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In silico Analysis of Antibacterial Activity of *Eclipta alba* against *Brucella melitensis*: A Bioinformatics Approach

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Abstract: *Brucella melitensis* is a facultative intracellular pathogen and one of the etiological agents of brucellosis, a highly common bacterial zoonosis worldwide. A facultative intracellular pathogen and one of the causes of brucellosis that is extremely prevalent around the world is *Brucella melitensis*. People prefer to employ natural substances derived from plants because synthetic drugs can have side effects. The study strongly supports the medicinal use of *Eclipta alba* (L.) Hassk. phytocompounds as a possible herb that can be considered to treat brucellosis caused by *Brucella melitensis*. Docking various medicinally significant chemical entities to the precise target sites offers a significant strategy having marvelous importance in a drug design procedure. This study aims to identify the phytocompounds that can be effective enough in inhibiting the antimicrobial activity of the *Brucella melitensis* with the aid of a bioinformatics approach. Based on the molecular docking results of methionyl-tRNA synthetase protein, ursolic acid showed the least binding energy of -8.5 Kcal/mol, followed by eclalbasaponin with -7.8 Kcal/mol and that of Brucella protein TcpB, wedelolactone, and ursolic acid showed the least binding energy with -7.5 Kcal/mol. These compounds can be considered further for in vitro evaluation and synergistically used as potential anti-brucellosis drugs.

Keywords: *Brucella melitensis*; *Eclipta alba*; molecular docking; phytocompounds.

1. Introduction

Human brucellosis typically results from *Brucella melintensis* and presents a wide range of clinical symptoms, including headache, arthralgia, weakness, sweating, and irregular fever [1]. One of the etiological agents of brucellosis is the facultative intracellular pathogen *Brucella melitensis*, a highly contagious bacterial zoonosis that can infect humans and animals and cause significant economic loss [2,3]. Brucellae are small, nonsporulating, nonmotile, nonencapsulated Gram-negative capnophilic coccobacilli. Growth happens aerobically, and many strains need more CO_2 for optimal growth. Although Brucellae can develop at

temperatures as high as 40°C, 37°C is the ideal temperature for growth. The ideal pH ranges between 6.6 and 7.4. Only a few changes have been made to the treatment approaches employed for more than 50 years, despite the development of new antibiotics and treatment plans. However, the treatment of brucellosis is still problematic due to emerging resistance, which yields high rates of treatment failure and relapses [1-3].

Only after extensive chemical and pharmaceutical testing has modern medicine emerged from folk medicine and the conventional system. The development of synthetic substances in modern medicine has resulted in a decrease in the utilization of plants [4]. Synthetic medicine, on the other hand, might have negative side effects, thus people choose to use natural substances derived from plants. As a result, plants continue to be a key source of therapeutic chemicals. *Eclipta alba* (L.) Hassk. (also known as *Eclipta prostrata* Roxb.) belongs to the Asteraceae family and is commonly known as bhringraj in India and a false daisy in English [4-6]. Table 1 describes the taxonomy of *E. alba*. The plant is classified as hepatoprotective by the Indian Ayurvedic Pharmacopoeia. *E. alba* has a wide range of biological activities, including treating memory ailments, fevers, edema, rheumatic joint aches, hepatitis, skin disorders, enlarged spleen, and digestion. Several compounds were isolated and reported, such as dimethyl wedelolactone, wedelolactone, and stigmasterol [6-8]. The coumestans, flavonoids, alkaloids, polyacetylenes, glycosides, and triterpenoids are only a few of the plant's many active compounds. The leaves contain a-terthienyl methanol, stigmasterol, dimethyl wedelolactone, wedelolactone, and dimethyl wedelolactone-7-glucoside 4. The plant's roots include polyacetylene-substituted thiophenes, while its aerial parts contain a phytosterol, β-glucoside of phytosterol, a glucoside of a triterpenic acid, β-amyrin in the n-hexane extract and luteolin-7-glucoside, and wedelolactone in the polar solvent extract. *A methanolic preparation of the plant's aerial portions inhibited S. aureus, S. epidermis, and Salmonella typhimurium*. The antibacterial activity of wedelolactone, extracted from the ethyl acetate fraction of aerial parts, was increased, suggesting that it could be the cause of the observed antimicrobial effects. Another phytochemical component of the plant, eclalbasaponin, has been found to be responsible for the plant's inhibitory activity against *B. subtilis* and *P. aeruginosa* [6-11].

Table 1. Taxonomic hierarchy of *E. alba*.

Kingdom	Plantae
Subkingdom	Viridaeplantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Asteranae
Order	Asterales
Family	Asteraceae
Genus	Eclipta L.
Species	*Eclipta alba* (L.) Hassk.

