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Research Article

IDENTIFICATION OF PHYTOCOMPOUNDS FROM *ARGEMONE MEXICANA* AS INHIBITORS OF EPSTEIN-BARR NUCLEAR ANTIGEN TO COMBAT INFECTIOUS MONONUCLEOSIS

MOHAMMED SHAFIQ K, ROHIT HARISH

Department of B. Tech Biotechnology, Sri Shakthi Institute of Engineering and Technology, Coimbatore, Tamil Nadu, India.
Email: Shafiq62001@gmail.com

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ABSTRACT

Objectives: Mono or infectious mononucleosis (IM) is often referred to as the kissing illness. Epstein-Barr virus (EBV), which causes mono, is spread by saliva. Kissing, sharing a drink, or eating utensils with a person who has mononucleosis can transmit the disease to healthy individuals. This study investigates several bioactive compounds derived from plants to forecast how effective plant-based ligands will be at preventing IM.

Methods: The purpose of the current study was to use computational techniques to assess the effectiveness of several phytochemicals against the EBV. The virtual screening tool PyRx was used to systematically perform molecular docking. The top 6 phytocompounds from *Argemone mexicana* were chosen among them to test their compatibility with the EBV nuclear antigen. Using ADMET filters, the ligands' pharmacological evaluation was performed.

Results: The phytocompounds Coptisine, Sanguinarine, and Dihydrosanguinarine from the plant *A. mexicana* were discovered to be the most potent antagonistic for the proteins EBV Nuclear Antigen 1 and EBV nuclear antigen 2.

Conclusion: All of these bioactive chemicals could be considered of as deserving candidates for the suppression of IM due to their strong affinity for the protein. Among the top ligand, the phytoconstituent Coptisine demonstrated better binding with both targets.

Keywords: Infectious mononucleosis, *Argemone mexicana*, Epstein-Barr Nuclear Antigen 1 and Epstein-Barr virus nuclear antigen 2 antigen.

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INTRODUCTION

Epstein-Barr virus (EBV), a double-stranded DNA virus from the Herpes family, is the main contributor underlying infectious mononucleosis (IM), a common condition with a 90% prevalence rate [1]. Fever, lymphadenopathy, and pharyngitis are among the symptoms of IM, also known as glandular fever, which typically shows after an incubation period of four to seven weeks [1,2]. The vast majority of IM patients have a good prognosis and are self-limiting, though acute complications such as splenic rupture, hepatitis, and severe tonsil enlargement with airway obstruction do occur on occasion [3,4]. EBV has long been suspected of being involved in diseases other than IM. A detailed overview of the literature reveals a long list of correlations, including those between thyroid conditions, autoimmune diseases such as inflammatory arthritis and multiple sclerosis, as well as lymphoproliferative disorders, head and neck cancer, breast cancer, systemic lupus erythematosus, Vitamin D deficiency, and chronic fatigue syndrome [5].

EBV is responsible for 90% of IM cases, with the remaining 10% being brought on by human herpesvirus 6, herpes simplex virus type 1, cytomegalovirus, and human immunodeficiency virus (HIV). Since EBV is present in saliva, it can transmit through coughing, sharing food, and kissing (thus the informal term "kissing disease"). EBV can be found in trace amounts in the saliva of infected individuals for the rest of their lives [3-5]. However, a study has revealed that patients may remain extremely contagious for up to 180 days following the onset of symptoms, and possibly even beyond that. Peak levels are reported during the acute phase of the infection [1].

In latently infected cells, EBV DNA is often carried as circular DNA or episomes and only rarely integrates into the genome of the host cell. The replication of viral DNA before mitosis and the distribution of episomes into progeny cells during cell division are therefore required by EBV [2]. To carry out these functions, EBV Nuclear Antigen 1 (EBNA 1) first initiates replication by binding to the episome with its COOH-terminal domain and then acts as a protein anchor by cross-linking the episome to mitotic chromosomes [6]. As a result, in healthy EBV carriers and all EBV-associated malignancies, EBNA 1 is expressed in proliferating cells. The multifunctional protein EBNA 1 is encoded by the EBV. It is widely known that EBNA 1 controls viral gene transcription and mediates EBV genome synthesis and non-random partitioning to daughter cells to retain EBV in latently proliferating cells [7]. The functions of EBNA 1 in altering the host cell to give EBV-carrying cells a selection advantage is less well known [8].

