Bangladesh J. Bot. **55**(1): 45-52, 2026 (March) DOI: https://doi.org/10.3329/bjb.v55i1.88597

ANTIOXIDANT, ANTICHOLINESTERASE INHIBITORY POTENTIAL AND MOLECULAR DOCKING OF ISOLATED BIOACTIVE COMPOUNDS FROM CALLUS OF *SALVIA SANTOLINIFOLIA* (BOISS)

TOUR JAN*

Department of Botany University of f Malakand, Chakdara, Dir Lower, KP, Pakistan

Keywords: Compounds, Antioxidant, Anticholinesterase, Alzheimer, Molecular docking

Abstract

The chemical constituents used in the medical industry are primarily derived from medicinal plants. The medicinal plant *Salvia santolinifolia* contains chemicals with cytotoxic, antibacterial, and antifungal properties. Callus culture presents a viable alternative to ensure a consistent supply of raw materials throughout the year. We investigated three compounds: salvialactomine (1), pentatriacontanoic acid 1, 3-dihydroxyprophle ester (2), and 5-methylflavone (3), for their potential as antioxidants and anticholinesterase inhibitors. Compound 3 was the most effective at suppressing the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. Compounds 1 and 3 showed modest ABTS cation radical scavenging activity in the ABTS assay, while only 3 were active in the DPPH assay. Molecular docking investigations of the isolated compounds revealed different binding interactions inside the enzymes' binding pocket, supporting the bioactivity data. These findings suggest that 5-methylflavone (3) shows promise as a potential medication for Alzheimer's disease based on its strong activity against acetylcholinesterase and butyrylcholinesterase in both theoretical and experimental research.

Introduction

The potential of many plants as antioxidants has been investigated. Natural antioxidants, in the form of chemical components or crude extracts, can block the harmful effects of oxidative stress (Lalitha and Jayanthi 2012). Free radicals are the body's biological response to aging, inflammation, immunosuppression, atherosclerosis, cancer, diabetes, neurological diseases, and ischemic heart disease (Chanda *et al.* 2011). The human body has a blockade system to restrict free radicals, such as protein, superoxide dismutase, glutathione peroxidase, and catalase. Phytonutrients are naturally occurring antioxidants used as self-protective mediators to fight free radicals inside the living body (Sies 1993). Selenium, vitamin E, β-carotene, lycopene, vitamin C, lutein, and diverse types of carotenoids have been consumed as extra antioxidants (Adeshina *et al.* 2010). Epidemiological research has shown that regular utilization of natural antioxidants is associated with a lower risk of cardiovascular illness and cancer (Renaud *et al.* 1998). The drugs to prevent cholinesterase (AChE and BChE) enzymes are the preferred therapeutic method for regulating neurodegenerative illnesses (Orhan *et al*. 2007). In Alzheimer's, the key is enzymes AChE and BChE; thus, the prevention of AChE and BChE increases communication and decreases the symptoms of Alzheimer's illness (Köse *et al*. 2015). Recent studies on antialzheimer and antidiabetic disease showed that natural compounds in herbal sources and food have amplified (Tang *et al*. 2019). Natural compounds have numerous properties, including the antienzymatic property of enzymes such as tyrosinase, α-glucosidase, α-amylase, AChE, and BChE (Placines *et al*. 2020). They can be obtained from different natural resources, i.e., medicinal plants (Gokhan *et al*. 2015). The chemistry of these natural compounds is complicated and based on the nature of action and the target site. Recent studies are concentrated on compounds whose action constrains glucose adjustment in the body by acting on sugar-humiliating enzymes (Taslimi and Gulçin 2017). The practice of plant constituents, including compounds from natural sources, has great

*Author for correspondence: <tour_jan@yahoo.com>.