Due to the growing popularity of natural products and their accessibility at reasonable prices, the use of plant products for pharmaceutical purposes has steadily risen in developing and developed nations. Researchers have reported the antimicrobial activity of *E. alba* extracts against bacterial and fungal infections. [8,10]. As a result, the search for novel antimicrobial drugs from medicinal plants could be considered a positive approach, particularly in developing countries where the frequency of infectious diseases increases quickly [12,13]. Due to complications and the economic cost of the experimental methods for finding complex

structures, different computational methods, such as molecular docking, are preferred to predict putative binding modes and affinities. The molecular docking technique can represent the atomic level interaction between a ligand and a protein, allowing us to define small molecule behavior in target protein binding sites and elucidate key biochemical processes. The docking procedure consists of two main steps: predicting the ligand structure as well as its position and orientation within these sites (known as pose) and determining the binding affinity. This approach can help drug development and medicinal chemistry by revealing molecular recognition. Docking has become crucial in computer-assisted drug design and development (CADDD). In the structure-based drug-designing process, docking small molecular compounds into the binding location or sites of a receptor and estimating the binding affinity of the complex is pivotal [14]. Docking various medicinally significant chemical entities to the precise target sites offers a significant strategy having marvelous importance in a drug design procedure [15,16]. This study aims to identify the phytocompounds that can be effective enough in inhibiting the antimicrobial activity of the *Brucella melitensis* with the aid of a bioinformatics approach.

2. Materials and Methods

2.1. Protein preparation and validation.

Based on a literature review, three-dimensional structures of the target proteins were retrieved from the protein data bank (PDB) (Figures 1 and 2). These structures were then validated using UCLA-DOE LAB — SAVES v6.0 web server. The PROCHECK program of this server was run to determine the percentage of residues in the allowed region [16].

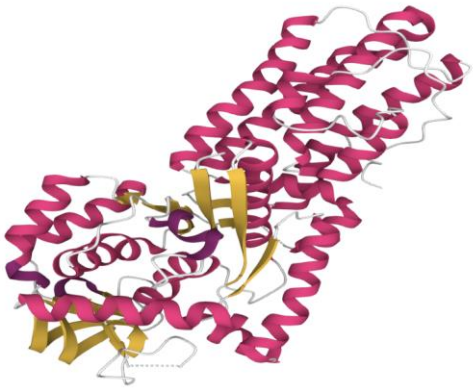


Figure 1. Crystal structure of methionyl-tRNA synthetase MetRS from *Brucella melitensis* (PDB ID: 4DLP)[17].

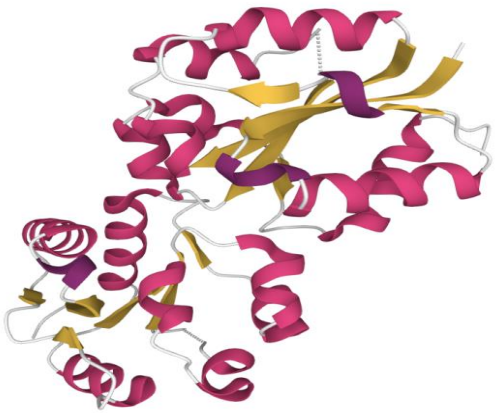


Figure 2. The crystal structure of the Brucella protein TcpB represented in ribbon format (PDB ID: 4LQC) [18].

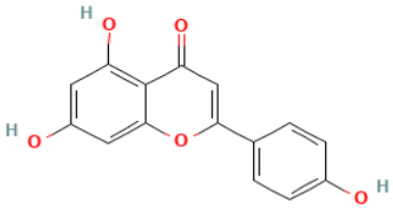
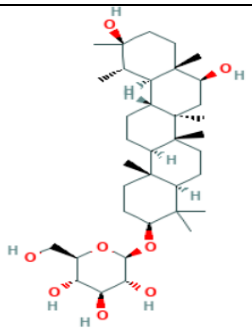
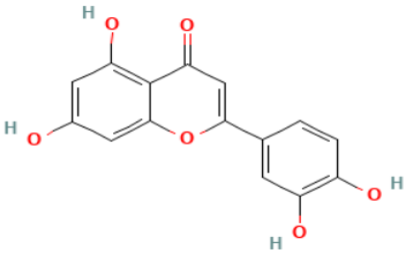
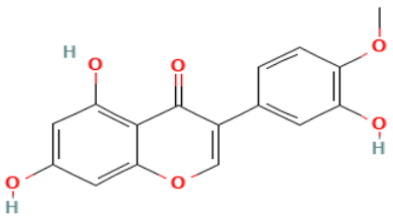
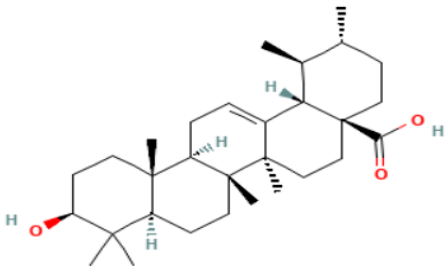
2.2. Binding site prediction.

