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In Silico Antibacterial Activity of Polyacetilene Derivatives Against *Mycobacterium tuberculosis* and In-Vitro Antioxidant Properties from Ethanol Extraction of Blackjack (*Bidens pilosa* L.)

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Abstract

Blackjack (*Bidens pilosa* L.) has a bioactive compound, one of which is polyacetylene, which can inhibit the growth of antimicrobials in general. This study aims to conduct an in silico study to determine the antibacterial activity against *Mycobacterium Tuberculosis* of 20 derivatives polyacetylene; TPC was determined by Folin–Ciocalteu colorimetric method using gallic acid as standard, and various concentrations of the extract solutions were measured at 741 nm. TFC was calculated using an aluminum chloride colorimetric assay. Quercetin was used as standard, and the absorbance was measured at 426 nm. Antioxidant activity was evaluated using DPPH scavenging 517 nm and Frap Assay 596 nm. The molecular docking results showed that the compounds (5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene, Glucopyranosyloxy-3-hydroxy-6(E)-tetradecane-8,10,12-triyne and Phenylhexa-1,3,5-triyn-1-yl acetate were most potentially anti-tuberculosis on the target protein InhA with the binding energy produced <-6.55 Kcal/mol and the lowest inhibitory cost of 1.838×10^{-6}. The compound has amino acids similar to natural ligands and standard compounds of isoniazid. The given ADME-T predictions showed good pharmacokinetic properties and safe levels of toxicity (5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene, Glucopyranosyloxy-3-hydroxy-6(E)-tetradecane-8,10,12-triyne in hepatotoxic and LD_{50}. The highest antioxidant properties in blackjack are produced by FRAP assay 722.905±7.985 mMTE/gr. This proves that blackjack has antioxidant activity and is required for bioassay-guided isolation testing against *Mycobacterium tuberculosis*.

1. Introduction

Tuberculosis is one of the diseases that can be transmitted through the air and is caused by the *Mycobacterium tuberculosis* bacteria (MTB). Tuberculosis cases are one of the leading causes of death worldwide [1]. Indonesia has the second-highest in terms of tuberculosis infections, following India. This is evident from the incidence rate in 2022, which reached 969 thousand people and increased 17% compared to 2020 [1]. This case shows the challenges associated with tuberculosis treatment, such as drug resistance and adverse side effects, which contribute to patient non-compliance during the course of therapy.

Treatment of tuberculosis is a comprehensive and ongoing treatment. It takes at least six months with a combination of at least four kinds of anti-tuberculosis drugs (OAT) [2]. Resistance to OAT occurs due to mutations in the *Mycobacterium tuberculosis* InhA gene, so this resistance is the driver for developing anti-tuberculosis drugs [3, 4].

One of the resistances that gives rise to mutations in the OAT target coding gene is the inhA gene for isoniazid [5]. Active isoniazid binds to the inhA-NADH complex. This complex will deactivate enoyl reductase and inhibit mycolic acid biosynthesis in the cell wall of *Mycobacterium tuberculosis*. The alternation of amino

acids at the NADH bond site of inhA results in isoniazid resistance by preventing mycolic acid biosynthesis. The substitution of Ser94Ala results in a decrease in the affinity of inhA bonds to NADH; thus, inhibition of mycolic acid synthesis occurs. InhA promoter mutations are more common at positions - 24(G-T), -16(A-G), or - 8(T-G/A) and -15(CT) [6, 7]. These promoter mutations produce inhA overexpression and trigger isoniazid resistance. This spurred the development of anti-tuberculosis drugs. One way to do this is to use active compounds from plants that can potentially be used as anti-tuberculosis agents [8].

The development of natural ingredients has the potential to support tuberculosis therapy blackjack (*Bidens pilosa* L.), which became one of the innovations in its development. Blackjack are wild plants that, ethnobotanically in Jombang, West Java, are used as vegetables, whereas in China, it is processed and utilized as herbal tea [9, 10]. Blackjack plants have bioactive compounds such as phenolics, flavonoids, polyacetylene, chalcones, okanine glycosides, and terpenoids [11]. In their activity, blackjack plants have been proven to be antimalarial, anti-inflammatory, antihypertensive, colorectal cancer, and antibacterial in modulating immunity [12, 13, 14, 15].

Polyacetylene compounds are potential candidates for producing antibacterial activity against Gram-positive and Gram-negative bacteria [16]; however, further development of anti-tuberculosis activity has yet to be carried out.

This research screens polyacetylene derivatives in blackjack, tested through molecular docking as an initial step in developing new drug ingredients. Additionally, the antioxidant properties of the ethanol extract of blackjack are examined as supporting data to assess the provided antioxidant characteristics. Molecular docking generates an energy affinity output that describes the bond strength resulting from the interaction between ligands and receptors.

2. Experimental

The research method used was in silico experimental research of polyacetylene derivative compounds from the blackjack plant. This included applying the Lipinski Rule of Five, conducting molecular docking studies, and predicting the pharmacokinetics and toxicity of the active compounds to assess their potential as anti-tuberculosis agents. Additionally, the antioxidant properties of the ethanol extract of the blackjack plant were evaluated using UV-Vis spectrophotometry.

2.1. Materials

This study employed blackjack plants, ligands, and receptors for molecular docking. The ligands comprised 20 polyacetylene-derived compounds obtained from blackjack plants (refer to Table 1) [12, 17, 18, 19, 20, 21, 22, 23]. Isoniazid was utilized as the comparison compound, and the receptor chosen was the InhA protein of MTB bacteria (PDB: 1eny); InhA protein plays an essential role in the survival of Mycobacterium, especially in the synthesis of mycolic acid [24]. Materials needed for measurement of antioxidant properties were gallic acid (Sigma-Aldrich, USA), ethanol (C_2H_6O) (Sigma-Aldrich, USA), Folin-Ciocalteu reagent (Sigma-Aldrich, USA), sodium hydroxide (NaOH) (Sigma-Aldrich, USA), quercetin (Sigma-Aldrich, USA), aluminum chloride (Sigma-Aldrich, USA), sodium acetate, 1,1-diphenyl-2-picrylhydrazil (DPPH) (Sigma-Aldrich, USA), standard trolox (Sigma-Aldrich, USA), hydrochloric acid (HCl), ferric chloride ($FeCl_3$) (Sigma-Aldrich, USA), TPTZ (Sigma-Aldrich, USA), and HCl (Sigma-Aldrich, USA).