significance as it decreases Alzheimer's disease progression by the prevention of AChE and BChE (Ferreira *et al*. 2006). *Salvia santolinifolia* is a small herb that is classified as a part of the Lamiaceae family. It is one of the highly varied and geographically diverse plant families. The *S. santolinifolia* medicinal value is mainly due to the presence of volatile oil (Sarac and Ugur 2007). Essential oils and chemical composition of the *Salvia* species have been previously reported (Abak *et al*. 2018). The crude extract of the whole plant of *S. santolinifolia* contained cytotoxic properties (Duru *et al*. 2012). The essential oil of *Salvia* has significant antioxidant, antibacterial, and antifungal activities (Çolak *et al*. 2018). Many important secondary metabolites that are part of different chemical groups, like essential medicinal oils, terpenoids, and phenolic moieties containing compounds, have been extracted from the *Salvia* genus and are featured in different medicinal literature across the globe (Ulubelen and Topou 1992). The structure elucidations of three compounds, namely, salvialactomine (1), pentatriacontanoic acid 1, 3-dihydroxyprophle easter (2), and 5-methylflavone (3), obtained from the callus culture of *S. santolinifolia* (Fig. 1) have been reported (Jan *et al*. 2018). The anticholinesterase and antioxidant inhibitory activities were studied along with their Molecular docking.

Materials and Methods

Callus was developed on a previously established protocol (Jan *et al*. 2018). In brief, the required plant parts were removed from healthy, field-grown plants. The excised branches were placed in running tap water for a minute. Then they were sterilized with a solution containing 0.05% mercuric chloride ($HgCl_2$) and a few drops of Tween-20, followed by four rinses with sterilized distilled water. The excised segments were inoculated on MS (Murashige and Skoog 1962) medium (pH 5.5), sugar (3%), agar (0.6%), and plant growth regulators (NAA): 0.1, 0.5 and 1.0 mg/l, NAA/BA (0.5/1.5 mg/l) for induction and multiplication of callus.

Previous chemical investigation of the crude extract of callus of this plant species led to the isolation and identification of three compounds, namely, salvialactomine (1), pentatriacontanoic acid 1,3-dihydroxyprophle ester (2), and 5-methylflavone (3) (Jan *et al*. 2018). The compounds (1-3) were used to test their bioactivities (Fig. 1).

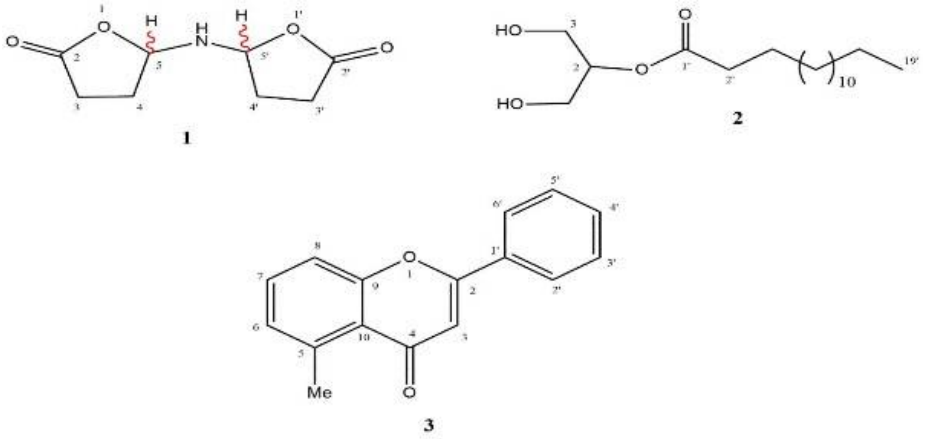

Fig. 1. Chemical structures of compounds 1-3 tested in this study.

The ABTS (2, 2'-azino-bis3-ethylbenzthiazoline-6-sulfonic) cation radical decolorization experiment: The ABTS assay was done to determine the ability of compounds for the ABTS free radical scavenging activity. Approximately 5 ml ABTS (14 mM) was mixed with 5 ml potassium

persulfate (4.9 mM) solution and kept in the dark for 16 hrs at room temperature. ABTS solution (1 ml) was added to 0.1 ml of each sample, and the changes in absorbance were recorded at intervals of 3 min at a single wavelength of 734 nm. For the comparison, the Trolox compound was used as a standard (Boonchum *et al*. 2011). The inhibition percentage of test samples was measured using the following equation:

$$Inhibition\ \% = \left[\frac{A0 - A1}{A0}\right] \times 100$$

In this equation, the absorbance of the control is *A0*, and the test sample is A1. The results of ABTS cation radical scavenging action are expressed in the form of IC_{50} (μg/ml). BHT and α-tocopherol are the positive controls.