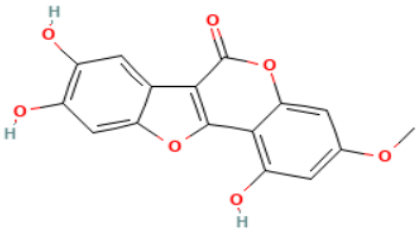
Computed Atlas of Surface Topography of proteins (CASTp) 3.0 webserver was used to determine the binding residues of the target proteins. It identifies pockets and cavities analytically. It also determines the area and volume of the pocket [15].

2.3. Ligand preparation.

The principal Phytochemicals available in the literature from *Eclipta alba* were identified and used in docking studies to inhibit the target proteins of *Brucella melitensis*. Table 2 consists of these compounds along with their 2D structures. The structures of these phytochemicals were retrieved from the PubChem database.

Table 2. Two-dimensional structures of the selected phytochemicals present in *E.alba.*

Phytocompounds of *A.calamus*	**2D Structures**
Apigenin	
Eclalbasaponin	
Luteolin	
Pratensein	
Ursolic acid	

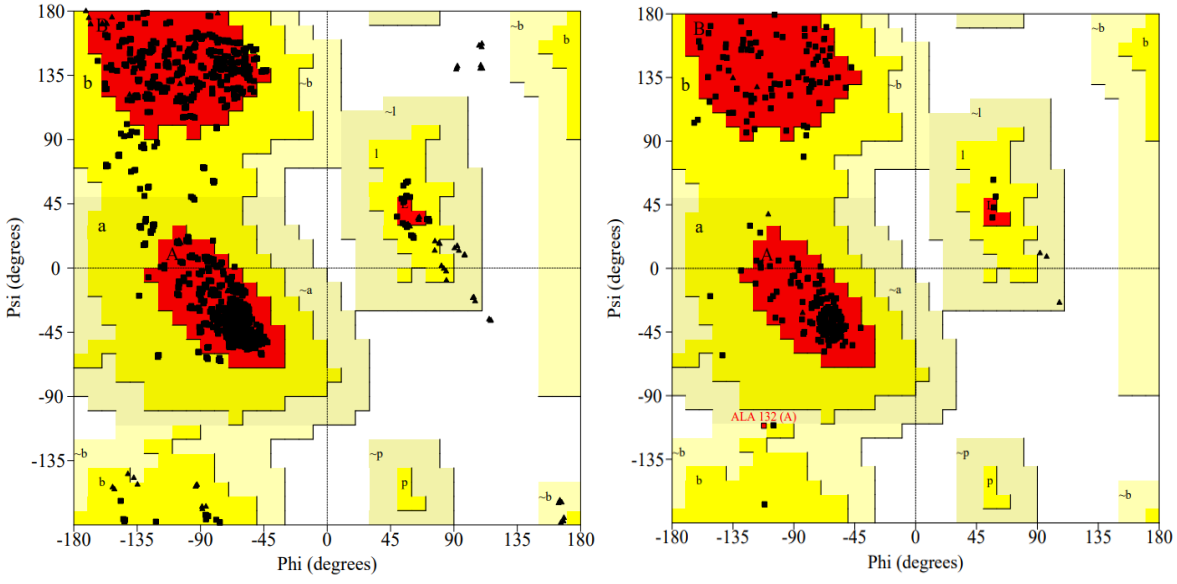
Phytocompounds of *A.calamus*	2D Structures
Wedelolactone	

2.4. Molecular docking.

The docking process determines the binding of the phytochemicals with the binding site of the target protein, which shows the nonbonded interactions based on binding affinity. Using the AutoDock Tools, protein structures were prepared for molecular docking by removing the co-existing ligands. Water molecules were removed, Gasteiger charges were added, and missing residues were checked, repaired, and converted to pdbqt format [15]. The downloaded 3D structures of the ligands were converted from sdf format to pdb format, which is the required file format for docking using OpenBabel GUI. These prepared files were loaded to the PyRx 0.8v workspace. PyRx is a virtual screening tool to carry out molecular docking analysis. The binding residues determined by CASTp software were selected, and a grid box was generated surrounding the proteins for the ligands to bind [15,16,19].

