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Lipophilic Compounds from Tunisian *Sarcocornia fruticosa*: Characterization, Acetylcholinesterase Docking and Drug-likeness Evaluation

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Abstract

Sarcocornia fruticosa is a halophytic shrub from coastal salt marshes valued for its ecological role and rich content of minerals and bioactive compounds. This study combined pharmacokinetic, *in silico*, and *in vitro* approaches to evaluate the antioxidant potential of its lipophilic extract. Gas chromatography/mass spectrometry identified 22 lipophilic compounds, mainly saturated and polyunsaturated fatty acids (62%), followed by alcohols (13%), hydrocarbons (8%), and phytosterols (7%). LE exhibited moderate antioxidant and anti-acetylcholinesterase (AChE) activities, with half maximal inhibitory concentration (IC_{50}) values of 479 µg/mL 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 566 µg/mL ferric reducing antioxidant power (FRAP) assay, 672 µg/mL $NO^{\bullet}$ scavenging assay and 588 µg/mL anti-AChE activity. Docking studies highlighted phytosterols (stigmasterol, *δ*-sitosterol, stigmastanol) as the strongest AChE inhibitors, followed by phytol, while fatty acids andhydrocarbons showed weak binding due to poor fit within the catalytic gorge. Pharmacokinetic analyses revealed significant limitations: phytosterols exhibited poor gastrointestinal absorption, high molar mass, rigidity, and low polarity, violating several drug-likeness filters. Phytol showed a slightly better profile due to its lower molar mass and flexibility, but its high lipophilicity and low solubility may still limit absorption. All four compounds were predicted as P-glycoprotein substrates, indicating reduced systemic exposure, and potential CYP2C9 inhibition suggested possible drug–drug interactions. Overall, integrating these approaches provides a comprehensive view of the bioactivity and pharmacokinetic constraints of *S. fruticosa* lipophilic compounds.

Keywords

Sarcoconia fruticosa, acetylcholinesterase activity, *in vitro* study, *in silico* study, pharmacology evaluation

1 Introduction

Edible glasswort, or *Sarcocornia fruticosa* (L.) A.J. Scott, is a perennial succulent halophyte that grows throughout the Mediterranean Basin in salt flats and saline coastal marshes, including well-known stands in Tunisia [1, 2]. Under high external salinity, this species' characteristic morphological xerohalophytic features – marked succulence, reduced leaf surface area, and thickened stems – allow tissue dilution and sequestration of excess salts, lowering ionic toxicity and preserving cellular water status. *S. fruticosa* has garnered increasing attention due to its nutritional and biotechnological potential, which goes beyond stress physiology. The species' traditional use as food and its growing significance as a source of antioxidant, antimicrobial, and nutraceutical compounds are supported by analyses conducted across *Sarcocornia* and closely related genera, which document notable levels of minerals, dietary fiber, phenolics, and other bioactives [3, 4]. Nonetheless, while phenolic and polar metabolites have been increasingly characterized, the lipophilic fraction and detailed lipidomic signatures remain comparatively underexplored in Tunisian halophyte populations – a gap that limits full evaluation of both ecological function and nutraceutical potential. Lipophilic constituents are central to membrane stability and signaling, and several are nutraceutically relevant due to their cardioprotective and neuroprotective effects [5, 6]. Fatty acids (FA),

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phytosterols, terpenoids, tocopherols, and long-chain alcohols are examples of lipophilic substances that are essential for structural, antioxidant, and nutraceutical functions. They control permeability, stability, and fluidity, making them essential to membrane architecture. While phytosterols stabilize membranes [7], unsaturated fatty acids support redox membrane regulation *via* oxylipin-mediated signaling [8], while tocopherols function as strong chain-breaking antioxidants that inhibit lipid peroxidation [9]. Free radicals are also scavenged by phytosterols and terpenoids [10]. Lipophilic compounds, proved else a potent anti-inflammatory, anti-cancer, neuroprotective, and cardioprotective qualities when taken as nutraceuticals. Indeed, tocopherols prevent neurodegenerative oxidative damage, unsaturated fatty acids (UFAs) promote cardiovascular and neuronal health, and phytosterols lower low-density lipoprotein (LDL) cholesterol [11]. Yet, a detailed characterization of these compounds in Tunisian populations is still lacking. In addition to chemical profiling, computational tools such as molecular docking offer valuable insights into the potential pharmacological activities of plant-derived compounds. Among therapeutic targets, acetylcholinesterase (AChE) – the enzyme responsible for hydrolyzing acetylcholine in cholinergic synapses – is of particular interest, as its inhibition is a key strategy in the management of Alzheimer's disease and other neurodegenerative disorders [12, 13]. Natural metabolites with AChE inhibitory activity are considered promising scaffolds for drug development [14], but, to date, no study has assessed the docking interactions of *S. fruticosa* metabolites with AChE. Therefore, the present study aims to:

1. characterize the lipophilic fraction of Tunisian halophyte (*S. frutiocsa*) by chromatography-mass spectrometry (GC/MS) approach;
2. evaluate *in vitro* antioxidant and anti- acetylcholinesterase activities of its lipidic fraction;
3. to investigate the molecular interactions between selected lipophilic compounds from *S. fruticosa* and acetylcholinesterase through molecular docking studies;
4. to identify promising natural inhibitors of AChE that could serve as scaffolds for future drug development, particularly in the context of neurodegenerative diseases such as Alzheimer's disease.