However, the idea of using EBNA 1 as a therapeutic target is new. The first small-molecule EBNA 1 inhibitors were just recently found, and two EBNA 1 crystal structures are available to the general public. On the structural specifics of EBNA 1 "druggable" binding sites; however, no comprehensive investigations have been reported [9].

The EBNA-2 is one of the six EBV viral nuclear proteins expressed in latently infected B lymphocytes and is a transactivator protein. EBV Nuclear Antigen-2 (EBNA 2) is involved in the regulation of latent viral transcription and contributes to the immortalization of EBV-infected cells [10]. EBNA 2 acts as an adapter molecule that binds to cellular sequence-specific DNA-binding proteins, JK recombination signal-binding protein, and PU.1 as well as working with multiple members of the RNA polymerase II transcription complex [11].

Medicinal plants contain certain therapeutic properties. In native areas, they serve as an easily accessible source of healthcare [12]. *Argemone mexicana* was used in this study. Although it is toxic to grazing animals and is rarely eaten, it is used for a variety of medicinal purposes. It is used to treat non-healing wounds, constipation, malaria, chronic fever, and other ailments. A single plant can be used to create a multitude of novel drugs and act as a foundation for those discoveries [13]. This

plant, a weed that is easily accessible and requires no special care, serves as the foundation for the development of a medicine that is affordable to everyone. *A. mexicana* has pharmacological properties such as anti-diabetic, anti-cancer, anti-HIV, and CNS-related capabilities, wound healing action, antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, anti-fertility, anti-allergic and allelopathic effects, and so on. Numerous microorganisms, including viruses, bacteria, and harmful fungi, are inhibited by the *A. mexicana* plant [14,15]. The most adaptable unicellular pathogens, bacteria commonly cause a wide range of infectious diseases in both humans and animals. They are spread by soil, water, air, and food. Such illnesses are treatable with a wide variety of natural substances derived from healing plants. *A. mexicana* had powerful antiviral effects against a variety of viruses, according to numerous investigations. The active components of *.cana* successfully prevented viral replication while enhancing the immune system in Pacific white-leg shrimp. Due to the pharmacological activities displayed by the bioactive phytocompounds of *A. mexicana,* the bioactive constituents were investigated for their therapeutic efficacy against Epstein-Barr Nuclear Antigenic protein targets [15].

METHODS

Retrieval of ligands

The IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) database (https://cb.imsc.res.in/imppat/) was used to look for potential ligands [16]. The canonical smiles of all the ligands selected for the current experiment were recorded. The top 24 ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in. SDF format [17].

Retrieval of proteins

The Crystal structure of EBNA-1 with PDB ID 6NPP was downloaded from the PDB databank (https://www.rcsb.org/structure/6NPP). The protein was downloaded in. PDB format. The resolution of the protein downloaded is 1.35Å and the method of retrieval of the protein is X-ray diffraction [18].

Homology modeling

In contrast to all other approaches, comparative homology modeling is the sole technique that can precisely build a 3-D model of a protein from its provided amino acid composition. Swiss Model (https://swissmodel.expasy.org/interactive/2fPUee/models/) was employed to build EBNA-2 protein 3D model [19]. The FASTA sequence of the protein was retrieved from the UniProt database (https://www.uniprot.org/). The template 2n2j.1.A Epstein-Barr nuclear antigen 2 with a sequence identity of 98.28% was selected to build the model. The best model was selected based on their GMQE (Global Mean Quality Estimate) and QMEANDisCo values. The nuclear antigen proteins (EBNA-1 and EBNA-2) were purified.

