Metabolomic Profiling and Hypoglycemic Potential of *Spirulina platensis* Extracts of Infusion and Decoction Techniques through *in silico* α-Amylase and α-Glucosidase Inhibition

Firdaus Muhamad*, Yahya Yahya, Priambodo Ahmad Faris and Gaisani Rizqia Zuhri Arifa
Faculty of Fisheries and Marine Sciences, Brawijaya University, Jl. Veteran, Malang – 65145, INDONESIA
*muhamadfir@ub.ac.id

Abstract
Spirulina platensis contains a group of metabolomes that can inhibit glucose absorption. This study aimed to identify metabolites in the infusion and decoction of S. platensis and evaluate their ability as inhibitors of α-amylase and α-glucosidase through the in silico method. The samples were analyzed using HPLC-HRMS by untargeted method for metabolite identification while molecular docking studies were conducted using PyRx 0.8 and physicochemical properties were analyzed using SwissADME. The results showed 120 metabolites identified, with 84 from decoction and 67 from infusions. Molecular docking studies revealed that zearalenone and adenosine have a strong binding affinity for α-amylase and α-glucosidase, with an affinity energy of -8.6 kcal/mol, lower than that of acarbose.

Zearalenone forms a hydrogen bond with Ile230 residue on α-amylase while adenosine forms a hydrogen bond with His332 and Arg400 residues on α-glucosidase. Analysis of physicochemical properties according to Lipinski's rule shows that both compounds meet the criteria for good bioavailability. Further molecular and in vivo dynamics studies are needed to strengthen the results of this research.

Keywords: Docking study, drug-likeness, glucose absorption inhibition, metabolome, *Spirulina platensis.*

Introduction

Hypoglycemic agents are substances that can control blood glucose levels. Their mechanisms include inhibiting amylase and glucosidase activities, increasing secretion and insulin sensitivity and increasing glucose uptake. The inhibition of amylase and glucosidase activity has been linked to many health maintenance efforts[7]. Many macro- and microalgae substances are used as hypoglycemic agents[15]. It is known that microalgae are widely consumed and are used to prevent the increase of blood sugar levels[12]. *S. platensis* has been recognized as a hypoglycemic agent in *in vitro*[17], *in vivo*[9] and clinical studies[10]. *S. platensis* is a microalga with many health benefits for humans. Its nutrients and metabolome provide physiological effects and prevent and treat diseases, including metabolic disorders. It is known that this microalga controls blood sugar levels by inhibiting *α*-amylase[18] and *α*-glucosidase activities[25]. The polyphenol of *S. platensis* plays a role in inhibiting the activity of these enzymes[23].

Extraction is a process of separating metabolome in the tissue of a material using a solvent. Infusion and decoction are techniques that can be used to extract compounds. Infusion is the maceration of the target material with boiling water for a short time, while decoction is done for a long time. Both techniques are mainly aimed at obtaining water-soluble and heat-resistant materials. The results are usually in the form of an extract or a thick solution ready for oral use[8]. Natural materials as hypoglycemic agents have been developed[7]. Most previous studies have focused on the general hypoglycemic properties of *S. platensis* without examining in depth the specific compounds responsible for the inhibiting effects of amylase and glucosidase.

Further research can help to identify the main compounds in water extracts, especially infusions and decoction, that contribute to this enzymatic activity and increase its effectiveness as a hypoglycemic agent. This study aimed to identify the metabolome contained in the infusion and decoction of *S. platensis* and evaluate their metabolome's ability as *α*-amylase and *α*-glucosidase inhibitors based on the *in silico* method.

Material and Methods

Materials: *S. platensis* powder was obtained from the Brackishwater Aquaculture Development Center, Situbondo District, East Java, Indonesia. For identity analysis, water, acetonitrile and formic acid were in HPLC grade. The structure of the metabolome and acarbose selected as ligand control was downloaded from the PubChem database. *α*-amylase (1smd) and *α*-glucosidase (3wy1) were obtained from protein data bank (http://www.rscb.org/).