2 Materials and methods

2.1 Study area

Aerial part of *Sarcocornia fruticosa* (L.) plants was collected in 27 May 2025 from the sebkha soils near to Gammarth city characterized by pH values ranging from 7.9 to 8.6 across the 0–40 cm profile (Fig. 1). Electrical conductivity indicated a strong salinity, with values between 8–12 dS/m in the surface layer (0–10 cm) and increasing to 18–25 dS/m at 20–40 cm depth. Na^+ was the dominant soluble cation, with concentrations rising from approximately 2,000–3,500 mg/L in the upper horizon to 4,500–6,000 mg/L in deeper layers. In contrast, K^+ levels remained comparatively low, ranging from 80–120 mg/L at the surface to 120–200 mg/L at depth. The harvested plants were identified at the Biotechnology center of the Technopark of Borj-Cedria by Pr Abderrazek SMAOUI. Voucher specimens [SFM24] was deposited in the herbarium of the Laboratory of Extremophile Plants (LEP).

2.2 Lipophilic metabolite profiling by gas chromatography/mass spectrometry

2.2.1 Preparation of lipophilic extract

100 g dried aerial part (AP) fennel powder was extracted with n-hexane (500 mL, three times) at room temperature for 72 h using magnetic stirring. 3 g hexane extract was obtained by filtering the mixture and removing the hexane with a rotary vacuum evaporator [15]. In order to increase the volatility of the compounds, and thus the sensitivity of the gas chromatography/mass spectrometry (GC/MS) analysis, approximately 20 mg dried hexane extract from fennel was dissolved in 1000 µL $CHCl_2$. This mixture was then silylated by the addition of 250 µL pyridine, 250 µL N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), and 50 µL

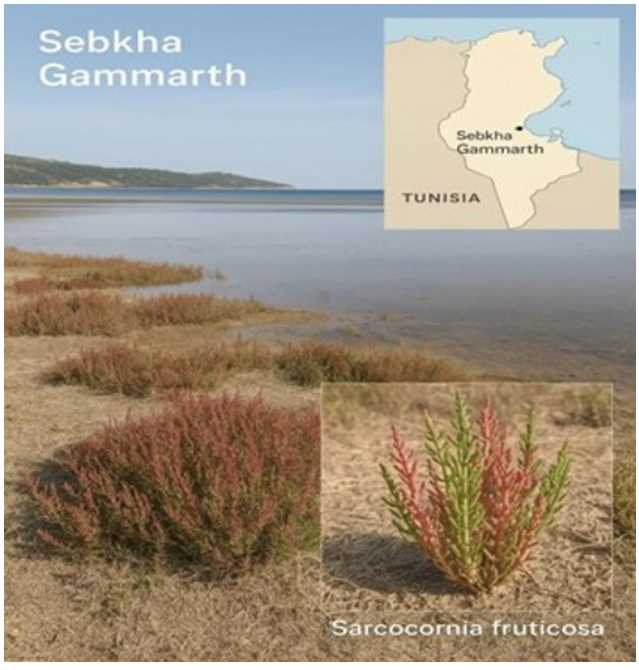

Fig. 1 Photograph of *Sarcocornia fruticosa* (L.) plants

trimethylsilyl chloride (TMSCl), as described by Rahmouni et al. [15]. The mixture was incubated at 70 °C for 30 min and then immediately injected into the GC/MS.

2.2.2 Gas chromatography–mass spectrometry

A Shimadzu Gas Chromatograph QP2010 Ultra with Autosampler AOC-20i was used for the GC/MS analysis. The ion source was an electronic impact high-performance quadrupole mass filter. The separation of compounds was carried out in a DB-5J&W capillary column (30 m × 0.25 mM inner diameter, 0.25 µM film thickness) using helium as the carrier gas (35 cm/s). The chromatographic conditions were as follows: start time at 6.5 min; initial temperature, 90 °C for 4 min; temperature rate, 16 °C/min up to 180 °C, followed by temperature rate, 6 °C/min up to 250 °C; followed by temperature rate, 3 °C/min up to 300 °C which was maintained for 5 min; injector temperature, 320 °C; transfer-line temperature, 300 °C; split ratio, 1:50. The mass spectrometer was operated in the electron impact (EI) mode with energy of 70 eV, and data were collected at a rate of 1 scan s^{-1} over a range of *m*/*z* 33–750. The ion source was kept at 250 °C.

2.2.3 Characterization of lipophilic extract components

By comparing the retention times and mass spectra data of the standard compounds injected under the same chromatographic conditions, the peaks were identified from the total ion chromatogram by comparing their mass spectra with the mass spectral libraries (NIST 14 Mass Spectral and Wiley Registry™ of Mass Spectral Data [16]), with MS spectra and MS fragmentation pattern that had been published in the literature. The retention index in relation to *n*-alkanes (C_5–C_{36}) was also compared with published data retention indexes whenever possible.

2.3 *In vitro* study

2.3.1 The evaluation of 2,2-diphenyl-1-picrylhydrazyl assay

The capacity of *S. fruticosa* AP extracts to quench 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) was assessed by the described protocol of Wasli et al. [17]. Briefly, 0.25 mL DPPH MeOH solution was mixed with 0.05 mL of different concentrations of plant extract ranging from 75 to 1000 µg/mL. After incubation in the dark for 30 min at room temperature, optical densities were measured at 517 nm using a UV–Vis spectrophotometer, with vitamin C (Sigma-Aldrich, St. Louis, MO, USA) as a positive control. The percentage of DPPH inhibition was calculated according to Eq. (1):

$$\begin{aligned}&\text{DPPH}^{\bullet}\text{ discoloration: \% DPPH}^{\bullet}\text{ scavenging}\\&=\left(A_{\text{control}}\left(0\right)\right)\left(\text{at } t=0\ \text{ min}\right)\\&-A_{\text{extract}}\left(t\right)\left(\text{at } t=30\ \text{ min}\right)/\text{Ac}\left(0\right)\times 100.\end{aligned} \quad (1)$$

Based on the graphic values of the percentage of DPPH• inhibition *vs*. extract concentration, the IC_{50} (concentration of the extract able to inhibit the 50% of the DPPH) of each extract was calculated. Ascorbic acid was used as a reference.