Protein purification

Proteins are purified before docking and the following protocol was followed: The crystallographic structure does not match the free energy of the water molecule. Water molecules were completely removed before docking because they can have an impact on docking scores. To accelerate binding with the ligands chosen for the study, the prebound ligands are removed from the crystal structures. The protein structures were simplified by removing extra chains while leaving chain A intact for analysis. Polar hydrogen atoms are introduced to improve purified structures. The protein purification was achieved in DS Biovia Discovery Studio [20].

Pharmacological studies

To assess the ligands' pharmacological characteristics, SwissADME (http://www.swissadme.ch) analysis was used [21]. The physicochemical qualities evaluated are Lipophilicity, Polarity, Insolubility, Size, Flexibility, and Instauration. The best ligands are then chosen using the LIPINSKI rule of five. ADMETLAB 2.0 (https://admetmesh.scbdd.com) was employed to evaluate the toxicity of the ligands [22,23].

Molecular docking

The purified protein (EBNA-1 and EBNA-2) was uploaded into PyRx as a macromolecule and the plant's phytocompounds were loaded into PyRx as ligands [24]. The ligands were converted from.SDF format to.PDB format using the OPENBABEL [22]. Grid dimensions of center X=0.4482, center Y=4.3588, and center Z=14.7741 (EBNA-1) and center X=1.4015, center Y=1.2140, and center Z=4.1008 (EBNA-2) were chosen for the active site. The ligands were docked independently against EBNA-1 and EBNA-2 using the PyRx web server and energy reduction was done. The most effective compounds, chelerythrine, sanguinarine, protopine, dihydrosanguinarine, benzophenanthridine, and coptisine, were chosen for further research based on their binding affinity with the target protein after the docking findings were received.

Visualization

Using Dassault Systems BIOVIA Discovery Studio Visualizer, the conformations with the highest binding scores were downloaded in.PDB format, and the 2D and 3D models were produced [20].

RESULTS

Protein structure analysis

The Ramachandran plot is used to see the energetically permissible areas where amino acid torsions are angled against one another in a protein structure. The Alpha-synuclein Ramachandran plot, displayed in Figs. 1 and 2, was created using PROCHECK. The sterically permissible regions on the graph that allows for stable peptide conformation are represented by the red areas on the graph. 107 amino acid residues, or 96.4%, fall in the preferred region, while 0 residues, or 0.0%, fall in the prohibited zone. The sub-regions account for the remaining 3.6%. Out of the 137 residues, 111 are non-proline and non-glycine residues, 11 are glycine residues, 14 are proline residues, and 1 is an end residue (Fig. 1). 42 amino acid residues, that is, 93.3%, fall in the favored region, while 0 residues, that is, 0.0%, fall in the prohibited zone. The sub-regions account for the remaining 6.7%. Out of the 58 residues, 45 are non-proline and non-glycine residues, 5 are glycine residues, 6 are proline residues, and 2 are end residues (Fig. 2).

As depicted in Fig. 3, the protein EBNA-1's predicted secondary structure includes 1 sheet, 1 beta hairpins, 2 beta bulges, 5 strands, 4 helices, 3 helix-helix, 7 beta turns, and 1 gamma turn according to the PDBsum data as shown in Fig. 3.

As depicted in Fig. 4, the protein EBNA-2's predicted secondary structure includes 2 sheets, 1 beta-hairpin, 1 beta bulge, 4 strands, 1 helix, 5 beta turns, and 3 gamma turns according to the PDBsum data as shown in Fig. 4.

