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Analyzing Phytocompounds, Antioxidants, and In-Silico Molecular Docking of Plant-Derived Potential *Andrographis paniculata* Inhibitory Action to Managed Beta Thalassemia

Soumya Khare[1,2,*], Tanushree Chatterjee[1], Shailendra Gupta[3] and Ashish Patel[2]

[1]*Department of Biotechnology, Raipur Institute of Technology, India*

[2]*Chhattisgarh Swami Vivekanand Technical University, India*

[3]*Department of Systems Biology and Bioinformatics, University of Rostock, Germany*

Abstract: It has long been known that medicinal plants are vital to human health and have been used for therapeutic purposes since the beginning of civilization. The goal of this study is to evaluate the scientific data supporting the use of the herbal remedy *Andrographis paniculata* in the management of beta thalassemia. Phytocompounds, one of the bases of traditional use, were assessed both qualitatively and quantitatively in five different extracts with varying solvent properties (methanol, chloroform, hexane, ethyl acetate, and aqueous), providing the fundamental explanation for the therapeutic relevance of this plant. The presence of flavonoids, phenols, tannins, saponins, and alkaloids in plant extracts was assessed. It was shown that ethanol extract had the highest concentration of phytocompounds among the various extracts, while chloroform extract had the lowest concentration. The plant's antioxidant activity was also assessed, and the results showed that the methanolic extract has potential antioxidant activity, as shown by the DPPH's lowest half-inhibitory concentration (IC50) values. To further evaluate the phytocompounds for their in-silico study, molecular docking techniques were employed. These phytocompounds (DL-alphatocopherol, 3,19-O-diacetylanhdroandrographolide, and 14-acetylandrographolide) to the fetal hemoglobin target protein PDBID:4MQJ were also found to have interactions with significantly minimum binding energies of −12.52 kcal/mol, −11.22 kcal/mol, and −11.08 kcal/mol, where DL-alphatocopherol was found to have the highest binding affinity (−12.524 kcal/mol) and interaction with the active site HIS97. The SwissADME was used to assess the drug-likeness and ADMET characteristics of phytocompounds derived from *Andrographis paniculata*. Based on chemical characteristics, drug-likeness score, and ADMET model, the present study selected and screened the phytocompounds DL-alphatocopherol, 3,19-O-diacetylanhdroandrographolide, and 14-acetylandrographolide, which are expected to have better drug-like features with improved toxicity profiles. Therefore, it makes sense to send the compounds reported in this work to an in-vivo analysis of therapeutic applicability due to their significant biological features.

Keywords: beta thalassemia, *Andrographis paniculata*, phytocompounds, fetal hemoglobin, molecular docking

1. Introduction

Since the dawn of human history, plants have been used for medicinal purposes. Over the world, the medicinal plant has been used for a very long time to create natural medicines that treat a wide range of illnesses and problems [1]. Due to a lack of access to modern medications and poverty, the World Health Organization estimates that up to 80% of the world's population receives their basic medical care from conventional or traditional medicine [2]. Around the world, the use of plants or other natural compounds as therapeutic approaches for a wide range of illnesses has expanded with the development of new medications. Because they are less hazardous or toxic than current generic pharmaceuticals and because their side effects are less well-known, herbal treatments are primarily used in rural areas [3]. India is home to a wealth of ancient indigenous traditional knowledge on medicinal plants. Traditionally, the treatment of beta thalassemia in the traditional medical system has greatly benefited from ethnomedicine, or the use of medicinal plants [4]. Herbal remedies have also gained a lot of interest recently because they are easily found in nature for free or at a minimal cost, and they pose less of a risk than synthetic medicines [5]. Thalassemia is a class of anemias caused by a hereditary malfunction in the synthesis of hemoglobin. Due to the inability of their cells to generate the beta polypeptide chain of human hemoglobin, thalassemia patients do not produce enough HbB [6]. Those suffering from β-thalassemia are unable to manufacture β chains. The main hemoglobin seen during pregnancy is fetal hemoglobin (HbF). Gamma globin, which