2.3.2 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) of *S. fruticosa* leaf extracts was assessed by their capacity to convert Fe^{3+} to Fe^{2+}, forming a blue Fe^{2+}–ligand complex. 200 µL *S. fruticosa* extracts (ranging from 0.05 to 2 mg/mL) were combined with 200 µL phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (200 µL; 1%), then incubated at 50 °C for 20 min. Following the addition of trichloroacetic acid (200 µL; 10%), mixtures were centrifuged at 3000 g for 10 min. Aliquots of the supernatant (75 µL) were mixed with distilled water (75 µL) and $FeCl_3$ (15 µL; 0.1%), and absorbance was recorded using an ELX800 Microplate (Thermo Fisher Scientific, Waltham, MA, USA) reader [18]. Reducing activity was expressed as EC_{50} (mg/mL), corresponding to the extract concentration yielding an absorbance of 0.5, calculated by linear regression. Butylated hydroxyanisole (BHA) was used as the positive control.

2.3.3 Nitric oxide scavenging assay

The NO• scavenging method was released basing on the experimental method of Rarison et al. [19]. 100 µL of serial dilutions of *S. fruticosa* samples were mixed with 100 µL of sodium nitroprusside (3.33 mM in 100 mM sodium phosphate buffer, pH 7.4; Acros Organics, Hampton, NH, USA) and incubated for 15 min under a fluorescent lamp (Tryun 26 W). Next, 100 µL of Griess reagent (consisting of 0.5% sulfanilamide, Acros Organics, Hampton, NH, USA) and 0.05% N-(1-naphthyl)-ethylenediamine dihydrochloride (VWR, Radnor, PA, USA) in 2.5% H_3PO_4 was added to the mixture, which was incubated for another 10 min at room temperature, in the dark. The optical density was then measured at 562 nm. The NO• scavenging capacity was computed as the sample concentration capable of scavenging 50% of the radical. Ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) was used as the reference compound.

2.3.4 Evaluation of anti-acetylcholinesterase activity

The anti-AChE activity was assessed according the procedure of Wasli et al. [20]. Briefly, 0.06 mL of sample extract

was mixed with 0.425 mL of Tris-HCl buffer (0.1 M, pH 8) and 0.025 mL of enzyme solution (0.28 U/mL). The mixture was incubated at 25 °C for 15 min. After pre- incubation, the reaction was initiated by adding 0.475 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; 0.059 g dissolved in 50 mL buffer) and 75 µL of substrate solution (0.005 g acetylthiocholine iodide in 10 mL). Following 30 min of incubation, the absorbance of the reaction mixture was measured spectro-photometrically.

A control was prepared under the same conditions by replacing the sample extract with Tris-HCl buffer. The percentage of enzyme inhibition was calculated according to Eq. (1). The inhibitory activity was expressed as IC_{50} values.

2.4 *In silico* study

2.4.1 Molecular docking procedure

Molecular docking was performed to investigate the interactions between the selected ligands and acetylcholinesterase. The 3D structure of acetylcholinesterase (PDB: 4ey7) was obtained from the Protein Data Bank and prepared using AutoDock Tools by removing water molecules and co-crystallized ligands, adding polar hydrogens, and assigning charges [21]. Ligands were energy-minimized and converted into the required format for docking. The binding sites were defined based on the active site residues or co-crystallized ligands, and docking simulations were conducted using AutoDock Vina. For each ligand, the best binding pose with the lowest docking score was selected and analyzed for hydrogen bonding, hydrophobic interactions, and other non-covalent contacts using Python Molecule Viewer (PMV) [22] and Discovery Studio Visualizer [23]. The docking protocol was validated by redocking the native ligand, and the results provided insights into the potential binding affinities and key interactions responsible for the observed *in vitro* biological activities.

2.5 Pharmacokinetic evaluation of the most active compounds

The physicochemical and pharmacokinetic properties were predicted using the SwissADME platform [24], using chemical structures obtained in SMILES format from the PubChem database [25]. The tool allowed to evaluate gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate status, potential interactions with major cytochrome P450 isoforms as well as classical drug-likeness rules (Lipinski, Ghose, Veber, Egan and Muegge) [25]. In addition, the bioavailability radar, based on six criteria (lipophilicity, molecular size, polarity, insolubility, degree of unsaturation and structural flexibility), was generated to visualize and compare the profiles of the four compounds [25].

2.6 Data analysis

Statistical analyses were carried out using GraphPad Prism (version 10) [26] (GraphPad Software, San Diego, CA, USA). Differences among means were assessed through one-way ANOVA, and multiple comparisons were subsequently tested using Tukey's post hoc procedure. Pearson's correlation analysis and hierarchical clustering were performed with Orange software (version 3.4.5) [27] (University of Gafsa, Tunisia).