2.2. Tools

The software used included ChemDraw version 16.0, Chem 3D version 16.0, AutoDockTools version 1.5.6, and Discovery Studio 2019. The hardware employed was the Asus TUF Gaming F15 featuring an Intel (R) Core (TM) i5-10300H CPU @2.50 GHz and 8 GB RAM. UV-Vis spectrophotometry (Shimadzu, Japan) was utilized as the required instrument for testing antioxidant properties.

2.3. Preparation of Protein and Native Ligand

Protein preparation involved isolating proteins in their active form, specifically those binding to native ligands, utilizing AutoDockTools. Eliminating the water component during protein preparation was essential to prevent interference with the ligand process. Then, polar hydrogen and Kollman charges were added, and the results were saved in .pdb format.

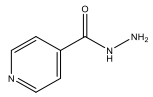
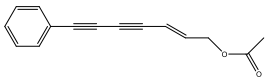
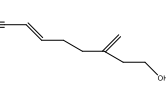
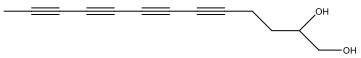
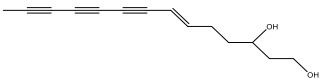
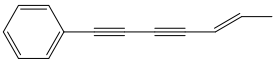
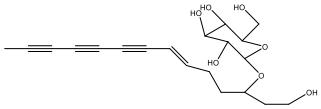
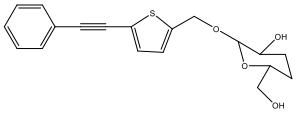
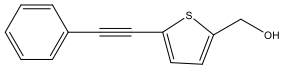
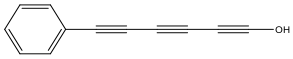
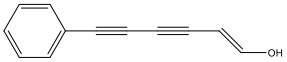
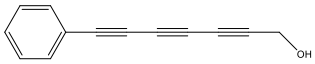
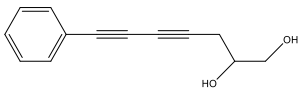
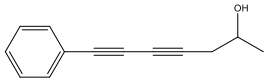
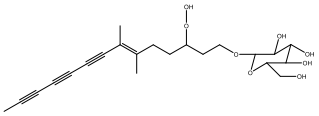
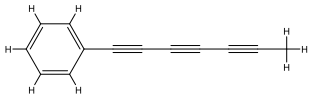
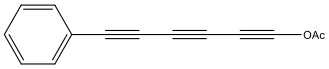
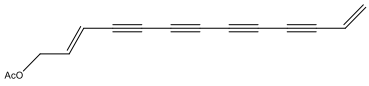
2.4. Validation of Molecular Docking Method

The molecular docking method was validated using natural ligands with target proteins that have undergone preparation using the AutoDockTools program. The grid box arrangement was used to determine the ligand-bound space to be in-docking, obtained grid box dimensions (X: 40, 50; Z: 36) with spacing amstrong (0.375 Å) and natural ligand coordinates (x: -3.047; y: 32.422; z: 14.205). The docking result obtained the banding energy value and Root Mean Square Deviation (RMSD). The docking validation method is said to be valid if the RMSD value is ≤ 2 Å [25]. The docking simulation was conducted using AutoDock4 software, which was configured with AutoDockTools and executed via the Command Prompt. The docking parameters were set using the "Genetic Algorithm (GA)" parameter, the number of GA runs was generated 100 times the ligand-receptor interaction, and the parameter settings were set by default. The output of the docking simulation was stored based on the Lamarckin GA settings, and the docking simulation was run using the command prompt.

2.5. Molecular Docking Analysis

All test ligands were docked using the same methodology as in the validation process, maintaining identical grid box dimensions and positioning for each receptor. The docking procedure, performed using AutoDockTools, yielded two key parameters: binding affinity (ΔG; kcal/mol) and ligand-receptor interactions, which were derived from interactions with similar amino acids [26].

Table 1. Isoniazid as a ligand standard and derivative of polyacetylene compounds

Structure	Name
	Isoniazid (ligand standard)
	(2E)-7-Phenylhept-2-ene-4-6-diyn-1-yl acetate
	(6E-12E)-3-Oxo-tetradeca-6-12-dien-8-10-diyn-1-ol
	1,2-Dihydroxytrideca-5,7,9,11-tetrayne
	1,3-Dihydroxy-6(E)-tetradecene-8,10,12-triyne
	1-Phenyl-hept-5t-ene-1,3-diyne
	2-b-D-Glucopyranosyloxy-1-hydroxy-5(E)-
	5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene
	5-(2-Phenylethynyl)-2-thiophene methanol
	6-phenylhexa-1,3,5-triyn-1-ol
	6-phenylhexa-1-en-3,5-diyn-1-ol
	6-phenylhex-1-en-3,5-diyn-1-yl acetate
	7-Phenyl-hepta-2,4,6-triyn-2-ol
	7-Phenyl-hepta-4,6-diyn-1,2-diol
	7-Phenyl-hepta-4,6-diyne-2-ol
	7-Phenyl-heptene-4,6-diyn-1-ol
	β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne
	Phenylheptatriyne (1-phenylhepta-1,3,5-triyne)
	Phenylhexa-1,3,5-triyn-1-yl acetate
	Trideca-1,11-dien-3,5,7,9-tetrayne
	Trideca-2,12-dien-4,6,8,10-tetrayn-l-yl acetate

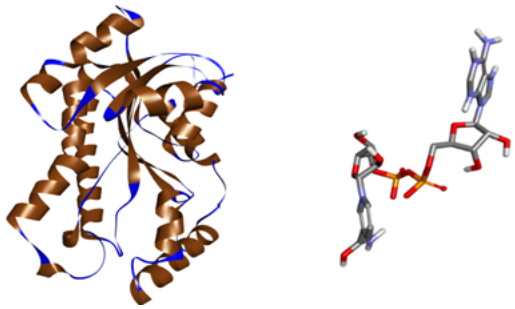


Figure 1. Separation of proteins and natural ligands

2.6. ADME-T Prediction

ADME prediction was conducted using Swiss ADME, accessed through the website http://www.swissadme.ch/index.php. For toxicity prediction, PrTox-II and pKcsm were utilized via the websites https://tox- new.charite.de/protox_II/index.php?site=compound_input and https://biosig.lab.uq.edu.au/pkcsm/prediction, respectively. To obtain the SMILES notation for the new compound, translation services provided by https://cactus.nci.nih.gov/translate/ were employed.