*Corresponding author: Soumya Khare, Department of Biotechnology, Raipur Institute of Technology and Chhattisgarh Swami Vivekanand Technical University, India. Email: soumyashrivastava82@gmail.com

combines to generate HbF, stabilizes the β globin chain in thalassemic individuals. Because of the high amounts of HbF synthesis, there is less hemolysis and more hemoglobin because less alpha and beta chains are produced by methods for boosting HbF concentration and reactivating it [7]. Many efforts have been undertaken to find both naturally occurring inducers and pharmaceutical treatments that can boost fetal hemoglobin (HbF) synthesis and fetal γ-globin gene expression. Fetal hemoglobin (HbF) reactivation by chemical agents becomes an effective intervention to treat human β-hemoglobinopathies, and reactive fetal γ-globin gene production of HbF has been proposed as a viable therapy for β-thalassemia patients [8]. The authenticity, safety, and therapeutic efficacy of medicinal plants have been studied using modern scientific methods, and the identification of natural remedies for β-thalassemia-boosting HbF-inducing activities has been made. Ayurvedic and traditional oriental medicine make use of *Andrographis paniculata*. There are roughly 40 species in the Acanthaceae family's genus Andrographis. For millennia, the plant *Andrographis paniculata* has been utilized with great success in traditional Asian treatments. Known as the "king of bitters", *Andrographis paniculata* is an annual plant that grows to a height of 1–3 feet. It is also known by the English name Create. It is grown in many other Asian nations and is used in China, Hong Kong, the Philippines, Malaysia, Indonesia, and Thailand as a traditional herbal remedy. Studies on phytocompounds have shown that *Andrographis paniculata* includes a variety of chemicals, including flavonoids, labdane diterpenoid lactones, and other substances. There is evidence that a broad range of pharmacological properties hold promise as chemotherapy drugs [9]. It is thought to be an effective treatment for leprosy, gonorrhoea, scabies, boils, skin eruptions, and both seasonal and chronic fevers because of its blood-purifying properties. Furthermore, *Andrographis paniculata* has pharmacological and immune-stimulatory properties as well as astringent, carminative, cytotoxic, and cardiovascular effects [10].

The organic chemicals obtained from medicinal plants that seem to have a preset physiological effect on the human body are called phytocompounds, or secondary metabolites. These compounds include flavonoids, phenols, alkaloids, saponins, and tannins. These secondary metabolites are incredibly varied compounds with unknown actions both chemically and taxonomically [11]. These different plant-derived phytocompounds have a wide range of positive effects on humans because of their medicinal qualities, which make them very advantageous for the human healthcare system. Antimicrobial, anti-inflammatory, antifungal, and antioxidant qualities are possessed by phytocompounds, which include phenolic and flavonoid compounds [12]. The presence of certain phytocompounds within plants is primarily responsible for their therapeutic properties. The effectiveness of phytocompounds in treating a wide range of diseases may be attributed to their antioxidant properties, as these compounds help to reduce the effects of oxidative stress, which is directly linked to numerous pathogenic mechanisms [13]. The injection of antioxidants provides efficient protection against free radicals and reactive species. Hematologists have long been interested in the fetal-to-adult hemoglobin flip and silencing fetal hemoglobin (HbF) because clinical stimulation of HbF production offers great promise in alleviating the clinical symptoms of beta-thalassemia [14]. A better comprehension of beta-globin regulation has enabled the use of pharmacologic agents for HbF induction and the identification of novel HbF-inducing techniques. The process of finding new drug leads through medicinal plant research is fraught with difficulties, such as obtaining plant parts, choosing and executing bioassays, and scaling up active molecules. Our research is based on the in-silico analysis of phytocompounds from the medicinal plant *Andrographis paniculata*. We use the AutoDock 4 molecular docking studies to identify the phytocompounds extracted from *Andrographis paniculata* and to assess antioxidant properties using the DPPH radical scavenging method. It was investigated how the phytocompounds from *Andrographis paniculata* interact molecularly with the target protein of fetal hemoglobin in beta thalassemia.

2. Research Methodology

2.1. Preparation of different plant extracts

For this investigation, *Andrographis paniculata* leaf effective plant components were used. After being thoroughly cleansed with distilled water and allowed to dry for 10–15 days in the shade, the plant sample was coarsely ground using a cutter mill or grinder machine to create a fine powder. Using aqueous solvents, methanol, chloroform, hexane, and ethanol, five distinct plant extracts were made. Additionally, the following extraction techniques were used in the current study to analyze the phytocompounds, antioxidant activity, and qualitative and quantitative data of five distinct extracts (ME: methanolic extract; CE: chloroform extract; HE: hexane extract; EE: ethanol extract; and AE: aqueous extract).