The HPLC-HRMS Thermo Scientific Dionex UltiMate 3000 RSLCNano using a Hypersil GOLD aQ column (50 × 1 mm × 1.9 µm particle size) was used to identify the metabolome from the sample. HP Intel® Core TMi3-5005U with a Microsoft Windows 10 operating system was used for the *in silico* analysis. This study used the Open Babel GUI version 2.4.1 to prepare the ligands and the PyRx 0.8 (Scripps Research Institute) to prepare the macromolecules and docking analysis. Biovia Discovery Studio 2019 (Dassault Systèmes Biovia Corp.) and PyMol (Schrödinger v1.7.4) were used to visualize the docking results[5]. The database in

SwissADME was used to determine the physicochemical properties of metabolome[13].

Metabolome identification: Samples were boiled in water (1:7: w/v) for 10 min at 90 °C for infusion and 30 min for decoction. After cooling to room temperature, the filtrate was obtained and filtered with Whatmann no. 42 paper. The filtrate was diluted with water containing 0.1% formic acid, vortexed for 1 min and filtered with a 0.22 µm filter syringe. This supernatant was then poured into the vial of an HPLC-HRMS autosampler (Thermo Scientific™) and injected into this apparatus's column. Metabolome identification was performed using the untargeted method.

The solvent system used in this analysis consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). The mobile phase flow rate of 40 µm L/min was run in a gradient ratio of solvents A and B (95:5 at minutes 0–15, 40:60 at minutes 15–22 and 5:95 at minutes 22–25). The setting temperature of the column was 30 °C. The identity of the metabolome was determined based on the similarity of detected compounds and compound information contained in the Compound Discoverer, mzCloud MS/MS Library[6].

Docking studies: The 3D structure of *S. platensis* metabolome and acarbose was obtained from https://pubchem.ncbi.nlm.nih.gov/ in an SDF format before changing into PDB using Open Babel[5]. Ligands (metabolome and acarbose) were optimized with Open Babel and included in the PyRx software. The macromolecules were α-amylase (ID: 1smd) and α-glucosidase (ID: 3wy1), downloaded in PDB format and obtained from http://www.rcsb.org/[11]. Acarbose was the inhibitor of α-amylase and α-glucosidase used as a control[22].

Macromolecules in PDB format were converted into PDBQT format using the PyRx software. Before the docking process, the energies of the identified compounds of *S. platensis* and acarbose were minimized to optimize their conformation before being formatted into PDBQT using the Open Babel. Docking analysis between α-amylase and ligands was executed via a grid-box size of x = 55.93 Å, y = 72.08 Å and z = 56.47 Å, with a grid center of x = 8.34 Å, y = 58.71 Å and z = 19.12 Å.

Meanwhile, docking analysis between α-glucosidase and ligands was executed via a grid-box size of x = 51.49 Å, y = 54.65 Å and z = 80.91 Å, with a grid center of x = −9.31 Å, y = −12.59 Å and z = 11.65 Å. An exhaustiveness search parameter of 8 was used to predict the binding affinities due to the probability of finding the global minimum of the scoring functions. Each ligand was flexible, interacting with the macromolecule under rigid conditions.

AutoDock Vina was used to simulate the docking of acarbose and ligands against α-amylase and α-glucosidase[16]. The docking results were evaluated and the best value (ΔG was the most negative) was observed in the area of the ligands attached to the macromolecule. Interactions in hydrogen bonds, hydrophobic bonds and electrostatic bonds and bond distances were visualized with Discovery Studio[24].

Physicochemical properties determination: The physicochemical or drug-likeness properties of metabolome were determined using the database available in SwissADME. The canonical SMILES descriptor metabolome contained in PubChem was used as input to obtain information about the physicochemical properties in the SwissADME database. The drug-likeness of the metabolome was determined based on Lipinski's rules[13].