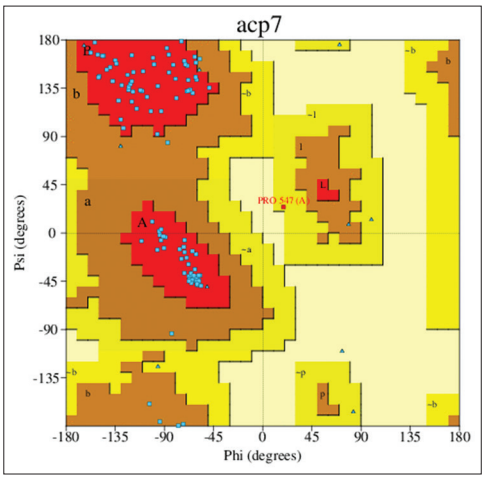

Fig. 1: Ramachandran plot of EBNA-1 protein using PDBsum

Drug likeliness analysis

Important requirements for a compound to be employed in drug products include its physicochemical features, ADMET properties, and Lipinski rule of 5. For the production of medicine with the fewest side effects, toxicity prediction and aggregate data are also crucial. Therefore, the phytocompounds retrieved from *A. mexicana* were subjected to pharmacological studies to determine their drug-likeness properties.

Pharmacological studies

The phytoconstituents from *A. mexicana* were subjected to physicochemical screening based on the parameters listed in Table 1 and the physicochemical properties of the top ligands are listed in Table 2.

Lipinski filter analysis

The Lipinski rule has five parameters, which are listed in Table 3, and a compound must adhere to at least four of them. As per the results (Table 4), it is evident that the top five ligands fulfill the Lipinski Rule of 5 without any violations.

ADME analysis

An ADME analysis has four key characteristics. The extent of the substance's intracranial movement is constrained by the Blood–Brain Barrier (BBB). This information is essential for creating a medicine. The amount of gastrointestinal (GI) adsorption should be substantial to maximize the drug's efficacy. In addition, the material must be easily soluble. Less negative solubility levels are consequently accepted. As per the results (Table 5), it is evident that all the ligands have high gastrointestinal absorption and they also have good solubility and bioavailability.

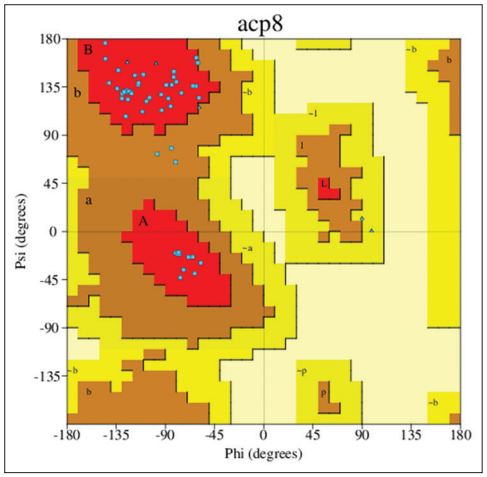

Fig. 2: Ramachandran plot of EBNA-2 protein using PDBsum

Table 1: Parameters for physicochemical properties

Properties		Optimal range
Lipophilicity	xLogP	−0.7–+5.0
Size	MW	150–500 g/moL
Polarity	TPSA	20–130
Saturation	Sp3 hybridization	Not<0.25
Flexibility	Rotatable bonds	Not more than 9

Toxicity prediction

The following are some of the key characteristics of toxicity prediction. Skin sensitivity, carcinogenicity, respiratory toxicity, AMES toxicity, Rat Oral Acute Toxicity, FDAMDD, hERG blockers, H-HT, and DILI parameters were evaluated to determine the toxicity of the top five ligands (Table 6).

Molecular docking

The binding affinity of all the selected ligands toward the EBNA-1 and EBNA-2 protein as obtained by PyRx is enlisted in Tables 7 and 8, respectively.

For further analysis, the docking conformation that had the maximum binding energy was taken into consideration. The phytocompounds above −6.2, or the compounds coptisine, sanguinarine, and dihydrosanguinarine, were shown to have the highest binding energy in this investigation. Therefore, these three are considered for further analysis.

Visualization

The ligand Copstine demonstrated the least binding with both the target proteins (EBNA1 and EBNA2). Therefore, the binding interactions of copstine with ENBA1 (Fig. 5) and EBNA2 (Fig. 6) were visualized. From the 2D and 3D interaction diagram, it is evident that the ligand interacts with the protein by establishing a bond with significant amino groups such as PRO, LEU, TRP, TYR, and ARG.