2.7. Preparation of Ethanol Extraction

Blackjack was found in the Banyumas area, Central Java, and had been proven to be authenticated. The dry powder of blackjack plants was extracted using 70% ethanol (1:10) by stirring and allowed to stand for 24 hours, and the macerated sample was filtered. The residue was remacerated with the same solvent. The filtrate obtained was evaporated using a rotary vacuum evaporator to obtain a thick extract.

2.8. Determination of Total Phenolic and Total Flavonoid Content

The ethanol extract of blackjack was utilized to determine total phenolic and total flavonoid levels [21]. To measure the total phenolic content (TPC), 1 mL of the extract or standard solution was combined with 5 mL of Folin-Ciocalteu reagent at room temperature for 8 minutes. Subsequently, 4 mL of 1% NaOH was added, and the mixture was left to stand for 40 minutes, followed by measuring the absorption at a wavelength of 741 nm. The total phenolic content is expressed in mgGAE/gram.

To measure total flavonoid content (TFC), 0.5 mL of the extract solution or standard was mixed with 1.5 mL of ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of CH_3COONa, and 2.8 mL of distilled water. The mixture was allowed to stand for 15 minutes at room temperature, and the wavelength was measured at an absorption of 426 nm. Total flavonoid content was reported in mgQE/gram.

2.9. Determination of Antioxidant Activity

Antioxidant analysis was carried out using DPPH scavenging and FRAP Assay following the method [27]. DPPH analysis was performed by taking 0.5 mL of extract, or standard solution reacted with 5 mL of DPPH (25 µg/mL ethanol), allowed to sit in a dark place for 30 minutes, and measured at a wavelength of 517 nm. DPPH scavenging was reported in mMTE/gr.

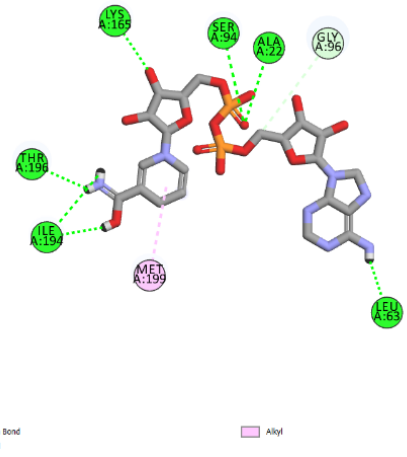


Figure 2. Interaction of amino acid residues and hydrogen bonds of isoniazid

The FRAP assay was conducted by mixing 0.21 mL of the extract solution or standard with 3.99 mL of FRAP reagent (300 mM acetate buffer, 10 mM TPTZ in HCl, and 20 mM $FeCl_3$ (ratio 10:1:1 pH 3.6)). The mixture was then allowed to stand in a dark place for 40 minutes before being measured at a wavelength of 596 nm. The results were reported in mMTE/gr.

2.10. Analysis Result

Data analysis encompassed several parameters, including bond energy and inhibition constant (Ki). Visualization tools, such as Discovery Studio, were utilized to observe interactions between ligands and amino acid residues. The formula for calculating the inhibition constant (IC) is described in Equation (1).

$$IC = e\frac{\Delta G}{RT} \quad (1)$$

Where ΔG is the binding energy (kcal/mol), R is the gas constant (1.986 kcal/mol), and T is the temperature (2981.5 K).

Data analysis of the total phenolic and flavonoid content, as well as antioxidant activity measured by DPPH scavenging and FRAP assay, was conducted by performing three repetitions with standard deviation (±SD) and calculated using the assay Equation (2).

$$C = \frac{c \times V \times DF}{1000 \times W} \quad (2)$$

Where, C is the TPC, TFC, and antioxidant level, c is the concentration obtained from the calibration curve mg/mL, V is the volume of extract in mL, DF is the dilution factor, and W is the mass of extract in gram.

Table 2. RMSD and free binding energy of natural ligands

PDB	RMSD	Binding energy (ΔG)	Amino acid residue
1ENY	1.964	-6.53	Ile194; Thr196; Met199; Lys165; Ser94; Ala22; Leu63; Gly96

Table 3. Simulation results of docking of standard compounds and polyacetylene derivatives of blackjack against MTB protein

PDB	Compound	Binding energy (ΔG) (kcal/mol)	Inhibition constant (μM)
1ENY	Isoniazid	-5.03	2.045×10^{-4}
	(2E)-7-Phenylhept-2-ene-4-6-diyn-1-yl acetate	-6.40	2.023×10^{-5}
	(6E-12E)-3-Oxo-tetradeca-6-12-dien-8-10-diyn-1-ol	-5.26	4.728×10^{-4}
	1,2-Dihydroxytrideca-5,7,9,11-tetrayne	-4.30	7.017×10^{-4}
	1,3-Dihydroxy-6(E)-tetradecene-8,10,12-triyne	-5.36	1.171×10^{-4}
	1-Phenyl-hept-5t-ene-1,3-diyne	-5.45	1.006×10^{-4}
	2-b-D-Glucopyranosyloxy-1-hydroxy-5(E)-	-5.48	9.565×10^{-5}
	5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene	**-7.60**	**2.665×10^{-6}**
	5-(2-Phenylethynyl)-2-thiophene methanol	-6.13	3.191×10^{-5}
	6-phenylhexa-1,3,5-triyn-1-ol	-5.57	8.216×10^{-5}
	6-phenylhexa-1-en-3,5-diyn-1-ol	-5.49	9.405×10^{-5}
	6-phenylhexa-1-en-3,5-diyn-1-yl acetate	-6.47	1.797×10^{-5}
	7-Phenyl-hepta-2,4,6-triyn-2-ol	-6.14	3.138×10^{-5}
	7-Phenyl-hepta-4,6-diyn-1,2-diol	-5.79	5.667×10^{-5}
	7-Phenyl-hepta-4,6-diyne-2-ol	-5.90	4.706×10^{-5}
	7-Phenyl-heptene-4,6-diyn-1-ol	-6.11	3.301×10^{-5}
	β -D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne	**-7.82**	**1.838×10^{-6}**
	Phenylheptatriyne (1-phenylhepta-1,3,5-triyne)	-5.35	1.191×10^{-4}
	Phenylhexa-1,3,5-triyn-1-yl acetate	**-6.55**	**1.570×10^{-5}**
	Trideca-1,11-dien-3,5,7,9-tetrayne	-4.68	3.694×10^{-4}
	Trideca-2,12-dien-4,6,8,10-tetrayn-l-yl acetate	-5.52	8.940×10^{-5}

3. Results and Discussion

3.1. Validation of Molecular Docking Method

The target protein utilized in the study was the InhA GDP, obtained from the rcsb.org website with a PDB ID: 1ENY. This protein complex included one natural ligand: Nicotinamide-Adenin-Dinucleotide. InhA protein is crucial for the survival of *Mycobacterium*, particularly in the synthesis of mycolic acid [23]. Method validation is performed by separating the protein structure from the natural ligand (Figure 1).