2.2. Qualitative screening or analysis of phytocompounds in different extracts of *Andrographis paniculata*

All five extracts—ME, CE, HE, EE, and AE—were evaluated for the content of various secondary metabolites, including flavonoids, phenols, tannins, saponins, and alkaloids, using a traditional methodology [15].

2.2.1. Test for flavonoids sulfuric acid test

A few drops of H2SO4 were added to the crude extracts. Flavonoids were detected by the emergence of orange. Lead acetate test: Extracts were subjected to a few drops of lead acetate solution in order to conduct the test. Flavonoids can be detected by the development of a yellow precipitate [16].

2.2.2. Test for phenols ferric chloride test

A few drops of ferric chloride solution were added to 10 mg of extracts. The presence of phenol is indicated by the emergence of a bluish-black tint [16].

2.2.3. Test for tannins ferric chloride test

In the water bath, a tiny quantity of extract was combined with water and brought to a boil. After the mixture was filtered, ferric chloride was added to the filtrate. The outcome was a dark green tint, which suggests that tannins are present [16].

2.2.4. Test for saponins foam test

A mixture of 5 ml of distilled water and 0.5 mg of extract was used. The production of foam—a foamy mist of tiny bubbles—signals the presence of saponins [17].

2.2.5. Test for alkaloids Mayer's test

When a few drops were applied to the plant extracts, the formation of cream-colored precipitates indicated the presence of alkaloids [17].

2.2.6. Test for carbohydrates Molisch test

One milliliter of concentrated sulfuric acid was carefully placed along the test tube's walls after two drops of an alcoholic α-naphthol solution was added to 2 ml of aqueous extract. Carbohydrates are present when a violet ring forms at the junction.

2.2.7. Tests for protein and amino acids Biuret's test

In a test tube, the extract was heated and treated with 1 ml of a 10% sodium hydroxide solution. To the mixture mentioned above, a drop of 0.7% copper sulfate solution was added. The development of a violet or pink hue signifies the existence of proteins.

2.2.8. Tests for glycosides Borntrager's test

Dilute sulfuric acid was added to 3 ml of test solution, allowed to boil for 5 min, and then filtered. An equal volume of either benzene or chloroform was added to the cold filtrate, and it was thoroughly shaken. Ammonia was added to the organic solvent layer after it had been separated. The ammonical layer's formation of a pink to red tint shows the presence of anthraquinone glycosides [17].

2.2.9. Tests for triterpenoids and steroids Salkowski's test

Chloroform was applied to the extract, and then it was filtered. A few drops of strong sulfuric acid were added to the filtrate, agitated, and left to stand. There are sterols present if the bottom layers become red. Triterpenes are present when a golden yellow layer is present at the bottom [17].

2.3. Quantitative screening or analysis of phytocompounds in different extracts of total flavonoid and phenolics content

2.3.1. Determination of total phenolics

The total phenolic content of the extracts was ascertained using the Folin–Ciocalteu method, which was modified and published by Wolfe et al. [18]. An aliquot of the extract was combined with 2 mL of sodium carbonate (75 g/L) and 2 mL of Folin–Ciocalteu reagent (previously diluted with water 1:10 v/v). For color development, the tubes were vortexed for 15 seconds and then let to stand at 25°C for 20 min. Next, absorbance was measured using a UV spectrophotometer set at 760 nm (Shimadzu, USA). Extract samples with final concentrations of 0.1 and 0.15 mg/mL were assessed. Gallic acid equivalent, or GAE (standard curve equation: $y = 0.091\times + 0.167$, $R2 = 0.994$), mg of GA/g of dry extract, was used to express the total phenolic contents. At each concentration, the experiment was conducted three times [18].

2.3.2. Determination of total flavonoids

The aluminum chloride colorimetric assay, as reported by Jia et al. [19], was used to quantify the total flavonoids. 2.5 mL of distilled water and 150 µL of 5% sodium nitrate were added to 0.5 mL of samples/standard. 0.3 mL of 10% AlCl3 was added after 5 min. After adding 0.55 mL of distilled water and 1 mL of 0.001 M NaOH, the mixture was kept at room temperature for 15 min. This was done at 6 min. The mixes' absorbance was calculated at 510 nm. At final concentrations of 0.1 and 0.15 mg/mL, extract samples were assessed. The catechin equivalent (CAE) (standard curve equation: $y = 0.000\times + 0.001$, $R^2 = 0.998$) was used to express the total flavonoid concentration in milligrams of CA/g of dry extract. Each concentration saw three iterations of the experiment [19].

