations was 25, 00,000 was set. The rest of the parameters were set to default with 0.02 rate of gene mutation, 0.8 rate of cross over with maximum number of generation was 27,000. The results were generated in.glg and .dlg file were further studied for the ligand and protein interaction. The final outcomes of docking were compiled from free energy (Binding energy) and inhibition constant (Ki). The best docked structures were developed using the Accelry's Discovery Studio Visualizer 2020.

4. RESULTS AND DISCUSSION

4.1 Phytochemical screening of B. aristata extracts

The sequentially extracted fractions were analysed for the presence or absence of different phytochemicals using standard protocols. The results have been reported in Table 1.

Table 1: Phytochemical profiling of sequentially extracted *B. aristata* extracts

S. No.	Phytochemical groups	*B. aristata* stem extracts				
		n-Hex	EtOAc	DCM	MeOH	Aqu.
1.	Tannins	+	+	+	+++	++
2.	Flavonoids	+	+	++	+++++	++++
3.	Quinones	++	+++	+++++	++++	+
4.	Terpenoids	+++++	+	++	+++	++++
5.	Cardiac glycosides	-	-	-	-	-
6.	Coumarins	-	-	+	++++	+++
7.	Steroids	+++	++++	+++++	-	+

*(n-hexane, ethyl acetate, dicholoromethane, methanol and aqueous)

Phytochemical analysis showed presence of secondary metabolites was higher in methanol and aqueous extracts of *B. aristata* including flavonoids, quinones, terpenoids, tannins etc.

4.2 Antioxidant activity of *B. aristata* extracts

4.2.1 DPPH free radicals scavenging activity of *B. aristata* extracts

The sequentially extracted fractions (0-200 µg/ml) were analysed for the DPPH free radical scavenging activity using standard protocols. All five extracts showed significant DPPH scavenging activity when compared to standard drug, ascorbic acid. The results have been reported in Figure 1.

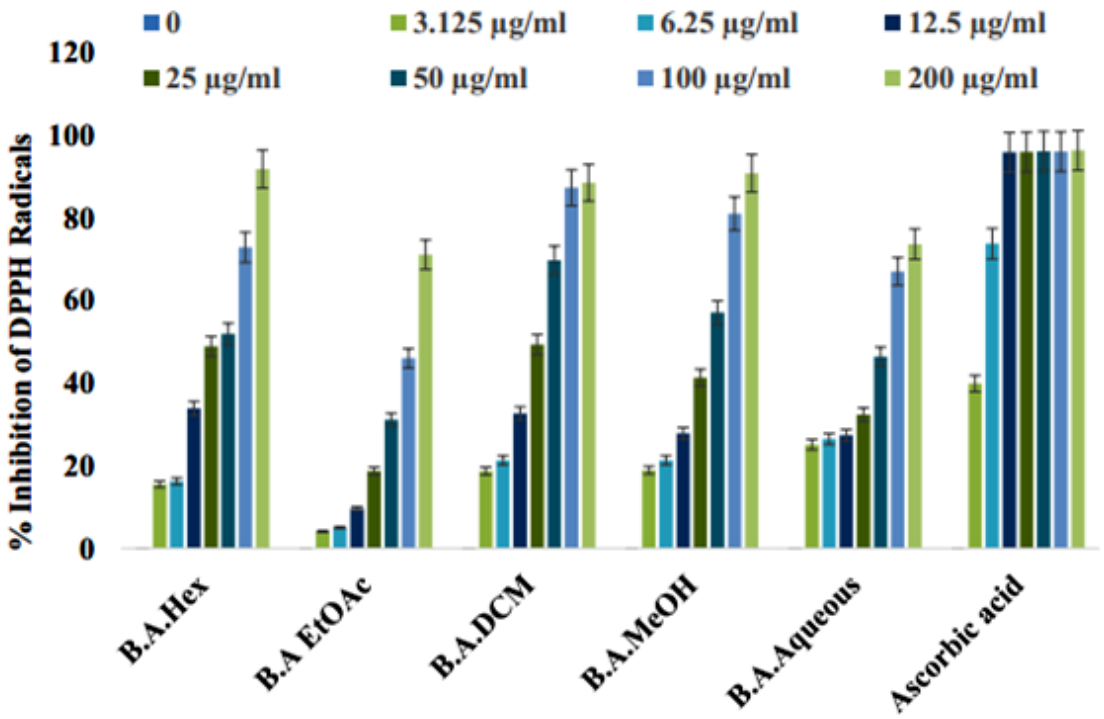

Figure 2: DPPH free radicals scavenging activity of *B. aristata* extracts.