Results and Discussion

Metabolome identification: The samples contained 120 metabolomes, of which 67 were for infusion and 84 were for decoction. The decoction technique produces more compounds than the infusion. Metabolome of *S. platensis* infuse has: DL-Arginine, 3,4-Dihydroxyphenyl propionic acid, Lactamide, L-Pyroglutamic acid, Cytosine, L-Histidine, D-Serine, Inosine, Valylproline, Threonine, L-Aspartic acid, 4-Hydroxybenzaldehyde, Biopterin, Acetohydroxamic acid, Glycyl-L-leucine, Zearalenone, Diaminopimelic acid, N6,N6,N6-Trimethyl-L-lysine, L(-)-Carnitine, Glycylproline, 3-Hydroxypicolinic acid, Cytidine, Indole, Tolycaine, Salicylamide, L-Ergothioneine, L-Kynurenine, Adenosine, Cytidine 5'-monophosphate (hydrate), 3,5-di-tert-Butyl-4-hydroxy benzaldehyde, Urocanic acid, N-Desmethylselegiline, 2'-Deoxyinosine, Skatole, N1-Hydrazino[3-(methylthio) anilino] methylidene benzene-1-sulfonamide and 2-(Cyclo hexylmethylidene)-1,2,3,4-tetrahydronaphthalen-1-one.

Metabolome of *S. platensis* decoct has: Uracil, Levetiracem, Guanine, 1-Methylguanine, Betaine, Y-L-Glutamyl-L-glutamic acid, N6-Methyladenine, Amfepramone, L-Phenylalanine, Triethanolamine, 6-Methyl[1,2,4] triazolo[4,3-b] pyridazine-8-ol, 2,3,5,6-Tetramethyl pyrazine, 2,4-Diaminotoluene, Piracetam, 4-Hydroxy ephedrine, Eicosatetraynoic acid, 2-Hydroxyphenylalanine, DL-Stachydrine, 3-Hydroxypyridine, 4-Aminophenol, D-(+)-Camphor, Uric acid, PPG n5, 5-Hydroxyindole, Kanosamine, 3-(1-hydroxyethyl)-2,3,6,7,8,8a-hexahydro pyrrolo[1,2-a]pyrazine-1, 4-dione, PPG n4, Verrucarol, Indole-3-acrylic acid, Isoamylamine, (+/-)-C75, Coumarin, N3,N4-Dimethyl-L-arginine, DL-Lysine, 2-Aminonicotinic acid, 5-Amino-2-methyl phenol, MMAI, N1-(3-Pyridylmethyl)-3-(3,4-dichlorophenyl) acrylamide, Muramic acid, 1,2,3,4-Tetramethyl-1,3-cyclopentadiene, D-Glucosamine, Heptanophenone, DL-Leucineamide, Prolinamide, 2-Isopropyl-6-methyl-4-pyrimidinol, Propyl hexedrine, 2-Naphthylamine, Cyclo(leucylprolyl), 8-Hydroxyquinoline, 4-Phenylbutyric acid, (3aR,8R,8aR, 9aR)-8-Hydroxy-8a-methyl-3,5-bis (methylene) decahydro naphtho [2,3-b] furan-2(3H)-one, DL-α-Aminocaprylic acid and N-Acetyltyramine.

Metabolome of *S. platensis* obtained both by infusion and decoction techniques has: L-Norleucine, Hypoxanthine, Pipecolic acid, Trans-3-Indoleacrylic acid, Proline, Valine, 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO), L-Glutamic acid, 4-tert-Butylcyclohexyl acetate, Dibutyl phthalate, Thymine, Pyridoxamine, n-Pentyl isopentyl phthalate, 7-Methylxanthine, N,N-Diisopropylethylamine (DIPEA), (-)-Camphor, 4-Methoxycinnamic acid, Diethyl phthalate, 4-Indolecarbaldehyde, Caprolactam, Tetranor-12(S)-HETE, Prolinamide, 1-Tetradecylamine, 6-Methylquinoline, Citral, 4-(Dimethylamino)benzophenone, Acetophenone, Choline, 4-Hydroxycoumarin, DEET and 2,6-Dimethylpyrazine.

