In-Silico Identification Of Phytochemical Ligands As A Drug Candidate For The Treatment Of Spinal Muscular Atrophy

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "*In-Silico* Identification of Phytochemical Ligands as a Drug Candidate for the Treatment of Spinal Muscular Atrophy" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr.Sudhakar Singh, working as Assistant Professor, in the School of Bioengineering & Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "*In-Silico* Identification of Phytochemical Ligands as a Drug Candidate for the Treatment of Spinal Muscular Atrophy" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the School of Bioengineering & Biosciences, is a research work carried out by Pankaj Bagga, 42200285 , is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Spinal muscular atrophy (SMA) is an autosomal recessive inherited neuromuscular condition distinguished by the deterioration of alpha motor neurons within the spinal cord. It is a major hereditary cause of newborn and early childhood death. The estimated incidence is one in every 6,000 to 10,000 live births, with a carrier frequency of 1/40-1/60. The telomeric or SMN1 gene, which determines spinal muscular atrophy and the centromeric or SMN2 gene are two nearly similar SMN genes found on chromosome 5q13.2.More than 95% of patients suffer from a homozygous deletion of the *SMN1* gene, which encodes the SMN protein. Insufficient SMN protein in neurons can cause muscular weakness, atrophy, respiratory failure, and even death. SMN2's coding sequence changes from SMN1 by a single nucleotide (C to T in exon 7). This nucleotide change causes modification of splicing, resulting in the exclusion of exon 7 from *SMN2* mRNA and the generation of SMNΔ7, a shortened and unstable protein. SMA severity ranges from mild to severe based on SMN protein levels, indicating an inverse link.

The SMA is categorized into five severity grades: SMA 0 (This is a rare form that affects the fetus prior to birth. Fetal movements are often diminished prior to birth. Type 0 newborns suffer severe muscular weakness at birth and frequently die from respiratory failure. Death typically occurs at birth or within the first month of life), SMA I (Type 1, also known as Werdnig-Hoffman disease, accounts for approximately 60% of all SMA cases. Symptoms appear within the first six months of birth and include reduced head control and muscular tone (hypotonia). Infants with type 1 SMA have difficulties swallowing and breathing. Without breathing support, children with type 1 SMA die before reaching their second birthday), SMA II (Symptoms of type 2 SMA (also known as Dubowitz disease) occur between six to 18 months of age. Symptoms include hypotonia and increased muscle weakness, which primarily

affects their legs. Children with type 2 SMA may be able to sit up but not walk. Around 70% of people with type 2 diabetes will live to the age of 25, with some even reaching their 30s. Respiratory disorders are the leading cause of death), SMA III (The symptoms of type 3 SMA (also known as Kugelbert-Welander disease) occur after a child's first 18 months. Lower limb muscle weakness, which causes difficulties walking, is one of the Type 3 symptoms. People with type 3 MSA rarely experience respiratory problems, and it usually has little effect on life expectancy) and SMA IV (This is the mildest variant of SMA. It normally appears after the age of 21. Muscle weakening symptoms develop gradually; therefore most persons with type 4 remain mobile. It rarely has an impact on life expectancy). Human testing indicates that a number of therapeutic techniques are promising and three drugs have been authorized as far by the Food and Drug Administration (FDA) of the United States. Between 2016 and 2020, the United States Food and Drug Administration (FDA) approved therapies that can considerably improve the course of SMA. They include disease-modifying treatments (These drugs increase the production of SMN2 protein. The FDA has approved Nusinersen (Spinraza®) for use in both children and adults. A healthcare provider injects the drug into the area surrounding your spinal canal. Risdiplam (Evrysdi®) is a different drug that can help those with SMA who are two months or older. Risdiplam is taken orally once day). Gene replacement therapy (Children under the age of two may benefit from a single intravenous (IV) infusion of the medicine onasemnogene abeparvovec-xioi (Zolgensma®). This therapy replaces a missing or malfunctioning SMN1 gene with a functional one).

Notwithstanding the shown potential of these authorized treatments, there are a number of significant drawbacks, chief among them being their cost. The FDA-approved medications are among the priciest medicines available worldwide and are rather costly. The cost is still too high and could put patients under financial strain. Herein, we have applied bioinformatics approaches to predict the target protein sequence

using NCBI's GenBank/ UniProt, domain prediction using PROSITE, SMART, and InterProScan databases and secondary structure analysis using PSIPRED and SOPMA. After that we analyze the structure of our taget protein avaiable on PDB and structure validation using PROCHECK and ProSA- Web. Active/binding Site predictions have been determined using CASTp and PDBsum. PubChem database was used to Identification of ligands from selected medicinal plants (*Brucea javanica* and *Tripterygium wilfordii).* The Autodock tool was used to perform virtual screening of phytochemicals.ADMETLab3.0 tool was used for pharmacological properties analysis of phytochemicals. Molecular dynamics (MD) simulations by GROMACS (Groningen Machine for Chemical Simulations) 2022.3 to explore the structural flexibility of the apo-protein and protein-phytochemical complexes obtained from the virtual screening and ADMET property analysis.

Currently, there are limited therapeutic options and there is an imminent medical need for new medications based on phytochemicals. The growth of computer methods and the explosion of biomedical data have created new avenues for the development of SMA treatments. This study focuses on the potential of phytochemicals derived from *Brucea javanica* and *Tripterygium wilfordii* as alternative agents for the treatment of SMA. Using molecular docking, a dataset of 115 phytochemicals from two plants was used to determine binding affinities with the SMN1 protein. A 1000ns molecular dynamics (MD) simulation were performed on the high-binding affinities compounds to elucidate their stability and interaction dynamics in a physiological state. Yadanzioside-M and Triptersinine-A were two leading phytochemicals that developed sustained interactions with the SMN1 protein. Specifically, Yadanzioside- M formed hydrogen bonds with W92, S143, C146, D147, and finally D134 and S103. Its multi-cyclic structure exhibited pi-pi interactions with aromatic amino acids that enhanced the stability of protein-ligand. Likewise, Triptersinine- A established hydrogen bonds with R133, Y130, G129 and G131, as well as pi-pi interactions inside the

Tudor domain's aromatic cage, indicating a high binding affinity. These findings highlight the utility of the phytochemicals as neuroprotective agents with a prolonged bind to the target protein, suggesting that these are potential therapeutic agents for SMA. Further experimental and clinical research is needed to validate their efficacy and safety for therapeutic application.

Keywords: Spinal Muscular Atrophy, Molecular docking, Molecular dynamics (MD) simulations, *Brucea javanica*, *Tripterygium wilfordii*.

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Pankaj Bagga

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List of Abbreviations

SMA	Spinal Muscular Atrophy
FDA	Food and Drug Administration
SMART	Simple Modular Architecture Research Tool
InterPro	The integrative protein signature database
SMN	Survival Motor Neuron
SMN1	Survival Motor Neuron 1
SMN2	Survival Motor Neuron 2
ISCCSMA	The International Standard of Care Committee for SMA
snRNPs	Small ribonucleoproteins
EMG	Electromyography
ACOG	American Congress of Obstetricians and Gynecologists
CNS	central nervous system
NHS	National Health Service
TCM	Traditional Chinese Medicine
BDNF	Brain-derived neurotrophic factor
NGF	Nerve growth factor
TCM-DAP	Traditional Chinese Medicine Database and Analysis Platform

IMPPAT	Indian Medicinal Plants, Phytochemistry and Therapeutics
NCBI	National Center for Biotechnology Information
OMIM	Online Mendelian Inheritance in Man
UCSC	The University of California Santa Cruz
SOPMA	Self-Optimized Prediction method with Alignment
PDB	Protein Data Bank
PSVS	The Protein Structure Validation Software
MD	Molecular Dynamics
GROMACS	Groningen Machine for Chemical Simulations
SMILE	Simplified Molecular Input Line Entry System.
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
OPLS-AA	Optimized Potentials for Liquid Simulations, All-Atom
NVT	Constant number of Particles ,Volume and Temperature
NPT	Constant number of Particles ,Pressure and Temperature
SASA	Solvent-Accessible Surface Area
RMSD	Root Mean Square Deviation

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CHAPTER 1: INTRODUCTION

1.1 Disease Overview

Spinal Muscular Atrophy (SMA) is a severe, progressive neurodegenerative disorder characterized by motor neuron loss leading to muscle weakness, hypotonia, and atrophy. It is one of the most common genetic causes of neonatal and early childhood mortality, with a prevalence ranging from 1 in 6,000 to 1 in 10,000 births (Zhang et al., 2024).