To determine the DPPH free radical destruction potential of the sample, a DPPH free radical test was performed. The test samples of various concentrations (100-800 μM) were prepared in ethanol. To the 0.4 ml test sample, we added 2 ml of the DPPH (0.004%) solution that was prepared in ethanol. An equal volume of the compound (2 ml) and DPPH (2 ml) was mixed and allowed to react at 25°C for 30 min. A spectrophotometer was used to measure the absorbance at the wavelength of 517 nm. In this assay, BHT and α-tocopherol were used as positive controls. The scavenging action of the test compounds was measured in triplicate, and values were obtained by putting in the following mathematical formula (Sahaa *et al*. 2008):

$$\%\ scavenging = \{\frac{Abs\ control - Abs\ sample}{Abs\ control}\} \times 100$$

In this equation, 'Abs control' represents the "absorbance of DPPH + respective solvent," while the 'Abs sample' represents the absorbance of DPPH along with the sample/ standard.

The commercially available electric eel AChE (acetylcholinesterase) and BChE (butyrylcholinesterase) from *Equine* serum were utilized as enzyme receptors to test the inhibitory action of the tested samples according to Ellman's assay with a slight modification (Ellman *et al*. 1961). Test samples were dissolved in ethanol and diluted to obtain various concentrations (100-800 μM). For the experiment, acetylcholinesterase (518 U/mg) and butyrylcholinesterase (7-16 U/mg) were taken, and their solution was made in 0.1 M phosphate buffer (pH 8.0) with a final concentration of 0.03 U/ml (AChE) and 0.01 U/ml (BChE). In deionized water, the coloring reagent DTNB (0.2273 mM), and the substrates AChCl (acetylcholine chloride) (0.5 mM) and BChI (butyrylcholine iodide) (0.5 mM) solutions were made and placed in the refrigerator at 8°C. 5 μl of each respective enzyme solution was placed in a cuvette, DTNB reagent (5 μl) and test sample (205 μl) were added to them for each assay and placed at 30°C for 15 min in a water bath, following the supplementation of substrate solution (5 μl). A double-beam spectrophotometer (Thermo Electron Corporation, USA) was used to measure the absorbance at a wavelength of 412 nm. Galantamine served as a positive control in this assay. The absorbance was recorded at a temperature of 30°C for 4 min during the reaction time. The measurements were recorded in triplicate. The percent enzyme performance and its destruction by test samples and control were measured from the amount of absorption with variation in time ($V=\Delta Abs/\Delta t$) as follows:

Where enzyme inhibition (%) = 100 - percent enzyme activity, Enzyme activity (%) = 100 × V/Vmax (Vmax is the enzyme activity in the absence of inhibitor compound).

The coordinates of crystal structures of acetylcholinesterase (AChE) with protein data bank (PDB) code 1EVE having a resolution of 2.5 Å (Kryger and Sussman 1999) and human peroxiredoxin-5 having PDB ID 1HD2 at 1.5 Å resolution (Declercq *et al*. 2001) were downloaded from the Protein Data Bank. The unnecessary hetero-atoms and water molecules in

the PDB file were deleted, and the required hydrogen atoms were added to the respective receptor proteins using MOE 2014.09.146 software. Before performing the docking calculations, an energy minimization using the AMBER99 force field was executed on the two selected enzymes as described previously in the literature (Khan *et al.* 2016).

Results and Discussion

The *in vitro* Inhibition effects of the selected compounds (1-3) on the AChE and BChE enzymes were investigated. These two are the main enzymes related to Alzheimer's disease. The IC_{50} values have been shown, and inhibition values are highlighted in Table 1. Compound 3 exhibited better inhibitory potential than compound 2, while compound 1 was inactive. The galantamine was the positive control in this experiment.

Table 1. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of the compounds (1-3).