Decoction could dissolve more compounds than infusion, which was possible because decoction was more optimal at dissolving compounds in a matrix of materials. The *Juncus acutus* rhizome was dissolved with 37.33% more decoction than dissolved by infusion[1]. However, the extract from artichoke (*Cynara cardunculus* L. subsp. *cardunculus*) leaves dissolved by infusion gave more results than a decoction [19]. It shows that the number of compounds dissolved due to decoction or infusion depends on the thickness and complexity of the matrix of the dissolved material.

Moreover, zearalenone and adenosine have a stronger affinity bond against α-amylase and α-glucosidase. Zearalenone is a macrolide comprising of a 14-membered lactone fused to 1,3-dihydroxybenzene, a potent estrogenic metabolite. One of the biological roles of this compound is to play as an α-glycosidase inhibitor[4]. α-amylase is an enzyme that hydrolyzes starch into glucose and is classified as hydrolase[20]. Adenosine is a ribonucleoside composed of a molecule of adenine attached to a ribofuranose moiety via a beta-N(9)-glycosidic bond. This compound is an endogenous nucleoside found in all body cells and is a potent vasodilator in most vascular beds. In high concentrations, this compound can inhibit endothermic glucosidase activity[4].

Docking studies: The samples comprised of five infuse metabolomes and two decoct metabolomes capable of inhibiting α-amylase and α-glucosidase activities. The energy affinity of zearalenone (-8.6 kcal/mol) is lower for α-amylase than for acarbose (-7.6 kcal/mol), indicating that zearalenone has the potential to form bonds with α-amylase. Meanwhile, adenosine (-8.6 kcal/mol) has a lower energy affinity for α-glucosidase than acarbose (-8.2 kcal/mol).

Amylase (1smd) has a ligand (pyroglutamic acid) that forms hydrogen bonds with the residues of this enzyme in Lys227 and Ile230[3]. Glucosidase (3wy1) has a ligand (3R, 5R, 7R)-octane-1,3,5,7-tetracarboxylic acid) that forms hydrogen bonds to the His332 and Arg400 residues of the enzyme residue[4]. Zearalenone and adenosine have the potential to bind to α-amylase or α-glucosidase receptors. It aligns with energy enthalpy theory, where the lower binding energy between a substance and a receptor (product) is exothermic than the compound itself, making it easier to form bonds between compounds and receptors[3].