DISCUSSION

EBV was discovered 36 years ago by Epstein, Achong, and Barr using electron microscopy on cells cultured from Burkitt's lymphoma tissue. Over 90% of people worldwide have been infected with the herpesvirus known as EBV. Cell-associated and cell-free EBV are present in peripheral blood during primary EBV infection, such as IM. In a latent infection, some altered B cells seep into the blood. Virion production occurs in plasma cells during lytic infection, and encapsidated viral genomes (virions) are released into the peripheral circulation. In addition, cell-free EBV DNA from apoptotic cells may be seen in peripheral blood as fragmented or bare DNA. Hence, PBMCs and cell-free blood (serum or plasma) can both contain EBV DNA [3,5].

A clinical condition known as IM is characterized by pharyngitis, swelling of the cervical lymph nodes, fatigue, and fever. There is no seasonal preference in the disease's occurrence worldwide. For reasons that are not entirely clear, it is most frequently diagnosed among teens and young adults from wealthy countries. Lack of awareness of the illness in preadolescents is one factor in the explanation [4]. In young infants, especially those under the age of four, the heterophile antibody test is frequently inaccurate. EBV DNA testing is not necessary for the diagnosis of IM because it is a self-limiting illness that is typically identified by clinical signs and symptoms as well as serology. IM, on the other hand, shows a correlation between the disease severity and EBV

Table 2: Physicochemical properties

Ligand	MW	Fraction Csp3	Rotatable bonds	TPSA	Lipophilicity
Chelerythrine	348.37	0.19	2	40.8	4.58
Sanguinarine	332.33	0.15	0	40.8	4.45
Coptisine	320.32	0.21	0	40.8	3.49
Benzophenanthridine	229.28	0	0	12.89	4.73
Protopine	353.37	0.35	0	57.23	2.79
Dihydrosanguinarine	333.34	0.2	0	40.16	4.12