4.2.2 Super oxide radical (O2.-) scavenging activity of *B. aristata* extracts

The sequentially extracted fractions (0-200 µg/ml) were analysed for the super oxide radical scavenging activity using standard protocols. All five extracts showed significant super oxide radical scavenging activity when compared to standard drug, ascorbic acid. The results have been reported in Figure 2.

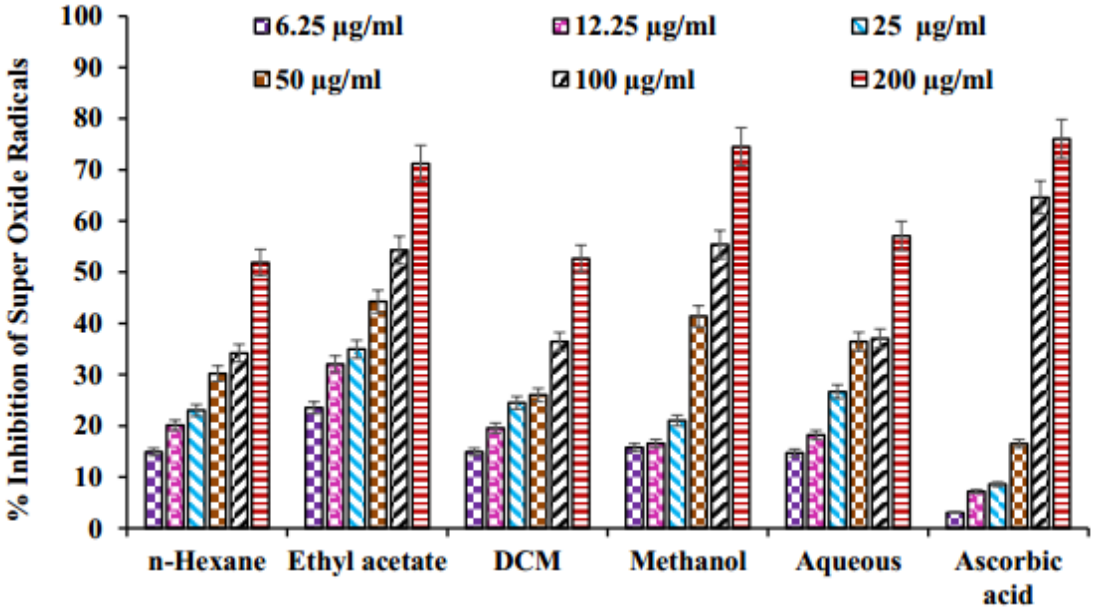

Figure 3: Superoxide radicals scavenging activity of *B. aristata* extracts.

4.3. *In silico* pharmacokinetic analysis of active metabolites of *B. aristata* and molecular docking analysis against AChE

Based on available literature six active compounds of *B. arsitata* have been compiled for *in silico* analyses (Table 2)

Table 2: Active metabolites of *B. arsitata*

S. No.	CompoundID	CompoundName
1.	2353	Berberine
2.	10170	Berbamine
3.	11066	Oxyberberine
4.	122728	Aromoline
5.	630739	Karachine
6.	19009	Palmatine

Pharmacokinetic study of compiled six compounds have been analysed for ADME/Tox and Lipinski's properties. All the six compounds fulfilled less or more all the descriptors of ADME/tox (Table 3). Furthermore these compounds were subjected to Lipinski's profiling. The results exhibited that two compounds named berbemine and aromoline failed in drugability test (with nviolation 2). It was found that the only four (Table 4) compounds showed no violation of all the five rules i.e. not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight of compounds less than 500, partition coefficient (log P) less than 5, rotatable bonds less than 10, topological polar surface area (TPSA) of not greater than 140. Pharmacokinetcally screened four active compounds of *B. aristata* subjected to molecular docking analysis against AChE enzyme of Alzheimer's disease.