3. Results and Discussion

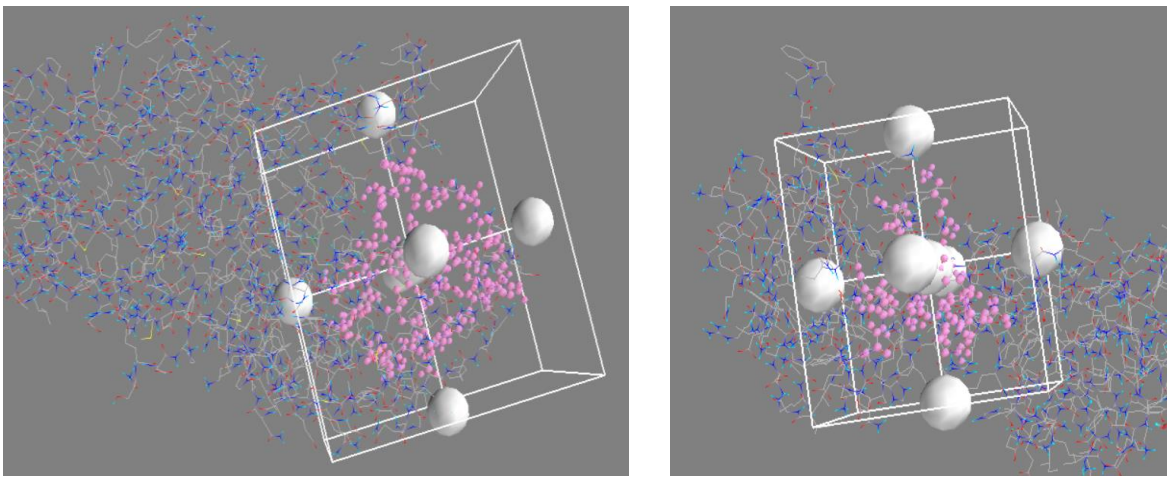
The three-dimensional structures of the target proteins of *Brucella melitensis* were validated using the PROCHECK program. Based on the results obtained, 92.4% of residues were in the most favored region for both the protein IDs 4DLP and 4LQC (Figure 3). To predict potential inhibitors of *E.alba* binding to the active site of the target proteins of *Brucella melitensis*, molecular docking was carried out. PyRx software predicted 9 poses of each phytochemical estimating different binding affinity (Kcal/mol). Figure 4 represents the grid box surrounding the binding site residues selected in the PyRx virtual screening tool workspace.



(a) **(b)**

Figure 3. Ramachandran plot validation of three-dimensional structures of proteins a) 4DLP and b) 4LQC.

The grid box for the methionyl-tRNA synthetase protein had dimensions of x=35.12, y=37.84, z=33.46, and for that of TcpB protein, the values were x=25.0, y=28.41, z=25.0. To analyse molecular docking results, the docked pose with the least binding affinity was considered. Lowering the binding affinity value is better than the docking result. These docked poses were then visualized for nonbonded interactions such as hydrogen bonds, van der Waals, pi-pi, n-Alkyl, pi-sigma, etc., using BIOVIA Discovery Studio Visualizer between the phytocompound and binding residues of the protein.



(**a**) (**b**)

Figure 4. The binding site residues (pink colored) surrounded by a grid box for the ligands to bind the target protein A) 4DLP B) 4LQC.

CASTp 3.0 computed a single binding pocket for each protein. For the *Brucella melitensis* methionyl-tRNA synthetase protein, a pocket of an area (SA) of 429.335 and a volume (SA) of 714.543 was identified (Figure 5). This protein was predicted to have a total of 35 amino acid residues at the active site. For the *Brucella protein* TcpB, a pocket of an area (SA) of 104.514 and a volume (SA) of 69.854 were identified, with a total of 20 amino acid residues at the active site (Figure 6).

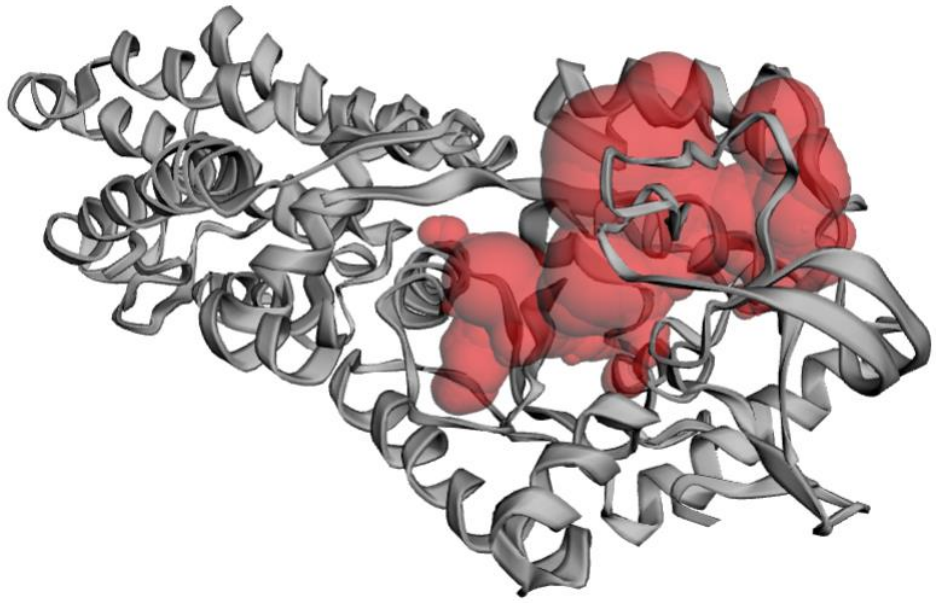


Figure 5. Representation of binding pocket on the surface of methionyl-tRNA synthetase protein as computed using CASTp 3.0v.