1.2 Molecular Pathophysiology and Mechanism of SMA

Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disorder primarily caused by deletions or mutations in the survival motor neuron 1 (*SMN1*) gene located on chromosome 5q13.2. The *SMN1* gene encodes the survival motor neuron (SMN) protein, which is essential for the maintenance and function of motor neurons in the brainstem and spinal cord. Deficiency of the SMN protein due to *SMN1* loss leads to degeneration of alpha motor neurons, resulting in muscular weakness, atrophy, and progressive motor dysfunction. SMA is recognized as one of the most common hereditary causes of early childhood mortality (Zhang et al., 2024) (Weststrate et al., 2022) (Ou et al., 2021).Humans possess a nearly identical paralogous gene, *SMN2*, which differs from *SMN1* by only a few nucleotides most notably a C to T substitution in exon 7. This single nucleotide difference causes most *SMN2* transcripts to undergo alternative splicing that excludes exon 7, leading to the production of predominantly truncated and unstable SMN protein. As a result, *SMN2* produces only approximately 10% of the functional full-length SMN protein. The number of *SMN2* gene copies varies among individuals and significantly modulates the clinical severity of SMA: patients with higher *SMN2* copy numbers generally exhibit milder disease phenotypes due to increased amounts of functional SMN protein (Chong et al., 2021) (Cances et al., 2022) (Wirth et al., 2020).Beyond the critical neurodegenerative features, the SMN protein plays diverse roles in fundamental cellular processes, including ribonucleoprotein assembly, spliceosome formation, mRNA transport, cellular bioenergetics, cytoskeleton organization, endocytosis, and autophagy (Menduti et al., 2020). The widespread SMN deficiency in

SMA patients explains the multisystemic impact of the disease, affecting organs such as the heart, pancreas, and components of the immune system, in addition to motor neurons (Mayer et al., 2023).This integrated understanding of *SMN1* gene deletion and the partially compensatory but insufficient role of *SMN2* underpins current therapeutic strategies targeting SMN protein restoration and highlights crucial gaps that necessitate the development of novel treatments.

1.3 Clinical Spectrum

Spinal Muscular Atrophy presents as a heterogeneous disorder with severity ranging from neonatal lethality to milder adult-onset forms. Traditionally, SMA has been classified into five major types (0–IV) based on age at onset and highest motor function achieved (Wirth et al., 2020). Type 0 is the rarest and most severe, presenting prenatally with limited survival. Type I, manifesting within the first six months of life, is marked by profound hypotonia, respiratory failure, and early mortality if untreated (Cances et al., 2022) (Zhang et al., 2023). Type II typically appears between 6–18 months, with affected children able to sit independently but unable to walk unaided, and survival often extending into early adulthood (Weststrate et al., 2022) (Balaji et al., 2023). Types III and IV represent milder forms, with later childhood or adult onset, and patients generally retain the ability to walk, often achieving near-normal lifespan (Ou et al., 2021) (Keinath et al., 2021).

1.4 Current Treatments, Challenges and Therapeutic Gaps

In December 2016, the US Food and Drug Administration (FDA) approved Nusinersen (Spinraza), the first treatment for all clinical subtypes of spinal muscular atrophy (SMA), followed by European approval six months later (Hensel et al., 2020). Nusinersen is an antisense oligonucleotide (ASO) that cannot penetrate the blood-brain barrier, necessitating direct delivery into the central nervous system (CNS) through lumbar puncture into the cerebrospinal fluid (CSF). This drug increases SMN protein levels by modulating SMN2 pre-mRNA splicing (Neil & Elizabeth, 2019). In 2019, the FDA approved Onasemnogene Abeparvovec (Zolgensma), a gene replacement therapy using an Adeno-associated virus 9 (AAV9) vector to deliver a functional SMN1 gene and it is systemically administered to children under the age of two (Hoy, 2019). In 2020, Risdiplam (Evrysdi) was the first oral drug approved by the FDA for

the treatment of SMA that targets SMN2 to increase the levels of functional SMN protein (Paik, 2022) but the cost of these three treatments is very high (Dangouloff et al.,2021). Chemical drugs have side effects (Zhong et al., 2023) therefore; there is a need to find out a medication based on the phytocompounds from the medicinal plants that can act as a potential drug candidate with low cost and negligible side effects.

1.4 Importance of Phytochemicals

Phytochemicals constitute naturally occurring compounds with considerable interest because they show diverse biological activities, which can be segmented into anti-inflammatory effects, antioxidant effects, and neuroprotection. Such properties make such subjects of interest in research for neurodegenerative conditions like SMA. The belief is that some phytochemicals influence pathways related to the health and survival of neurons, which may better benefit from the traditional treatments. For example, flavonoids, terpenes, and alkaloids show neuroprotective effects in animal models (Hassan & Raghava, 2021).

Phytochemicals attract special attention within modern drug discovery due to their origin being from natural sources, chemical diversity, and often much lower side effect profiles in comparison to synthetic drugs (Egbuna et al., 2019). The difference here with most synthetic drugs is that phytochemicals have innate biocompatibility, thereby limiting the risk of extreme adverse effects (Shah & Amini-Nik, 2017) Moreover, this variability in structure provides opportunities to investigate novel mechanisms of action which can work synergistically or as supplements to the current SMA therapy available today.

The potential benefits of phytochemicals to SMA are multifaceted. For example, some of the plant-derived compounds are known to have neuroprotective properties, where their administration could help save the motor neuron function and stop or slow down the progression of SMA. These compounds may mediate through several pathways that reduce oxidative stress, modulate the inflammatory response, or promote the survival of neurons. If used in these mechanisms, they will play a critical role in protecting motor neurons in addition to improving muscle functionality in SMA patients (Brahmachari, 2017).

In addition, phytochemical-based treatments align well with the developing trend in personalized medicine. The different types of compounds will therefore allow a customization of treatment approach by a consideration of genetic, biochemical, and environmental factors relevant to the patient. The effectiveness of such treatment shall be boosted with fewer side effects in place, making it even more of a patient-centered intervention in treating SMA (Onukwuli et al., 2024).

1.5 Computational Screening and Drug Discovery Innovations

New developments in *in-silico* approaches also pave the way for the scientific study of the discovery of phytochemicals as SMA drugs (Makhouri & Ghasemi, 2018). The *in-silico* approaches facilitate high-throughput screening of huge libraries of plant-derived compounds at extremely low costs (Mahto & Mahato, 2024). Through the application of *in-silico* methods like molecular docking and pharmacophore modeling, researchers can predict the way through which phytochemicals would interact with a given biological target, like SMN protein or any other molecular pathway implicated in SMA (Makhouri & Ghasemi, 2018).

1.7 Research Scope and Significance

The results of this study have great potential and wide applicability in several scientific and medical disciplines, thus representing a paradigm shift in drug discovery, treatment development, and disease understanding. This research, by focusing on *in-silico* identification of SMA potential drug candidates as its target, offers new insights at the same time it sets itself up for transformational levels of advancement in pharmacology, neurobiology, medicinal chemistry, and the overarching field of drug discovery in general.

In pharmacology, this research is going to make a great impact, as it will facilitate the design of safer and more effective treatments for SMA. Designing and understanding drug actions are at the heart of pharmacology and thus the identification of plant-derived compounds with potential therapeutic benefits provides an invaluable contribution to this field. These natural origin phytochemicals with their diverse array

of bioactive properties are likely to provide safer alternatives that are not linked to severe side effects and costly production for synthetic drugs. This forms the foundation for developing medicines that alleviate the symptomatic burden of SMA while also targeting its molecular causes, thereby enhancing long-term patient outcomes and quality of life (Egbuna et al., 2019) (Makhouri and Ghasemi, 2018).

This research provides neurobiologists with an opportunity to gain a more profound grasp of the pathophysiology of SMA. Neurobiology seeks to comprehend the interactions of molecular, cellular, and systemic processes within the nervous system. This study enhances the fundamental understanding of the effects of phytochemicals on neuronal proteins, specifically the survival motor neurone (SMN) protein, and their influence on cellular pathways crucial for motor neurone survival (Makhouri & Ghasemi, 2018).The focus on *in-silico* methodologies in drug development indicates a pivotal role for computational biology and bioinformatics in contemporary science. The incorporation of sophisticated computational tools into drug discovery enhances the efficiency of this intricate process (Niazi & Mariam, 2023).

In-silico techniques, including molecular docking, virtual screening, and pharmacophore modelling, forecast the interactions of phytochemical ligands with specific biological targets, such as SMN proteins. These methodologies significantly decrease the time, cost, and materials required for preliminary drug screening in comparison to traditional experimental procedures. The research surpasses its objective of identifying novel potential ligands for SMA.It makes a milestone contribution towards the understanding of the mechanism of diseases, processes for drug discovery and usage of natural products in contemporary medicine and is significant to other fields of science and learning (Chong et al., 2021). Interestingly, this study can further be helpful in the treatment map of SMA by discovering some novel nature-derived drug candidates. Through a combination of phytochemistry and computational biology, this research offers new therapeutic possibilities that are safe, accessible, and sustainable as well. The work will benefit the life of patients with SMA while pushing forward natural compounds for use in modern medicine.

This study is relevant due to the high possibility of remedying severe challenges posed by SMA and innovation in drug discovery through the identification of in-silico phytochemical ligands. SMA still remains a severe challenge for patients, their families, and health systems worldwide. Though there has been advancement in the treatment of SMA, existing therapies are limited in their effectiveness, accessibility, and long-term impacts. This study will mark a groundbreaking contribution to change because it identifies novel plant-based drug candidates that might have the potential to change SMA treatment and create avenues of approach for other genetic diseases of this sort.