3 Results and discussion

3.1 GC/MS identification of fatty acids and lipophilic compounds in *Sarcocornia fruticosa*

In recent years, research has increasingly focused on halophytic species as potential sources of lipid-rich plant biomass. In this study, oils were extracted from the aerial parts of *Sarcocornia fruticosa* using *n*-hexane in a Soxhlet apparatus, yielding 2.5% (Table 1, dry mass basis). This lipid content is relatively high compared to other *Sarcocornia* species, such as *S. perennis* and *S. europea*, which typically produce 0.5–1.5% lipids under similar environmental conditions. The elevated lipid accumulation in the aerial tissues of *S. fruticosa* may result from a combination of factors, including the high salinity and mineral composition of coastal Tunisian soils, plant developmental stage and tissue maturity, and the efficiency of the solvent-based lipid extraction method employed [28]. Fatty acid analysis showed that saturated fatty acids (SFA, 30.6%) and polyunsaturated fatty acids (PUFA, 30.5%) contributed almost equally to the total pool. The dominance of palmitic acid (C16:0, 22.3%) within the SFA fraction is consistent with its widespread role in membrane phospholipids and cuticular waxes, and aligns with reports for halophytes such as *Sarcocornia perennis* and *Salicornia ramosissima*, where palmitic acid constitutes >20% of total fatty acids [29]. Within the PUFA fraction, linolenic acid (C18:3n-3, 15.0%) and linoleic acid (C18:2n-6, 9.4%) were the most abundant, giving a PUFA/SFA ratio of ~1.0. This ratio is markedly higher than those reported in other *Sarcocornia* aerial parts (PUFA/SFA often <0.5 [29]), suggesting that *S. fruticosa* may allocate a greater proportion of unsaturated lipids to vegetative tissues. Comparisons across the genus reinforce the uniqueness of this profile. Seed oils of *Sarcocornia ambigua* are richer in unsaturated

Table 1 Characterization of lipophilic compunds expressed in dry mass of *S. fruticosa*

Fatty acids		Phytosterols		Alcohols	
Palmitic acid C16:0	0.56	Stigmasterol	0.052	1-Nonadecanol,	0.016
Stearic acid C18:0	0.047	*δ* sitosterol	0.103	Heptacosanol	0.21
Tetracosanoic acid	0.03	Stigmastanol	0.044	1-Hexacosanol	0.105
Arachidic acid C20:0	0.03	∑	**0.20**	∑	**0.33**
Docosanoic acid	0.037	Terpenoids		Hydrocarbones/alkanes/alkenes	
Pentacosanoic acid	0.065	Phytol	0.069	Pentacosane	0.014
∑ **Saturated fatty acids (SFA)**	**0.77**	∑	**0.069**	Heptacosane	0.021
Linolenic acid C18:3n−3	0.37	Tetradecanal	0.106	1-Octadecene	0.017
1,21-Docosadiene	0.06	∑	**0.106**	Docos-1-ene	0.16
1,19-Eicosadiene	0.066			1-Nonadecene	0.01
Linolelaidic acid	0.03			∑	**0.22**
∑ **Polyunsturated fatty acids (PUNFA)**	**0.76**				
Ratio unsaturated/satuarted					
Yield %	**2.5**				

fatty acids, especially linoleic (~43%) and oleic acids (~18%), and total lipid yields may exceed 13% of dry mass [30]. Residual biomass (shoots plus seed coats) of *S. ambigua* produces ~5.2% oil, dominated by SFA (>50%) and with markedly lower PUFA proportions [31].

Aerial parts of *S. perennis* also exhibit high palmitic acid content and SFA dominance, frequently surpassing 50% of total fatty acids [29]. On the other hand, *S. fruticosa*'s aerial tissues have an abnormally high level of n-3 fatty acids, especially linolenic acid, in addition to a more balanced SFA/PUFA profile. Because n-3 fatty acids improve membrane fluidity and shield photosynthetic equipment from oxidative stress and salinity, this has ecological significance. In fact, stress enhanced the unsaturation of C18 fatty acids to stabilize membranes and preserve chloroplast function, demonstrating lipid remodeling under salinity in *S. fruticosa* physiotypes [32].

Palmitic acid also supports the biosynthesis of cuticular wax, which protects the aerial tissues from UV rays and transpirational water loss. Therefore, the coexistence of high SFA and PUFA indicates a dual adaptive strategy: dynamic stabilization of internal membranes (*via* PUFA) and rigidification of external barriers (*via* SFA-derived waxes). The ecological success of *S. fruticosa* in extreme coastal salt marshes may be explained by these metabolic adaptations. The oil's multi-functionality is further highlighted by its unsaponifiable fraction. There were many phytosterols (7.9%), with *δ*-sitosterol, stigmasterol, and stigmastanol predominating. These sterols are vital components of membranes that also modulate fluidity and permeability, especially in stressful situations. In humans, they also have anti-inflammatory and decrease intestinal cholesterol absorption [33]. Additionally, the fraction included aldehydes (4.2%, mainly tetradecanal), which may aid in chemical defense and stress signaling, and terpenoids (2.8%, mainly phytol), an alcohol derived from chlorophyll with antioxidant and antimicrobial properties. Remarkably, hydrocarbons (1.4%) and long-chain aliphatic alcohols (13.1%), such as hexacosanol and heptacosanol, made up a significant portion. As well-known constituents of epicuticular waxes, these extremely long-chain alcohols provide hydrophobicity and defense against pathogen attack, desiccation, and salinity [34]. The importance of cuticular adaptations in halophyte survival is highlighted by their high abundance in the aerial parts of *S. fruticosa*.