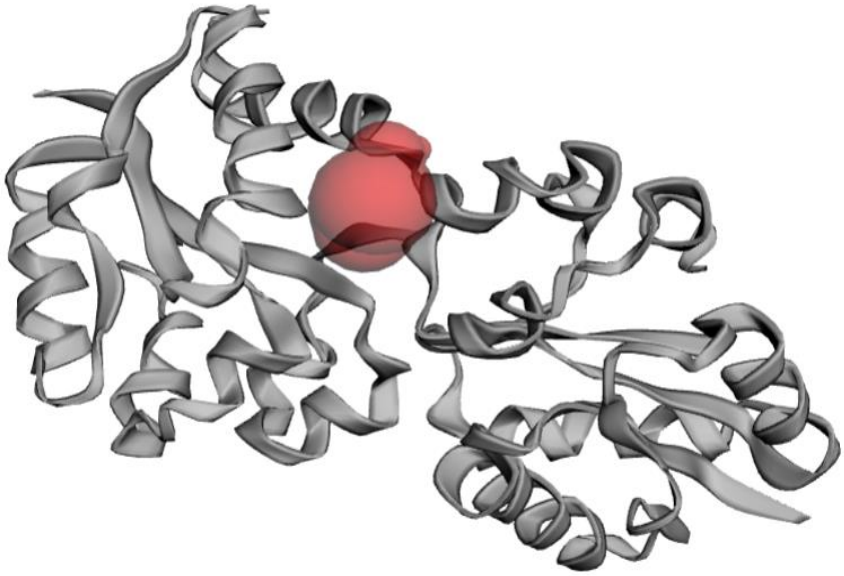
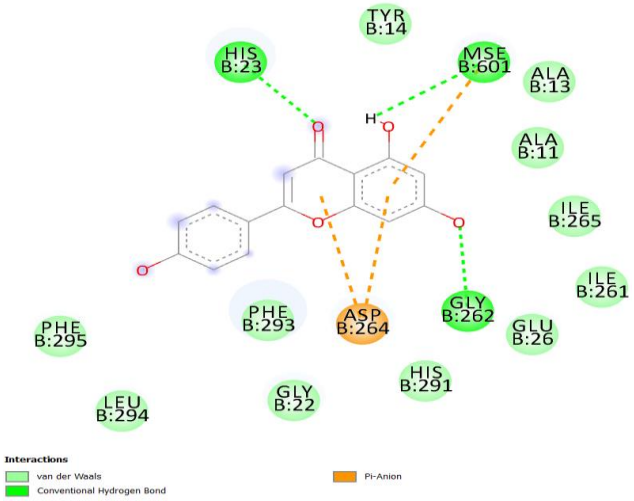
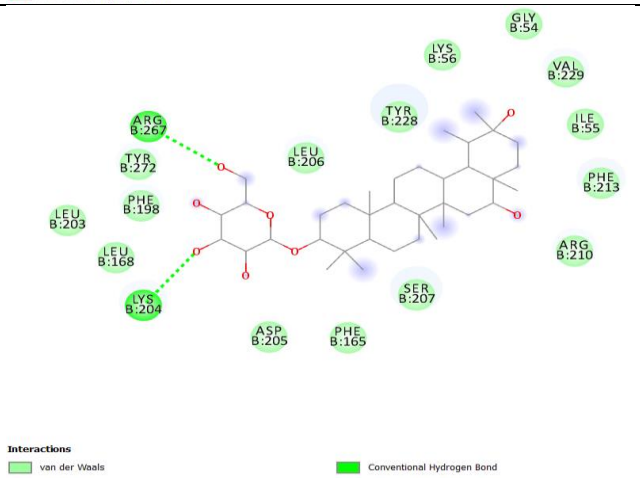
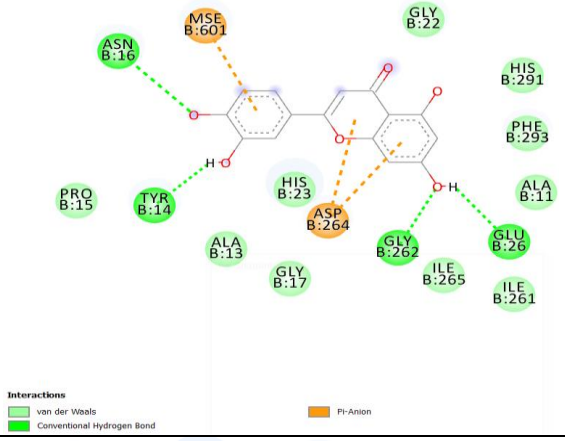
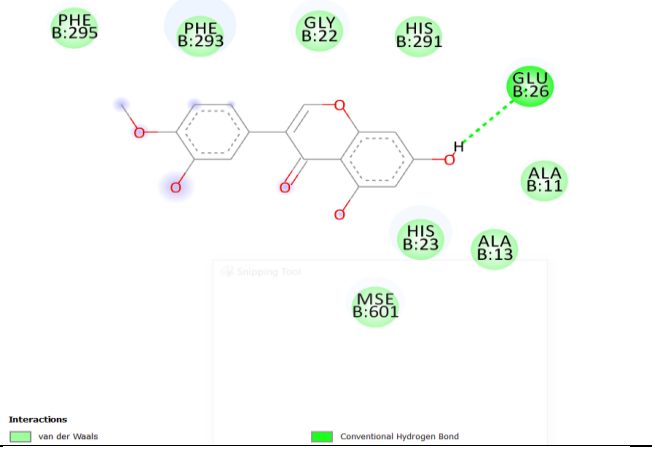
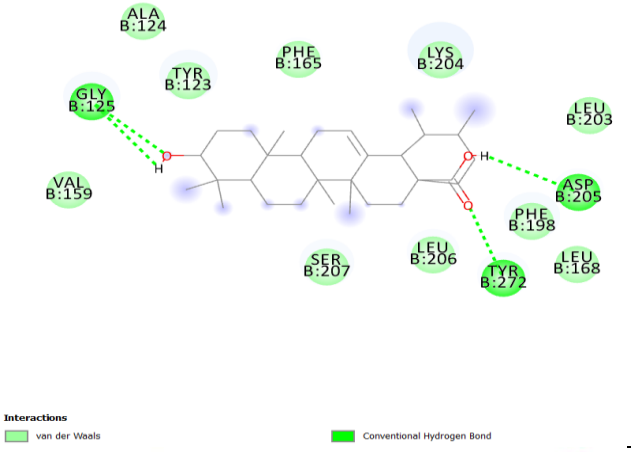
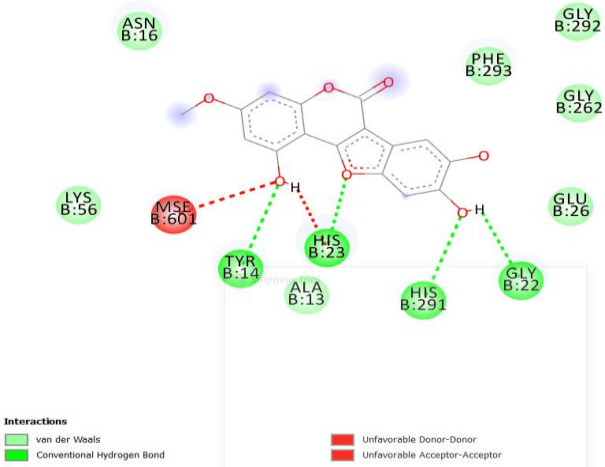