Medicinal plants like *Brucea javanica* and *Tripterygium wilfordii* are rich in phytochemicals as per earlier available ethnopharmacological data that can be used for the treatment of Spinal Muscular Atrophy (Khan et al., 2021). There is a gap in the knowledge regarding the identification of phytocompounds from the proposed plant sources for the treatment of Spinal Muscular Atrophy. In this current study, we identified different phytochemical compounds from medicinal plants like *Brucea javanica* and *Tripterygium wilfordii* for the identification and Characterization of ligand(s) as a drug candidate for the treatment of Spinal Muscular Atrophy and *Brucea javanica* and *Tripterygium wilfordii* had showed potential role in the treatment of spinal muscular atrophy.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Brief about SMA

Spinal Muscular Atrophy (SMA) is a rare and debilitating genetic disorder that primarily exerts influence on the motor neurons in the spinal cord leading to muscle atrophy and weakness. This disorder has garnered significant attention in recent years due to the development of groundbreaking treatments such as gene therapies which have the potential to change the prognosis for individuals affected by SMA (Bagga et al., 2024). SMA is a heterogeneous group of inherited neuromuscular disorders characterized by the progressive degeneration of motor neurons in the spinal cord. The condition's prevalence and incidence can vary significantly based on geographic and ethnic factors making it a complex subject for epidemiological study (Lapp et al., 2023).The estimated global prevalence of SMA ranges from 1 in 6,000 to one in 10,000 live births with variations in different populations and regions. This range represents a significant burden for affected families and healthcare systems (Toro et al., 2023). This condition is an autosomal recessive disorder resulted by mutations in the survival motor neuron 1 (*SMN1*) gene. The incidence of SMA depends on the carrier frequency of these mutations in a given population (Savad et al., 2023). SMA is classified into different divisions based on the age of onset and clinical severity which also have implications for epidemiology. SMA Type I with an early outbreak and severe phenotype is often the most common while SMA Type IV with adult onset and milder symptoms is rarer (Toro et al., 2023). There may be variations in the prevalence and incidence of SMA across different countries and regions. For example some studies suggest a higher prevalence in certain European populations. Research is ongoing to understand these variations better (G. Whitney et al., 2023) . Addressing the impact of SMA on families and healthcare systems necessitates a multifaceted approach that includes comprehensive support services, caregiver education and training, psychosocial interventions, financial assistance programs and healthcare system reforms to improve access to specialized care (McMillan et al., 2021) (Murrell at al., 2017) Collaboration among healthcare providers, lawmakers, advocacy

organizations and community stakeholders is critical for reducing the effect of SMA and improving the well-being of afflicted individuals and their families (Chan et al., 2023).

2.2. Clinical Classification of SMA

In 1891, SMA first came to light through the observations of Guido Werdnig in two infant brothers (Werdnig, 1891). Over the subsequent nine years, Johann Hoffmann documented an additional seven cases. The traditional framework for classifying SMA was built upon the timing of symptom onset and the highest level of motor function achieved. While the cases they scrutinized had intermediate symptom severity, the term "Werdnig-Hoffmann disease" was coined to denote the more severe manifestations of SMA (Nakevska & Yokota, 2023). The year 1899 saw Sylvestre and Beevor describe severe forms of SMA, further delineating the spectrum. In 1964, Dubowitz contributed to the field by detailing intermediate forms of SMA in 12 patients and naming this variant "Dubowitz disease" (Nakevska & Yokota, 2023) (Awano et al., 2014) (Dubowitz, 1964). Additionally, in 1955, the discovery of a milder form of SMA was made, with Kugelberg and Welander providing a comprehensive description one year later (Wohlfart et al., 1955). This division of kinds of SMA is based on the age of symptom when its starts, the highest motor function achieved and the severity of muscle weakness. Research into the epidemiology of SMA is vital for understanding the wide prevalence of disease, its occurrence and natural evolution. These studies provide beneficial insights into the biological, ecological and demographic factors that drive the development and progression of SMA in populations. The International Standard of Care Committee for SMA (ISCCSMA) has classified SMA into five primary types as shown in the (**Figure 2.1),** which include:

2.2.1 SMA Type 0: This is the most drastic and rarest form of SMA with onset in womb or within the first few days of life. Neonate with SMA Type 0 often exhibit severe muscle weakness and may not survive beyond a few months (Shimizu-Motohashi et al., 2023).

2.2.2 SMA Type I (Werdnig-Hoffmann Disease): This is the most common form of SMA. Symptoms typically appear before first six months of life and affected newborn

may never will have the ability to sit independently or stand. SMA Type I is characterized by severe muscle weakness, respiratory difficulties and a shortened lifespan if not treated (Cintas, 2023).

2.2.3 SMA Type II: This form of SMA has a later onset, typically occurring between six and eighteen months of age. Affected individuals often get the ability to sit but may struggle with standing or walking. The rate of disease progression varies among individuals (Awano et al., 2014).

2.2.4 SMA Type III (Kugelberg-Welander Disease): This type of disorder has an onset after 18 months of age. Individuals with this form may achieve the ability to walk independently, but muscle weakness and atrophy progress slowly. Some individuals may experience a relatively normal lifespan (Oskoui et al., 2023).

2.2.5 SMA Type IV: This is the mildest form of SMA with an adult onset of symptoms. Affected individuals may experience muscle weakness, twitching and exercise intolerance. The progression of the disorder is slow and life aging is normal (Belter et al., 2023) . Twenty cases with SMA type 4 were found in a Brazilian cohort of 227 SMA patients. This study includes the biggest cohort of SMA type 4 patients and provides practical, genetic, radiological and neurophysiological aspects that may serve as biomarkers for future SMA-specific genetic therapeutics (Souza et al., 2021).

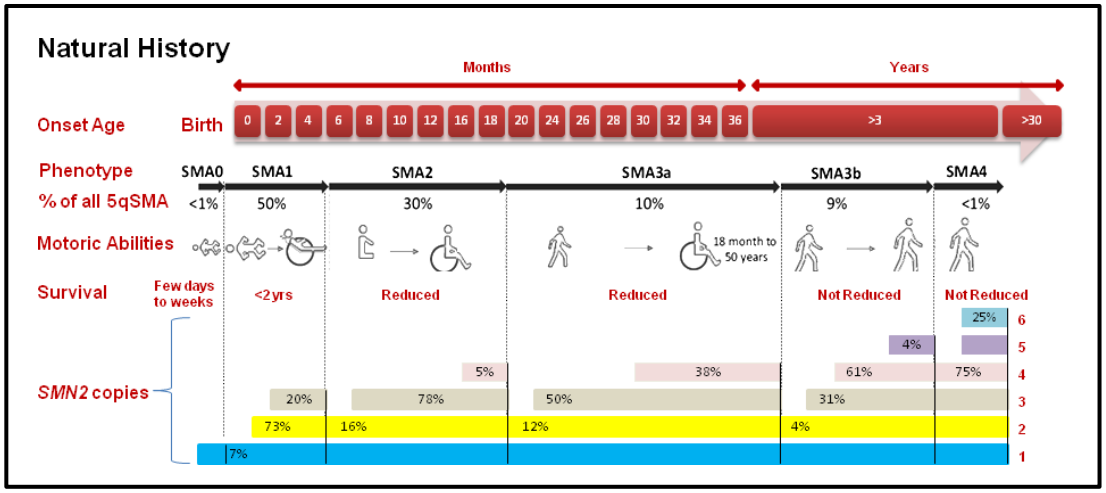


Figure 2.1. Comprehensive insight into the progression of SMA. The top panel categorizes the five types of SMA (0, 1, 2, 3, 4) which is based on the onset age and

achieved motor capabilities. SMA type 3 is further subdivided upto 3a (onset <3 years) and 3b (onset >3 years). Furthermore, the figure presents the total count of SMN2 gene copies for each SMA type.