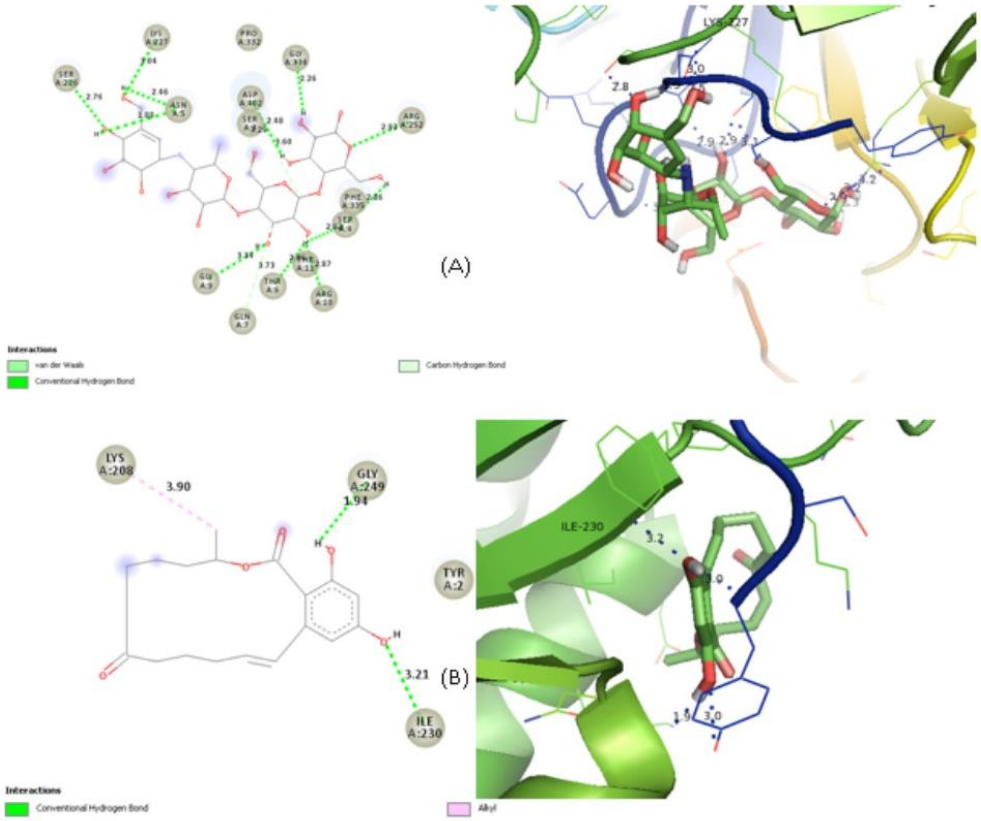

Figure 1: Interaction between amylase and (A) acarbose or (B) zearalenone

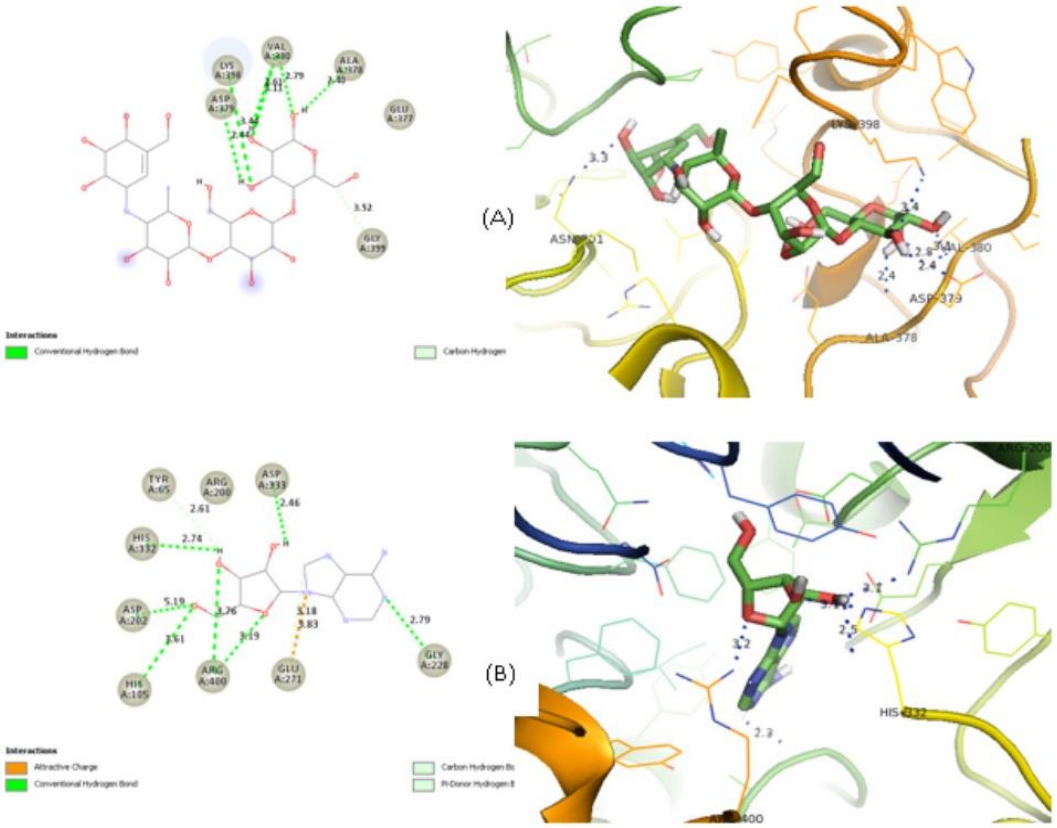

Figure 2: Interaction between glucosidase and (A) acarbose or (B) adenosine

As shown in figure 1A, acarbose has no bond in the two α-amylase residues, while zearalenone shows the presence of hydrogen bonds in Ile230 (Figure 1B). It suggests that the presence of hydrogen bonds in zearalenone with amylase residues contributes to the inhibition of enzyme activity and the similarity of this linkage indicates that zearalenone is a competitive inhibitor of amylase. The formation and strength of hydrogen bonds between ligands and residues of the enzyme's active site affect the inhibition of amylase activity[14].

As shown in figure 2A, acarbose does not bind with the two residues while adenosine shows the presence of hydrogen bonds in the two α-glucosidase residues (Figure 2B). It suggests that adenosine can inhibit glucosidase activity. The formation of hydrogen bonds between ligands and glucosidase helps to inhibit this enzyme. The similarity in the formation of hydrogen bonds by adenosine and (3R, 5R, 7R)-octane-1,3,5,7-tetracarboxylic acid shows that adenosine is a potent inhibitor of this enzyme[2, 21].

Physicochemical properties determination: The physicochemical properties of the metabolome are determined according to Lipinski's rule. Table 1 shows the characteristics of *S. platensis* metabolome which indicate α-amylase and α-glucosidase inhibitors. As presented in table 1, the metabolome of *S. platensis* can be absorbed, distributed, metabolized and excreted by cells. These substances can penetrate the cell membrane and dissolve in cell fluids since they have a molecular weight of < 500 Da, log P < 5, < 5 hydrogen bond donors and < 10 hydrogen bond acceptors[14].

