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Computational Insights into the Interaction of Silver Nanoparticles from *Mimosa pudica* linnaeus with Tumor Necrosis Factor-*α* and Heat Shock Protein 60: An *In-Silico* Approach

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ABSTRACT

Mimosa pudica linnaeus has been recognised for its diverse range of bioactive compounds, with antibacterial, anti-inflammatory, hepatoprotective, and antiparasitic effects. This study aims to investigate the inhibitory potential of bioactive compounds from *Mimosa pudica linnaeus* against *Blastocystis sp*., TNF-*α,* and HSP60 using an *in-silico* method. The analysis involved predicting biological activity using PASS, evaluating drug-likeness according to Lipinski's Rule of Five, and assessing the ADME properties and toxicity profiles of *Mimosa pudica linnaeus* phytochemicals. The PASS analysis showed that Apigenin, p-Hydroxybenzoic acid, Quercetin, and Naringenin had the highest probability of activity across various categories. Among the 18 compounds analysed, 13 met all Lipinski's criteria, while 17 showed high gastrointestinal absorption. However, 9 compounds exhibited high toxicity effects. Molecular docking analysis revealed that Jasmonic acid, Naringenin, and Monoamidomalonic acid exhibited lower binding affinities than metronidazole (a standard drug) against *Blastocystis sp*. Luteolin and Naringenin also showed potential as competitive inhibitors of TNF-*α.* However, 4-(2-phenylethyl) phenol and Fisetin showed the lowest binding affinity scores of -7.47 and -7.75 kcal/mol, respectively, against HSP60. Molecular docking results showed that the ligands interacted with the target proteins via hydrophobic and hydrogen-bond interactions, suggesting the formation of stable protein-ligand complexes. These results show that bioactive compounds of *Mimosa pudica linnaeus* have potential as therapeutic candidates with promising anti-inflammatory properties.

Keywords: Molecular docking, Tumor Necrosis Factor-*α*, Heat Shock Protein 60, *Blastocystis Sp*

Introduction

Blastocystis sp. is a frequently occurring intestinal protozoan detected in human and animal faeces, particularly in tropical and subtropical regions[1]. This protozoan can disrupt gut microbiota balance and trigger inflammation, leading to both acute and chronic diarrhea[2]. Several studies have shown that diarrhea remains a high global health issue, with approximately two billion cases annually and a high mortality rate, particularly in children[3]. Among Indonesian children under 5, the disease is a leading cause of morbidity and mortality, with the highest prevalence observed in those aged 6–11 months[4,32,33]. *Blastocystis sp*. infection elicits an immune response by activating Toll-like Receptors (TLRs), leading to the production of pro-inflammatory cytokines, including TNF-*α*, IL-6, and IL-1*β*[5,6].

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This response exacerbates intestinal inflammation, worsening diarrheal symptoms[7], and further disrupting gut microbiota homeostasis[8]. In addition, excessive inflammation driven by TNF-*α* and Heat Shock Protein 60 (HSP60) can impair intestinal barrier function, aggravating the patient's condition[9,10]. According to previous studies, *Mimosa pudica linnaeus* has been identified as a potential antidiarrheal plant due to its pharmacological properties, including antimicrobial, anti-inflammatory, and immunomodulatory activities. The flavonoids in this plant have been shown to inhibit TNF-*α* production, reduce inflammation, and disrupt cell membrane integrity [11, 12]. In addition, tannins and alkaloids in *Mimosa pudica linnaeus* play a crucial role in inhibiting pathogen development and enhancing the immune response against infection[13, 14, 31.]

In recent years, silver nanoparticle (AgNP) technology has been developed as an innovative approach for treating *Blastocystis sp*. infection. These nanoparticles penetrate the parasite's cell membrane, induce metabolic dysfunction, and enhance macrophage activation to eliminate the pathogen[15,16]. The combination of *Mimosa pudica* extract and AgNPs exerts a synergistic effect by inhibiting microbial growth and suppressing key inflammatory pathways that require TNF-α and HSP6017, [18].

Several *in silico* analyses have been conducted to evaluate the molecular interactions between the bioactive compounds of *Mimosa pudica linnaeus* and target proteins in the inflammatory mechanisms triggered by *Blastocystis sp*. infection. Molecular docking methods were employed to assess the binding potential of flavonoids to TNF-*α* and HSP60, both of which play crucial roles in regulating the immune

response. Simulation results showed that Luteolin and Fisetin exhibited low binding energies, suggesting strong interactions with the active sites of the target proteins [19, 20]. Molecular dynamics analysis also showed that compound-protein complexes maintained high stability under physiological conditions, further supporting their effectiveness in modulating inflammatory pathways[21]. Therefore, the combination of *Mimosa pudica linnaeus* and nanotechnology presents a promising alternative treatment that is safer and more effective compared to conventional therapies, which often face challenges related to drug resistance. This study aims to employ a computational approach to investigate the interaction potentials of silver nanoparticles from *Mimosa pudica linnaeus* with Tumour Necrosis Factor-*α* and Heat Shock Protein 60.

Materials and Methods

Bioactivity Score Prediction
A total of 19 compounds in *Mimosa pudica linnaeus* extract, with reported antibacterial and anti-inflammatory, hepatoprotective, and antiparasitic properties, were used as ligands for this study. Their three-dimensional structures were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Subsequently, these compounds were analysed using the Prediction of Activity Spectra for Substances (PASS) software in MOL format to assess their potential antibacterial, anti-inflammatory, hepatoprotective, and antiparasitic activities[33,34]

Evaluation of Drug Likeness Potentials Based on Lipinski's Rule of Five
The pharmacokinetic-relevant molecular characteristics of the ligands were analysed using Lipinski's Rule of 5 through an online platform in SDF format. A compound was considered to meet the drug-likeness criteria when it fulfilled at least 3 out of the 5 parameters defined in the rule. These parameters included a molecular weight under 500 Daltons, a Log P value below 5 (showing favorable lipophilicity), a maximum of 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a molar refraction[33,34]

ADME and Toxicity Prediction
ADME (absorption, distribution, metabolism, and excretion) prediction describes the disposition and fate of bioactive compounds within an organism, particularly in the human body. The ADME profile was predicted using the LLMD Admetsar webserver, with SMILES structures sourced from PubChem (https://pubchem.ncbi.nlm.nih.gov) as input. Furthermore, the toxicity of bioactive compounds in *Mimosa pudica linnaeus* extracts was predicted using the Pro-Tox II webserver, which classifies toxicity levels based on parameters such as cytotoxicity, carcinogenicity, and mutagenicity [33,34].