2.3. Genetics of Spinal Muscular Atrophy

The *SMN1* gene encodes the survival motor neuron (SMN) protein, which is essential for the normal functioning of motor neurons in the spinal cord (Nishio et al., 2023) (Younger & Mendell et al., 2023) .SMN, a foundational protein within the SMN-Gemin multiprotein complex, serves as a core component. Additionally, it actively engages in various physiological functions, including responding to cellular stress, facilitating axon transport, regulating cytoskeletal dynamics, modulating mitochondrial and bioenergy pathways and participating in ubiquitin pathways. Consequently SMN emerges as a significant molecule, intricately involved in a multitude of essential activities that underpin human existence (Du et al., 2022) . These *SMN* genes are found within the 5q13 region, which harbors inverted repeats and multiple gene copies (Nishio et al., 2023) (Kataoka et al., 2023) (Lefebvre et al. 1995). The telomeric version of SMN1, with its nine exons, generates a functional 294-amino acid, 38 kDa SMN protein as shown in **(Figure 2.2)** (Lapp et al., 2023). Typically, this protein is found in both organelles that is the cytoplasm and nucleus, specifically in the Gemini of Coiled Bodies compartment, which forms Cajal bodies holding high concentrations of small ribonucleoproteins (snRNPs) along with pre-mRNAs (Ponomarev et al., 2023). SMN contains crucial and highly conserved domains that are essential for its cellular functions. Any kind of mutations occurring within these domains of SMN1 result in the production of an inefficient protein (Liu & Dreyfuss, 1996). The*SMN2* is a centromeric gene which is a paralog of SMN1, having almost identical sequences with *SMN1* except for 5 nucleotide differences. To understand the contributions of the Survival Motor Neuron 2 (SMN2) gene to Spinal Muscular Atrophy (SMA) pathology, it's important to grasp its differences in alternative splicing compared to SMN1 and how these differences impact disease severity and progression. The one of these changes leads to the exclusion of exon number 7 in approximately 90% of the transcripts through alternative splicing (Aslesh & Yokota,

2022). *SMN2* is located 875 kb far from *SMN1* and originates from a duplication of an ancestral gene which is unique to the human lineage (Chen et al., 2020). Both SMN1 and SMN2 genes encode the Survival Motor Neuron (SMN) protein, which is crucial for the survival and function of motor neurons. However, a critical difference between SMN1 and SMN2 lies in a single nucleotide difference within exon 7, resulting in a C-to-T transition in SMN2. This single nucleotide change in SMN2 affects the alternative splicing pattern, leading to the exclusion (skipping) of exon 7 in a significant proportion of transcripts. Exon 7 skipping results in the production of an isoform of the SMN protein lacking exon 7 (SMNΔ7), which is less stable and less functional compared to the full-length SMN protein produced by SMN1.The exclusion of exon 7 in a substantial portion of SMN2 transcripts results in reduced levels of functional SMN protein in cells, contributing to the pathogenesis of SMA. While SMN2 can partially compensate for the loss of SMN1 function, the lower levels of full-length SMN protein produced by SMN2 are insufficient to fully support motor neuron survival and function. The severity and progression of SMA are influenced by the number of copies of SMN2 present in the genome. Individuals with fewer copies of SMN2 typically produce lower levels of functional SMN protein and tend to have more severe forms of the disease, whereas those with more copies of SMN2 may produce higher levels of functional SMN protein and exhibit milder symptoms. The unique alternative splicing pattern of SMN2 has made it a primary target for therapeutic interventions aimed at increasing the production of full-length SMN protein. SMA symptoms manifest when there is a deficiency of proper functional SMN protein, usually stemming from minimum one copy of the *SMN1* (Prior et al., 2024). However, around 10% of full-length SMN2 transcripts, often present in multiple copies within the genome, provide some degree of protection against motor neuron degeneration (Chaudhary et al., 2022). The more *SMN2* copies a patient possesses, the more they can compensate for the absence of *SMN1* (Arikan et al., 2022). Consequently, in rare cases, individuals with 6 or more copies can exhibit milder symptoms appearing after the age of 30, characterized by mild muscle weakness and retained full mobility. Most type I SMA patient‘scarries either one or two *SMN2* copies

(Blasco-Pérez et al., 2021). While the number of *SMN2* gene copies strongly correlates with disease severity, some studies suggest that it may not always be a definitive indicator of severity, especially in SMA patients who retain one *SMN1* allele (Yamamoto, et al., 2014). Additionally, even when *SMN* is expressed normally, point mutations in *SMN* can affect protein functionality and stability leads to the disorder along with genetic and epigenetic factors, as well as environmental influences may modulate disease (Takarada et al., 2017). Approaches such as antisense oligonucleotide (ASO) therapy and small molecule drugs target the splicing machinery to promote the inclusion of exon 7 in SMN2 transcripts, thereby increasing the production of functional SMN protein. These therapies aim to augment the levels of functional SMN protein in motor neurons, potentially ameliorating disease symptoms and improving outcomes for individuals with SMA.

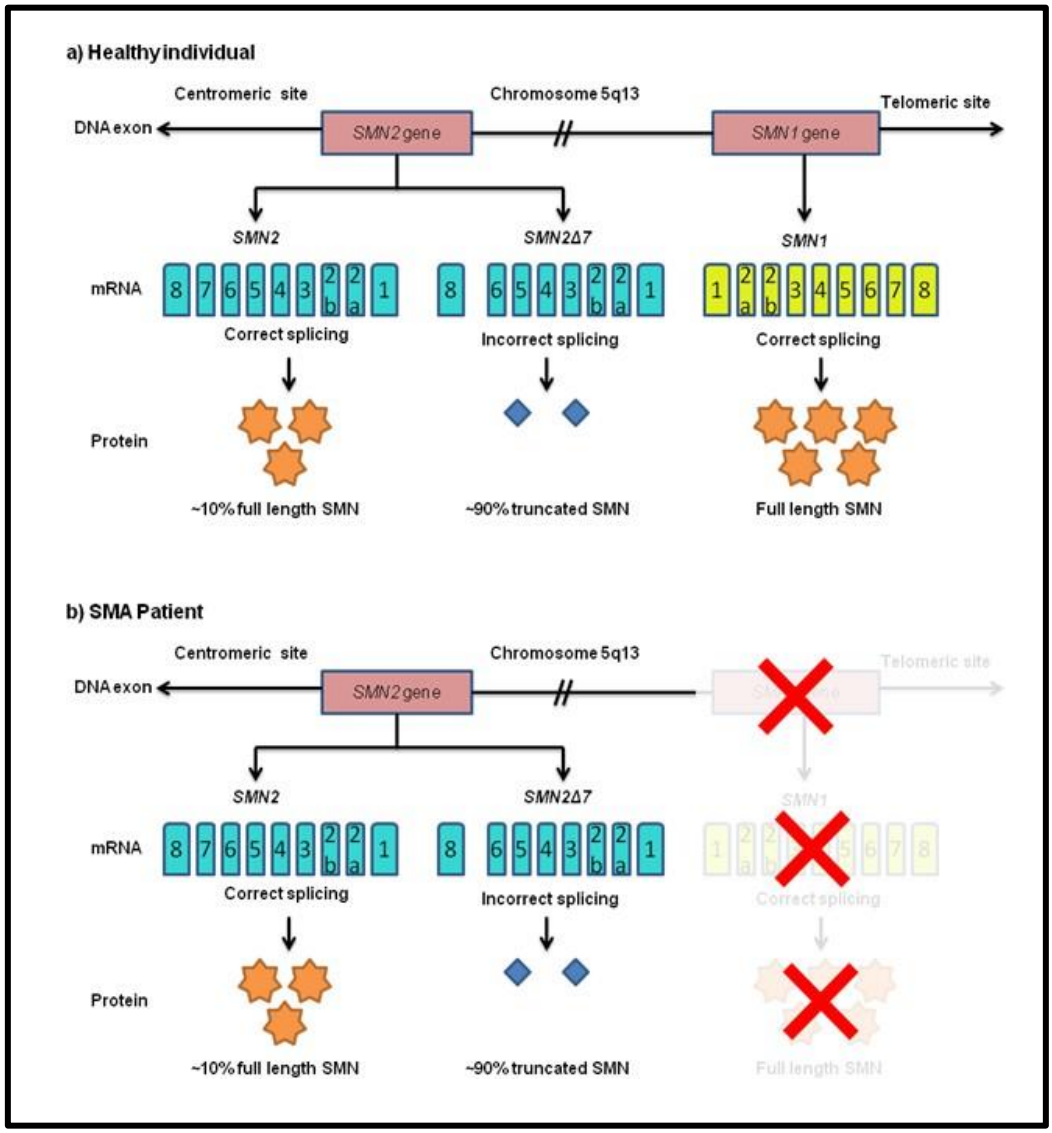


Figure 2.2. Human survival motor neuron (SMN) gene expression is shown in a schematic diagram for both healthy people and those with SMA. The chromosome 5q13 region (long arm of chromosome 5) has been explicitly identified as the location of the telomeric *SMN1* and centromeric *SMN2* genes. Full-length, functional SMN (FL-SMN) protein is produced by the *SMN1* gene whereas the *SMN2* gene, owing to incorrect splicing, produces 90% truncated SMN protein (SMNΔ7) and only 10% of FL-SMN protein. **(a)** Both SMN genes are present in healthy individuals. **(b)** The *SMN1* gene is absent in SMA patients due to mutations which prevent *SMN1* from producing FL-SMN

protein (this condition is denoted by a red 'X'). Because production is completely dependent on the *SMN2* gene, there is inadequate production.