2.4. In-vitro antioxidant assay

This study aimed to assess and evaluate the in-vitro antioxidant activity of different extracts of *Andrographis paniculata* using the 2,2-diphenyl-1-picrylhydrazyl scavenging assay. Specifically, the antioxidant potential of all extracts (methanolic, chloroform, hexane, ethanol, and aqueous) was investigated for free radical scavenging.

2.4.1. DPPH radical scavenging assay

The DPPH radical scavenging assay, as reported by Blois [20], was used to examine the extracts' capacity to scavenge-free radicals. The capacity of the plant extractives to donate hydrogen atoms was assessed by observing the decolorization of the 2,2-diphenyl-1-picrylhydrazyl methanol solution (DPPH). When antioxidants are present, the violet/purple color that DPPH creates in methanol solution fades to shades of yellow. A 0.1 mM DPPH solution in methanol was made, and 1.6 mL of extract in methanol at various concentrations (12.5–150 µg/mL) was combined with 2.4 mL of this solution. For 30 minutes, the reaction mixture was vortexed completely and kept at room temperature. Using spectrophotometry, the mixture's absorbance was calculated at 517 nm. Using the following formula, the percentage DPPH radical scavenging activity was determined:

{(A0− A1)/A0} × 100 represents the percent DPPH radical scavenging activity, where A0 represents the absorbance of the control and A1 represents the absorbance of the extractives/standard. After that, the percentage of inhibition was plotted against concentration, and the IC50 was determined using the graph. At each concentration, the experiment was conducted three times [21].

2.5. In-silico studies

Andrographis paniculata phytocompounds have a wide range of therapeutic uses. It is made up of phenol, alkaloids, terpenoid, steroid, and flavonoid compounds. These particular compounds were chosen as the ligand for analyzing the beta thalassemia inducer characteristics.

2.5.1. Ligand preparation

GC-MS analysis was utilized to identify ten phytocompounds from *Andrographis paniculata*, which were then utilized to create ligands. The Pubchem databases were used to download the three-dimensional (3D) structure, NCBI website (http://pubchem.ncbi.nim.nic.gov). These ligands were computed as 3D coordinates, and the prepared ligand protocol of AUTODOCK4.0 was used to construct the ligand conformation. Using ChemSketch, the ligand structures that were not found in Pubchem were initially sketched. It includes tools for creating, viewing, and modifying chemical structures and may be found at (http://www.acdlabs.com/resources/freeware/). Using http://openbabel.org/wiki, the ligands that were originally retrieved in .mol format were converted to .pdb format.

2.5.2. Protein preparation

Target protein fetal hemoglobin's (3D) structure was found in the RCSB protein database (http://www.rcsb.org). It is made up of heteroatoms, many of which are cofactors, water molecules, metal ions, and co-crystallized ligands. Energy was then reduced to SPDBV and hydrogen was optimized using the exhaustive sampling method (http://spdbv.vital-it.ch).

2.5.3. Active site identification

Using metapocket 2.0 (projects.biotec.tu-dresden.de>metapocket), the active site analysis of PDBID: 4MQJ was carried out. This web-based platform allows for the automated recognition of residues with 3D coordinates.

2.5.4. Molecular docking analysis

Docking is the process of virtually screening a phytocompound database and determining which binder is stronger by using a variety of score systems. Following ligand production and preparation, the AutoDock 4 (http://autodock.scripps.edu) was used for the docking investigations. Using AutoDock 4.0, the binding mode and interaction of PDBID: 4MQJ with phytocompounds were investigated. Docking was done on ten phytocompounds that were thought to be worth more research. To comprehend the protein–ligand interactions, the docking results were evaluated using the Discovery studio Visualization software (www.3dsbiovia.com).

2.5.5. ADMET analysis

SwissADME online version was used to estimate the drug-likeness and pharmacokinetic characteristics of phytocompounds [22]. All phytocompounds demonstrated moderately soluble and soluble gastrointestinal (GI) absorption, with no BBB permeability, according to the pharmacokinetic parameters; nonetheless, the bioavailability score indicated drug-likeness.

2.6. Statistical analysis

Each statistical analysis was carried out three times, and the findings were assessed as mean±SD or as an average value with standard deviations [23]. Using Prism graph pad software, an ANOVA was performed to determine the mean difference between the values of phytocompounds and antioxidant activity. To ascertain the overall significant difference ($p < 0.05$) among all of the extracts, the one-way ANOVA test was also utilized [24].