Zearalenone and adenosine have a high potential for bioavailability to the body. A ligand can quickly diffuse into the cell membrane if its molecular weight is < 500 Da. Soon, the body absorbs a ligand in the direction of the target protein if the distribution ratio (log P) of the ligand to the organic solvent and water is < 5. The number of donors and acceptors of hydrogen-bonding protons are related to the stability of the conformation of the binding protein. The conformation of a protein is declared stable if there are sufficient hydrogen bonds between the ligands and the target protein[21].

Conclusion

The decoction technique on *S. platensis* produces more metabolite compounds than the infusion technique where the decoction produces 84 metabolites while the infusion produces 67 metabolites. This is possible because the deoxy process is more optimal in dissolving compounds in the material matrix. From the results of the docking analysis, it was found that zearalenone and adenosine have strong potential as inhibitors of α-amylase and α-glucosidase enzymes, shown with lower affinity energy compared to acarbose as a control. Zearalenone forms a hydrogen bond with a residue of Ile230 on α-amylase, while adenosine forms a hydrogen bond with both residues on α-glucosidase.

Table 1
Physicochemical properties of *S. platensis* metabolome

Compounds	MW (g/mol)	Log P	H bond donor	H bond acceptor
Valylproline	214.26	-0.27	2	4
Zearalenone	318.36	2.86	2	5
Adenosine	267.24	-1.61	4	7
N1-Hydrazino[3-(methylthio) anilino] methylidene benzene-1-sulfonamide	336.43	2.09	3	4
2-(Cyclohexylmethylidene) -1,2,3,4-tetrahydro naphthalen-1-one	240.34	4.20	0	1
Y-L-Glutamyl-L-glutamic acid	401.41	0.41	5	7
(3aR,8R,8aR,9aR)-8-Hydroxy-8a-methyl-3,5-bis (methylene) decahydronaptho [2,3-b] furan-2(3H)-one	248.32	2.35	1	3
Acarbose	645.60	-6.22	14	19

Based on the physicochemical properties according to Lipinski's law, the two compounds meet the criteria to be well absorbed, distributed, metabolized and excreted by cells because they have a molecular weight of <500 Da, a log P <5, a hydrogen bond donor <5 and a hydrogen bond acceptor <10. However, molecular dynamics and *in vivo* studies need to be conducted to reinforce the results of this study.