Ligand Preparation
Ligands identified through PASS prediction and Lipinski's Rule of 5 criteria were prepared using the Avogadro software. These compounds, obtained from the PubChem database, were formatted and saved as SDF files. Metronidazole was used as the reference anti-inflammatory ligand for this study.

Receptor Preparation
The receptor structures used were obtained from the Protein Data Bank (https://www.rcsb.org/) in PDB format: the covalent-specific ERK2 inhibitor (PDB ID: 8AOJ) and the KCL802 fragment in complex with MAP kinase p38-alpha (PDB ID: 6SP9), both of which are proteins implicated in inflammatory mechanisms. Subsequently, ChimeraX (https://www.rbvi.ucsf.edu/chimerax/) was used to eliminate water molecules, native ligands, and non-standard residues from the receptor structures[33,34].

Molecular Docking Simulation
In this study, the docking simulations were executed using the AutoDock Tools software suite (https://autodock.scripps.edu/). The receptor was loaded into the AutoDock Tools workspace and converted into a macromolecule. After the conversion, the ligand tab was used to select and convert the ligand into AutoDock Ligands. The grid coordinates were set using AutoGrid 4, and AutoDock 4 was chosen as the docking algorithm. The molecular docking process was completed by following the docking steps, where results with an RMSD value below 2.00 Å were selected. Furthermore, docking complexes obtained were explored for their 2D and 3D interaction profiles using Biovia Discovery Studio (https://www.3ds.com/products/biovia/discovery-studio), 2021. The binding residue interactions formed during the molecular docking analysis were also examined[33,34].

Data Analysis
Molecular docking results data were analysed by comparing the binding scores of the test ligands with those of the standard ligand and the target proteins. Compounds from *Mimosa pudica linnaeus* extract that exhibited a lower binding score than the standard ligand were considered a potential anti-inflammatory agent against *Blastocystis sp*. infection by acting as a competitive inhibitor of the standard ligand. The molecular docking results were visualised and analysed according to the types of interactions formed between the compounds and the target proteins. All molecular docking results were presented in tabular format.

Results and Discussion

The Prediction of Activity Spectra for Substances (PASS) is a powerful web-based computational method that researchers have used to predict the biological activity of chemical compounds with potential for drug development based on their 2D structure. In this study, using PASS, compounds in *Mimosa pudica linnaeus* extract that exhibited antibacterial activity were ranked by the largest predicted probability of activity (Pa), with Apigenin having the highest Pa value (0.507). These 19 compounds showed potential antibacterial, anti-inflammatory, hepatoprotective, and antiparasitic properties as predicted by the PASS software. Their molecular and phytochemical properties were further analysed using Lipinski's Rule of 5 to determine bioactive compounds suitable for *in silico* evaluation. As shown in Table 1, PASS predictions indicate that 18 of the analysed compounds have the potential to suppress inflammatory responses. The PASS values were interpreted as follows: (i) A Pa value greater than 0.7 suggested a strong likelihood of biological activity and potential similarity to known drugs. (ii) A Pa value between 0.5 and 0.7 showed moderate activity with a possibility of differing structurally from current pharmaceuticals. (iii) A Pa value below 0.5 reflected a low probability of exhibiting biological activity. This study demonstrated that the bioactive compounds in *Mimosa pudica linnaeus* extract possess antibacterial, anti-inflammatory, hepatoprotective, and antiparasitic properties, indicating potential as therapeutic agents. Based on bioactivity analysis using PASS software, the identified compounds exhibited a diverse range of activities, with Apigenin, p-Hydroxybenzoic acid, Quercetin, and Naringenin emerging as the primary candidates with high activity potential. According to Pa (Probability of Activity) interpretation, compounds with Pa > 0.7 were highly likely to exhibit significant experimental activity, while compounds with Pa < 0.5 had a lower probability of biologically relevant activity. These results were consistent with previous studies, which reported that flavonoids such as Apigenin and quercetin possess significant anti-inflammatory and antioxidant properties[19].
Similarly, the Lipinski prediction test for bioactive compounds was conducted using the SCFBio website. The results showed that 13 compounds met all 5 Lipinski criteria, 4 met 4 criteria, and 1 met 3, totalling 18 that fulfilled Lipinski's Rule of 5. However, a compound, Monoamidomalonic acid, produced an error when docking was performed without silver. The predicted Lipinski test results for bioactive compounds in *Mimosa pudica linnaeus* extract show that most compounds in the extract met the criteria for good drug-likeness, with 13 compounds fulfilling all five rules, as presented in Table 2. Only a few compounds met 4 or 3 criteria, suggesting that most had favourable potential as drug candidates with appropriate pharmacokinetic

properties. Previous studies have also reported that flavonoids in *Mimosa pudica linnaeus* meet Lipinski's criteria, making them promising candidates for drug development with potential for human bioavailability [20]. Further ADME analysis showed that most compounds exhibited high gastrointestinal absorption, while some did not cross the blood-brain barrier (BBB), potentially limiting their distribution to the brain. These results were consistent with a previous study by Menezes *et al*., which reported that compounds in *Mimosa pudica linnaeus* extract possess a favourable ADME profile for pharmaceutical applications.[14]

Table 1: Possible bioactivity of bioactive compounds in *Mimosa pudica linnaeus* extract based on PASS test