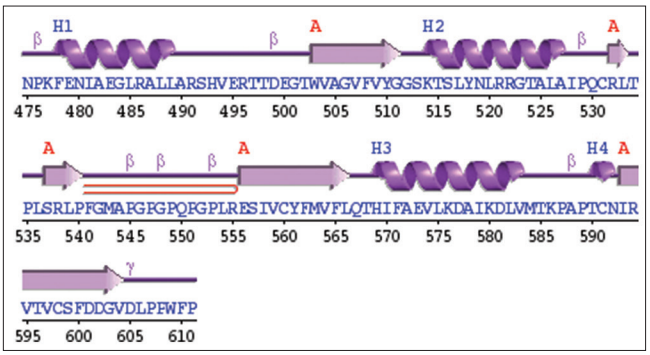

Fig. 3: Secondary structure of protein EBNA-1 using PDBsum

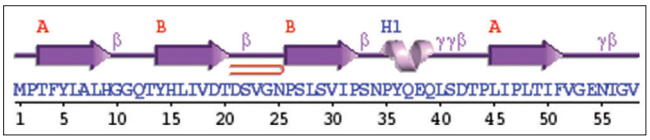

Fig. 4: Secondary structure of protein EBNA-2 using PDBsum

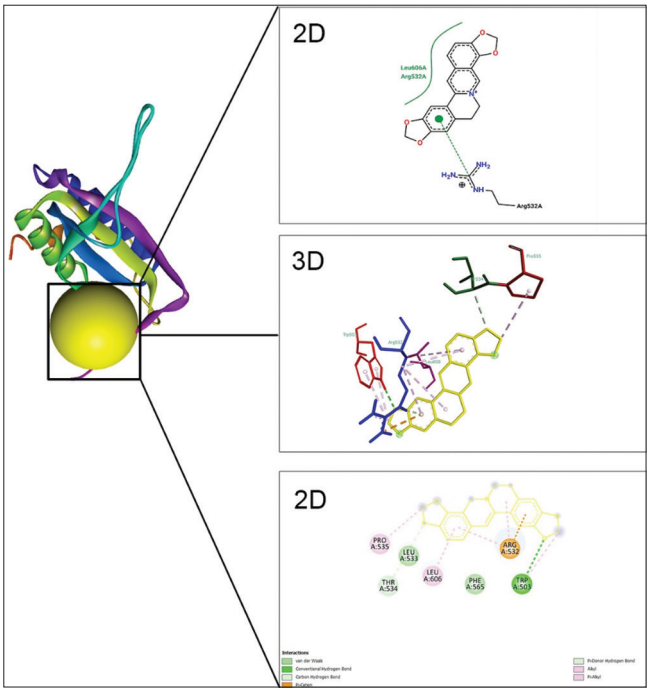

Fig. 5: Visualization of molecular interactions of EBNA-1 with Coptisine ligand

load in serum or plasma. The hemophagocytic syndrome can sometimes be brought on by primary EBV infection [6]. This condition, also known as hemophagocytic lymphohistiocytosis, is uncommon in Western nations but widespread in eastern Asia. Early detection and aggressive treatment are required because EBV-associated hemophagocytic syndrome can be a serious, even fatal, and condition. Both the PBMCs and the serum of patients with EBV-associated hemophagocytic syndrome exhibit extremely high viral loads. For assessing the effectiveness of the treatment, monitoring EBV DNA in serum is helpful [1].

Epstein-Barr nuclear antigen 1, or EBNA-1, is required for the replication of the EBV genome as an extrachromosomal element and is a key transcriptional regulator of this virus's latent gene expression. In addition to affecting EBV lytic infection, EBNA 1 plays several significant roles in EBV latent infection. EBNA 1 interacts with particular DNA

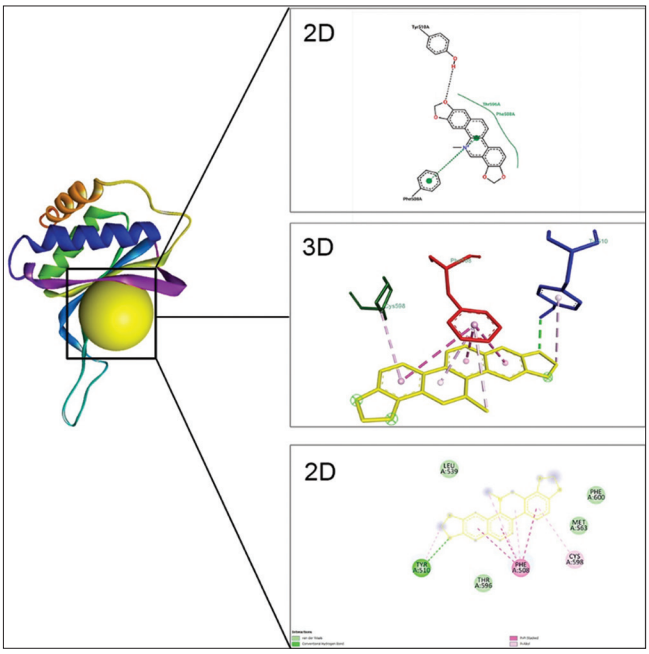

Fig. 6: Visualization of molecular interactions of EBNA-2 with Sanguinarine ligand

Table 3: Lipinski rule parameters

Properties	Optimal range
MW	150–500 daltons
MlogP	<4.15
H donors	<5
H acceptors	<10
MR	40–130

sequences in the viral episomes to stimulate the expression of other EBV latency genes and is necessary for the stable persistence of the EBV genomes in latent infection [7,9].