Figure 6. Representation of binding pocket on the surface of *Brucella* protein TcpB as computed using CASTp 3.0v.

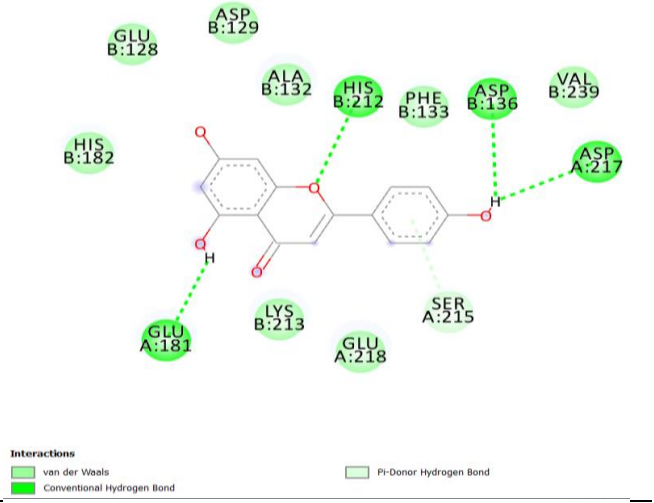
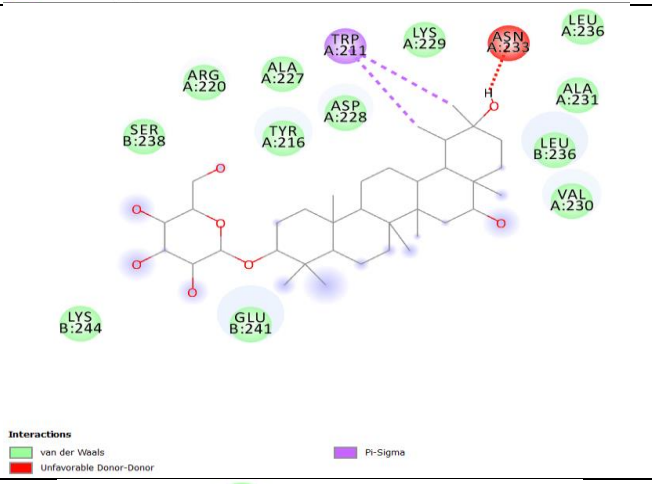
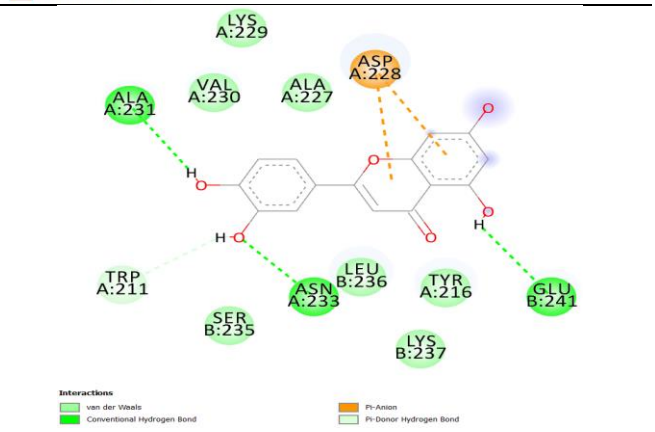
Table 3. Molecular docking results of methionyl-tRNA synthetase protein (4DLP) with phytocompounds of *E.alba* determining the binding affinities and amino acid residues forming the hydrogen bonds.