No	Compound	Bioactivity	Probable activity	Degree of activity
1	2-Tert-butyl-2-phenyl1,3-	Antibacterial	0.189	Very Low
	Dioxolane	Antiinflamation	0.316	Very Low
		Hepatoprotectant	0.207	Very Low
		Antiparasitic	0.190	Very Low
2	4-(2-Phenylethyl)- phenol	Antibacterial	0.000	Very Low
		Antiinflamation	0.285	Very Low
		Hepatoprotectant	0.219	Very Low
		Antiparasitic	0.295	Very Low
3	4-Phenylbutan-2-ol	Antibacterial	0.206	Very Low
		Antiinflamation	0.474	Very Low
		Hepatoprotectant	0.302	Very Low
		Antiparasitic	0.174	Very Low
4	1-Naphthalenecarboxylic acid	Antibacterial	0.327	Very Low
		Antiinflamation	0.581	Low
		Hepatoprotectant	0.361	Very Low
		Antiparasitic	0.284	Very Low
5	Ferulic acid	Antibacterial	0.496	Very Low
		Antiinflamation	0.604	Low
		Hepatoprotectant	0.621	Low
		Antiparasitic	0.377	Very Low
6	Myoinositol	Antibacterial	0.422	Very Low
		Antiinflamation	0.590	Low
		Hepatoprotectant	0.000	Very Low
		Antiparasitic	0.392	Very Low
7	Caffeic acid	Antibacterial	0.486	Very Low
		Antiinflamation	0.651	Low
		Hepatoprotectant	0.461	Very Low
		Antiparasitic	0.432	Very Low
8	Tyrosinamide	Antibacterial	0.266	Very Low
		Antiinflamation	0.354	Very Low
		Hepatoprotectant	0.283	Very Low
		Antiparasitic	0.000	Very Low
9	2-Hydroxybenzeneethanol	Antibacterial	0.280	Very Low
		Antiinflamation	0.477	Very Low
		Hepatoprotectant	0.338	Very Low
		Antiparasitic	0.416	Very Low
10	p-Hydroxybenzoic acid	Antibacterial	0.405	Very Low
		Antiinflamation	0.743	Hight
		Hepatoprotectant	0.417	Very Low
		Antiparasitic	0.369	Very Low
11	Luteolin	Antibacterial	0.501	Low

		Antiinflamation	0.661	Low
		Hepatoprotectant	0.658	Low
		Antiparasitic	0.401	Very Low
12	Fisetin	Antibacterial	0.373	Very Low
		Antiinflamation	0.642	Low
		Hepatoprotectant	0.637	Low
		Antiparasitic	0.334	Very Low
13	Apigenin	Antibacterial	0.507	Low
		Antiinflamation	0.644	Low
		Hepatoprotectant	0.627	Low
		Antiparasitic	0.441	Very Low
14	Gallic acid	Antibacterial	0.432	Very Low
		Antiinflamation	0.640	Low
		Hepatoprotectant	0.504	Low
		Antiparasitic	0.309	Very Low
15	Quercetin	Antibacterial	0.421	Very Low
		Antiinflamation	0.689	Low
		Hepatoprotectant	0.706	Hight
		Antiparasitic	0.381	Very Low
16	Jasmonic acid	Antibacterial	0.313	Very Low
		Antiinflamation	0.639	Low
		Hepatoprotectant	0.593	Low
		Antiparasitic	0.293	Very Low
17	3-Fluoro-p-anisidine	Antibacterial	0.292	Very Low
		Antiinflamation	0.482	Very Low
		Hepatoprotectant	0.171	Very Low
		Antiparasitic	0.000	Very Low
18	Naringenin	Antibacterial	0.413	Very Low
		Antiinflamation	0.660	Low
		Hepatoprotectant	0.721	Hight
		Antiparasitic	0.443	Very Low

Table 2: The result of the evaluation of drug likeliness based on Lipinski's Rule of Five

		Lipinski Rules of Five					
No	**Compounds**	**Molecular weight (<500)**	**LogP (<5)**	**Hydrogen Bond donor (<5)**	**Hydrogen Bond acceptor (<10)**	**Molar Refractivity (>40)**	**Lipinski Status**
1	1-Naphthalenecarboxylic acid	172	2.53	1	2	50.9	Yes, zero violation
2	2-Hydroxybenzeneethanol	138	0.92	2	2	38.89	No, 1 violation
3	2-Tert-butyl-2-phenyl1,3-Dioxolane	206	2.93	0	2	59.47	Yes, 0 violations
4	3-Fluoro-p-anisidine	141	1.41	2	2	37.36	No, 1 violation
5	4-(2-Phenylethyl)- phenol	198	3.17	1	1	61.95	Yes, 0 violations
6	4-Phenylbutan-2-ol	150	2	1	1	46.44	Yes, 0 violations
7	Apigenin	270	2.41	3	5	70.81	Yes, 0 violations
8	Caffeic acid	180	1.19	3	4	46.44	Yes, 0 violations
9	Ferulic acid	194	1.49	2	4	51.32	Yes, 0 violations

10	Fisetin	286	2.3	4	6	72.38	Yes, 0 violations
11	Gallic acid	170	0.5	4	5	38.39	No, 1 violation
12	Jasmonic acid	210	2.41	1	3	57.52	Yes, 0 violations
13	Luteolin	286	2.12	4	6	72.47	Yes, 0 violations
14	Myoinositol	180	-3.83	6	6	36.04	No, 2 violations
15	Naringenin	272	2.5	3	5	70.19	Yes, 0 violations
16	p-Hydroxybenzoic acid	138	1.09	2	3	35.06	No, 1 violation
17	Quercetin	302	2.01	5	7	74.05	No, 1 violation
18	Tyrosinamide	180	-0.25	5	4	48.98	No, 1 violation

The ADME prediction test was conducted using the LMMD Admetsar web server. According to the study's results, 17 compounds demonstrated high gastrointestinal (GI) absorption capacity, facilitating efficient absorption in the digestive tract, and the ADME prediction further showed that none of the compounds crossed the BBB. However, 2 compounds were classified as P-gp substrates, potentially influencing drug distribution and bioavailability. Drug metabolism primarily occurs in the liver, where cytochrome P450 enzymes play a crucial role. Some tested compounds were identified as inhibitors of the cytochrome P450 enzyme. Hence, the pharmacokinetic parameters revealed that all active compounds in *Mimosa pudica linnaeus* exhibited favourable drug-binding properties. The toxicity prediction results showed that 9 compounds exhibited high toxicity. However, five compounds, namely 3-Fluoro-p-anisidine, Fisetin, Luteolin, Naringenin, and Quercetin, showed cytotoxicity, carcinogenicity, mutagenicity, and clinical and nutritional toxicity. The predicted ADME and toxicity test results for bioactive compounds in *Mimosa pudica linnaeus* extract are presented in Tables 3 and 4.