3.2 Evaluation of antioxidant activities (DPPH•, FRAP, NO•)

The lipophilic extract of the aerial parts of *Sarcocornia fruticosa* exhibited a moderate antioxidant activities observed in DPPH• (IC_{50} = 479 µg /mL), FRAP (EC_{50} = 566 µg /mL), and NO• (IC_{50} = 672 µg /mL) (Table 2) assays can be rationalized by this chemical composition as compared to other halophyte species. Indeed, the lipophilic fractions of *Thalassia testudinum* exhibited a DPPH• IC_{50} of 312 µg/mL and inhibited NO• production by 58.6% at 400 µg/mL, indicating

Table 2 Antioxidant activities of *Sarcoconia fruticosa* lipophilic extracts

Tests / *S. fruticosa* lipophilic extract	IC_{50}/EC_{50} (µg/mL)
DPPH•	479 ± 12
FRAP	566 ± 3
NO•	672 ± 2

slightly higher activity. In contrast, hexane extracts of *Halodule pinifolia* and *Mesembryanthemum nodiflorum* showed much weaker radical scavenging, with DPPH• IC_{50} values of approximately 2.378 µg/mL and 1.970 µg/mL, respectively. Lipophilic extracts of seaweeds such as *Himanthalia elongata* also displayed very low activity, with DPPH IC_{50} values ranging from 980 to 2900 µg/mL. Similarly, the hexane fraction of *Suaeda mollis* exhibited modest antioxidant capacity but significant NO• inhibition at higher concentrations (~160 µg/mL).

On the other hand, the moderate antioxidant activity observed for the lipophilic extract of *Sarcocornia fruticosa* is consistent with reports on other Mediterranean edible halophyte species, such as *Salicornia europaea*, *Sarcocornia perennis* and *Crithmum maritimum*, where hexane or lipid-rich fractions generally exhibit weaker radical-scavenging activity compared to polar extracts [10, 29, 35].

The moderate antioxidant activity of the lipophilic extract of *Sarcocornia fruticosa* could be attributed to its specific chemical composition and the mechanisms by which its constituents act [32]. Indeed, the omni- presence of PUFAs, certain SFAs, phytosterols, and terpenoids could contribute to antioxidant mechanisms, albeit less efficiently in standard *in vitro* assays. For example, linolenic and linoleic acids may scavenge lipid radicals or act *via* chain-breaking antioxidant mechanisms in lipid systems, whereas phytosterols like *δ*-sitosterol and stigmasterol can provide mild radical stabilization and membrane-protective effects [36].

Long-chain alcohols (heptacosanol, 1-hexacosanol) and terpenoids such as phytol may also contribute weakly to electron donation or quenching of reactive oxygen species (ROS), explaining the measurable but relatively high IC_{50} values. Additionally, hydrocarbons and aldehydes, which are present in minor amounts, generally do not contribute directly to radical scavenging but may influence lipid- phase antioxidant activity by stabilizing membranes or interacting with radical intermediates [37].

3.3 Acetylcholinesterase inhibitory activity

Beyond its antioxidant effects, *S. fruticosa* extracts demonstrated also AChE inhibitory activity, with an IC_{50} of 588 µg/mL (Table 3). Although these values remain considerably higher than those of the standard drug "galantamine", they still indicate a notable inhibitory effect. This moderate inhibition suggests neuroprotective potential through the preservation of acetylcholine levels, which can be indirectly linked to antioxidant defense since reduced oxidative stress improves neuronal function. Such activity is in the same range as leaf extracts of *Rosmarinus officinalis* plants ($IC_{50} \approx 150$ µg/mL) [38].

Table 3 *In vitro* acetylcholinesterase inhibitory activity of *Sarcoconia frutiocsa* lipophilic extract

S. fruticosa lipophilic extract	IC_{50} (µg/mL)
AChE	588 ± 7
Galantamine	13 ± 0.4

4 *In silico* study

4.1 Molecular docking of lipophilic molecules targeting acetylcholinesterase (PDB: 4EY7)

To better understand the relationship between the *in vitro* biological activities of the tested ligands and their structural features, molecular docking studies were conducted on acetylcholinesterase. Each of the 24 identified compounds was analyzed individually to precisely identify the most active ligands and to understand how their structural features contribute to binding affinity and potential enzyme inhibition. This approach provided detailed information on the key interactions stabilizing the ligands within the AChE active site, thereby explaining the observed variations in biological activity.

These analyses revealed significant variations in binding affinities among the lipophilic compounds, which were largely dependent on their chemical class (Table 4). Among all compounds, phytosterols exhibited the best docking scores, with stigmasterol (−7.9 kcal/mol), *δ*-sitosterol (−7.6 kcal/mol), and stigmastanol (−7.6 kcal/mol) standing out as the most promising ligands. This strong affinity can be attributed to the bulky and rigid steroidal skeleton of these molecules, which fits well within the aromatic gorge of AChE and allows extensive hydrophobic interactions, including van der Waals contacts and alkyl interactions with Trp532, Phe295, Tyr337, Leu536, Leu540, Val367/Val370, and Pro235/Pro537. Additionally, the hydroxyl group at C3 of the Sitosterol can form hydrogen bonds with polar residues such as Thr238, further stabilizing the ligand within the active site (Fig. 2 (a) to (c)). These interactions collectively stabilize the ligand within the active site and explain the relatively high docking scores compared to other lipophilic compounds [20].