2.4. Molecular Mechanisms of SMA

The underlying molecular mechanisms of SMA revolve around the loss of functional SMN protein and its impact on motor neurons and muscle cells. The reduction in functional SMN protein in motor neurons results in their degeneration (Valsecchi et al., 2020). Motor neurons are responsible for transmitting signals from the spinal cord to muscles and without proper functioning SMN protein, these neurons become vulnerable to damage and eventual death. This leads to muscle weakness and atrophy (Butchbach, 2021).SMN protein plays a crucial role in the assembly of snRNPs, which are essential for mRNA splicing (Malik et al., 2021). Impaired snRNP assembly due to SMN deficiency leads to widespread splicing defects in various genes, further exacerbating motor neuron dysfunction (Singh et al., 2022).While SMA is primarily a disorder of motor neurons, the resulting muscle atrophy and weakness are critical clinical features. The lack of neural input from affected motor neurons causes muscle disuse, contributing to muscle wasting (Navarrete-Opazo et al., 2021).The neuromuscular junction, where motor neurons communicate with muscle cells is also affected in SMA (Iyer et al., 2021). The loss of functional motor neurons disrupts this communication, leading to muscle weakness and contractures (Courtney et al., 2019).

2.5. Diagnostic Approaches of Spinal Muscular Atrophy

SMA presents with a spectrum of clinical features, ranging from severe, early-onset forms to milder, adult-onset forms. The key clinical manifestations include muscle weakness, atrophy and hypotonia. Patients with SMA may also exhibit respiratory difficulties, joint contractures and scoliosis (Strauss et al., 2022). It is essential to recognize these clinical signs early, as timely intervention can significantly impact the prognosis and quality of life for affected individuals.

2.5.1 Clinical Evaluation: A thorough clinical evaluation is the initial step in diagnosing SMA. The healthcare provider takes a detailed medical history, conducts a physical examination and assesses motor function (Mercuri et al., 2022).The clinical evaluation

includes a review of family history to identify any known cases of SMA or related neuromuscular disorders. It is important to consider that SMA may not be the first suspicion in cases with mild or atypical symptoms (Faravelli et al., 2020) .

2.5.2 Electromyography (EMG): Electromyography is a diagnostic technique used to assess the electrical activity of muscles and nerves (Dos Santos et al., 2022) . In SMA, EMG may reveal neurogenic changes, denoting motor neuron dysfunction. EMG can help distinguish SMA from other neuromuscular disorders and provide information about the extent of motor neuron involvement (Waldrop & Elsheikh , 2020) .

2.5.3 Nerve Conduction Studies (NCS): Nerve conduction studies evaluate the function of peripheral nerves. In SMA, NCS can be normal or show mild abnormalities. These tests help rule out other neurological conditions and provide additional information to support the diagnosis of SMA (Aziz et al., 2022).

2.5.4 Muscle Biopsy: While muscle biopsy is not the primary diagnostic tool for SMA, it can be used to confirm the absence of muscle pathology, ruling out conditions like muscular dystrophy. Muscle biopsies typically show atrophy and denervation in SMA, supporting the diagnosis (Pera et al., 2020).

2.5.5 Serum Creatine Kinase (CK) Levels: Measuring serum CK levels can be useful in differentiating SMA from muscular dystrophies. In SMA, CK levels are usually within the normal range or only mildly elevated, whereas in muscular dystrophies, CK levels are significantly elevated (Pera et al., 2020).

2.5.6 Newborn screening: Newborn screening for Spinal Muscular Atrophy (SMA) is a critical and rapidly evolving aspect of pediatric healthcare aimed at early detection and intervention for this devastating genetic disorder. One of the primary benefits of newborn screening for SMA is the early identification of affected infants. Unlike in the past, when diagnosis often occurred after the onset of symptoms, newborn screening allows for early intervention and treatment (Shih et al., 2021).

2.5.7 Genetic Testing:Genetic testing is the gold standard for diagnosing SMA. It provides a definitive diagnosis, identifies the specific genetic mutation and helps determine the severity of the condition. Genetic testing typically involves the following

approaches : (a) The primary genetic test for SMA involves analyzing the *SMN1* gene. Most SMA cases result from deletions or mutations in this gene, leading to reduced SMN protein levels. This test is highly specific and sensitive and can diagnose SMA with a high degree of accuracy (Niba et al., 2021). (b) In addition to *SMN1* analysis, counting the number of copies of the *SMN2* gene can provide information about the disease severity. SMA patients with more *SMN2* copies tend to have milder forms of the disease, while those with fewer copies typically have more severe forms (Milligan et al., 2021).

2.5.8 Next-Generation Sequencing (NGS): NGS is a powerful tool for identifying rare or typical mutations in the SMN1 gene. It can be especially useful in cases where standard genetic tests do not yield a diagnosis. NGS can also detect other rare genetic conditions that may mimic SMA (Yang et al., 2024).

2.5.9 Prenatal Testing: Genetic testing can be performed during pregnancy to identify SMA in the fetus. This can be done through chorionic villus sampling or amniocentesis. Early diagnosis allows for informed reproductive decisions and early intervention if the fetus is affected (Zhang et al., 2020). Prenatal testing for Spinal Muscular Atrophy (SMA) raises several ethical considerations, including issues related to informed consent, autonomy, disability rights and the potential for discrimination. It's crucial that expectant parents fully understand the purpose, benefits, limitations and potential consequences of SMA prenatal testing. They should have access to comprehensive information about SMA, including its prognosis, available treatments and the emotional impact of receiving a positive result. Current recommendations by the American College of Medical Genetics (ACMG) include offering SMA carrier screening to all couples, regardless of race or ethnicity, before conception or early in pregnancy. Current recommendation by the American Congress of Obstetricians and Gynecologists (ACOG) do not advise preconception and prenatal screening for SMA be offered to the general population and advice testing offered to general population (Carré & Empey 2016). Some disability rights advocates argue that prenatal testing for conditions like SMA perpetuates ableism and sends a message that individuals with disabilities have less value or are not worthy of

existence. This perspective challenges the notion that certain disabilities should be actively prevented or eliminated through selective abortion.

2.6. Disease-Modifying Treatments and current implications

Several treatment approaches are available for Spinal Muscular Atrophy (SMA), as shown in Table 2.1, which has been previously published (Bagga et al., 2024).

Nusinersen (marketed as Spinraza®) was the first FDA-approved disease-modifying treatment for SMA having obtained approval in December 2016 and by the EMA in 2017 for both infant and adult (Chen 2020). This innovative therapeutic approach, which involves the intrathecal administration of a 2′-O-methoxyethyl phosphorothioate modified antisense oligonucleotide (ASO), focuses on enhancing the incorporation of exon 7 into mRNA transcripts of *SMN2* (Jablonka et al., 2022). The intrathecal route of administration is crucial for nusinersen‘s effectiveness in treating SMA because it allows for targeted delivery to the site of pathology, bypasses the blood-brain barrier, optimizes concentration at the target site, minimizes systemic side effects and provides a longer duration of action within the central nervous system (Jablonka et al., 2022). By administering nusinersen directly into the central nervous system (CNS) through the intrathecal route, the medication effectively suppresses the activity of certain splice-factors and binds to a specific intronic splice-silencing site within intron 7 of *SMN2 (*Studzińska et al., 2024). This intervention substantially increases the probability of exon 7 being included in the mRNA, ultimately enabling the translation of a more substantial quantity of fully functional SMN protein (Aslesh & Yokota 2022).This enhanced production has demonstrated significant improvements in both survival and the overall condition of various experimental models of SMA. Importantly, nusinersen‘s journey to approval and commercialization has been bolstered by a multitude of studies confirming its efficacy, without any notable drug-related adverse events (Day et al., 2022). Due to ASOs‘ inability to traverse the blood-brain barrier, nusinersen was consistently administered intrathecally in all clinical trials. During the initial loading phase, it was administered four times over two months and in the maintenance phase, it was given onceevery four months (Przymuszała et al., 2022). The standard dosage of nusinersen typically amounts to 12 milligrams (Łusakowska et al., 2023). Studies on nusinersen have

revealed the potential for some patients to regain lost abilities, such as sitting up, standing and walking, without the need for therapy. Furthermore, early initiation of this treatment has demonstrated positive outcomes in individuals with SMA types I, II and III (Gavriilaki et al., 2022). It is worth noting that a notable drawback of this therapy is the possibility of side effects, including constipation and upper and lower respiratory tract infections (Hoy, 2017).