Table 3: The result of the toxicity prediction

No	**Compounds**	**Toxicity Prediction**					
		Toxicity Class	**Cito toxicity**	**Carcino Genic**	**Mutagenic**	**Clinical Toxicity**	**Nutritional Toxicity**
1	1-Naphthalenecarboxylic acid	4	Inactive (0.87)	Inactive (0.63)	Inactive (0.61)	Inactive (0.69)	Inactive (0.89)
2	2-Hydroxybenzeneethanol	4	Inactive (0.89)	Inactive (0.74)	Inactive (0.82)	Inactive (0.54)	Inactive (0.68)
3	2-Tert-butyl-2-phenyl1,3-Dioxolane	5	Inactive (0.82)	Active (0.50)	Inactive (0.65)	Inactive (0.73)	Inactive (0.74)
4	3-Fluoro-p-anisidine	4	Active (0.54)	Active (0.76)	Active (0.84)	Active (0.50)	Inactive (0.86)
5	4-(2-Phenylethyl)-phenol	4	Inactive (0.92)	Inactive (0.83)	Inactive (0.85)	Inactive (0.73)	Inactive (0.88)
6	4-Phenylbutan-2-ol	5	Inactive (0.88)	Inactive (0.78)	Inactive (0.91)	Inactive (0.65)	Inactive (0.84)
7	Apigenin	5	Inactive (0.87)	Inactive (0.62)	Inactive (0.57)	Inactive (0.54)	Inactive (0.55)
8	Caffeic acid	5	Inactive (0.86)	Active (0.78)	Inactive (0.98)	Active (0.50)	Inactive (0.77)
9	Ferulic acid	4	Inactive (0.88)	Inactive (0.61)	Inactive (0.96)	Active (0.52)	Inactive (0.82)
10	Fisetin	3	Inactive (0.98)	Active (0.71)	Active (0.71)	Inactive (0.54)	Active (0.67)
11	Gallic acid	4	Inactive (0.91)	Active (0.56)	Inactive (0.94)	Active (0.55)	Inactive (0.83)
12	Jasmonic acid	2	Inactive (0.66)	Inactive (0.74)	Inactive (0.88)	Active (0.53)	Inactive (0.78)

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13	Luteolin	5	Inactive (0.99)	Active (0.68)	Active (0.51)	Inactive (0.53)	Active (0.63)
14	Myoinositol	6	Inactive (0.84)	Inactive (0.55)	Inactive (0.60)	Inactive (0.53)	Inactive (0.83)
15	Naringenin	4	Active (0.59)	Inactive (0.62)	Inactive (0.83)	Active (0.50)	Active (0.52)
16	p-Hydroxybenzoic acid	5	Inactive (0.51)	Inactive (0.51)	Inactive (0.99)	Inactive (0.60)	Inactive (0.95)
17	Quercetin	3	Inactive (0.99)	Active (0.68)	Active (0.51)	Inactive (0.53)	Active (0.63)
18	Tyrosinamide	4	Inactive (0.70)	Inactive (0.70)	Inactive (0.75)	Active (0.55)	Inactive (0.63)

Table 4: The result of the ADME prediction activity

No	Compounds	GI *Absorption*	BBB *Permeant*	Pgp Substrate	CYP 1A2	CYP 2C19	CYP 2C9	CYP 2D6	CYP 3A4	Bioavaibility Score
1	1-Naphthalenecarboxylic acid	High	Yes	No	No	No	No	No	No	0.85
2	2-Hydroxybenzeneethanol	High	Yes	No	No	No	No	No	No	0.55
3	2-Tert-butyl-2-phenyl1,3-Dioxolane	High	Yes	No	Yes	No	No	Yes	No	0.55
4	3-Fluoro-p-anisidine	High	Yes	No	No	No	No	No	No	0.55
5	4-(2-Phenylethyl)- phenol	High	Yes	No	Yes	Yes	No	Yes	No	0.55
6	4-Phenylbutan-2-ol	High	Yes	No	No	No	No	No	No	0.55
7	Apigenin	High	No	No	Yes	No	No	Yes	Yes	0.55
8	Caffeic acid	High	No	No	No	No	No	No	No	0.56
9	Ferulic acid	High	Yes	No	No	No	No	No	No	0.85
10	Fisetin	High	No	No	Yes	No	No	Yes	Yes	0.55
11	Gallic acid	High	No	No	No	No	No	No	Yes	0.56
12	Jasmonic acid	High	Yes	No	No	No	No	No	No	0.85
13	Luteolin	High	No	No	Yes	No	No	Yes	Yes	0.55
14	Myoinositol	Low	No	Yes	No	No	No	No	No	0.55
15	Naringenin	High	No	Yes	Yes	No	No	No	Yes	0.55
16	p-Hydroxybenzoic acid	High	Yes	No	No	No	No	No	No	0.85
17	Quercetin	High	No	No	Yes	No	No	Yes	Yes	0.55
18	Tyrosinamide	High	No	No	No	No	No	No	No	0.56

The docking study of the bioactive constituents of *Mimosa pudica linnaeus* against *Blastocystis* suggested that Jasmonic acid, Naringenin, and Monoamidomalonic acid exhibited potential as competitive inhibitor candidates against the standard ligand, metronidazole. This was because these 3 compounds exhibited lower binding affinities than the standard ligand (-5.11 kcal/mol). Monoamidomalonic acid could bind to *Blastocystis* with a binding affinity of -7.35 kcal/mol, Naringenin with -7.07 kcal/mol, and Jasmonic acid with -7.10 kcal/mol. The molecular docking analysis of bioactive compounds from *Mimosa pudica linnaeus* with TNF-α suggested that Luteolin and Naringenin were competitive inhibitor candidates against the standard ligand metronidazole, with binding affinity values lower than that of metronidazole (-3.83 kcal/mol). Luteolin binds to TNF-α with a binding affinity of -7.16 kcal/mol, while Naringenin has a binding affinity of -7.02 kcal/mol. The results of the docking of *Mimosa pudica linnaeus* compounds with HSP60 showed that 4-(2-Phenylethyl)-phenol and Fisetin had the potential to act as competitive inhibitors against the standard ligand metronidazole, with lower binding affinity values compared to the control ligand Metronidazole (-5.22 kcal/mol). 4-(2-Phenylethyl)-phenol bound to HSP60 with a binding affinity of -7.47 kcal/mol, while Fisetin showed an even lower binding affinity of -7.75 kcal/mol. From the docking results (Tables 5 and 6), a lower RMSD value indicates a smaller deviation of the test ligand from its original conformation, suggesting greater interaction stability.