Similarly, the terpenoid phytol showed a high binding energy (−7.5 kcal/mol), likely due to its long hydrophobic chain, which promotes insertion into the binding pocket. Its terminal hydroxyl group contributes specifically to hydrogen bonding with Arg296, while the rest of the molecule engages in van der Waals interactions, carbon-hydrogen bond, pi-sigma and alkyl contacts (Fig. 2 (d)).

Table 4 Binding energy of the docked compounds in the binding cavity of acetylcholinesterase (PDB:4EY7)

Class	Compound	Binding energy (kcal/mol)
Fatty acids	Palmitic acid	−5.5
	Stearic acid	−5.8
	Tetracosanoic acid	−4.4
	Archidic acid	−4.1
	Docosanoic acid	−5.9
	Pentacosanoic acid	−6
	Linoleic acid	−5
	Linolenic acid	−5.3
	Linolelaidic acid	−5.5
Phytosterols	Stigmasterol	−7.9
	δ-Sitosterol	−7.6
	Stigmastanol	−7.6
Terpenoid	Phytol	−7.5
Alcohols	1-Nonadecanol	−4.3
	Heptacosanol	−4.5
	1-Hexacosanol	−4
Hydrocarbons/Alkanes/Alkenes	Pentacosane	3.8
	Heptacosane	−3
	1,21-Docosadiene	−4.3
	1,19-Eicosadiene	−4.1
	1-Octadecene	−4.2
	Docos-1-ene	−4.5
	1-Nonadecene	−4.8
Aldehyde	Tetradecanal	−4.1
Standard	Galantamine	−8.6

In contrast, fatty acids displayed moderate docking scores ranging from −4.1 to −6.0 kcal/mol. The highest affinities were recorded for very long-chain saturated fatty acids, such as pentacosanoic acid (−6.0 kcal/mol) and docosanoic acid (−5.9 kcal/mol) (Table 4), suggesting that chain length and hydrophobic surface area favor interactions with the hydrophobic gorge [39]. However, the carboxyl group of fatty acids, which is negatively charged at physiological pH, is not optimal for the catalytic site that typically favors cationic or aromatic ligands, which may limit their inhibitory potential in biological systems. Unsaturated fatty acids such as linoleic (−5.0 kcal/mol) and linolenic acid (−5.3 kcal/mol) showed slightly lower affinities (Table 4), consistent with their higher flexibility and less optimal packing within the binding pocket [40].

Other classes of compounds, including unsaturated hydrocarbons (−4.3 to −5.1 kcal/mol), long-chain alcohols (−4.0 to −4.9 kcal/mol), alkanes (−4.2 to −5.0 kcal/mol), and the aldehyde tetradecanal (−4.4 kcal/mol), exhibited relatively weak binding energies (Table 4). Their lack of polar functional groups and limited capacity to form specific interactions with key residues of the catalytic gorge explain their weaker docking performance, suggesting that they are unlikely to significantly contribute to AChE inhibition [41]. Importantly, although phytosterols and phytol demonstrated relatively high binding affinities, none of the individual compounds surpassed the standard reference inhibitor galantamine, which exhibited a binding energy of −8.6 kcal/mol. This observation indicates that while natural lipophilic compounds may contribute to AChE inhibition, their individual potency remains lower than that of clinically used inhibitors. These results highlight the significance of hydrophobic bulk and selective polar functionality in achieving strong and stable interactions with the active site of AChE, and suggest that further structural optimization would be necessary for these natural compounds to approach the efficacy of galantamine [42].