The method validation results were carried out by looking at the RMSD parameters. RMSD is the deviation distance between the binding position of natural ligands and proteins after docking against the actual position of natural ligand bonds. Docking validation is carried out by determining the grid box, which is the area where interactions between natural ligands and proteins occur [28]. The results of the visualization of natural proteins and ligands in RMSD and bond energy can be seen in Table 2.

Based on the validation results, it produces an RMSD value of 1.47 Å, which means that the molecular docking method used is validated and reliable for docking other test ligands [29]. The parameter used to assess docking results is the binding energy, which evaluates a drug's capacity to bind to receptors. A lower ΔG value indicates a stronger interaction between receptors and ligands, signifying stronger attachment. This is reflected in the ΔG value, where a smaller value indicates stronger binding affinity [30]. The inhibition constant (Ki) indicates the concentration required of ligands in inhibiting the target protein. A lower Ki value indicates a stronger complex formed by the ligand and the protein. This is attributed to the rise in torsional energy within the complex, contributing to the stability of the enzyme-ligand complexes [31]. The results obtained from isoniazid standard compounds demonstrate the smallest bond energy and inhibition constant among other standard compounds, specifically -6.53 kcal/mol and 2.045×10^{-4} μM (Table 3).

Table 4. Similarity of amino acid residuals

Parameter	Nicotinamide-Adenine-Dinucleotide	Isoniazid (standard)	5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene	β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne	Phenylhexa-1,3,5-triyn-1-yl acetate
ΔG (kcal/mol)	-6.53	-5.03	-7.60	-7.82	-6.55
Amino acid residue	Ile194	-	-	-	
	-	Ile122	Ile122	-	Ile122
	-	Ile95	Ile95	Ile95	Ile95
	-	-	Ile16	-	-
	-	-	-	Ile21	-
	-	Phe41	Phe41	-	Phe41
	-	Val65	Val65	-	Val65
	-	Asp64	-	-	-
	-	-	-	-	Ser20
	Ser94	-	-	-	
	Leu63	-	Leu63	Leu63	-
	Gly96	-	Gly96	Gly96	-
				Gly14	
	-	-	-	Thr39	-
	Thr196		-	-	
	Met199	-	-	Met199	-
	Ala22	-	-	Ala22	-
	-	-	-	Pro193	-
	Lys165	-	-		-
The similarity of amino acid residues to natural ligands (%)		0	22.22	44.44	0
The similarity of amino acid residues to standard compounds (%)			80	12.50	80

The bond energy value of isoniazid plays a significant role in its interaction with proteins, particularly through intramolecular interactions such as hydrogen bonds and van der Waals forces. The amino acid residue produced by isoniazid is Ile21, Ile95, Phe41, Val65, and Asp64 (Figure 2). Hydrogen bonds are typically stronger than van der Waals bonds, as they can form even when the distance between the ligand and the receptor is relatively far.

3.2. Molecular Docking Analysis

Polyacetylene compounds exhibit antibacterial properties against both Gram-negative and Gram-positive bacteria [16], suggesting their potential as candidates for tuberculosis treatment. Molecular docking analysis was conducted on 20 derivatives of polyacetylene compounds obtained from blackjack plants, revealing three compounds —5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene, β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne, and Phenylhexa-1,3,5-triyn-1-yl acetate—with notably lower bond energies and inhibitory constants (Table 3).

The results show higher activity than the standard molecule, namely isoniazid (-5.03 Kcal/mol). The lower the bond energy value, the energy required to interact between ligands and proteins becomes more stable. The compound has hydrogen bonds formed respectively 5- (2-Phenylethynyl)-2-b-glucosylmethyl-thiophene (5 hydrogen bonds), β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecane-8,10,12-triyne (5 hydrogen bonds) and Phenylhexa-1,3,5-triyn-1-yl acetate (1 hydrogen bond) respectively (Figure 3). The formation of more hydrogen bonds is predicted to increase the activity of ligands as anti-tuberculosis agents.

5-(2-Phenylethynl)-2-b-glucosylmethyl-thiophene forms hydrophobic bonds with InhA proteins via amino acids Ile95, Ile122, Ile16, Val65, Leu63, Gly96, Phe41 as well as hydrogen binding via amino acids Gly96, Asp64, Leu63 and Val65 (Figure 3B). The most common type of amino acid residue is hydrophobic amino acids except Asp64, including hydrophilic amino acids [32]. Specifically, 5-(2-Phenylethynl)-2-b-glucosylmethyl-thiophene exhibits a 22.22% similarity with natural ligands and an 80% similarity of amino acid residues with isoniazid standard compounds (Table 4).