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Table 5: The result of molecular docking

No	Compounds	Molecular Docking Result								
		Blastocystis			TNF-α			HSP 60		
		Binding Affinity	Inhibition Constant (uM)	RMSD	Binding Affinity	Inhibition Constant (uM)	RMSD	Binding Affinity	Inhibition Constant (uM)	RMSD
1	1-Naphthalenecarboxylic acid	-6.94	8.22	0.8	-5.23	146.79	0.16	-6.4	20.27	1.22
2	2-Hydroxybenzeneethanol	-4.66	383.18	0.94	-4.16	898.66	0.61	-4.69	365.97	1.5
3	2-Tert-butyl-2-phenyl1,3-Dioxolane	-5.49	94.18	0.14	-6.16	30.27	0.2	-6.83	9.78	0.25
4	3-Fluoro-p-anisidine	-4.58	442.91	1.55	-4.2	839.49	0.89	-5.44	102.46	0.14
5	4-(2-Phenylethyl)-phenol	-5.73	63.29	0.31	-6.41	19.99	1.01	-7.47	3.34	0.57
6	4-Phenylbutan-2-ol	-5.49	94.59	1.19	-6.15	30.92	1.36	-6.05	36.89	0.62
7	Apigenin	-6.79	10.60	0.23	-6.95	7.99	0.16	-5.52	89.88	0.36
8	Caffeic acid	-5.35	119.45	0.67	-5.95	43.41	0.38	-4.85	277.09	1.17
9	Ferulic acid	-5.71	64.84	0.37	-5.94	44.58	0.56	-5.05	198.61	1.05
10	Fisetin	-5.83	53.40	1.95	-6.88	3.12	0.77	-7.75	2.07	2.31
11	Gallic acid	-4.19	841.57	1.85	-3.86	1.49	0.39	-4.7	355.93	1.99
12	Jasmonic acid	-7.1	6.29	1.94	-6.27	25.56	1	-4.79	309.59	1.41
13	Luteolin	-6.09	34.17	1.3	-7.16	5.64	1.65	-6.89	8.87	0.19
14	Monoamidomalonic acid	-7.35	4.11	1.56	-4.26	750.06	1.72	-5.25	142.38	0.32
15	Myoinositol	-3.95	1.27	0.72	-7.02	7,19	1.9	-6.75	11.31	0.21
16	Naringenin	-7.07	6.52	0.28	-5.3	129.25	0.27	-4.92	245.89	1.93
17	p-Hydroxybenzoic acid	-5.14	172.15	0.29	-5.61	76.91	1.63	-6.71	12	1.53
18	Quercetin	-6.27	25.16	0.9	-5.7	66.44	1.59	-6.2	28.46	1.15
19	Tyrosinamide	-6.28	25.13	1.27	-5.23	146.79	0.16	-6.4	20.27	1.22

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Table 6: The result of the interaction between ligand and receptor

No	Compounds	Amino Acid Residue					
		Blastocystis		TNF-α		HSP 60	
		Hydrophobic Bond Interaction	Hydrogen Bond Interaction	Hydrophobic Bond Interaction	Hydrogen Bond Interaction	Hydrophobic Bond Interaction	Hydrogen Bond Interaction
1	1-Naphthalenecarboxylic acid	Lys230	Val53, His4, Ile3		Leu26, Ile136	Ala369, Val172, Lys370	Lys192, Lys370, Gly190, Met191, Ala369
2	2-Hydroxybenzeneethanol	Ile3, Leu54, Lys230	Glu5, Arg58, Val53, His4, Gln52	Tyr59	Ser60, Leu120	Lys370, Val172, Ala369, Lys370	Lys192
3	2-Tert-butyl-2-phenyl1,3-Dioxolane	Lys230	Arg58, Val53	Lys98, Pro117	Tyr115, Ser99	Val172, Lys370, Ala369	Lys370, Lys192
4	3-Fluoro-p-anisidine	Lys230, Leu54	Ala55	Tyr59, Tyr119	Tyr115, Leu120	Ile247, Leu260, Val271, Ile225, Ala249, Ala252, Ala273	
5	4-(2-Phenylethyl)-phenol	Lys50, Cys110, Val48, Val113, Leu227	Lys550, Cys110, Val113, Gln20	Val17, Pro20, Leu29, Arg32, Val17	Arg32, Glu146, Ala18	Leu219, Leu237, Phe299, Leu219, Val311, Leu316	Gln312, Ala234, Val311, Leu308
6	4-Phenylbutan-2-ol	Gly111, Val113, Pro218, Leu38, Cys45	Lys114, Glu216	Phe124, Leu93	Gln125, Leu93	Ile247, Ala273, Ile225, Ala249, Val252, Ala273, Val252, Ala256, Leu260	Ala249, Glu250
7	Apigenin	Lys230	Asn2	Val17, Ala18, Pro20	Ala33, Gly148	Ala273, Val252, Ile231, Ile247, Leu260, Val271, Ala273	Ala249, Val274
8	Caffeic acid	Val158, Phe156, Lys173	Lys157, Lys173, Gln170, Leu155, Glu153, Lys173	Arg82, Leu93, Val91	Gln125, Asn92	Ala369, Lys370	Glu366
9	Ferulic acid	Lys230	Gly57, Arg58, Asp228, Ile3	Val17	Ala18, Val150, Ala33	Val172, Ala369, Lys370	Gly190, Met191
10	Fisetin	His4	Glu5, Gly57, Arg58, Asp228, Ile3, Ala51	Ala33, Val91, Val17, Arg32	Ala18, Gln149, Val150, Ala33, Arg32	Ala293, Met191, Leu331	Lys370, Leu331
11	Gallic acid	Val48, Val71, leu227, Leu73	Lys550, Val113, Val71, leu227	Leu93	Gln125, Leu93	Gul366, Lys192, Lys370, Met191, Lys192, Lys 370, Ala369	
12	Jasmonic acid	Val48, Leu227	Lys50, Val113, Gly111, Gln20, leu227		Ser95, Gln125		Gly30. Gly51, Thr87, Thr89, Asp85, Thr28