Compounds	AChE assay IC_{50} (µM)[a]	BChE assay IC_{50} (µM)[a]
1	NA	NA
2	1016 ± 50	1159 ± 92
3	509.7 ± 33	361.7 ± 5.5
Galantamine [b]	4.48 ± 0.78	46.03 ± 0.14

[a]Values expressed are mean ± SEM of three parallel measurements ($p < 0.05$). [b]Reference compound, [c]NA: not active

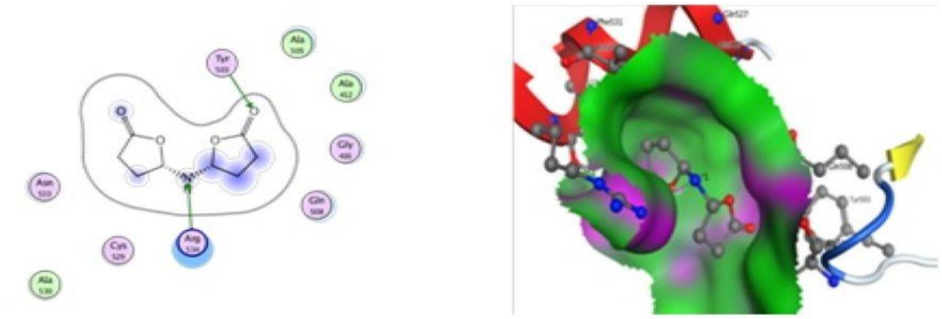

Fig. 2. The figure on the left side shows a two dimensional (2D) picture of the binding pocket of the acetylcholinesterase enzyme interacting with compound 1 through hydrogen bonding, while the figure on the right side is a 3D view of the same binding pocket.

The compound 1 expressed two hydrogen bonding interactions with the acetylcholinesterase enzyme. The oxygen atom of the keto group of one of the five-member lactone accepts a hydrogen bond from Tyr503, while the nitrogen atom of the secondary amine bonded to the two five-member lactones in Compound 1 accepts a hydrogen bond from Arg534 of the acetylcholinesterase (Fig. 2). Compound 1 on docking with acetylcholinesterase, showed a docking score of -10.444 kcal/mol and a binding energy of -9.222 kcal/mol. Compound 2 expressed four interactions of hydrogen bonding with the following residues of protein: Asn131, Asp128, and Tyr134, with a good docking score of -12.432 kcal/mol and binding energy -11.432 kcal/mol (Fig. 3). The two oxygens of the hydroxyl group are the acceptors of hydrogen bonding from Asn131. The two hydrogen atoms of the same two hydroxyl alcohol groups of Compound 2 act as a hydrogen bond donor to Asp128 and Tyr134 of the respective protein receptor. Compound 3 showed two interactions, one of which is a hydrogen bond with the residues Tyr70, where

tyrosine 70 is the hydrogen bond donor, and the keto group of the compound acts as a hydrogen bond acceptor. The second interaction is the arene-arene interaction between Trp279 of the protein and the phenyl group directly attached to the six-member lactone ring, as shown in Fig. 4. It has a docking score of -8.323 kcal/mol and a binding energy of -7.445 kcal/mol.

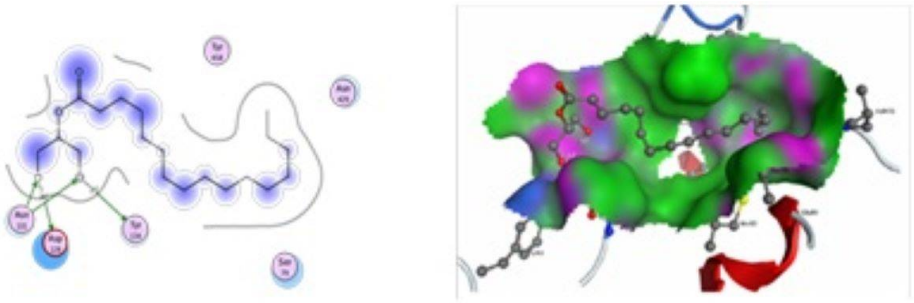

Fig. 3. The figure on the left side shows a two-dimensional (2D) picture of the binding pocket of acetylcholinesterase enzyme interaction with compound 2 through hydrogen bonding while the figure on the right side is a 3D view of the same binding pocket.