Table 5. Lipinski's Rule of Five

Compound	MW (≤ 500 Da)	Log P (< 5)	nHBD (≤ 5)	nHBA (≤ 10)	TPSA (<140 Å²)	MR	Log Kp (cm/s)	Log S	nRotB
5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene	344.42 g/mol	3.18	2	4	87.16 Å²	92.93	-6.55	-3.69	4
β -D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne	422.47 g/mol	3.87	5	8	128.84 Å²	109.24	-7.91	-2.72	9
Phenylhexa-1,3,5-triyn-1-yl acetate	208.21 g/mol	3.12	0	2	26.30 Å²	60.83	-4.38	-4.18	1

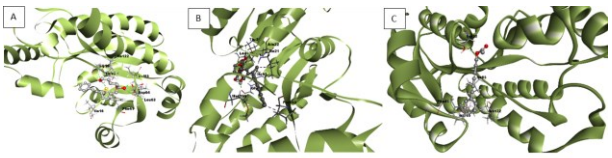


Figure 3. The interaction of ligands with tuberculosis proteins: (A) 5-(2-Phenylethynl)-2-b-glucosylmethyl-thiophene, (B) β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecane-8,10,12-triyne, and (C) Phenylhexa-1,3,5-triyn-1-yl acetate

β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne binds to InhA protein hydrophobically via the amino acids Pro193, Ile21, Ile95, Ala22, Gly96, Gly14, Leu63 and hydrogen binds via amino acids Gly96, Ile95, Gly14, Leu63, Thr39. The predominant amino acid residues are hydrophobic, except for Thr39, which includes hydrophilic amino acids, and Met199, which encompasses amphipathic amino acids [32] (Figure 3C). β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecane-8,10,12-triyne has amino acid similarity to compounds from natural ligands by 44.44%. It has amino acid residue similarity with standard compounds by 12.5% (Table 4).

Phenylhexa-1,3,5-triyn-1-yl acetate binds hydrophobically to InhA proteins via Val65, Ile95, and Ile122, and hydrogen binds via Ser20. All amino acids are hydrophobic except Ser20, including hydrophilic ones (Figure 3A) [32]. The amino acid residue between (2E)-7-Phenylhept-2-ene-5-6-diyn-1-yl acetate has a standard compound confidence of 80% (Table 4).

Residues produced by selected active compounds of blackjack plants against target proteins are known to be hydrophilic, and most amino acid residues are hydrophilic. Hydrophobic amino acids have properties that are difficult to dissolve in water. On the other hand, amino acids are hydrophilic, with ionized side chains easily soluble in water [33]. The similarity of polyacetylene-derived amino acid residues between natural ligands and the standard isoniazid indicates a similarity in the type of interaction and binding to the target protein. Pratama *et al.* [34] stated that the higher similarity of amino acid residues indicates a high probability that the active compound's ligand will interact similarly with natural ligands.

3.3. ADME-T Prediction

Lipinski's Rule of Five is utilized to assess the drug-likeness and oral activity potential of active compounds. Selected polyacetylene derivatives, including 5-(2-Phenylethynl)-2-b-glucosylmethyl-thiophene, β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne, and Phenylhexa-1,3,5-triyn-1-yl acetate, comply with all Lipinski criteria (MW ≤ 500 Da, LogP < 5, nHBD ≤ 5, nHBA ≤ 10, and TPSA < 140 Å²). However, the Log S values for these three derivative compounds are - 3.69, -2.72, and -4.18, respectively, indicating poor water solubility [35] and excellent absorption in the intestine is shown by TPSA < 140 [36] (Table 5). The bioavailability of oral compounds with good membrane permeability and hydrophobicity of drug molecules is indicated by Log P, TPSA, MW, HBA, and HBD values.

The toxicity prediction for selected compounds indicates excellent human intestinal absorption values for 5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene and Phenylhexa-1,3,5-triyn-1-yl acetate, ranging from 91.795% to 100%. However, β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne exhibits poor human intestinal absorption at 21.821%, falling below the recommended minimum threshold of 30%. The BBB's goal is to maintain CNS homeostasis by controlling the flow of material, nutrients, and cells from the blood to the brain and back. The authorized values for BBB are as follows: strong absorption to the Central Nervous System (log BBB > 0.3), moderate absorption to the CNS (log BBB -1.0 - 0.3), and low absorption to the CNS (log BBB < -1.0) [25, 37]. The drug might be able to penetrate the CNS unless the log Ps falls within the range of -3>log Ps>-2. The results suggest that the compound is highly likely to cross the barrier, except for the compound β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne (Table 6).

The enzymatic metabolism of the drug indicates the biotransformation of a drug in the body; it is essential to consider drug metabolism. The superclass of cytochrome P450 enzymes plays a vital role in drug metabolism. The CYP families responsible for drug metabolism include 1A2, 2C9, 2C19, 2D6, and 3A4 (Table 6). The compounds 5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene and Phenylhexa-1,3,5-triyn-1-yl acetate have lower total clearance values than the standard compounds of 0.275 and 0.658 (Table 6).

Table 6. Prediction of ADME-T properties of selected compounds

Compound	Absorption Intestinal Human (%)	Distribution		Metabolism substrate inhibitors CYP							Total clearance	Hepatotoxic	LD_{50} in rat (mol/kg)
		Log BB	LogPS	2D6	3A4	1A2	2C19	2C9	2D6	3A4			
Isoniazid	92.601	0.002	-3.351	No	No	No	No	No	No	No	0.722	Active	2.304
5-(2-phenylethynyl)-2-b-glucosyl methyl-thiophene	91.795	0.065	-2.369	No	Yes	Yes	Yes	No	No	No	0.275	Inactive	2.569
β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne	21.821	-1.056	-3.997	No	No	No	No	No	No	No	1.889	Inactive	2.321
Phenylhexa-1,3,5-triyn-1-yl acetate	100	0.304	-1.409	No	Yes	Yes	No	No	No	No	0.658	active	1.436

Drug toxicology is one of the important areas in preclinical investigation, as toxicity is a major factor leading to reduced drugs at the discovery and development stage [38]. Therefore, reliable prediction of compound toxicity in silico plays a crucial role in minimizing costs and saving time throughout the drug discovery and development process [39, 40]. The risk of toxicity for compounds 5-(2-phenylethynyl)-2-b-glucosylmethyl-thiophene, β-D-glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne, and Phenylhexa-1,3,5-triyn-1-yl acetate is shown in Table 6. Three toxicity descriptors, namely AMES and Hepatotoxic LD_{50}, become the main estimated component. The AMES test is a test to detect the mutagenicity of a compound that can suppress genetic damage and mutations [41]. According to the findings, 5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene exhibits mutagenic properties, while the other compounds do not demonstrate mutagenic properties (Table 6).

Hepatotoxicity is a major factor leading to reduced drugs. Unfortunately, a quarter of marketed drugs were recalled due to their adverse effects on the liver. The phenylhexa-1,3,5-triyn-1-yl acetate compound can provide hepatotoxic effects, while the other two compounds do not exhibit hepatotoxic effects (Table 6). Determination of acute toxicity is another important task in the goal of drug discovery and development. Acute toxicity expressed in median lethal dose (LD_{50}); the number of doses of the test compound that killed 50% of treated animals within a given time [42]. LD_{50} of compounds 5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene and β -D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne indicate low acute toxicity, as they are higher than those of standard compounds, measured at 2.569 and 2.321 mol/kg, respectively, in rat models (Table 6).