13	Luteolin	Leu54, Lys230	Glu5, Arg58, Val53, His4	Leu93	Gln125, Leu93		Asp194, Lys370, Pro277
14	Monoamidomalonic acid	Pro218, Cys45	Ala112, Lys114, Cys45, Glu216, Asp220, Pro218	Leu93, Val91	Gln125, Leu93, Asn92	Met29, Lys49	Gly30, Gly51, Thr88, Pro31, Lys94
15	Myoinositol		Gly57, Arg58		Lys98, Ser99, Tyr115, Glu116, Pro117		Lys63, Glu522, Thr521, Glu522, Ther521 Val520
16	Naringenin		Gln170, Asp177, Lys157	Leu93	Gln125, Asn92	Met29, Lys49	Gly30, Gly51, Thr88, Pro31, Lys49, Thr28
17	p-Hydroxybenzoic acid	Val158, Phe156, Lys173, Leu174	Lys157, Lys173, Gln170, Leu155, Glu153	Val17	His15, Leu93, Ala33	Ile247, Ile225, Ile231, Val252, Ala 273	Val274, Glu250
18	Quercetin	Leu54	Glu5, Arg58, Val53, Ile3, Gln52	Leu55	Gln125, Gly121	Val172, Lys192, Lys370	Asp194, Glu366, Lys170, Gly190, Gly171, Gly374, Lys370
19	Tyrosinamide	Pro218, Lys114	Lys114, Pro43, Glu216, Asp220	Leu93, Val91	Gln125, Asn92, Leu93	Gly334, Met191, Ala293	Gly190, Lys370, Leu371, Asp373, Gly374

The molecular docking visualisation showed that the standard ligand metronidazole formed hydrogen bonds with Leu26 and Ile136, which could enhance its stability against the target protein. 1-Naphthalenecarboxylic acid had hydrophobic interactions with Tyr59 and formed hydrogen bonds with Ser60 and Leu120, potentially increasing its affinity for the target. 2-Hydroxybenzeneethanol exhibited hydrophobic interactions with Lys98 and Pro117, as well as hydrogen bonds with Tyr115 and Ser99, suggesting its potential to stabilise the ligand-protein complex. 4-Phenylbutan-2-ol exhibited hydrophobic interactions with Val17, Ala18, and Pro20, alongside hydrogen bonds interactions with Ala33 and Gly148, showing good stability toward the target protein. Flavonoids such as Apigenin showed hydrophobic interactions with Arg82, Leu93, and Val91, as well as hydrogen bonding with Gln125 and Asn92, reinforcing their strong binding affinity with the target protein. Caffeic acid interacted with Val17 by hydrophobic bonds and formed hydrogen bond interactions with Ala18, Val150, and Ala33. Ferulic acid exhibited hydrophobic interactions with Ala33, Val91, and Val17, as well as hydrogen bond interactions with Ala18, Gln149, Val150, and Arg32, thereby demonstrating greater stability with the target protein. Gallic acid formed only hydrogen bonds with Ser95 and Gln125, while Luteolin exhibited hydrophobic interactions with Leu93 and Val91, alongside hydrogen bonding with Gln125, Leu93, and Asn92, showing stronger binding compared to other flavonoids. Myoinositol formed hydrogen bonds with Lys98, Ser99, Tyr115, Glu116, and Pro117, with high binding affinity. p-Hydroxybenzoic acid interacted hydrophobically with Val17 and formed hydrogen bonds with His15, Leu93, and Ala33, suggesting moderate binding potential. Quercetin exhibited hydrophobic interactions with Leu55 and formed hydrogen bonds with Gln125 and Gly121, resembling the interaction pattern of other flavonoids. Tyrosinamide formed hydrophobic interactions with Leu93 and Val91, in addition to hydrogen bonding to Gln125, Asn92, and Leu93, demonstrating strong stability as an inhibitor candidate. Based on hydrophobic and hydrogen-bond interactions, the compounds with the highest potential as inhibitor candidates included Apigenin, Luteolin, Quercetin, Ferulic acid, and 2-Tert-butyl-2-phenyl-1,3-Dioxolane. These compounds exhibited extensive interactions with the target residues, including both hydrophobic contacts and hydrogen bonds, thereby enhancing their potential to stabilise ligand-protein binding. Furthermore, Myoinositol showed strong potential despite relying solely on hydrogen bonds, owing to its interactions with key residues involved in the protein's biological activity. 3-Fluoro-p-anisidine also stood out for its numerous hydrophobic and hydrogen interactions, which increased affinity and binding stability. Overall, flavonoid compounds such as Apigenin, Luteolin, and Quercetin, as well as phenolic compounds like ferulic acid and Caffeic acid, showed the highest potential as competitive inhibitors due to their complex and strong interaction patterns with the target protein residues. Previous studies also showed that these compounds exhibit high affinity for various biological targets. For instance, Naringenin has been shown to possess significant antiparasitic activity against *Plasmodium falciparum*[22]. Furthermore, flavonoid compounds such as Luteolin, Naringenin, and Quercetin have shown strong affinity for TNF-*α*, making them potential candidates for inflammatory therapy. The hydrophobic interactions and hydrogen bonds formed between these compounds and their target proteins contribute to the stability of the complexes, underscoring their potential as effective inhibitors of various inflammatory and parasitic pathways.[23]

The molecular docking results for other compounds, such as Luteolin and Quercetin, showed high affinity for TNF-*α*, a cytokine in inflammatory processes. These compounds formed hydrogen bonds with key residues at the TNF-α active site, potentially inhibiting its interaction with its receptor and thereby reducing pro-inflammatory effects in the body.[24, 25] Luteolin, which also showed hepatoprotective effects, had the potential to mitigate liver damage caused by chronic inflammation.[26,27] This finding was consistent with previous studies, which report that flavonoids, including Luteolin and Quercetin, have the potential to act as TNF-α inhibitors in inflammatory therapy.[28,29] Metronidazole interacted with Ala369, Val172, and Lys370 via hydrophobic bonds while forming hydrogen bonds with Lys192, Lys370, Gly190, Met191, and Ala369, with strong stability. In this study, 1-naphthalenecarboxylic acid exhibited hydrophobic interactions with Lys370, Val172, and Ala369, as well as a hydrogen bond with Lys192, suggesting a moderate binding potential to the target protein. Furthermore, 2-hydroxybenzeneethanol formed hydrophobic interactions with Val172, Lys370, and Ala369, as well as hydrogen

bonds with Lys370 and Lys192, thereby enhancing its binding affinity. Apigenin exhibited hydrophobic interactions with Ala369 and Lys370, as well as a hydrogen bond with Glu366, suggesting a strong inhibitory potential. Caffeic acid exhibited hydrophobic interactions with Val172, Ala369, and Lys370, and formed hydrogen bonds with Gly190 and Met191, resulting in good stability. Ferulic acid exhibited hydrophobic interactions with Ala293, Met191, and Leu331, as well as hydrogen bonds with Lys370 and Leu331, indicating a high affinity toward the target protein.