3. Results and Discussion

Using GC-MS analysis, ten phytocompounds from *Andrographis paniculata* were discovered. Eight phytocompounds' 3D chemical structures were obtained from Pubchem databases, and two phytocompounds' structures were created with ChemSketch software. Although a plant makes phytocompounds to defend itself, current studies show that many of these substances can also prevent diseases in humans [25]. The immune system is strengthened by the antioxidant, anti-inflammatory, antibacterial, antimutagenic, and antiadhesion qualities of *Andrographis paniculata* plant [26]. Since the beginning of time, phytocompounds and secondary metabolites have been crucial for therapeutic purposes [27]. The process of screening the phytocompounds found in *Andrographis paniculata* has the potential to facilitate medication development and discovery. Table 1 displays the total phenolic and flavonoid content data. The maximum concentration of phenol and flavonoid (65.89 mg/gm and 0.89 mg/gm, respectively) was found in the ethanol extracts, followed by methanol. The chloroform extract had the lowest levels of flavonoids and phenols (13.67 and 0.13 mg/gm, respectively). Extracts from *Andrographis paniculata* leaves were discovered to contain the terpenoids, flavonoids, and tannins shown in Table 2. Additionally, ethanol, aqueous, methanol, ethyl acetate, and chloroform included carbohydrate, glycoside, alkaloid, fats and oil, amino acid, and phenol; however, terpenoid was absent from methanol and ethanol, fats and oil were absent from hexane, and amino acid was absent from aqueous extracts. Figure 1 displays the antioxidant activity values. The *Andrographis paniculata* leaf extracts to scavenge DPPH radicals in methanol, ethanol, hexane, ethyl acetate, and chloroform aqueous extracts. The methanolic extracts were found to have the highest level of antioxidant activity at a concentration of 100 mg/ml in 50.04 %. Ethyl acetate and chloroform came in second and third, respectively, at 34.8 % and 25.16 % (Figure 1).

The biological characteristics of *Andrographis paniculata* phytocompounds, such as phenolic and flavonoid compounds, are well-known to exhibit a wide range of pharmacological and biological activities, such as antibacterial, hepatoprotective, diuretic, antifungal, anticancer, and antidiabetic effects [28]. Furthermore, flavonoids are polyphenolic compounds with well-known characteristics like scavenging free radicals and inhibiting oxidative and hydrolytic

Table 1. Total phenolic content and total flavanoid content of *Andrographis paniculata*

S. no.	Solvent	Total phenol (Mg/gm)	Total flavanoid (Mg/gm)
1.	Aqueous	16.58	0.18
2.	Methanol	46.93	0.48
3.	Ethanol	65.89	0.89
4.	Hexane	22.34	0.23
5.	Chloroform	13.67	0.13

Table 2. The analysis of phytocompounds in the hexane, ethyl acetate, chloroform, ethanol, methanol, and aqueous extract of *Andrographis paniculata*

		Extracts used: Leaf					
S. no.	Tests employed	Aqueous	Methanol	Ethanol	Hexane	Ethyl acetate	Chloroform
1.	Saponin	−	+	+	−	+	+
2.	Tannin	+	+	+	+	+	−
3.	Flavanoid	+	+	+	−	−	−
4.	Phenol	+	+	+	+	+	+
5.	Alkaloid	+	+	+	+	+	+
6.	Glycosides	−	+	+	+	+	+
7.	Carbohydrate	+	+	+	+	+	+
8.	Terpenoid	+	−	−	+	+	+
9.	Amino acids and proteins	−	+	+	+	+	+
10.	Fats and oil	+	+	+	−	+	+

Note: * + = presence; − =absence

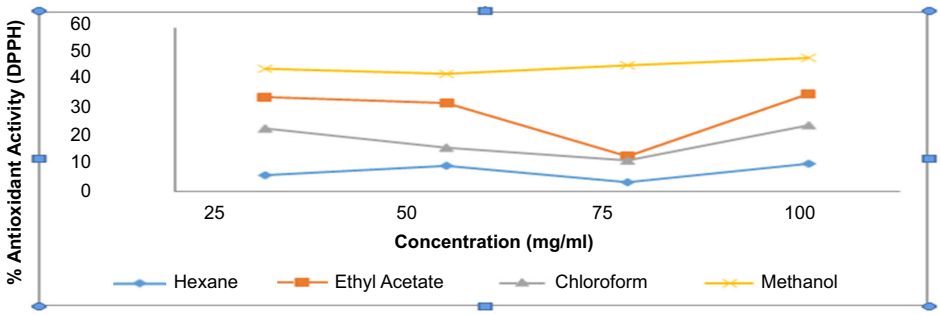

Figure 1. DPPH radical scavenging activity by *Andrographis paniculata* leaf hexane extract, ascorbic acid, ethyl acetate extract, chloroform extract, and methanol extract