3.4. Determination of Antioxidant Properties

The ethanol extracts of blackjack demonstrated a total phenolic content of 74.754±1.145 mgGAE/g and a total flavonoid content of 22.589±0.089 mgQE/g. Additionally, the antioxidant activity assessed by DPPH and FRAP assays yielded levels of 601.756±2.587 mMTE/g and 722.905±7.985 mMTE/g, respectively (Table 7).

Plant-derived natural antioxidants play a crucial role in reducing or mitigating the detrimental effects of oxidative stress. The DPPH assay is a handy method for assessing the antioxidant capacity of plants, among other assays. Flavonoids and phenols, which are antioxidant chemicals with hydrogen-donating groups, lead to reducing the methanolic DPPH solution by forming nonradical compounds [43]. In addition to their antioxidant capabilities, flavonoids and other phenolics also possess other biological activities, including antibacterial, antiviral, and anticancer effects [44]. The ability of these substances to bind proteins and scavenge free radicals is typically responsible for their biological and pharmacological effects [45]. Antioxidants are crucial compounds that can shield the body from damage caused by oxidative stress created by free radicals. Plant polyphenols function as reducing agents and antioxidants due to the hydrogen-donating characteristic of their hydroxyl groups [46].

Table 7. Determination of antioxidant properties

Sample	Total phenolic content (mgGAE/g)	Total flavonoid content (mgQE/g)	DPPH scavenging (mMTE/g)	FRAP assay (mMTE/g)
Ethanol extract of blackjack	74.754±1.145	22.589±0.089	601.756±2.587	722.905±7.985

4. Conclusion

The compounds that showed the most effective interaction with the target protein InhA from blackjack plants were 5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene, β -D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10 ,12-triyne, and Phenylhexa-1,3,5-triyn-1-yl acetate. Their respective ΔG values were -7.60, -7.82, and -6.55 Kcal/mol, while their inhibition constant values were 2.665×10^{-6}, 1.838×10^{-6} and 1.570×10^{-5} µM, respectively. All three compounds are similar to natural ligands and isoniazid comparison compounds, except that Phenylhexa-1,3,5-triyn-1-yl acetate compounds do not resemble natural ligands. ADME-T predictions in all three selected groups showed good pharmacokinetic properties and toxicity prediction properties, particularly 5-(2-Phenylethynyl)-2-b-glucosylmethyl-thiophene and β-D-Glucopyranosyloxy-3-hydroxy-6(E)-tetradecen-8,10,12-triyne, which exhibited safer and non-toxic predictions. Additionally, the antioxidant activity profile of blackjack plants exhibited higher levels using the FRAP method, measured at 722.905±7.985 mMTE/gr. The results of docking predictions for selected polyacetylene compounds indicate a strong potential for binding to the InhA protein, suggesting their potential as active agents against tuberculosis. Additionally, the antioxidant profile observed suggests further analysis for potential anti-tuberculosis activity.

References

[1] World Heart Organization, *Global Tuberculosis Report 2022*, World Health Organization, Geneva, 2022

[2] Kementerian Kesehatan Republik Indonesia, *Petunjuk Teknis Penatalaksanaan Tuberculosis Resisten Obat Di Indonesia*, Jakarta, 2020

[3] Irmayanti Irwan, Hajrah Hajrah, Yurika Sastyarina, Simulasi Docking Senyawa Napthoquinones Umbi Bawang Tiwai (*Eleutherine americana* Merr.) terhadap Bakteri *Mycobacterium tuberculosis*, *Proceeding of Mulawarman Pharmaceuticals Conferences*, 2021

[4] Ruswanto Ruswanto, *molecular docking* empat turunan *isonicotinohydrazide* pada *mycobacterium tuberculosis enoyl-acyl carrier protein reductase* (InhA), *Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-Ilmu Keperawatan, Analis Kesehatan Dan Farmasi*, 13, 1, (2015), 135-141

[5] Mara Imam Taufiq Siregar, Mekanisme Resistensi Isoniazid &Mutasi Gen KatG Ser315Thr (G944C) *Mycobacterium tuberculosis* Sebagai Penyebab Tersering Resistensi Isoniazid, *Jambi Medical Journal: Jurnal Kedokteran dan Kesehatan*, 3, 2, (2015), 119-131

[6] E. T. Y. Leung, P. L. Ho, K. Y. Yuen, W. L. Woo, T. H. Lam, R. Y. Kao, W. H. Seto, W. C. Yam, Molecular Characterization of Isoniazid Resistance in *Mycobacterium tuberculosis*: Identification of a Novel Mutation in *inhA*, *Antimicrobial Agents and Chemotherapy*, 50, 3, (2006), 1075-1078 https://doi.org/10.1128/aac.50.3.1075-1078.2006

[7] Evelyn Koeche Schroeder, Luiz Augusto Basso, Diógenes Santiago Santos, Osmar Norberto de Souza, Molecular Dynamics Simulation Studies of the Wild-Type, I21V, and I16T Mutants of Isoniazid-Resistant *Mycobacterium tuberculosis* Enoyl Reductase (InhA) in Complex with NADH: Toward the Understanding of NADH-InhA Different Affinities, *Biophysical Journal*, 89, 2, (2005), 876-884 https://doi.org/10.1529/biophysj.104.053512

[8] Michelle H. Larsen, Catherine Vilchèze, Laurent Kremer, Gurdyal S. Besra, Linda Parsons, Max Salfinger, Leonid Heifets, Manzour H. Hazbon, David Alland, James C. Sacchettini, William R. Jacobs Jr, Overexpression of *inh*A, but not *kas*A, confers resistance to isoniazid and ethionamide in *Mycobacterium smegmatis*, *M. bovis* BCG and *M. tuberculosis*, *Molecular Microbiology*, 46, 2, (2002), 453-466 https://doi.org/10.1046/j.1365-2958.2002.03162.x

[9] Lailatus Sholichah, Dinar Alfidhdhoh, Ethnobotany of the wild plant as a food crop in Mendiro Village, Wonosalam District, Jombang, *Jurnal Ilmu Pertanian Indonesia*, 25, 1, (2020), 111-117 https://doi.org/10.18343/jipi.25.1.111