Acknowledgement

PNBP scheme of the Faculty of Fisheries and Marine Sciences (No. 37/UN10.F06.06/2024), Universitas Brawijaya, supports this research financially.

References

1. Adouni K., Mekhelfi T., Daouadji M.Z.D. and Achour L., Decoction, Infusion and Ethanolic Extract of *Juncus acutus* Rhizome: Phytochemical Content and Antioxidant Properties. *Int. J. Pharm. Sci. Rev. Res*., **48(1)**, 148-152 **(2018)**

2. Ali F., Khan K.M., Salar U., Taha M., Ismail N.H., Wadood A., Riaz M. and Perveen S., Hydrazinyl arylthiazole based pyridine scaffolds: Synthesis, structural characterization, in vitro α-glucosidase inhibitory activity and in silico studies, *Euro. J. Med. Chem*., **138**, 255-272 **(2017)**

3. Borah P.K., Sarkar A. and Duary R.K., Water-soluble vitamins for controlling starch digestion: Conformational scrambling and inhibition mechanism of human pancreatic α-amylase by ascorbic acid and folic acid, *Food Chem*., **288**, 395-404 **(2019)**

4. El-Shora H.M., Ibrahim M.E. and Alfakharany M.W., Activators and Inhibitors of α-glucosidase from *Penicillium chrysogenum*, *Ann. Res. Rev. Bio*., **24(1)**, 1-9 **(2018)**

5. Firdaus M., Nurdiani R., Artasasta I.N., Mutoharoh S. and Pratiwi O., Potency of three brown seaweeds species as the inhibitor of RNA-dependent RNA polymerase of SARS-CoV-2, *Rev. Chim*., **71(11)**, 80-86 **(2020)**

6. Firdaus M., Nurdiani R., Rivai B., Hemassonida W.H., Badzliyah A. and Sugiat N.K., The glucose uptake of type 2 diabetic rats by *Sargassum olygocystum* extract: *In silico* and *in vivo* studies, *J Appl. Pharm. Sci*., **12(3)**, 132-139 **(2022)**

7. Governa P., Baini G., Borgonetti V., Cettolin G., Giachetti D., Magnano A.R., Miraldi E. and Biagi M., Phytotherapy in the Management of Diabetes: A Review, *Molecules*, **23**, 1-22, doi: 10.3390/molecules23010105 **(2018)**

8. Handa S.S., Khanuja S.P.S., Longo G. and Rakesh D.D., Extraction Technologies for Medicinal and Aromatic Plants, International Centre for Science and High Technology, Trieste, 21-54 **(2008)**

9. Hossain M.I., Akter S., Nipa N.S., Chowdury U.W.K., Bhuiyan A.S., Ali T. and Rafiq K., Reno-pancreas protective effects of *Spirulina platensis* in alloxan induced diabetic rats, *Pak. J. Pharm. Sci*., **33(6)**, 2511-2519 **(2020)**

10. Huang H., Liao D., Pu R. and Cui Y., Quantifying the effects of Spirulina supplementation on plasma lipid and glucose concentrations, body weight and blood pressure, *Diab. Met. Synd. Ob*.: *Tar. Ther*., **11**, 729–742 **(2018)**

11. Ibrahim M.A., Bester M.J., Neitz A.W. and Gaspar A.R.M., Rational *in silico* design of novel α-glucosidase inhibitory peptides and *in vitro* evaluation of promising candidates, *Biomed. Pharmacother*., **107**, 234-242 **(2018)**

12. Joventino I.P. et al, The Microalga *Spirulina platensis* Presents Anti-inflammatory Action as well as Hypoglycemic and Hypolipidemic Properties in Diabetic Rats, *J. Compl. Integ. Med*., **9(1)**, 1-24 **(2012)**