enzymes. Flavonoids are among the most common phytocompounds with antioxidant action, and they directly support the management of diseases linked to oxidative stress, such as beta thalassemia [29]. According to our research, *Andrographis paniculata*, particularly its leaves, are a very rich source of potential phytocompounds. Nonetheless, antioxidants act as scavengers of free radicals, shielding the body from a range of illnesses brought on by the generation of free radicals. They lessen the damage that free radicals inflict, offering defence against radical toxicity. Furthermore, because of their wide spectrum of antioxidant properties, plant-derived terpenoids and flavonoids have drawn a lot of attention lately. The main reason why plants can help in this field is because of the antioxidant properties of their flavonoid and phenolic components [30].

The basis of our research is the in-silico examination, employing AutoDock 4 molecular docking studies, of bioactive chemicals from extracts of medicinal plants *Andrographis paniculata*. Figure 2 illustrates the molecular interaction between the phytocompounds of *Andrographis paniculata* and the target protein, fetal hemoglobin, which is involved in beta thalassemia. Table 3 lists the active sites of fetal hemoglobin for the target protein in β-thalassemia. Upon analyzing phytocompounds containing the target protein's active sites (PDBID: 4MQJ), docking at the following sites for interaction was found: Glu27, Gly51, Pro124, His50, His112, Glu23, Glu30, Ser49, Val135, Arg141, Ser138, Lys139, Val1, Ser84, His97, Arg40, His97, Thr134, and Ser131.

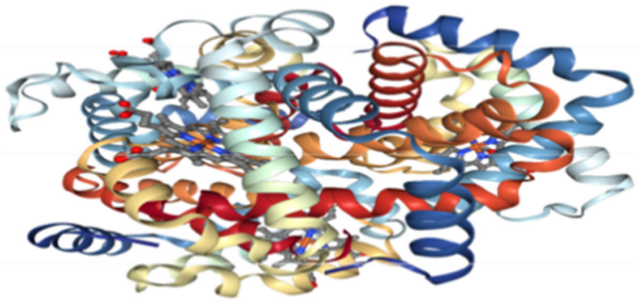

Figure 2. The crystal structure of target protein fetal hemoglobin (PDBID: 4MQJ)

Scoring functions are used by molecular docking systems to approximate the binding energies of anticipated ligand-receptor complexes. The most significant events involved in ligand-receptor binding, including as intermolecular interactions and entropic effects, are evaluated in order to predict binding energy [31]. Computational docking is necessary for drug discovery and development, as well as protein–ligand interaction. A target protein with a known structure that is of therapeutic interest is frequently the first step in the process. Next, the bond conformation and binding-free energy of the small molecules to the target protein are predicted [32]. Potency, affinity, effectiveness, and pharmacokinetic qualities (ADMET, absorption, distribution, metabolism, excretion, and toxicity) are assessed in terms of pharmacodynamic properties [33]. The effectiveness, safety, and legitimacy of natural treatments for beta thalassemia have been investigated through the stimulation of HbF-inducing activities by medicinal plants. Binding scores for interactions with acceptable values were noted. Table 4 displays the satisfactory interaction between 10 phytocompounds and the target protein of beta thalassemia, fetal hemoglobin (PDBID:4MQJ). These compounds, which include DL-alphatocopherol, 3,19-O-diacetylanhdroandrographolide, and 14-acetylandrographolide, were also discovered to interact with the fetal hemoglobin target protein PDBID:4MQJ with notably low binding energies of −12.52 Kcal/mol, −11.22 Kcal/mol, and −11.08 Kcal/mol. Three phytocompounds were selected and shown in Figure 3 based on their binding scores, which allowed for the necessary amounts of interaction with the target proteins. Treatment strategies that reactivate HbF include hydroxyurea, butyrates, and 5-azacytidine; however, these medications have poor specificity, minimal efficacy, and may even be carcinogenic. Research has been done on the efficacy, safety, and validity of using medicinal plants to stimulate HbF generating activities as a natural remedy for beta-thalassemia.