[10] Yi-Ming Chiang, Da-Yung Chuang, Sheng-Yang Wang, Yueh-Hsiung Kuo, Pi-Wen Tsai, Lie-Fen Shyur, Metabolite profiling and chemopreventive bioactivity of plant extracts from Bidens pilosa, *Journal of Ethnopharmacology*, 95, 2, (2004), 409-419 https://doi.org/10.1016/j.jep.2004.08.010

[11] Tran Dang Xuan, Tran Dang Khanh, Chemistry and pharmacology of *Bidens pilosa*: an overview, *Journal of Pharmaceutical Investigation*, 46, 2, (2016), 91-132 https://doi.org/10.1007/s40005-016-0231-6

[12] M. G. L. Brandão, A. U. Krettli, L. S. R. Soares, C. G. C. Nery, H. C. Marinuzzi, Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds, *Journal of Ethnopharmacology*, 57, 2, (1997), 131-138 https://doi.org/10.1016/S0378-8741(97)00060-3

[13] Shu-Lin Chang, Yi-Ming Chiang, Cicero Lee-Tian Chang, Hsu-Hua Yeh, Lie-Fen Shyur, Yueh-Hsiung Kuo, Tung-Kung Wu, Wen-Chin Yang, Flavonoids, centaurein and centaureidin, from Bidens pilosa, stimulate IFN- γ expression, *Journal of Ethnopharmacology*, 112, 2, (2007), 232-236 https://doi.org/10.1016/j.jep.2007.03.001

[14] Wen-Chin Lee, Chiung-Chi Peng, Chi-Huang Chang, Shiau-Huei Huang, Charng-Cherng Chyau, Extraction of Antioxidant Components from *Bidens pilosa* Flowers and Their Uptake by Human Intestinal Caco-2 Cells, *Molecules*, 18, 2, (2013), 1582-1601 https://doi.org/10.3390/molecules18021582

[15] Jianguo Wu, Zhongxian Wan, Jun Yi, Yanbin Wu, Wei Peng, Jinzhong Wu, Investigation of the extracts from *Bidens pilosa* Linn. var. *radiata* Sch. Bip. for antioxidant activities and cytotoxicity against human tumor cells, *Journal of Natural Medicines*, 67, 1, (2013), 17-26 https://doi.org/10.1007/s11418-012-0639-x

[16] Pinarosa Avato, Cesare Vitali, Piernatale Mongelli, Aldo Tava, Antimicrobial activity of polyacetylenes from *Bellis perennis* and their synthetic derivatives, *Planta Medica*, 63, 06, (1997), 503-507 https://doi.org/10.1055/s-2006-957751

[17] Rachel L. C. Pereira, Tereza Ibrahim, Leonardo Lucchetti, Antonio Jorge R. da Silva, Vera Lucia Gonçalves de Moraes, Immunosuppressive and anti-

inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L, *Immunopharmacology*, 43, 1, (1999), 31-37 https://doi.org/10.1016/S0162-3109(99)00039-9

[18] Bernhard Hoffmann, Josef Hölzl, Weitere acylierte Chalkone aus *Bidens pilosa*, *Planta Medica*, 54, 05, (1988), 450-451 https://doi.org/10.1055/s-2006-962497

[19] Li-Wha Wu, Yi-Ming Chiang, Hsiao-Ching Chuang, Sheng-Yang Wang, Ga-Wen Yang, Ya-Huey Chen, Ling-Ya Lai, Lie-Fen Shyur, Polyacetylenes Function as Anti-Angiogenic Agents, *Pharmaceutical Research*, 21, 11, (2004), 2112-2119 https://doi.org/10.1023/B:PHAM.0000048204.08865.41

[20] Laura Alvarez, Silvia Marquina, Maria Luisa Villarreal, Daniel Alonso, Eduardo Aranda, Guillermo Delgado, Bioactive polyacetylenes from Bidens pilosa, *Planta Medica*, 62, 04, (1996), 355-357 https://doi.org/10.1055/s-2006-957902

[21] Shu-Lin Chang, Cicero Lee-Tian Chang, Yi-Ming Chiang, Rong-Hong Hsieh, Chii-Ruey Tzeng, Tung-Kung Wu, Huey-Kang Sytwu, Lie-Fen Shyur, Wen-Chin Yang, Polyacetylenic Compounds and Butanol Fraction from *Bidens pilosa* can Modulate the Differentiation of Helper T Cells and Prevent Autoimmune Diabetes in Non-Obese Diabetic Mice, *Planta Medica*, 70, 11, (2004), 1045-1051 https://doi.org/10.1055/s-2004-832645

[22] Rui Wang, Quan-Xiang Wu, Yan-Ping Shi, Polyacetylenes and Flavonoids from the Aerial Parts of *Bidens pilosa*, *Planta Medica*, 76, 09, (2010), 893-896 https://doi.org/10.1055/s-0029-1240814

[23] R. A. Norton, *Bidens alba* (Smooth Beggar-Tick) and *Bidens pilosa* (Hairy Beggar-Tick), in: Y.P.S. Bajaj (Ed.) *Medicinal and Aromatic Plants III*, Springer Berlin Heidelberg, Berlin, Heidelberg, 1991, https://doi.org/10.1007/978-3-642-84071-5_8

[24] Rafael Baptista, Sumana Bhowmick, Jianying Shen, Luis A. J. Mur, Molecular Docking Suggests the Targets of Anti-Mycobacterial Natural Products, *Molecules*, 26, 2, (2021), 475 https://doi.org/10.3390/molecules26020475

[25] Ajay N. Jain, Anthony Nicholls, Recommendations for evaluation of computational methods, *Journal of Computer-Aided Molecular Design*, 22, 3, (2008), 133-139 https://doi.org/10.1007/s10822-008-9196-5

[26] Mohammad Rizki Fadhil Pratama, Hadi Poerwono, Siswandono Siswodihardjo, Introducing a two - dimensional graph of docking score difference vs. similarity of ligand - receptor interactions, *Indonesian Journal of Biotechnology*, 26, 1, (2021), 54-60 https://doi.org/10.22146/ijbiotech.62194