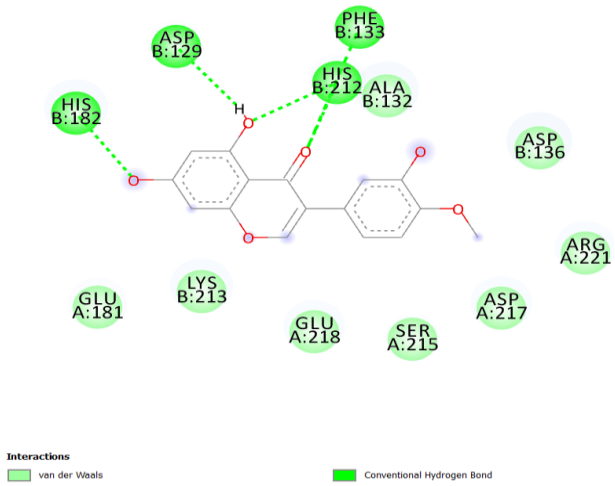
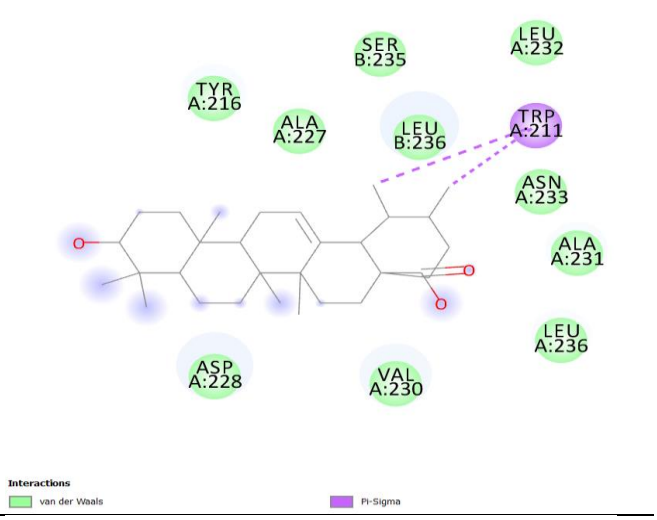
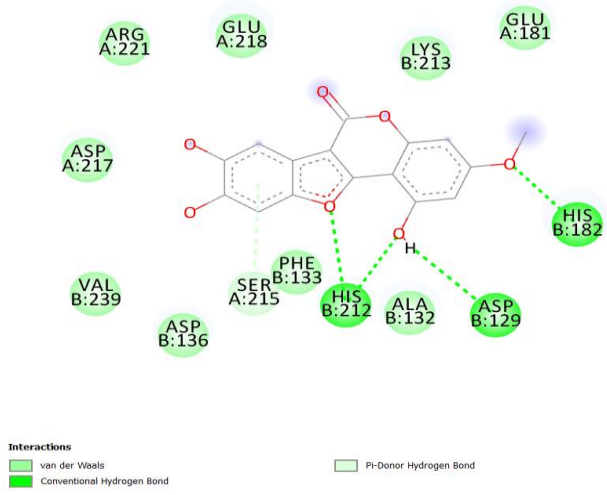
Phytocompounds	Binding affinity (Kcal/mol)	Hydrogen bonds	2D representation of the intermolecular nonbonded interactions between binding site residues of the protein and ligand
Apigenin	-7.4	TYR B:14, HIS B:23, GLY B:262	
Eclalbasaponin	-7.8	LYS B:204, ARG B:267	