Fisetin exhibited multiple hydrophobic and hydrogen-bonding interactions with Glu366, Lys192, Lys370, Met191, and Ala369, which could enhance its stability and binding affinity. Gallic acid formed hydrogen bonds only with Gly30, Gly51, Thr87, Thr89, Asp85, and Thr28, which could reduce its affinity for the target protein. Jasmonic acid also formed hydrogen bonds with Asp194, Lys370, and Pro277, but lacked hydrophobic interactions. Luteolin interacted with Met29 and Lys49 through hydrophobic interactions, while forming hydrogen bonds with Gly30, Gly51, Thr88, Pro31, and Lys94, with good binding affinity. Myoinositol formed hydrogen bonds with Lys63, Glu522, Thr521, and Val520, with possible low binding strength. Naringenin exhibited hydrophobic interactions with Met29 and Lys49 and hydrogen bonds with Gly30, Gly51, Thr88, Pro31, Lys49, and Thr28, indicating strong stability. p-Hydroxybenzoic acid showed hydrophobic interactions with Ile247, Ile225, Ile231, Val252, and Ala273, while forming hydrogen bonds with Val274 and Glu250, suggesting strong binding potential. Quercetin exhibited hydrophobic interactions with Val172, Lys192, and Lys370, and formed hydrogen bonds with Asp194, Glu366, Lys170, Gly190, Gly171, Gly374, and Lys370, showing high potential as an inhibitor. Furthermore, tyrosinamide exhibited hydrophobic interactions with Gly334, Met191, and Ala293, and formed hydrogen bonds with Gly190, Lys370, Leu371, Asp373, and Gly374, showing good binding affinity.

Molecular docking analysis showed that compounds such as Jasmonic acid, Naringenin, and Monoamidomalonic acid exhibited potential as competitive inhibitors against *Blastocystis*, showing stronger binding affinities for HSP60 than the standard ligand, metronidazole. These results showed that the compounds formed stable interactions with the target protein through hydrogen bonding and hydrophobic interactions. For instance, Naringenin formed a hydrogen bond with Glu53, a crucial residue in *Blastocystis* proteins, while Jasmonic acid interacts with Arg120, a key residue in the parasite's biological activity. With lower binding energies than metronidazole, these compounds could serve as promising alternatives to antiparasitic therapies, potentially offering enhanced efficacy.[22] From interaction analysis, several compounds showed strong potential as inhibitors. Quercetin, for instance, exhibits numerous hydrophobic and hydrogen-bonding interactions with key residues, such as Asp194, Glu366, Lys170, Gly190, and Gly374, thereby enhancing its affinity for the target protein. Fisetin also showed high potential due to extensive hydrophobic and hydrogen-bonding interactions, including those with Lys192, Lys370, Met191, and Glu366, which contributed to the stability of the protein-ligand complex. Luteolin, with its hydrophobic interactions with Met29 and Lys49, along with multiple hydrogen bonds, also suggested a strong binding affinity.

Apigenin, with hydrophobic interactions with Ala369 and Lys370 and a hydrogen bond with Glu366, showed potential as a potent inhibitor. Ferulic acid, on the other hand, exhibits promising inhibitor due to its hydrophobic interactions with Ala293, Met191, and Leu331, as well as hydrogen bonds with Lys370 and Leu331. Flavonoid-based compounds such as Quercetin, Fisetin, Apigenin, and Luteolin exhibited significant interactions with key residues, suggesting their potential as effective inhibitors. Similarly, phenolic acid-based compounds such as Ferulic and Caffeic acids also showed strong inhibitory potential. Therefore, these compounds could be considered primary candidates for the development of inhibitors targeting the examined protein. Results of molecular docking and protein interaction analysis show that the bioactive compounds from *Mimosa pudica linnaeus* have significant potential to inhibit the activity of various biological targets involved in inflammation, parasitism, and liver disease.[25,30] The strong hydrophobic interactions and hydrogen bonding between these compounds and key residues of target proteins provided further insight into their binding stability and strength, which is crucial in drug development. Therefore, these compounds could be considered as prime candidates for the development of inhibitors for various diseases.

Figures 1, 2, and 3 present the three-dimensional (3D) and two-dimensional (2D) molecular docking analyses between the bioactive compounds identified from *Mimosa pudica linnaeus* and three primary target proteins, namely *Blastocystis sp*., Tumor Necrosis Factor-α (TNF-α), and Heat Shock Protein 60 (HSP60). The docking results revealed that all compounds exhibited stable interaction patterns with low binding energy values, indicating a strong affinity toward the active sites of the respective proteins. In Figure 1, the interaction between the bioactive compounds and Blastocystis sp. protein formed stable hydrogen bonds and van der Waals forces, suggesting potential inhibition of enzymatic activity in the microorganism. Figure 2 shows that the *Mimosa pudica linnaeus* compounds interacted with TNF-α through key residues such as Lys98 and Glu116, which may interfere with the activation of pro-inflammatory cytokines. Meanwhile, Figure 3 demonstrates ligand binding with HSP60 at crucial residues including Lys155, Arg264, and Asp379, indicating possible modulation of chaperone protein function involved in maintaining cellular protein stability under oxidative stress conditions.

Overall, these in silico results support the hypothesis that the bioactive compounds contained in silver nanoparticles synthesized from *Mimosa pudica linnaeus* possess broad pharmacological potential, particularly as anti-inflammatory, cytoprotective, and antimicrobial agents. The interaction with TNF-α suggests a potential mechanism for suppressing inflammatory mediator expression, while the binding to HSP60 indicates enhancement of cellular defense mechanisms against stress-induced damage. In addition, the inhibitory effect on *Blastocystis sp.* protein implies antimicrobial activity against intestinal pathogens, which is relevant to acute diarrhea conditions. These findings are in agreement with previous studies reporting that *Mimosa pudica linnaeus* extract and its constituent *L-mimosine* possess strong antioxidant and anti-inflammatory properties [11].Therefore, this study provides a scientific basis for the development of *Mimosa pudica linnaeus* pudicaderived silver nanoparticles as promising natural therapeutic candidates for managing inflammation and gastrointestinal infections.