13. Lipinski C.A., Lead- and drug-like compounds: the rule-of-five revolution, *Drug Disc. Today: Tech*., **1(4)**, 337-341 **(2004)**

14. Lipinski C.A., Lombardo F., Dominy B.W. and Feeney P.J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Adv. Drug Del. Rev*., **46(1–3)**, 3–26, doi:10.1016/S0169-409X(00)00129-0 **(2001)**

15. Milović S., Kundaković T., Mačić V., Stanković J.A., Grozdanić N., Đuričić I., Stanković I. and Stanojković T., Anti α-glucosidase, antitumour, antioxidative, antimicrobial activity, nutritive and health protective potential of some seaweeds from the adriatic coast of Montenegro, *Farmacia*, **65(5)**, 731-740 **(2017)**

16. Nawaz M., Taha M., Qureshi F., Ullah N., Selvaraj M., Shahzad S., Chigurupati S., Waheed A. and Almutairi F.A., Structural elucidation, molecular docking, α-amylase and α-glucosidase inhibition studies of 5-amino-nicotinic acid derivatives, *BMC Chem*., **14**, https://doi.org/10.1186/s13065-020-00695-1 **(2020)**

17. Okechukwu P.N., Ekeuku S.O., Sharma M., Nee C.P., Chan H.K., Mohamed N. and Froemming A.G.R., *In vivo* and *in vitro* antidiabetic and antioxidant activity of Spirulina, *Pharm. Mag*., **15(62)**, 17-29 **(2019)**

18. Parikh P., Mani U. and Iyer U., Role of *Spirulina* in the control of glycemia and lypidemia in type 2 diabetes mellitus, *J. Med. Food*, **4**, 193-199 **(2001)**

19. Pistón M., Machado I., Branco C.S., Cesio V., Heinzen H., Ribeiro D., Fernandes E., Chisté R.C. and Freitas M., Infusion, decoction and hydroalcoholic extracts of leaves from artichoke (*Cynara cardunculus* L. subsp. cardunculus) are effective scavengers of physiologically relevant ROS and RNS, *Food Res. Int*., **64**, 150–156 **(2014)**

20. Ramasubbu N., Paloth V., Luo Y., Brayer G.D. and Levine M.J., Structure of human salivary alpha-amylase at 1.6 Å resolution: implications for its role in the oral cavity, *Acta Crystallogr. D: Struc. Biol*., **52**, 435-446 **(1996)**

21. Rudovich N., Pivovarova O., Traberth A., Sparwasser A., Weickert M.O., Bernigau W., Birkenfeld A.L., Arafat A.M., Bergmann A. and Pfeiffer A.F.H., Acarbose treatment enhances mid-regional pro-atrial natriuretic peptide concentrations in non-diabetic individuals: further evidence for a common cardiometabolic pathway?, *Diabetologia*, **55**, 3392-3395 **(2012)**

22. Scaglioni P.T., Quadros L., de Paula M., Furlong V.B., Abreu P.C. and Badiale-Furlong E., Inhibition of Enzymatic and Oxidative Processes by Phenolic Extracts from *Spirulina* sp. and *Nannochloropsis* sp., *Food Tech. Biotech*., **53**, 344-353 **(2018)**

23. Soonmin Ho and Naser Ahmed, An overview on spray pyrolysis deposition of metal oxide thin films, *Res. J. Chem. Environ.*, **27(11)**, 131-141 **(2023)**

24. Telagari M. and Hullatti K., *In-vitro* α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum*Linn. and *Celosia argentea* Linn. extracts and fractions, *Indian J. Pharm*., **47(4)**, 425 -429 **(2015)**

25. Wana X., Lia T., Zhonga R., Chen H., Xia X., Gao L., Gao X., Liu B., Zhang H. and Zhao C., Anti-diabetic activity of PUFAs-rich extracts of *Chlorella pyrenoidosa* and *Spirulina platensis* in rats, *Food Chem. Toxicol*., **128**, 233–239 **(2019)**.

(Received 27th October 2024, accepted 07th January 2025)