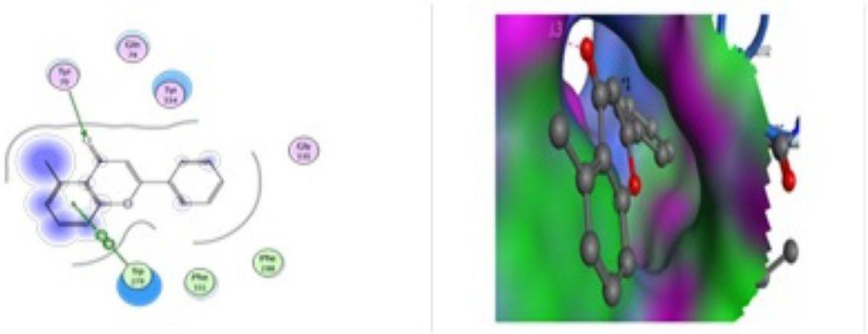

Fig. 4. The figure on the left side is the two-dimensional (2D) picture of the binding pocket of acetylcholinesterase enzyme interaction with compound 3 through hydrogen bonding while the figure on the right side is a 3D view of the same binding pocket.

The isolated purified compounds were analyzed for their anti-radical properties using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays (Table 2). The α-tocopherol and butylated hydroxyltoluene (BHT) were positive controls. In the ABTS assay, all three tested compounds, 1-3 possessed moderate $ABTS^{\bullet+}$ scavenging activity. In the DPPH assay, only compound 3 exhibited $DPPH^{\bullet}$ scavenging activity.

Table 2. Antiradical activities of compounds (1-3).

Compounds	ABTS Assay IC_{50} (mM)	DPPH Assay IC_{50} (mM)
1	10.15 ± 0.20	NA
2	69.52 ± 0.33	NA
3	5.6 ± 0.10	6.79 ± 0.25S
BHT [b]	2.91 ± 0.55*	54.97 ± 0.99*
α-Tocopherol [b]	4.87 ± 0.45*	12.26 ± 0.07*

[a]Values expressed are mean ± SEM of three parallel measurements ($p < 0.05$).
[b]Reference compounds *(IC_{50} value is expressed in µg/M), [c]NA: not active

The docking of Compound 1 with the antioxidant enzyme peroxiredoxin showed two hydrogen bonding interactions. The oxygen atom of the left side lactone moiety of compound 1 accepts hydrogen bonds from Asn122. The nitrogen atom of the bridge amine group between the two lactone groups of Compound 1 accepts a hydrogen atom from Asn76 of the peroxiredoxin enzyme (Fig. 5). The docking results provided a docking score of -8.234 kcal/mol and a binding energy of -8.121 kcal/mol. Compound 2 also makes two hydrogen bonding interactions on docking with the antioxidant enzyme peroxiredoxin. The oxygen atoms of the two hydroxyl groups of Compound 2 accept hydrogen bonds from each Asn76 and Arg134 of the peroxiredoxin receptor protein (Fig. 6). The docking results computed a docking score of -9.148 kcal/mol and a binding energy of -9.777 kcal/mol. When Compound 3 was docked with the crystal structure of the human peroxiredoxin receptor protein (Fig. 7), it showed only one interaction with the side chain phenyl group, made an arene-cation with Arg 86 of the protein receptor, and had a binding energy of -5.434 kcal/mol.

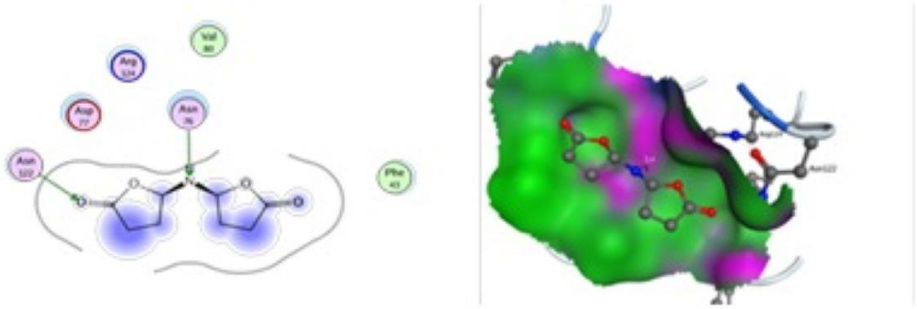

Fig. 5. The left panel shows the two-dimension (2D) picture of the binding pocket of PDB ID 1HD2 interaction with compound 1 through hydrogen bonding while the right-side figure is a 3D view of the same binding pocket.