Table 3. Active site of target protein selected for molecular docking studied

S. no.	Disease	Target protein	PDB ID	Active site
1.	Beta thalassemia	Fetal hemoglobin	4MQJ	HIS112, GLU23, ARG141, VAL1, LYS139, ARG30, GLU26, LYS120, ASP43, LEU96, LYS95, SER133, THR137, ALA130, HIS97, GLU121, VAL135, SER52, PRO124, HIS50, SER49, GLU30, SER131, LYS99, GLU119.

Analyses of the phytocompounds from *Andrographis paniculata* that were drug-like, physical-chemical, and ADMET-related were compared with the standard recommended range for each ADMET parameter in Table 5. Through ADMET analysis, these phytocompounds were further assessed for drug-likeness using Lipinski's rule 5. Promising ligands found through molecular docking studies were used to predict the cytotoxicity of medications using SwissADME, as seen in Table 5. The phytocompounds that showed promise were DL-alphatocopherol, 14-acetylandrograp holide, and 3,19-O-diacetylandroandrographolide.These compounds possessed a number of desirable properties, including being soluble or moderately soluble, having a minimum of five hydrogen bond donors and a maximum of ten hydrogen bond acceptors, and meeting Lipinski's rule. High GI absorption and the absence of blood–brain barriers were thought to be the ideal therapeutic ingredients.

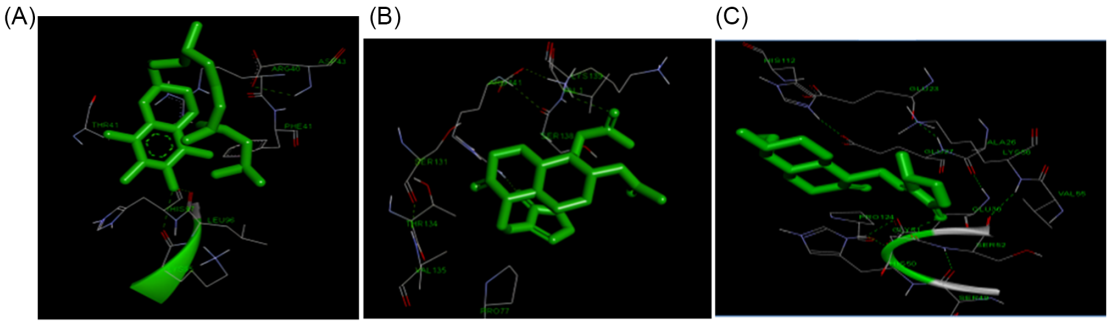

Figure 3. Interaction of DL-alphatocopherol, 14-acetylandrographolide, and 3,19-O-diacetylandroandrographolide with active site residue of the target protein 4MQJ

Table 4. Molecular docking scores of *Andrographis paniculata* phytocompounds with fetal hemoglobin protein PDBID4MQJ

S. no.	Phytocompounds	Ligand_pose	Binding_score
1	DL-alphatocopherol	5	−12.524
2	3,19-O-diacetylanhdroandrographolide	8	−11.228
3	14-acetylandrographolide	7	−11.087
4	3-pentadecylphenol	3	−10.416
5	Andrograpanin	1	−9.413
6	19-O-acetylanhdroandrographolide	3	−8.524
7	14-deoxy11,12-didehydroandrographolide	10	−8.389
8	Isoandrographolide	5	−8.329
9	Neoandrographolide	9	−8.321
10	Andrographolide	9	−8.19

Table 5. SwissADME values for bioactive compounds which have passed the drug-likeness screening (described values are prescribed in bracket)

S. no.	Phytocompounds	Mol.Wt. g/mol range (<500)	GI absorption	Hydrogen bond donor range (<5)	Hydrogen bond acceptor range (<10)	BBB per meant	Water solubility	Number of Lipinski's rules violations range (<4)
1	DL-alphatocopherol	416.66	Low	1	2	No	Poorly soluble	1
2	3,19-O-diacetylandrographolide	388.45	High	0	6	Yes	Soluble	0
3	14-Acetylandrographolide	364.40	High	2	6	No	Soluble	0
4	3-Pentadecylphenol	304.5	Low	1	1	No	Poorly soluble	1
5	Andrograpanin	290.40	High	1	3	Yes	Soluble	0
6	19-O-Acetylandrographolide	346.42	High	1	5	Yes	Soluble	0
7	14-Deoxy11,12-didehydroandrographolide	304.38	High	2	4	Yes	Soluble	0
8	Isoandrographolide	308.37	High	2	5	No	Soluble	0
9.	Neoandrographolide	480.59	High	4	8	No	Moderately soluble	0
10	Andrographolide	322.40	High	3	5	No	Soluble	0