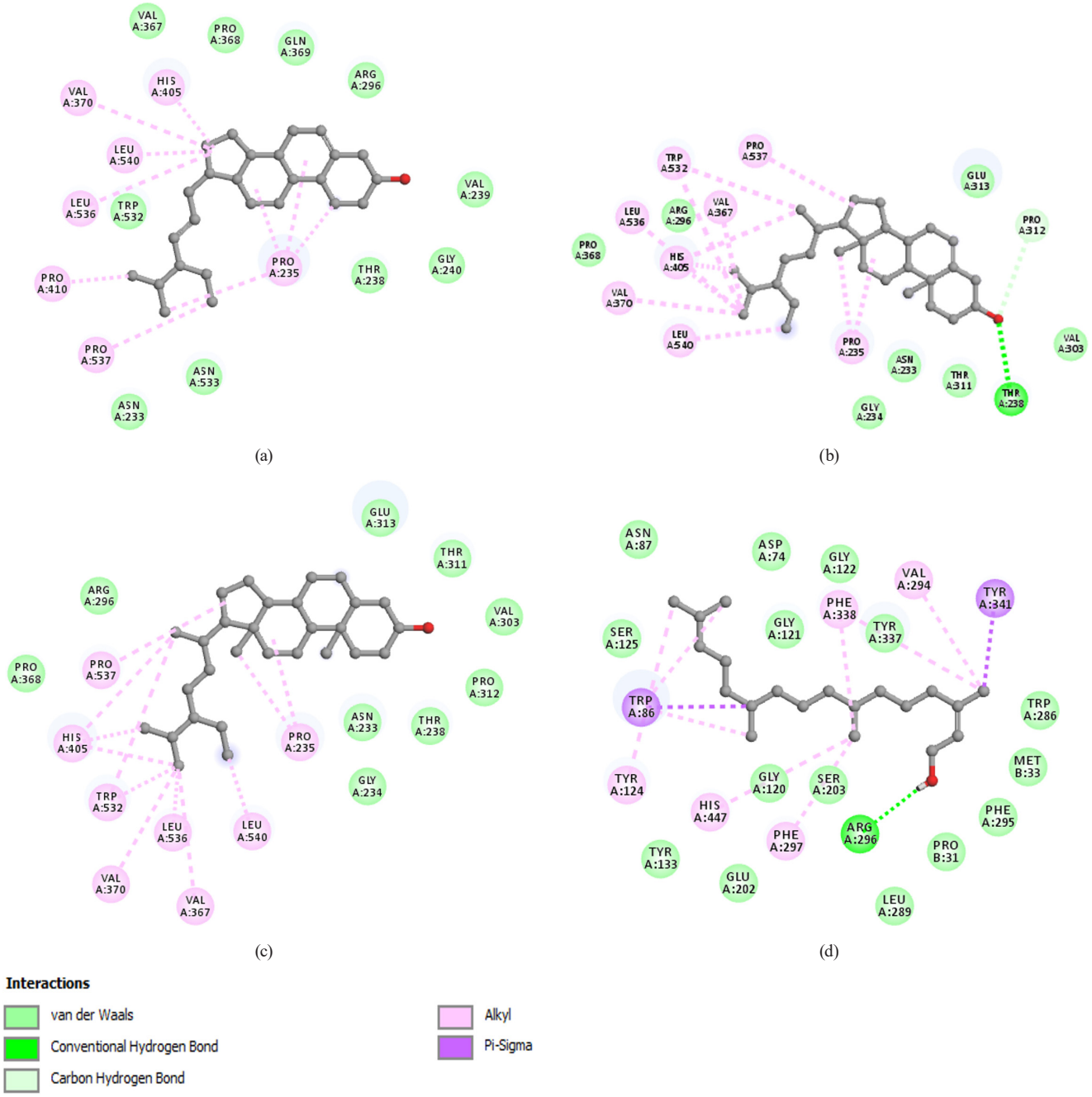

Fig. 2 Binding modes of compounds: the reference: (a) stigmasterol; (b) *δ*-sitosterol; (c) stigmastanol; and (d) phytol within the binding cavity of acetylcholinesterase (PDB: 4EY7)

4.2 Pharmacokinetic and drug-likeness evaluation of the most active lipophilic compounds

Lipinski's rule of five and the Ghose filter are commonly used drug-likeness criteria that provide an early indication of the oral bioavailability of small molecules. Lipinski's rule evaluates molar mass, lipophilicity, and hydrogen-bonding capacity, while the Ghose filter further considers molecular size, lipophilicity, and structural complexity. In this study, these rules were applied to rationalize the predicted absorption limitations of the most active lipophilic compounds.

The pharmacokinetic and drug-likeness properties of the most active compounds: phytol, stigmasterol, *β*-sitosterol and stigmastanol, were evaluated to better understand their potential bioavailability, metabolic behavior, and overall performance in the human body (Table 5).

Table 5 Pharmacokinetic and druglikeness properties of phytol, stigmasterol, β-sitosterol, and stigmastanol

Parameter	Phytol	Stigma sterol	β-Sitosterol	Stigma stanol
Chemical formula	$C_{20}H_{40}O$	$C_{29}H_{48}O$	$C_{29}H_{50}O$	$C_{29}H_{52}O$
Molar mass (g/mol)	296.53	412.69	414.71	416.73
Gastroint estinal absorption (GI absorption)	Low	Low	Low	Low
Blood– brain barrier permeati on (BBB permeant)	No	No	No	No
P-glycoprot ein substrate (P-gp substrate)	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
Lipinski (violations)	1	1	1	1
Ghose (violations)	0	0	0	0
Veber (violations)	0	1	1	1
Egan (violations)	0	1	1	1
Muegge (violations)	2	2	2	2
Bioavailability score	0.55	0.55	0.55	0.55

Phytol, with a molar mass of 296.53 g/mol, is considerably smaller than the three phytosterols, which all exceed 412 g/mol. All compounds exhibited low gastrointestinal absorption, suggesting limited oral bioavailability, and none were predicted to permeate the blood–brain barrier, indicating a low likelihood of central nervous system activity. This property may reduce their effectiveness in the treatment of neurological disorders such as Alzheimer's disease, thus limiting their *in vivo* efficacy [43]. Additionally, all four compounds were identified as substrates of P-glycoprotein, which may actively efflux them from cells and further reduce systemic exposure [44]. Regarding metabolism, only CYP2C9 was predicted to be inhibited by all compounds, while other major cytochrome P450 isoforms – including CYP1A2, CYP2C19, CYP2D6, and CYP3A4 – were not affected. This selective inhibition may influence the metabolism of drugs processed by CYP2C9, with potential implications for drug–drug interactions [41]. Drug-likeness evaluation further revealed that all four molecules violated at least one Lipinski's rule due to their high lipophilicity, although they passed the Ghose filter. The phytosterols failed also the Veber and Egan filters, primarily due to their high number of rotatable bonds and overall lipophilicity, and all compounds exhibited two violations of the Muegge filter, reflecting both excessive lipophilicity and a low number of heteroatoms. Despite these limitations, all compounds had a moderate bioavailability score of 0.55 suggesting that they could reach sufficient levels to be pharmacologically relevant [45]. Overall, these results indicate that while Phytol has a slightly more favorable pharmacokinetic profile due to its smaller size and flexibility, the three phytosterols share very similar properties characterized by high molar mass, poor gastrointestinal absorption, and multiple drug-likeness violations, which collectively may limit their therapeutic potential *in vivo*.