Onasemnogene abeparvovec (Zolgensma) an advanced gene therapy: In May 2019, the FDA granted approval to AVXS-101, also known as Zolgensma is a gene therapy approved for the treatment of SMA, developed by AveXis, a subsidiary of Novartis (Chen 2020). This approval followed the release of favorable outcomes from the phase one clinical trial known as START (Identifier: NCT01547871). This trial assessed the drug‘s safety and effectiveness when administered as a one-time infusion to infants with SMA symptoms appearing before six months of age. Subsequently, in March 2020, Zolgensma received conditional marketing authorization and in May 2020, it was granted approval by the European Medicines Agency (EMA) as well (Menduti et al., 2020). The adeno-associated virus 9 (AAV9) capsid is used to transport the SMN-encoding complementary DNA (cDNA) to the motor neurons that need it (Chong et al., 2021), (Hoy, 2019), (Hensel et al., 2020). A single dose of AVV9 administered intravenously (IV) is sufficient to transport a functional copy of the *SMN1* gene over the blood-brain barrier and into patient cells, where it may stimulate the production of SMN protein (Aslesh & Yokota 2022). The *SMN1* trans gene and synthetic promoter based on AVV9 are also crucial components in maintaining SMN protein synthesis throughout time (Van Alstyne et al., 2021). Although it successfully corrects the underlying molecular defect in SMA, it has a deleterious effect on the liver by elevating serum amino transferase (Abreu & Waldrop 2021). However, prednisone is effective at reducing elevated liver enzymes. Therefore, at least three months after administration, patients should be monitored for liver function (Ramdas & Servais 2020), (Gandhi et al., 2021). The long-term durability of the benefits of Zolgensma (onasemnogene abeparvovec) is still being actively studied and monitored. Zolgensma is a gene therapy approved for the treatment of spinal

muscular atrophy (SMA) in pediatric patients and it has shown remarkable efficacy in improving motor function and survival in clinical trials. While the initial data from clinical trials and real-world experience have shown sustained benefits of Zolgensma treatment over several years, including improvements in motor function and survival, more long-term follow-up is needed to fully understand the duration of these benefits. Clinical trials and observational studies are ongoing to assess the durability of Zolgensma‘s effects, including its impact on motor function, respiratory function, quality of life and survival rates over extended periods. These studies involve monitoring patients treated with Zolgensma for several years to track their progress and detect any potential changes in treatment outcomes over time. It‘s important to note that as research continues and more data become available, our understanding of the long-term benefits and potential limitations of Zolgensma treatment will continue to evolve. Patients and caregivers should work closely with healthcare providers to stay informed about the latest research findings and recommendations regarding the use of Zolgensma in the management of SMA (Chong et al., 2021), (René & Parks, 2023).

Risdiplam (Evrysdi™) was authorized by the FDA as of 7 August 2020, as the first oral medication for children as young as 2 months old and adults with SMA. It is a collaborative development effort involving Roche, PTC Therapeutics Inc., and the SMA Foundation, aimed at addressing spinal muscular atrophy (Chen 2020), (Dhillon, 2020). Risdiplam serves as a modifier of mRNA splicing that leads to an elevation in SMN protein expression (Li, 2022). It is a tiny molecule that changes the splicing of the *SMN2* by binding to two locations in the *SMN2* pre-mRNA. These sites are known as the 5′ splice site (5′ ss) of intron number 7 and the exon splicing enhancer 2 (ESE2) of exon 7 (Paik, 2022). Increases in full- length SMN mRNA and protein levels are caused by the unique specificity of binding two sites, which also reduces impact on other pre-mRNA splicing and prevents the likelihood of off-target effects (Kwon et al., 2022). According to preclinical studies, risdiplam can reach the central nervous system and peripheral organs *in vivo* and can result in a significant increase of SMN protein in the blood, brain and muscles, as well as an increase in survival in various SMA mouse models (Markati et

al., 2022). While risdiplam's systemic distribution in preclinical tests with oral administration allowed for the possibility of an impact on other tissues, nusinersen's intrathecal delivery method mostly limited its effect to motoneurons of the central nervous system (Ojala et al., 2021). Previous studies in human and murine models suggest that SMA may in fact be considered a multi-system disorder involving the neuromuscular junction, cardio-vascular system, lung, gastrointestinal-tract and liver (Yeo & Darras 2020). Risdiplam has shown significant improvements in motor function and has the advantage of being an oral therapy, making it a more convenient option for many patients (Brandsema & Cappa, 2021).

Table 2.1: Treatment options currently in use for spinal muscular atrophy.

Treatment	**Nusinersen (Spinraza)**	**Onasemnogen Abeparvovec (Zolgensma)**	**Risdiplam (Evrysdi)**
Class	Antisense oligonucleotide	Adeno-associated virus (AAV) based gene therapy.	Small molecule
Mechanism	Improves *SMN2* splicing to produce full-length SMN protein	Provides a functioning SMN transgene.	Improves *SMN2* splicing to produce full-length SMN protein
Administrative route	Intrathecal injection	Intravenous injection	Oral

FDA approved age categories	All	Greater than 2 years	More than two months
Frequency	Dosing schedule: 4 loading doses in the first 2 months then every 4 months	Just One Time Dose (Single Dose)	Daily
Problems with Current Treatments	Unable to get a lumbar puncture done	AAV9 antibodies present at the baseline	Interactions between drugs
FDA approval	December 2016	May 2019	August 2020
Cost	$125,000 per dosage(approx.)	$2.125 million per treatment	$100,000–$340,000 annually
Unfavourable outcomes	lumbar puncture problems, proteinuria, Thrombocytopenia,	Transaminitis, thrombocytopenia, troponemia and acute liver damage	Diarrhoea, rash and fever

SMA treatments have evolved notably in recent years particularly as previously stated gene therapies like Zolgensma and disease- modifying drugs like Spinraza (nusinersen). Much more significant perspective, which should consider insurance coverage availability across regions and efforts to improve access to SMA treatments. While these

drugs offer a promising treatment option for children with SMA they are highly costly, as seen in **Table 2.1**. According to studies certain governments have made efforts to design policies for such disease treatments such as: The Department of Revenue, Ministry of Finance, govt. of had issued Notification No. 46/2021- Customs dated 30.09.2021, which waives all Basic Customs Duty (BCD) and Integrated Goods and Services Tax (IGST) on drugs imported (personal use only) for the treatment of spinal muscular atrophy (SMA) rare disease, making medicines for SMA rare disease more affordable (https://pib.gov.in/Pressreleaseshare.aspx?PRID=1846230). Government of India also made a provision for financial assistance of up to Rs. 50 lakhs to patients suffering from any category of Rare Diseases such as SMA and for treatment at any of the Centres of Excellence (CoE) identified in the NPRD-2021, outside of the Rashtriya Arogaya Nidhi umbrella scheme (https://pib.gov.in/PressReleasePage.aspx?PRID=1846230). The National Health Service (NHS) England, for example, states that Biogen (the pharmaceutical company that manufactures treatment for SMA) will make the treatment for spinal muscular atrophy (SMA) available to the youngest and most severely affected (SMA type 1) patients immediately with NHS England offering funding contingent on the National Institute for Health and Care Excellence (NICE) publication of final guidance. In Singapore, the Rare Disease pay has been established to pay five drugs to treat three rare diseases. In Malaysia and Australia, qualifying patients are given discounted access to pricey and life-saving medications (https://rarediseases.mohfw.gov.in/uploads/Content/1624967837_Final-NPRD-2021.pdf) .

2.7. Rehabilitation (Care & therapies) and Disease Management for Spinal Muscular Atrophy

In addition to disease-modifying treatments, SMA management often involves a multidisciplinary approach as shown in (**Figure 2.3)** that focuses on addressing the symptoms and complications associated with the disease. Supportive care strategies aim to improve the quality of life for individuals with SMA and include:

2.7.1 Physical therapy: Physical therapy is essential in managing muscle weakness, contractures and maintaining range of motion (Dunaway et al., 2016), (Trenkle et al., 2021) . People affected by spinal muscular atrophy may experience limitations in their ability to fully articulate their joints due to muscle weakness. This can potentially lead to the development of contractures, characterized by muscle tightness that may become permanent and restrict mobility. Physical therapy often incorporates exercises and stretching routines designed to enhance flexibility and overall functionality (Yi et al., 2020). These interventions aim to minimize the likelihood of joint contractures, mitigate the progression of scoliosis and promote the maintenance of a healthy weight. Mounting evidence suggests that consistent participation in physical therapy sessions can yield positive outcomes in terms of both function and the progression of spinal muscular atrophy in patients (Mirea et al., 2022), (Yi et al., 2020).

2.7.2 Respiratory care: The management of the respiratory system plays a pivotal role in addressing spinal muscular atrophy (Bach et al., 2000). Weakness in the chest muscles can hinder one‘s capacity to breathe or cough effectively, consequently elevating the likelihood of infections (Chen et al., 2017) ,(Chen, et al., 2019). The available respiratory care solutions encompass both non-invasive and invasive methods:

(i) non-invasive respiratory care comprise strategies that aim to circumvent or postpone the necessity for invasive procedures (Bach et al., 2000). Specialized apparatus such as a ventilator or a bilevel positive airway pressure (BiPAP) machine can provide a constant airflow to the lungs using a mask that covers the mouth and/or nose. Additionally a home-based cough assist device may be utilized to facilitate coughing and the clearance of secretions. (ii) Invasive respiratory care establishes a protected passage to the lungs using an endotracheal tube inserted either through the mouth (intubation) or directly into the trachea via a minor neck incision (tracheotomy) (Blackwood et al., 2021), (Lewelt et al., 2012).