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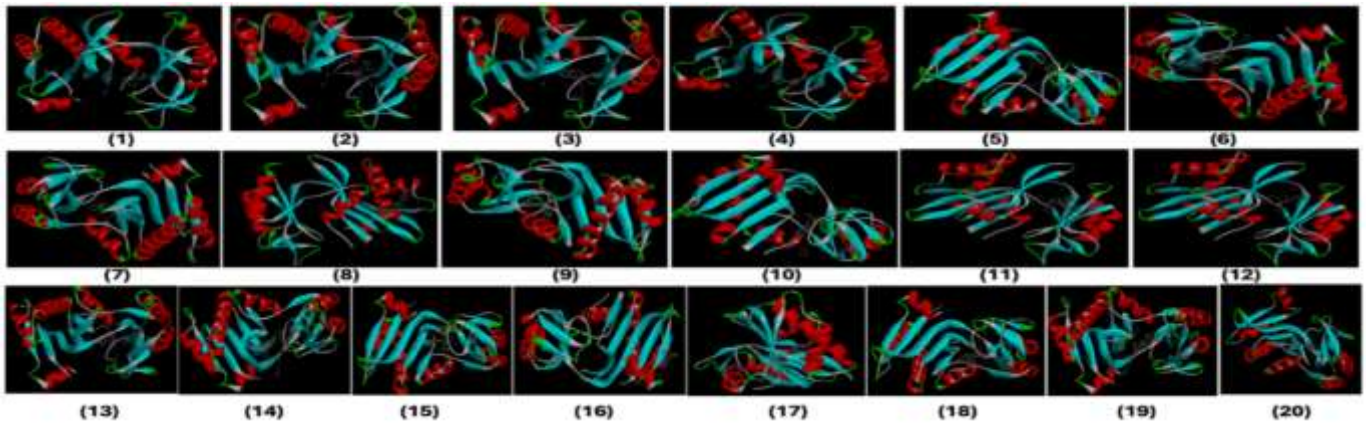

Figure 1: Interaction of 3D and 2D Protein *Blastocystis* (Based on the number of compounds)

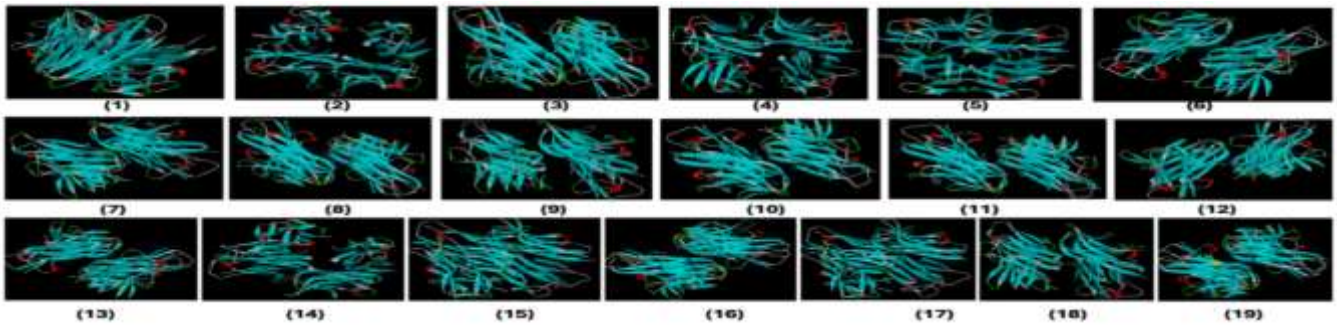

Figure 2: Interaction of 3D and 2D Protein TNF-α (Based on number of bioactive compounds)

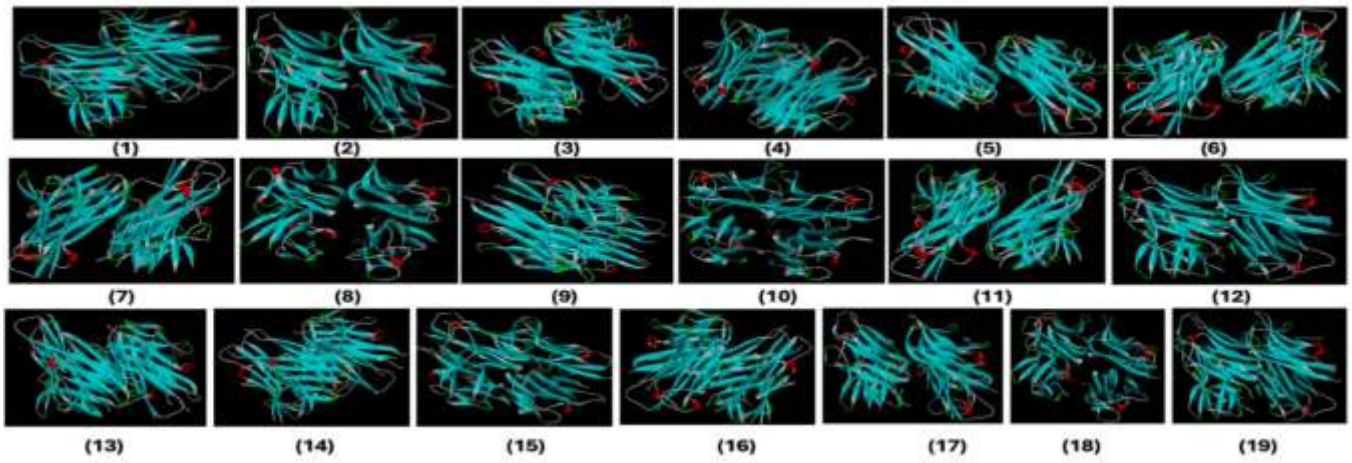

Figure 3: The analysis was conducted using the structure of 3D and 2D proteins (Based on the number of bioactive compounds)

Conclusion

In conclusion, this study identified 19 bioactive compounds in *Mimosa pudica linnaeus* extract, each with potential as an antibacterial, anti-inflammatory, hepatoprotective, and antiparasitic agent. Several compounds, including Apigenin, Luteolin, Quercetin, and Naringenin, exhibit significant potential based on bioactivity assays, ADME analysis, and molecular docking studies. These compounds exhibit strong interactions with target proteins, and some flavonoids and phenolic compounds show high inhibitory potential against various diseases, including parasitic infections, inflammation, and liver disorders. Despite their promising therapeutic potential, some compounds raise concerns about potential toxicity that must be addressed in further development. Therefore, additional *in vitro* and *in vivo* studies are necessary to evaluate their efficacy and toxicity comprehensively. This study provides strong evidence that *Mimosa pudica linnaeus* extract contains bioactive compounds that can be further developed as potential drug candidates for the treatment of bacterial infections, inflammation, and liver disorders.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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