This predicted profile was further also confirmed by the bioavailability radars, which visualize the main properties of the studied molecules according to six criteria: lipophilicity, molecular size, polarity, insolubility, degree of unsaturation, and structural flexibility, graded from 0 (low) to 5 (high). These analyses highlighted the low absorption potential of the four compounds. Phytol, being smaller and more flexible, showed slightly higher polarity but remains limited by its high lipophilicity and low aqueous solubility. The sterols (stigmasterol, β-sitosterol, and stigmastanol) display very similar profiles, characterized by large molecular size, structural rigidity, and an almost complete lack of polarity. Their main distinction lies in the degree of unsaturation, which is higher in stigmasterol, intermediate in β-sitosterol, and absent in stigmastanol. Overall, these properties reflect poor oral bioavailability and emphasize the need for tailored formulations to improve their absorption (Fig. 3).

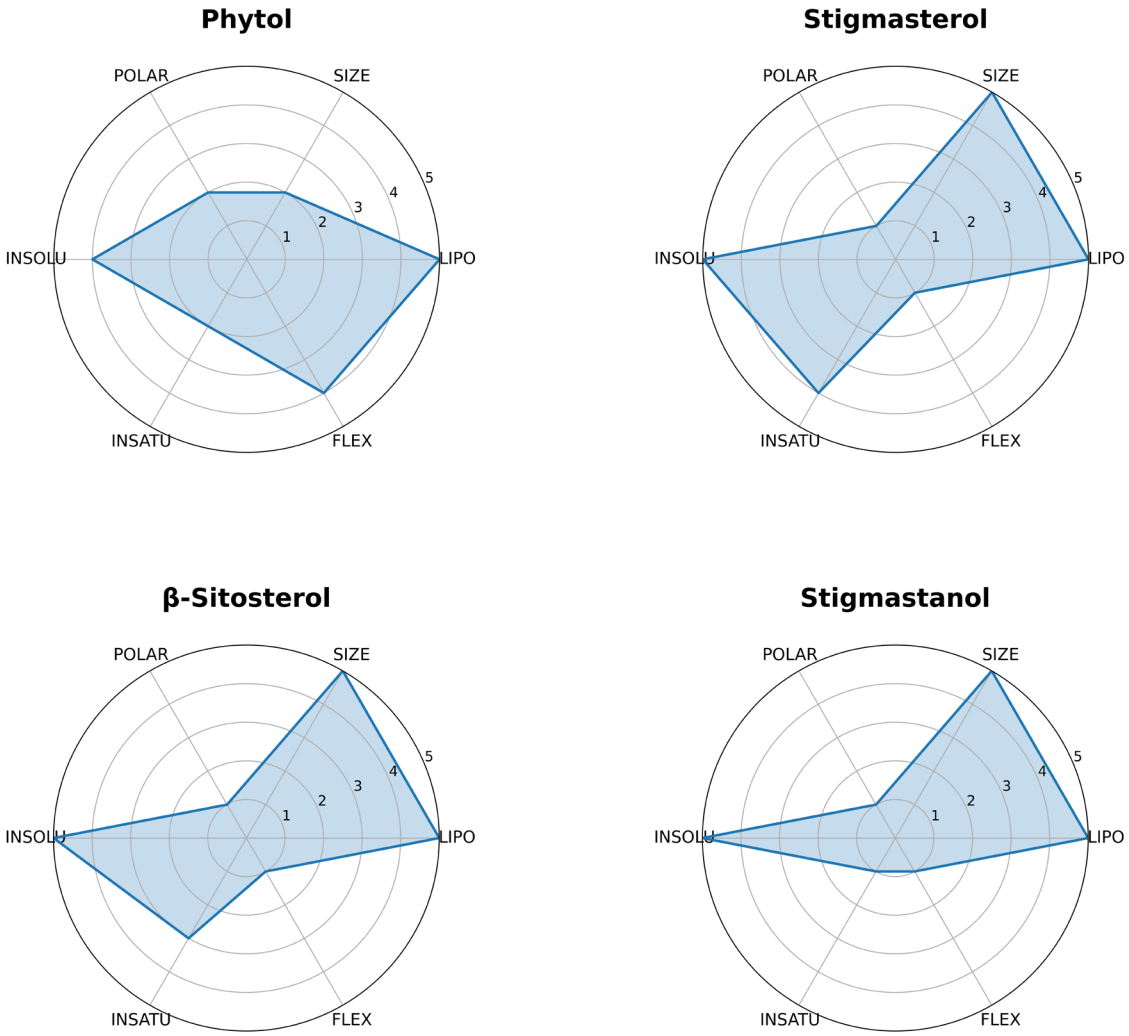

Fig. 3 Bioavailability radar charts of phytol, stigmasterol, *β*-sitosterol, and stigmastanol [lipophilicity (LIPO), molecular size (SIZE), polarity (POLAR), water insolubility (INSOLU), degree of unsaturation (INSATU), and molecular flexibility (FLEX)]

5 Conclusion

This study provides insights into the antioxidant mechanisms of lipophilic extracts from *P. fruticosa* leaves, using an integrative approach of pharmacology analysis and *in silico* and *in vitro* verification. Docking studies identified phytosterols (stigmasterol, *δ*-sitosterol, and stigmastanol) as the most promising AChE inhibitors, followed by phytol, while fatty acids and hydrocarbons showed weaker affinities. Integration with pharmacokinetic predictions revealed major limitations, including low gastrointestinal absorption, P-glycoprotein efflux, and multiple drug-likeness violations, with only phytol showing a slightly more favorable profile. Despite a moderate bioavailability score, these compounds display restricted pharmacokinetic suitability, highlighting the need for formulation strategies or structural optimization to enhance their therapeutic potential.

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