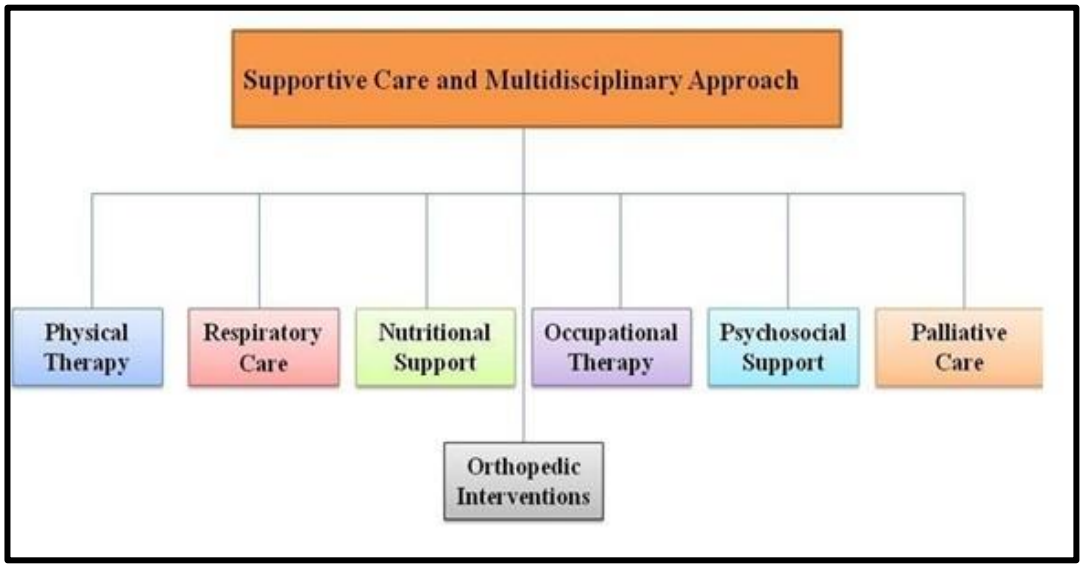


Figure 2.3: Supportive Care and Multidisciplinary Approach flow chart

2.7.3 Nutritional Support: The weakening of muscles can result in some individuals with SMA experiencing a decline in their ability to chew and swallow proficiently. This situation increases the risk of food or liquids being aspirated into the lungs, potentially leading to respiratory infections (Li et al., 2020), (Moore et al., 2016). To address this a temporary or permanent feeding tube may be inserted to ensure the essential intake of nutrition and hydration (Li et al., 2020). Broadly speaking, there are two categories of feeding tubes: those inserted through the nose and those placed in the abdominal area. Nasogastric (NG) tubes are inserted through the nasal passage and deliver nutrition directly into the stomach (Choi et al., 2020), (Corsello et al., 2021). These are typically employed for patients requiring short-term feeding tube access and are conveniently replaceable ((Choi et al., 2020). Gastrostomy (G) tubes are surgically implanted through the abdominal wall and directly into the stomach (Birnkrant et al., 1998) (Mercuri et al., 2018) (Wadman et al., 2021). Due to their ease of maintenance, they are often the favored choice for individuals in need of extended-term feeding assistance.

2.7.4 Orthopedic Interventions: Orthopedic surgery may be required in cases where there are severe joint contractures or scoliosis (Mercuri et al., 2018) (Katz et al., 2015). Scoliosis characterized by a spinal curvature, can be a challenge for individuals with

spinal muscular atrophy as a consequence of muscle weakness (Haaker & Fujak, 2013). An orthopedic specialist may propose postural support in the form of bracing or recommend surgical intervention to address scoliosis (Mercuri et al., 2018) (Haaker & Fujak ,2013).

2.7.5 Occupational therapy: Occupational therapists work with SMA patients to enhance their ability to perform daily tasks and improve their independence (Shank & Rahman 2021) (Salem & Jaffee Gropack, 2010). They assess patients‘ needs and recommend assistive devices or home modifications to facilitate daily activities. Their role includes: adaptive techniques means teaching individuals adaptive techniques and recommending assistive devices that enable greater independence (Kraskowsky & Finlayson, 2001). Home modifications means assessing home environments and suggesting modifications to make daily tasks more manageable (Renda & Lape, 2018) . Assisting with communication means in cases of severe SMA, occupational therapists can help individuals use communication devices to facilitate interaction with others (Van Stormbroek & Buchanan, 2019).

2.7.6 Psychosocial Support: SMA affects not only the physical health but also the emotional and psychosocial well-being of patients and their families (Ropars et al., 2020). Ensuring the holistic care of a patient with SMA and their family is insufficient without the monitoring and treatment of their psychosocial welfare. Requirements differ based on the patient‘s age and specific sub-types of SMA. Psychosocial health can be influenced by various elements, including social and emotional factors as well as treatment factors such as innovative therapies. Psychosocial care should encompass a wide range of characteristics, including social and cognitive development, quality of life and the impact on patient and family functioning in various situations such as home, school, or job. The care of SMA should include the involvement of a mental health practitioner, such as a psychologist, psychiatrist, or neuropsychologist, as well as a social worker who has specialized experience in assisting patients with chronic diseases. Assessments should be considered around the time of diagnosis, before entering school and after a change in functionality. Implementing psychologically informed care and

employing a range of interventions has the potential to mitigate psychological morbidity in both children and parents. During every multidisciplinary appointment, it is important to assess the individual‘s mental health and quality of life. If deemed essential, the mental health clinician will be involved in evaluating the psychological state of the patient as well as their parents and siblings (Ropars et al., 2020). Psychosocial support addresses these aspects and may include: (i) counseling: individual and family counseling can help individuals and caregivers cope with the emotional challenges of living with SMA (Ropars et al., 2020), (Inhestern et al., 2023) . (ii) Support groups: joining support groups either in person or online can provide a sense of community and shared experiences. (iii) Mental health services: access to mental health services is essential for addressing anxiety, depression and stress that may result from the condition (Inhestern et al., 2023) (Betancourt et al., 2013) .

2.7.7 Palliative care: For individuals with advanced SMA or those with severe complications, palliative care can provide symptom management, pain relief and emotional support to enhance quality of life (Hully et al., 2020).

2.7.8. Disease Management approaches for SMA Management:

2.7.8.1 Supportive care and Multidisciplinary Approach

The management of SMA requires a multidisciplinary team approach involving various healthcare professionals to address the diverse needs of individuals with the condition. The team may include neurologists, physical and occupational therapists, respiratory therapists, nutritionists, orthopedic surgeons and social workers. This collaborative approach ensures that the physical, emotional and psychosocial aspects of SMA are managed comprehensively (Zingariello et al., 2019). Supportive care is the fundamental aspect of clinical management in spinal muscular atrophy (SMA). The development of disease-modifying medications such as nusinersen, onasemnogene abeparvovecxioi and risdiplam has provided improved treatment choices for the most severe forms of the condition. These medications have increased survival rates and brought hope for a longer and better quality of life. Additionally, they have influenced the way healthcare is provided for these patients. Although there have been some improvements in the field,

adults living with SMA and those transitioning into adulthood have been somewhat overlooked, despite the emergence of studies and advancements such as enhanced respiratory care, home adaptations and devices that promote greater independence, like power wheelchairs and voice amplifiers. It is important for everyone to acknowledge and appreciate these achievements. The effects of fragmented care might be intensified for individuals migrating from pediatric to adult care, as they no longer receive the same degree of coordinated assistance provided in pediatric settings. According to reports, individuals who are transferring from pediatric to adult healthcare services face challenges in understanding and interacting with a complex health system and new specialists. They commonly describe this experience as challenging and intimidating.

In addition, adults with SMA may have difficulties during this phase of transition, particularly when they need to relocate (e.g., for higher education), as this necessitates them to become part of a new healthcare system. Although not prevalent within the SMA community as a whole, certain jurisdictions have acknowledged this issue and have adopted targeted measures to mitigate its effects. For instance, they have established transitional clinics where medical professionals from both adult and pediatric fields participate in appointments. We advocate for increased implementation of optimal methods and specialized procedures (such as established transitional care clinics, communication paths between pediatric and adult experts and a nationwide network of specialists) that streamline the transition to adult care and the transfer of knowledge (Waldrop & Elsheikh ,2020) .

2.7.8.2 Assistive Devices and Technology

The use of assistive devices and technology plays a significant role in SMA management. These devices help individuals with SMA lead more independent and fulfilling lives (Livingstone & Paleg, 2021). Examples include: wheelchairs, scooters and other mobility aids provide individuals with the freedom to move and explore their environments (Fujak et al., 2011). Augmentative and alternative communication (AAC) devices assist those with severe SMA in expressing themselves (Van Stormbroek & Buchanan, 2019). These systems enable individuals to control various aspects of their environment such as lights,

doors and appliances, through adapted technology. Adapting the home environment with features like ramps wider doorways and accessible bathrooms enhances accessibility (Renda & Lape, 2018). Adaptive technology and software allow individuals with limited physical mobility to use computers and access the internet (Kraskowsky & Finlayson, 2001).

2.8. Emerging therapies

Research into SMA continues with several promising therapies under investigation. These therapies include small molecule drugs, gene-editing technologies and exon-skipping therapies, among others. The goal is to further enhance the disease-modifying potential and offer a more comprehensive treatment approach for SMA. Advances in genetic testing have made it easier to diagnose SMA accurately and offer prenatal testing for at-risk pregnancies. These advancements include: NGS has become a powerful tool in identifying rare or atypical mutations in the *SMN1* gene and other related genes. It can uncover genetic variations that were previously challenging to detect (Odabas et al., 2022) (Lopez-Lopez et al., 2020). Techniques like chorionic villus sampling and amniocentesis allow for the diagnosis of SMA in the fetus, enabling informed reproductive decisions and early intervention if the fetus is affected (Calder et al., 2016) (Dangouloff & Servais, 2019) .

