Assessment of phytocompounds and antioxidants of in-silico ADMET of plant derived potential inhibitory activity of Andrographis paniculated to managed beta thalassemia

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ABSTRACT: Medicinal plants have been exploited for therapeutic purposes since the dawn of civilization and have long been acknowledged essential to humanhealth. The purpose of this research is to examine the scientific evidence for using the therapeutic herbal plants Andrographis paniculated to managed beta thalassemia illness. The fundamental explanation for the therapeutic relevance of these plants is phytocompounds, which were evaluated qualitatively and quantitatively in three separate extracts with different solvent properties (methanol, chloroform, hexane, ethyl acetate and aqueous) as one of the bases of traditional use. Flavonoids, phenols, tannins, saponins, and alkaloids were all evaluated for their presence in plant extracts, and it was observed that ethanol extract had the highest content of phytocompounds among different extracts whereas, the chloroform extract showed least amount of phytocompounds. Additionally, the antioxidant activity of this plant was also evaluated and methanolic extract was revealed with potential antioxidant activity, as also evidenced by the lowest half inhibitory concentration (IC50) values in the DPPH. The ADMET and drug-likeness properties of bioactive compounds from Andrographis panicluata were evaluated using the SwissADME . The present study chose and screened phytocopounds DL-alphatocopherol, 3,19-O-diacetylanhdroandrographolide, and 14-acetylandrographolide are projected to have better drug-like qualities with improved toxicity profiles based on chemical attributes, drug-likeness score, and ADMET model. Thus, the molecules identified in this study are having profound biological properties and hence worth forwarding them to the in vivo analysis of drug suitability.

Keywords - Beta thalassemia, Andrographis paniculated, phytocompounds, ADMET

I. 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 (WHO) 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 haemoglobin. Due to the inability of their cells to generate the beta polypeptide chain of human haemoglobin, thalassemia patients do not produce enough HbB [6]. Those suffering from β-thalassemia are unable to manufacture β chains. The main haemoglobin seen during pregnancy is fetal haemoglobin (HbF). Gamma globin, which 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 haemoglobin 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 haemoglobin (HbF) synthesis and fetal γ-globin gene expression. Fetal haemoglobin (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 utilised 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. 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 [12]. The injection of antioxidants provides efficient protection against free radicals and reactive species. 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 insilico analysis of phytocompounds from the medicinal plant Andrographis paniculata. We use ADMET 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 haemoglobin in beta thalassemia.

II. Research Methodology

(i) 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 ten to fifteen 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 analyse 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: aquoeus extract):

(ii) Preparation of plant extract by rotary orbital shaker method -The procedure described by Subashini and Rakshitha (2012) was used to make the extract. In order to create the extract, 20 g of powdered shade-dried plant parts, such as leaves and roots, were combined with 200 ml of each of the following solutions: methanol, chloroform, hexane, ethanol, and aqueous solution. The mixture was then placed in a 250 ml conical flask and shaken at 150 rpm for 24 hours.

(iii) Filtration or hot air oven drying up of plant extract - The solvent was mixed for 24 hours, and then the extracts were prepared by filtering the solvent through Whatmann Filter Paper No. 1 and letting it dry in a hot air oven at 37 °C for an additional 24 hours. After that, the crude extracts of each of the five solvents (ME: methanolic extract, CE: chloroform extract, HE: hexane extract, EE: ethanol extract, and AE: aqueous extract) were divided and stored in a refrigerator at 4 °C for additional chemical analysis [13].

(iv) 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 [14].

a. Test for flavonoids Sulphuric acid test: A few drops of H_2SO_4 were added to the crude extracts. Flavonoids were detected by the emergence of orange hue. 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 [15].

b. 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 [15].

c. 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 [15].

d. 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 [16].

e. 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 [16].

f. Test for carbohydrates Molish test: One millilitre of concentrated sulfuric acid was carefully placed along the test tube's walls after two drops of an alcoholic α-napthol solution were added to 2 ml of aqueous extract. Carbohydrates are present when a violet ring forms at the junction.

g. Tests for protein and amino acids Biuret's test: In a test tube, the extract was heated and treated with 1 millilitre of a 10% sodium hydroxide solution. To the mixture mentioned above, a drop of 0.7 percent copper sulphate solution was added. The development of a violet or pink hue signifies the existence of proteins.

h. Tests for glycosides Borntrager'stest: Dilute sulfuric acid was added to 3 ml of test solution, allowed to boil for 5 minutes, 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 [16].

i. Tests for triterpenoids & steroids Salwoski'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].

(v) Quantitative screening or analysis of phytocompounds in different extracts oftotal flavonoid and Phenolics content.

a. 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., 2003. 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 colour development, the tubes were vortexed for 15 seconds and then let to stand at 25°C for 20 minutes. 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× + 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].

b. Determination of total flavonoids

The aluminium chloride colorimetric assay, as reported by Zhisen et al., 1999 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 minutes. 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 minutes. This was done at 6 minutes. 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× + 0.001, R2 = 0.998) was used to express the total flavonoid concentration in milligrammes of CA/g of dry extract. Each concentration saw three iterations of the experiment [19].

(vi) 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.