Table 3: ADME/Tox properties of selected compounds.

		Toxicity			Absorption				Distribution		Metabolism
S.NO	**Compound**	**Mutagenicity (Ames Test)**	**Carcinogenicity**		**HIA**	**Caco 2**	**MDCK**	**Skin Permeability**	**PPB (Plasma Protein Binding)**	**BBB (Blood Brain Barrier)**	**CYP2D6 Inhibition**
			Mouse	**Rat**							
1.	Berberine	M	NC	NC	97.88	55.57	14.40	-4.373	58.54	0.693	Inh
2.	Berbamine	NM	NC	NC	97.52	52.69	0.043	-2.970	82.05	0.357	Non-Inh
3.	Oxyberberine	M	NC	NC	97.80	45.40	8.086	-4.374	83.87	0.197	Non-Inh
4.	Aromoline	NM	NC	NC	96.70	48.26	0.043	-2.985	80.33	2.265	Non-Inh
5.	Karachine	NM	NC	NC	97.44	48.80	55.62	-4.523	76.61	0.016	Inh
6.	palmatine	M	NC	NC	97.92	55.95	1.207	-4.097	97.92	0.972	Inh

Mutagenicity (NM=Non-mutagenic, M Mutagenic), bCarcinogenicity (NC=Non-Carcinogenic), cHIA=Percentage of human intestinal absorption, dPCaco-2=Cell permeability (Caco-2 in nm/sec), ePMDCK= Cell permeability Maden Darby Canine Kidney in nm/sec, fPSkin=Skin permeability (nm/sec), gPPB=Percentage of plasma protein binding, hBBB =Blood Brain Barrier (CBrain/CBlood), iCYP2D6= Cytochrome P450 2D6 binding (Non-inhibitor).

Table 4: Lipinski's parameters for *B. aristata* screened compounds.

S. No.	**Compound ID**	**Compound Name**	**miLogP**	**TPSA**	**natoms**	**MW**	**nON**	**nOHNH**	**nviolations**	**nrotb**	**volume**
7.	2353	Berberine	0.20	40.82	25	336.37	5	0	0	2	296.30
8.	10170	Berbamine	6.24	72.87	45	608.74	8	1	2	3	561.52
9.	11066	Oxyberberine	3.13	58.94	26	351.36	6	0	0	2	301.58
10.	122728	Aromoline	5.96	83.87	44	594.71	8	2	2	2	544.00
11.	630739	Karachine	3.36	57.24	32	433.50	6	0	0	2	386.11
12.	19009	Palmatine	-0.05	40.82	26	352.41	5	0	0	4	323.46

Selected compounds of *B. aristata* (Pharmacokinetically satisfied) were analysed for their binding affinity with the key enzymatic target of AD (AChE). All the tested compounds showed good binding affinity with protein. Among the all the compound palmatine followed by berberine showed best binding affinity. The molecular docking results are compiled in Table 5.

Table 5: Molecular docking results of selected compounds with AChE

S.No	Compound	Binding Energy Kcal/mol	Ki (nM)	Interacting Amino Acid
1.	Berberine	-9.24	168.95	PHE295: UNK0: HIS447: SER203:
2.	Karachine	9.22	174.38	ASN233:PRO410: ASN233:UNK0
3.	Oxyberberine	-8.84	331.12	GLY121:UNK0: SER203: PHE295:UNK0: HIS447:SER203
4.	Palmatine	-9.26	161.69	PHE295:UNK0: HIS447:SER203