Phytocompounds	Binding affinity (Kcal/mol)	Hydrogen bonds	2D representation of the intermolecular nonbonded interactions between binding site residues of the protein and ligand
Luteolin	-7.1	TYR B:14, ASN B:16, GLU B:26, GLY B:262	
Pratensein	-6.9	GLU B:26	
Ursolic acid	-8.5	GLY B:125, ASP B:205, TYR B:272	
Wedelolactone	-7.1	TYR B:14, GLY B:22, HIS B:23, HIS B:291	

Based on the above molecular docking results of methionyl-tRNA synthetase protein with phytocompounds of *E. alba* (Table 3), ursolic acid showed the least binding energy of -8.5 Kcal/mol followed by eclalbasaponin with -7.8 Kcal/mol. Ursolic acid formed the highest nonbonded interactions with a hydrogen bond, each with ASP B:205 and TYR B:272 and two hydrogen bonds with GLY B:125 and several var der Waals interactions. At the same time, eclalbasaponin displayed two hydrogen bonds, each with LYS B:204 and ARG B:267, along with several van der Waals interactions.

Table 4. Molecular docking results of *Brucella* protein TcpB (4LQC) with phytocompounds of *E.alba* determining the binding affinities and amino acid residues forming the hydrogen bonds.

Phytocompounds	Binding affinity (Kcal/mol)	Hydrogen bonds	2D representation of the intermolecular nonbonded interactions between binding site residues of the protein and ligand
Apigenin	-6.8	ASP B:136, GLU A:181, HIS B:212, ASP A:217	
Eclalbasaponin	-7.3	Nil	
Luteolin	-6.9	ALA A:231, ASN A:233, GLU B:241	

Pratensein	-6.7	ASP B:129, PHE B:133, HIS B:182, HIS B:212	
Ursolic acid	-7.5	Nil	
Wedelolactone	-7.5	ASP B:129, HIS B:182, HIS B:212	

According to the molecular docking results of *Brucella protein* TcpB with phytocompounds of *E.alba* (Table 4), wedelolactone and ursolic acid showed the least binding energy with -7.5 Kcal/mol in comparison with other ligands. As represented in the 2D diagram in table 3, wedelolactone forms a hydrogen bond, each with ASP B:129 and HIS B:182, and two H-bonds with HIS B:212 along with several van der Waals interactions and a pi-donor hydrogen bonds. Ursolic acid, on the other hand, does not form any hydrogen bond with the binding residues of the protein but can be seen having van der Waals and pi-sigma interactions with TYR A:216, ALA A:227, ASP A:228, VAL A:230, ALA A:231, LEU A:232, ASN A:233, SER B:235, LEU A:236, LEU B:236, and TRP A:211 respectively.

4. Discussion

Various chemical components extracted from Eclipta alba have been tested for antibacterial activity against *Shigella dysenteriae*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus epidermidis*, *S. aureus*, *B. subtilis* and *K.pneumoniae* in prior studies [8,9,20]. The leaf extract of *E. alba* was discovered to have antimalarial activities against *Plasmodium berghei* ANKA strain in mice in a study [10]. Ursolic acid, Eclalbasaponins, and ecliptasaponins are some of the triterpenoids identified from this plant that have been demonstrated to have anti-fibrotic, hypoglycemic, cytotoxic, and anti-osteoporotic properties [9]. Another chemical, wedelolactone, has been shown to have anti-venom properties against the venoms of South American crotalids [11]. *In vitro* antibacterial activity of 7 essential oils and 10 plant extracts against *B. melitensis* isolates was tested by Safi *et a*l.. *T. syriacus* and *C. zeylanicum* essential oils, as well as *L. nobilis* plant extract, were found to be bactericidal against *B. melitensis* [13]. According to Muhammad *et al*., the synergistic use of phytochemicals produced from *B. lyceum* could potentially protect against *B. melitensis*. Phytic acid was projected to be the most powerful inhibitor *in silico* screening, followed by jehlumine, barbamine, oxyberberine, and sindamine [14]. However, no reports of *E.alba* phytocompounds being tested against *B. melitensis* have been reported.

5. Conclusions

The use of plant-derived extracts to control diseases has been in practice for an unknown time. Medicinal plants are valuable sources of natural compounds effortlessly available for tackling health-related issues. This study was carried out to compare and analyze the anti-Brucella activity of different phytoconstituents to inhibit the predominant proteins involved in the brucella progression. All the ligands showed good binding affinity. In conclusion, our study strongly supports the medicinal use of *Eclipta alba* (L.) Hassk. phytocompounds as a possible herb that can be considered to treat brucellosis caused by *Brucella melitensis.* Further research may be performed on the phytocompounds, ursolic acid, wedelolactone, and eclalbasaponin to determine the exact action mechanism and further validate the obtained results. These compounds can be considered further for *in vitro* evaluation and synergistically used as potential anti-brucellosis drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

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