DPPH radical scavenging assay

The DPPH radical scavenging assay, as reported by Blois, 1953 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 colour 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 thirty 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 [20].

(vii) 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.

ADMET analysis- SwissADME online version was used to estimate the drug-likeness and pharmacokinetic characteristics of phytocompounds [21]. All phytocompounds demonstrated moderately soluble and soluble gastrointestinal absorption, with no BBB permeability, according to the pharmacokinetic parameters; nonetheless, the bioavailability score indicated drug-likeness.

(viii) 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 [22]. 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 [23].

III Results and Discussion

Using GC-MS analysis, ten phytocompounds from Andrographis paniculata were discovered. Eight phytocompounds' three-dimensional (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 [24]. The immune system is

strengthened by the antioxidant, anti-inflammatory, antibacterial, antimutagenic, and antiadhesion qualities of Andrographis paniculata plant [25]. Since the beginning of time, phytocompounds and secondary metabolites have been crucial for therapeutic purposes [26]. 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 mg/gm 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, ethylacetate, 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 ability of leaf extracts from Andrographis paniculata to scavenge DPPH radicals in aqueous extracts, methanol, ethanol, hexane, ethyl acetate, and chloroform. At a concentration of 100 mg/ml in 50.04%, the methanolic extracts were found to have the maximum antioxidant activity, followed by ethylacetate and chloroform in 34.8 % and 25.16 %, respectively.

Table1- Total phenolic content and total flavanoid content of Andrographis paniculata

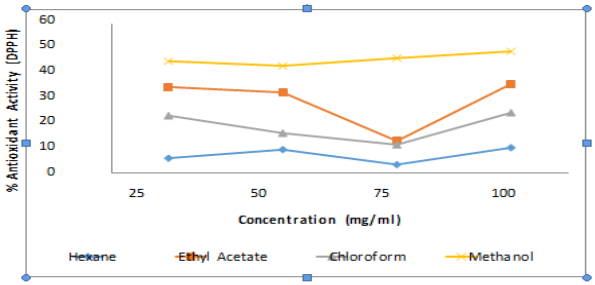
S.No	Solvent	Total Phenol (Mg/gm)	Total Flavanoid (Mg/gm)
1.	Aqueous	16.58	0.18
2.	Methanol	46.93	0.48
3.	Ethanol	65.89	0.89
4.	Hexane	22.34	0.23
5.	Chloroform	13.67	0.13

Table 2- The analysis of phytocomponds in the hexane, ethyl acetate, chloroform, ethanol, methanol, aqueous extract of Andrographis paniculate.

S.No	Tests Employed	Extracts Used: Leaf					
		Aqueous	Methanol	Ethanol	Hexane	Ethyl acetate	Chloroform
1.	Saponnin	-	+	+	-	+	+
2.	Tannin	+	+	+	+	+	-
3.	Flavanoid	+	+	+	-	-	-
4.	Phenol	+	+	+	+	+	+
5.	Alkaloid	+	+	+	+	+	+
6.	Glycosides	-	+	+	+	+	+
7.	Carbohydrate	+	+	+	+	+	+
8.	Terpenoid	+	-	-	+	+	+
9.	Aminoacids& Proteins	-	+	+	+	+	+
10.	Fats & oil	+	+	+	-	+	+

*** + = presence; - = absence**

Figure - 1 DPPH radical scavenging activity by Andrographispaniculataleaf hexane extract, ascorbic acid, ethyl acetate extract, chloroform extract, and methanol extract



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 [27]. Furthermore, flavonoids are polyphenolic compounds with well-known

characteristics like scavenging free radicals and inhibiting oxidative and hydrolytic 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. 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 [28].

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 3**. Through ADMET analysis, these phytocompounds were further assessed for druglikeness 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 3**. The phytocompounds that showed promise were DL-alphatocopherol, 14-acetylandrographolide, 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 gastrointestinal (GI) absorption and the absence of blood-brain barriers were thought to be the ideal therapeutic ingredients.

Table 3- 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 Permeant**	**Water Solubility**	**Number of Lipinsk'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	Andrographanin	290.40	High	1	3	Yes	Soluble	0
6	19-O-Acetylandrographolide	346.42	High	1	5	Yes	Soluble	0
7	14-Deoxy11,1	304.38	High	2	4	Yes	Soluble	0

	2-didehydroandrographolide							
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 thalasseima by improving the equilibrium between A globin chain and B globin chian [29]. Since HbF is the most potent regulator of the hematologic and clinical characteristics of beta thalasseima, where it can replace HbA, researchers have been looking for medications that can raise HbF levels [30]. In beta thalassemia, it has been discovered that the natural inducer HbF suppresses oxidative stress and stomach carcinogenesis. 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.

IV 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 current literature on in-silico studies, however, focuses primarily on the therapeutic efficacy of important phytocompounds. Of particular note is DL-alphatocopherol, which is a naturally occurring inducer for managing beta-thalassemia by stimulating HbF inducing activities by medicinal plants. The safety, efficacy, and authenticity of these medicinal plants' uses have been investigated. Using the SwissADME, bioactive compounds from Andrographis panicluata 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 panicluatamight be effective in treating the illnesses indicated above. 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.

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