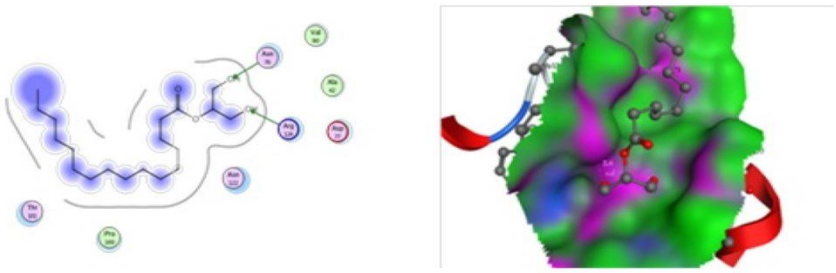

Fig. 6. The left panel shows the two-dimensional (2D) picture of the binding pocket of PDB ID 1HD2 interaction with compound 2 through hydrogen bonding, while the right figure is a 3D view of the same binding pocket.

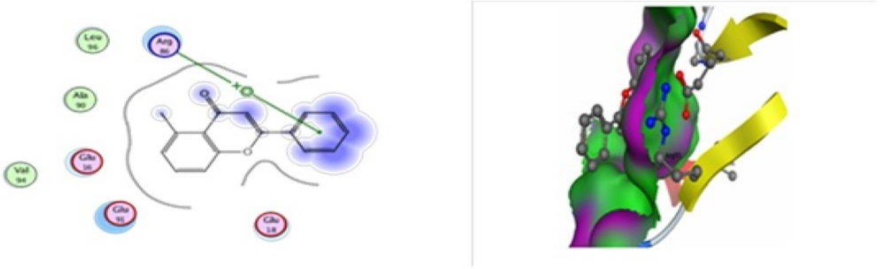

Fig. 7. The left panel shows the two-dimensional (2D) picture of the binding pocket of PDB ID 1HD2 interaction with compound 3 through arene-cation, while the right-side figure is a 3D view of the same binding pocket.

The molecular docking investigation of the isolated compounds against the human peroxiredoxin enzyme showed that compound 2 is a better drug candidate as compared to the other two compounds in terms of binding. These results are slightly different from the experiment two antioxidant assay results, which showed compound 3 as the ideal drug candidate for antioxidant activity. These differences are due to the mode of action of these compounds and the different enzymes involved in the theoretical and experimental work.

Acknowledgments

I am indebted to the University of Malakand, the Department of Botany, and HEJRICCBS for their continuous technical and instrumental support during this study.

References

Abak F, Yildiz G, Atamov V and Kurkcuoglu M 2018. Composition of the essential oil of *Salvia montbretii* Benth. from Turkey. Rec. Nat. Prod. **12**: 426-431.

Adeshina GO, Onaolapo JA, Ehinmidu JO and Odama LE 2010. Phytochemical and antimicrobial studies of the ethyl acetate extract of Alchornea cordifolia leaf found in Abuja, Nigeria. J. Medici. Plants Res. **4**(8): 649–658.

Boonchum W, Peerapornpisal Y, Vacharapiyasophon P, Pekkoh J, Pumas C, Jamjai U, Amornlerdpison D, Noiraksar T and Kanjanapothi D 2011. Antioxidant activity of some seaweed from the gulf of Thailand. Int. J. Agric. Biol. **13**: 95–99.

Chanda S, Kaneria M and Nair R 2011. Antibacterial activity of *Psoralea corylifolia* L seed and aerial parts with various extraction methods. Res. J. Micro. **6**(2): 124-131.

Çolak NU, Yıldırım S, Bozdeveci A, Yayli N, Çoşkunçelebi K, Fandaklı S and Yaşar A 2018. Essential Oil Composition, Antimicrobial and Antioxidant Activities of *Salvia staminea*. Rec. Nat. Prod. **12**: 86-94.