A. mexicana has a strong pharmacological profile and a long history of use. The entire plant has largely been used as an analgesic, antispasmodic, depurative, emmenagogue, psychedelic, sedative, and vulnerary. Since it includes alkaloids, it has some minor analgesic properties [12]. The aqueous extract's flavonoids, which are both antioxidants and free radical scavengers, have been shown to have anti-inflammatory benefits. An infusion of the leaves is administered to treat diseases of the spleen, and liver, and for jaundice or whooping cough. To treat asthma, cough, and fever, the young leaves or blooms are infused and administered orally. Numerous microorganisms, including viruses, bacteria, and harmful fungi, are inhibited by the *A. mexicana* plant [13]. The most adaptable unicellular pathogens, bacteria commonly cause a wide range of infectious diseases in both humans and animals. They are spread by soil, water, air, and food. Such illnesses are treatable with a wide variety of natural substances derived from healing plants. *A. mexicana* had powerful antiviral effects against a variety of viruses, according to numerous investigations. In Pacific white leg shrimp *Litopenaeus vannamei*, the active components of *A. mexicana* successfully suppressed viral replication and stimulated the immune system against the white spot syndrome virus [14].

The results of the current study suggest that the phytocompounds chelerythrine, sanguinarine, protopine, dihydrosanguinarine, benzophenanthridine, and coptisine displayed the best binding with both the protein targets (EBNA 1 and EBNA 2). Specifically, the phytocompound coptisine was an effective inhibitor of both EBNA 1 and EBNA 2 proteins.

Table 4: Data for the properties of Lipinski rule obtained using SwissADME

Ligand	MW	MLogP	H Donors	H acceptors	Molar refractivity
Chelerythrine	348.37	2.53	0	4	101.6
Sanguinarine	332.33	2.72	0	4	94.68
Coptisine	320.32	2.37	0	4	87.95
Benzophenanthridine	229.28	3.62	0	1	76.76
Protopine	353.37	1.9	0	6	97.49
Dihydrosanguinarine	333.34	2.86	0	4	96.7

Table 5: ADME data obtained using SwissADME

Ligands	BBB	GI Absorption	PGP substrate	Solubility (LOGSw-SILICOS IT)
Chelerythrine	Yes	High	Yes	−6.59
Sanguinarine	Yes	High	Yes	−6.09
Coptisine	Yes	High	Yes	−5.43
Benzophenanthridine	Yes	High	Yes	−7.06
Protopine	Yes	High	Yes	−5.38
Dihydrosanguinarine	Yes	High	Yes	−6.06

Table 6: Toxicity analysis

Ligands	hERG	H-HT	DILI	Ames	ROA	Carcinogenicity	Respiratory
Chelerythrine	0.067	0.119	0.908	0.905	0.097	0.957	0.779
Sanguinarine	0.037	0.096	0.897	0.954	0.047	0.966	0.865
Coptisine	0.132	0.057	0.533	0.642	0.095	0.957	0.884
Benzophenanthridine	0.356	0.177	0.933	0.878	0.151	0.814	0.953
Protopine	0.523	0.284	0.889	0.25	0.21	0.875	0.625
Dihydrosanguinarine	0.216	0.094	0.886	0.855	0.103	0.956	0.906

Table 7: Docking score of EBNA-1 protein with selected ligands

Ligand	Binding affinity with EBNA1
Chelerythrine	−6.6
Sanguinarine	−7.2
Coptisine	−7.6
Benzophenanthridine	−6.7
Protopine	−6.9
Dihydrosanguinarine	−7.1

Table 8: Docking score of EBNA-2 protein with selected ligands

Ligand	Binding Affinity with EBNA1
Chelerythrine	−6.2
Sanguinarine	−7.0
Coptisine	−7.2
Benzophenanthridine	−6.2
Protopine	−6.6
Dihydrosanguinarine	−7.0

CONCLUSION

In recent years, IM has become more common, typically affecting young adults. Numerous vaccines are being developed to eradicate this disease. Just as important as vaccinations are the medicinal plants that can be used to make medications to treat IM. One such plant with numerous therapeutic properties is *A. mexicana*. In this approach, improved prevention and treatment can be achieved with the help of several studies. Because of the growing use of medicinal plants in medication development, medicine will undergo a substantial change.

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AUTHORS CONTRIBUTION

All the authors have equally contributed to the research and manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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