[27] Dwi Hartanti, Shintia Lintang Charisma, Widya Agustina, Rizky Destya Sary, Denia Awanda Putri, Alwani Hamad, The *in-vitro* Antioxidant Properties of Crude Drugs Traditionally Used for Diabetes Management in Northern Banyumas, *Majalah Obat Tradisional*, 27, 3, (2022), 199-209 https://doi.org/10.22146/mot.76958

[28] Harry Noviardi, Fachrurrazie Fachrurrazie, Potensi Senyawa Bullatalisin Sebagai Inhibitor Protein Leukotrien A4 Hidrolase Pada Kanker Kolon Secara *In Silico*, *Fitofarmaka: Jurnal Ilmiah Farmasi*, 5, 2, (2015), 65-73 https://doi.org/10.33751/jf.v5i2.410

[29] Indah Wulan Sari, Junaidin Junaidin, Dina Pratiwi, Studi *Molecular Docking* Senyawa Flavonoid Herba Kumis Kucing (*Orthosiphon Stamineus* B.) Pada Reseptor α -Glukosidase Sebagai Antidiabetes Tipe 2, *Jurnal Farmagazine*, 7, 2, (2020), 54-60

[30] Karisma Enggar Saputri, Nurul Fakhmi, Erwinda Kusumaningtyas, Dedy Priyatama, Broto Santoso, Docking molekular potensi anti diabetes melitus tipe 2 turunan zerumbon sebagai inhibitor aldosa reduktase dengan autodock-vina, *Chimica et Natura Acta*, 4, 1, (2016), 16-20 https://doi.org/10.24198/cna.v4.n1.10443

[31] Usman Sumo Friend Tambunan, Samira Alamudi, Designing cyclic peptide inhibitor of dengue virus NS3-NS2B protease by using molecular docking approach, *Bioinformation*, 5, 6, (2010), 250-254

[32] Doroteja Vnučec, Andreja Kutnar, Andreja Goršek, Soy-based adhesives for wood-bonding – a review, *Journal of Adhesion Science and Technology*, 31, 8, (2017), 910-931 https://doi.org/10.1080/01694243.2016.1237278

[33] Muhammad Idrees, Afzal R. Mohammad, Nazira Karodia, Ayesha Rahman, Multimodal Role of Amino Acids in Microbial Control and Drug Development, *Antibiotics*, 9, 6, (2020), 330 https://doi.org/10.3390/antibiotics9060330

[34] Mohammad Rizki Fadhil Pratama, Evi Mulyani, Suratno Suratno, Molecular docking study of akar kuning (*Arcangelisia flava*) secondary metabolites as Src Inhibitor, *Indonesian Journal of Cancer Chemoprevention*, 10, 3, (2019), 122-130 http://dx.doi.org/10.14499/indonesianjcanchemoprev10iss3pp122-130

[35] Rafaelle Bonzanini Romero, Adriano Lopes Romero, Inibição de Ciclooxigenases 1 (COX-1) e 2 (COX-2) por Monoterpenos: um Estudo in Silico, *Journal of Health Sciences*, 16, 4, (2014), 307-316

[36] Jogoth Ali, Patrick Camilleri, Marc B. Brown, Andrew J. Hutt, Stewart B. Kirton, Revisiting the General Solubility Equation: *In Silico* Prediction of Aqueous Solubility Incorporating the Effect of Topographical Polar Surface Area, *Journal of Chemical Information and Modeling*, 52, 2, (2012), 420-428 https://doi.org/10.1021/ci200387c

[37] Yuan H. Zhao, Michael H. Abraham, Adam Ibrahim, Paul V. Fish, Susan Cole, Mark L. Lewis, Marcel J. de Groot, Derek P. Reynolds, Predicting Penetration Across the Blood-Brain Barrier from Simple Descriptors and Fragmentation Schemes, *Journal of Chemical Information and Modeling*, 47, 1, (2007), 170-175 https://doi.org/10.1021/ci600312d

[38] Congying Xu, Feixiong Cheng, Lei Chen, Zheng Du, Weihua Li, Guixia Liu, Philip W. Lee, Yun Tang, In silico Prediction of Chemical Ames Mutagenicity, *Journal of Chemical Information and Modeling*, 52, 11, (2012), 2840-2847 https://doi.org/10.1021/ci300400a

[39] Denis Mulliner, Friedemann Schmidt, Manuela Stolte, Hans-Peter Spirkl, Andreas Czich, Alexander Amberg, Computational Models for Human and Animal Hepatotoxicity with a Global Application Scope, *Chemical Research in Toxicology*, 29, 5, (2016), 757-767 https://doi.org/10.1021/acs.chemrestox.5b00465

[40] Bruce N. Ames, Joyce McCann, Edith Yamasaki, Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test, *Mutation Research/Environmental Mutagenesis and Related Subjects*, 31, 6, (1975), 347-363 https://doi.org/10.1016/0165-1161(75)90046-1

[41] B. Mathieu, L. Bertrand, V. Peyrusse, F. Schaffner, M. Bertrand, Culicidae and water mites: parasitism under Mediterranean climatic conditions, *Acarologia*, 47, 1-2, (2007), 55-61

[42] Tailong Lei, Youyong Li, Yunlong Song, Dan Li, Huiyong Sun, Tingjun Hou, ADMET evaluation in drug discovery: 15. Accurate prediction of rat oral acute toxicity using relevance vector machine and consensus modeling, *Journal of Cheminformatics*, 8, (2016), 6 https://doi.org/10.1186/s13321-016-0117-7

[43] Luciana L. Mensor, Fábio S. Menezes, Gilda G. Leitão, Alexandre S. Reis, Tereza C. dos Santos, Cintia S. Coube, Suzana G. Leitão, Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, *Phytotherapy Research*, 15, 2, (2001), 127-130 https://doi.org/10.1002/ptr.687

[44] Bent H. Havsteen, The biochemistry and medical significance of the flavonoids, *Pharmacology & Therapeutics*, 96, 2, (2002), 67-202 https://doi.org/10.1016/S0163-7258(02)00298-X

[45] Jean Fotie, The antiprotozoan potential of flavonoids, *Pharmacognosy Reviews*, 2, 3, (2008), 6-19

[46] Ali Aberoumand, S. S. Deokule, Comparison of phenolic compounds of some edible plants of Iran and India, *Pakistan Journal of Nutrition*, 7, 4, (2008), 582-585 https://doi.org/10.3923/pjn.2008.582.585