Declercq JP, Evrard C, Clippe A,Stricht DV, Bernard A and Knoops B 2001. Crystal Structure of Human Peroxiredoxin 5, a Novel Type of Mammalian Peroxiredoxin at 1.5 Å Resolution. J. Mol.Biol. **311**: 751-59.

Duru ME, Tel G, Oztürk M and Harmandar M 2012. Chemical Composition, Antioxidant and Anticholinesterase Activities of the Essential Oil of *Salvia chrysophylla*. Rec. Nat. Prod. **6**: 175-179.

Ellman GL, Courtney KD, Andres V and Featherston RM 1961. A new and rapid colorimetric determination of acetylcholin-esterase activity. Bioche. Pharmacol. **7**: 88-95.

Gokhan Z, Guler GO, Aktumsek A, Ceylan R, Picot CMN and Mahomoodally MF 2015. Enzyme inhibitory properties, antioxidant activities, and phytochemical profile of three medicinal plants from Turkey. Advanc. Pharmacologi. Sci. 410675.

Jan T, Qadri R, Naqvi B, Adhikari A, Nadeem S and Muhammad A 2018. A novel Salvialactomine from the callus culture of *Salvia santolinifolia* Boiss. Nat. Prod. Res. **32**: 749-754.

Khan H, Amin H, Ullah A, Saba S, Rafique J, Khan K, Ahmad N and Badshah SL 2016. Antioxidant and antiplasmodial activities of bergenin and 11-O-galloylbergenin isolated from *Mallotus philippensis*. Oxid. Med. cellu longe. 1051925.

Kose LP, Gülcin I, Gören AC, Namiesnik J, Martinez-Ayala AL and Gorinstein S 2015. LC–MS/MS analysis, antioxidant and anticholinergic properties of galanga (Alpinia officinarum Hance) rhizomes. Industrial Crops and Products. **74**: 712–721.

Kryger G, Silman I and Sussman JL 1999. Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs. Structure **7**: 297-307.

Lalitha TP and Jayanthi P 2012. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Asian J. Plant Sci. Res. **2**: 115-122.

Orhan I, Kartal M, Naz Q, Ejaz A, Yilmaz G, Kan Y and Iqbal CM 2007. Antioxidant and anticholinesterase evaluation of selected Turkish *Salvia* species. Food Chem. **103**(4): 1247-1254.

Placines C, Castañeda-Loaiza V, João RM, Pereira GC, Stefanucci A, Mollica A and Custódio L 2020. Phenolic profile, toxicity, enzyme inhibition, in silico studies, and antioxidant properties of Cakile maritima Scop. (Brassicaceae) from Southern Portugal. Plants. **9**(2):142.

Renaud SC, Gueguen R, Schenker J and d'Houtaud A 1998. Alcohol and mortality in middle-aged men from eastern France. Epidemiology **9**: 184-188.

Sarac N and Ugur A 2007. Antimicrobial activities and usage in folkloric medicine of some Lamiaceae speices growing in Mugla, Turkey. EurAsia J. BioSci. 4: 28–37.

Sies H 1993. Ebselen, a selenoorganic compound as glutathione peroxidase mimic. Free Radical Bio. Medi. **14**(3), 313-323.

Sies H 1993. Ebselen, a selenoorganic compound as glutathione peroxidase mimic. Free Radical Bio. Medi. **14**(3), 313-323.

Tang H, Song P, Li J and Zhao D 2019. Effect of Salvia miltiorrhiza on acetylcholinesterase: Enzyme kinetics and interaction mechanism merging with molecular docking analysis. I. J. Biol. Macromo.**135**:303–313.

Taslimi P and Gulçin İ 2017. Antidiabetic potential: *In vitro* inhibition effects of some natural phenolic compounds on α-glycosidase and α-amylase enzymes. J. Biochem. Mol. Toxi. **31**(10): 21956.

Ulubelen A and Topou G 1992. Abiean diterpenoids from *Salvia pomifera*. Phytochem. **31**: 3949-3951.

(Manuscript received on 04 June, 2025; revised on 19 February, 2026)