According to Table 5, the current study indicates that three phytocompounds—DL-alphatocopherol, 14-acetylandrographolide, and 3,19-O-diacetylandroandrographolide—had potent antioxidant and antimutagenic actions. These phytocompounds may have potential therapeutic uses. There has been a suggestion that elevated HbF levels may ameliorate the clinical characteristics of individuals with beta thalassemia by improving the equilibrium between A globin chain and B globin chain [34]. Since HbF is the most potent regulator of the hematologic and clinical characteristics of beta thalassemia, where it can replace HbA, researchers have been looking for medications that can raise HbF levels [35]. In beta thalassemia, it has been discovered that the natural inducer HbF suppresses oxidative stress and stomach carcinogenesis [7]. Cell stiffness is thought to be caused by HbF interfering with the polymerized globin chain's whole contact with one another. The study clearly shows that *Andrographis paniculata*, which contains the bulk of phytocompounds, has the highest therapeutic efficacy. The goal of in-silico research in medicine is to reduce the requirement for clinical trials and specialized laboratory work while increasing the rate of discovery. Because of their significant biological qualities, the compounds revealed in this study should be sent for an in-vivo evaluation of their appropriateness as drugs.

4. Conclusion

In recent times, the significance of phytocompounds derived from traditional medicines has grown significantly as targeted therapy for managing various health concerns. This has led to an excessive harvesting of traditional medicinal herbs for the purpose of extracting phytocompounds. Antioxidants found in nature have been proposed as a treatment and prophylactic. Based on the current study's findings, *Andrographis paniculata* methanolic extracts may be a promising source for new medications with strong antioxidant properties. Additionally, through binding to the HIS97 active site of DL-alphatocopherol (PDB ID: 4MQJ), in-silico studies anticipate the role of flavonoids as antioxidants, specifically DL-alphatocopherol, 3,19-O-diacetylanhdroandrographolide, and 14-acetylandrographolide. The safety, efficacy, and authenticity of these medicinal plants' uses have been investigated. Using the SwissADME, bioactive compounds from *Andrographis paniculata* were assessed for their drug-likeness and ADMET. Based on chemical attributes, drug-likeness score, and ADMET model, the phytocompounds DL-alphatocopherol, 3,19-O-diacetylanhydroandrographolide, and 14-acetylandrographolide were selected and screened in this study. It is anticipated that these compounds will have improved toxicity profiles and better drug-like qualities. The available evidence suggested that *Andrographis paniculata* might be effective in treating the illnesses indicated above. To promote the development of innovative modalities, it is essential to comprehend the intricate control of HbF. New pharmacologic approaches to raising HbF levels are currently the subject of research. However, functional studies are needed to confirm their proposed relevance in beta-thalassemia. To confirm the pharmacological effects of bioactive substances, additional controlled experimental study should be carried out. Consequently, it is thought that additional in-vitro and in-vivo studies are required to determine the pharmacological component or significance of these potent medicinal herbs.

5. Future Perspectives

Throughout the beginning of time, people have recognized the importance of medicinal plants for customary and native use. On the other hand, there is no information accessible on the production of medications in the future using specific medicinal plants that have not been scientifically verified. With this in mind, the present study centers on a contemporary methodology pertaining to this noteworthy regenerative plant, which may find application in the production of pharmacological drugs in the future. Our studies of the antioxidant and in-silico capabilities of *Andrographis paniculata* leaves indicate that DL-alphatocopherol from the methanolic extract should be employed in additional drug testing, including in-vitro and in-vivo evaluation, to help confirm its promise for scientific research. Furthermore, to validate more and more data on the effectiveness of these potent phytocompounds, the most potent phytocompounds derived from the corresponding study can be subjected to additional isolation and screening of other activities, as well as pharmacological evaluation in vivo (based on animal studies) or in vitro (in a lab setting; human cell line studies).

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Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

The data in the manuscript are generated by Using AUTODOCK 4.0 for computational analysis. AutoDock 4 (http://autodock.scripps.edu) was utilised for the docking research. The binding mode and interaction of PDBID: 4MQJ with AutoDock 4.0. The operating system used for the investigation of phytocompounds was Windows 10 Home Single Language 64-bit, powered by an Intel Ⓡ Core TM i5-6200U CPU running at 2.30 GHz.

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