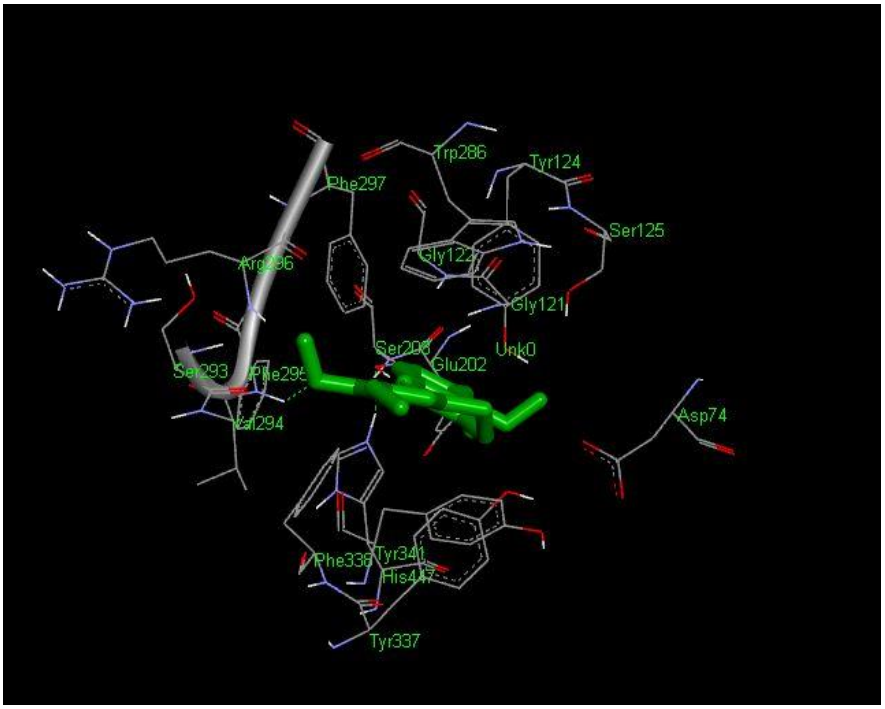

Figure 5: Interactive amino acids of AChE with Palmatine.

Oxidative stress induced by reactive oxygen species (ROS) can cause cell membrane disintegration, protein, lipid, and deoxyribose nucleic acid (DNA) damage which can further initiate or propagate the development of many chronic and degenerative diseases [10]. The uppermost concern in the mind of a sick person is always going to be whether or not full health can ever be achieved again. Sometimes the answer to that question is “yes,” and sometimes it’s “no”; sometimes it’s a very heavily qualified “yes” with lots and lots of imponderables. For some neurological issues, the outlook can be pretty good with treatment and adequate rehabilitation, while for others the prognosis can be grim. Rather than disability dwelling on a potentially unavailable cure, sometimes what’s called for is an adjustment to the patient’s lifestyle to better accommodate whatever is imposed by the affliction [5].

There has been enormous interest in natural antioxidants due to their ability to neutralize the effects of ROS that are not only responsible for alleviating the oxidative stress condition as well as neurological disorders. The growing interest to combat the side effect of the drugs available for diabetes leads to the development of green medicines due to their higher stability, higher antioxidant potential, low cost, and low cytotoxicity. Plants are rich sources of phytochemicals, which possess a variety of biological activities including antioxidant and antidiabetic potential both in vitro and in vivo [19].

In the current study, we evident the great antioxidant potential of *B. aristata* extracts. We found that methanolic and aqueous extracts exerted significant inhibition of DPPH radicals activity. These findings showed that *B. aristata* possess potent antioxidant activity almost comparable to ascorbic acid which is being used as standard for antioxidant assays [10]. In addition Methanolic and aqueous extracts also exhibited the good superoxide radical scavenging activity. This potent antioxidant potential of *B. aristata* encouraged us to precede further assays [10].

Furthermore the compiled active compounds of *B. aristata* showed good binding affinity with the key target of AD. Antioxidant potential of *B. aristata* is self-explanatory for their neuroprotective properties. Free radical generation in brain is responsible for the damage of nerve cells as well as neurotransmitters. Thus the antioxidant potential of this plant helps to ameliorate the effect of free radicals. Binding affinity of active compounds of *B. aristata* to AChE protein may be responsible to inhibit

the degradation of acetylcholine (one of the most neurotransmitter). Further in vitro studies are required to evaluate the exact mechanism of these compounds.

CONCLUSION

B. aristata is abundant of secondary metabolites, present study showed the presence of various secondary metabolites in different extracts. Methanol and aqueous extracts of *B. aristata* showed significant antioxidant potential in terms of DPPH and superoxide dismutase scavenging activity. *In silico* analyses of *B. aristata* compounds showed good binding affinity with AChE target of AD. Among the all the compound palmatine followed by berberine showed best binding affinity with AChE. Further, a thorough and full-fledged *in vitro* study is needed to explore the role of *B. aristata* in order to establish a better treatment approach to get rid of oxidative stress consequences and neurodegenerative diseases.

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