2.8.1 Gene-Editing Technologies: Emerging gene-editing techniques, such as CRISPR-Cas9, offer the potential to correct genetic mutations directly, providing a curative approach to SMA. These technologies are in the early stages of development and are being explored in preclinical studies. In addition to this, CRISPR technology, which expands the scope of genetic engineering and gene treatments, enables the treatment of a wide range of hereditary illnesses. Some prior research in the literature show that SMA can be treated using the CRISPR method. Homology directed repair (HDR)-based CRISPR technology, which produces a high rate of in-del (insertion-deletion) mutations rather than editing, has been proven unsuitable for therapeutic purposes. CRISPR-prime editing (PE) technology is a novel type of gene editing technique that enables precise genomic alterations without the need for double- strand breaks or donor DNA sequences.

The CRISPR-prime editing approach has also been employed in rare disorders like as sickle cell anaemia and Tay–Sachs and its effectiveness in editing diverse harmful variants has been proven. However, CRISPR Prime Editing- mediated gene editing for spinal muscular atrophy (SMA) has yet to be investigated (Odabas et al., 2022) .

2.8.2 Small Molecule Therapies: Small molecules that target specific pathways involved in SMA are also under investigation. These drugs aim to increase SMN protein production and improve motor function (Calder et al., 2016) .

2.8.3 Combination Therapies: Researchers are exploring the use of combination therapies, including a mix of *SMN2*-targeting drugs and other treatments, to enhance the efficacy of SMA management (Harada et al., 2020). Combining different therapeutic strategies to maximise SMA treatment outcomes is an exciting approach. Limited data supports the efficacy of expensive drug combinations in people, encouraging clinicians and scientists to examine all therapeutic options (Chen 2020), (Poletti & Fischbeck ,2020). A combination of SMN-dependent ASO-inducing *SMN2* exon inclusion and SMN-independent myostatin inhibition yielded positive results in a SMA animal model (Chen, 2020) (Zhou et al., 2020). A limited sample of patients was treated with a combination of Zolgensma and nusinersen, but the long-term benefits remain unclear. Zolgensma and nusinersen have distinct modes of action, making drug-to-drug interactions less common. Nusinersen targets an intron sequence to increase exon 7 inclusion. The transplanted Zolgensma gene lacks introns and hence should not interfere with nusinersen translation. Zolgensma treatment should be approached with caution due to the reported adverse event of thrombocytopenia associated with nusinersen. Long-term follow-up data, particularly in pre-symptomatic patients is needed to evaluate the effectiveness and hazards of combination therapy (Chen, 2020). Combination therapies, treatments and advocacy initiatives have played an important role in determining research orientations and legislative changes in the management of spinal muscular atrophy (SMA). Advocacy groups such as cure SMA, SMA foundation and fight SMA have been instrumental in catalyzing SMA research. By raising awareness, funding research initiatives and fostering collaborations among scientists, these organizations have

accelerated the pace of discovery in understanding the underlying genetic mechanisms of SMA, identifying potential therapeutic targets and developing innovative treatment strategies.

2.8.4 Early Intervention and Pre-Symptomatic Treatment: Research has shown the benefits of early intervention, even before the onset of symptoms, in infants with SMA (Sampaio et al., 2018). The nurture study demonstrated that early treatment with nusinersen in pre-symptomatic infants significantly improved motor function and developmental outcomes (Webster et al., 2018).

2.8.5 Patient and Caregiver Advocacy: SMA patient and caregiver advocacy groups have played a crucial role in raising awareness, driving research and improving access to care and treatments. Their efforts have been instrumental in advancing the SMA field (Paracha et al., 2022).

In addition to the historical use of plants in treating neurological conditions, contemporary research has increasingly focused on exploring the pharmacological potential of phytochemicals as drug candidates for neurodegenerative diseases, highlighting the relevance of ancient wisdom in modern therapeutic strategies (Wink, 2020).

Sections 2.1–2.8 provide a comprehensive, evidence-based overview of Spinal Muscular Atrophy, covering its clinical spectrum, genetic mechanisms, diagnostic methodologies and recent therapeutic developments. While these treatments have improved patient outcomes, challenges such as high costs, limited access and incomplete efficacy persist. These gaps highlight the urgent need for exploring additional therapeutic avenues. Phytochemicals, characterized by diverse biological activities and favorable safety profiles, present promising candidates. Consequently, the following sections (2.9 onwards) explores deeper into the role of plant-derived compounds in neuroprotection and their potential application in SMA management.

2.9. Plants in Medicine: A Historical Look at Neuroprotective Compounds

While Chapter 1 briefly outlined the importance of phytochemicals in neuroprotection, this section provides a more detailed account of their role and relevance to Spinal Muscular Atrophy. Subsections 2.9.1–2.9.5 trace the development of plant-based neuroprotection, starting from traditional medicine and advancing to contemporary

scientific validation, thereby emphasizing their significance in bridging ancient knowledge with modern therapeutic strategies. Plants have been important to human health for thousands of years. They are the basis of traditional medicine in many cultures. Ayurveda, Traditional Chinese Medicine, Unani, and Indigenous healing are some of the practices that have used the therapeutic properties of plants to treat a wide range of illnesses. Not only did these old medicines keep people well, they also provided us early hints about how to treat what we now call neurological illnesses. Researchers are still interested in how plants can protect the brain (Brahmachari, 2018) (Maher, 2021).

2.9.1 Preliminary Identification of Plant Neuroprotective Capabilities

For a long time, herbal medicine has been utilised to aid with mental and brain health. Ayurveda has historically used plants like *Withania somnifera* (ashwagandha), *Centella asiatica* (Gotu kola) and *Bacopa monnieri* (brahmi) to help with memory, focus, and stress. Medicines like *Ginkgo biloba* and *Panax ginseng* have also been used in Traditional Chinese Medicine to purify the mind and help with memory loss that happens as people become older. Native American tribes have also employed plants like *Salvia divinorum* to change their minds and affect their brains (Ortega et al., 1982) (Beshara et al., 2019) (Mahadevan & Park, 2008).

These old customs realised how vital it was to maintain the brain healthy, therefore they sought for herbs that are good for the brain since they are relaxing, anti-inflammatory, and antioxidant. For instance, people utilised ashwagandha to soothe "nervous exhaustion," which is like what we now term brain strain caused by stress. People appreciated gotu kola because it made them feel peaceful and sharp, which might mean that it protects the brain (Grey et al., 2016) (Kuboyama et al., 2014).

2.9.2 Contemporary Verification of Traditional Neuroprotective Medicines

People have used herbs like ashwagandha, brahmi and ginkgo to help their brains for hundreds of years. These plants are doing so well that contemporary science is now catching up and figuring out why. To learn more about how these plants protect the brain at the molecular level, scientists have been looking at the components of the plants that are active. Ashwagandha works because it has withanolides, which decrease inflammation, fight off harmful free radicals, and even assist nerve cells heal and grow

again (Dar & Choudhary, 2017).

Bacosides are what give Brahmi its brain-boosting properties. They help with memory and thinking by changing how brain cells interact to each other and modulating neurotransmitters (Aguiar & Borowski, 2013). *Ginkgo biloba* is another plant that has been studied a lot. There are a lot of terpenoids and flavonoids in it. These compounds stop neurones from dying, lower oxidative stress, and make more blood flow to the brain. Because of this, it is a good choice for those with Alzheimer's (DeFeudis, 2003).

Not only do these studies confirm what traditional healers always knew, but they also provide us new ways to treat difficult disorders like Alzheimer's or spinal muscular atrophy, which is when nerve cells die (Tutar et al., 2020).

2.9.3 Plant-Based Compounds: Key Neuroprotective Mechanisms

For a long time, people have used plants to keep their brains and neurological systems healthy. This is especially true for those with serious diseases like spinal muscular atrophy (SMA), which progressively kills nerve cells. Phytochemicals are the natural compounds in plants that protect neurones and help them perform well in a number of clever ways.

One key way these plant components help is by protecting cells from oxidative stress. This happens when there are too many free radicals, which are harmful molecules, and they go in the way of the body's natural defences. This can damage neurones, but phytochemicals like flavonoids and polyphenols are like superheroes that safeguard brain cells by getting rid of free radicals. This maintains the brain's environment stable and protects cells from breaking down in ways that might create big problems (Pietta, 2000). Then there's inflammation, which can hurt a lot of nerves in diseases like SMA. Chronic inflammation damages neurones and makes it hard to move, but plant chemicals like terpenoids and alkaloids can help. They inhibit the body from making inflammatory signals and quiet down pathways that are excessively active. This keeps neurones healthy and helps them do their jobs. This anti-inflammatory activity is highly crucial for slowing down diseases that do well when there is constant inflammation (Calderon et al., 2017).

Plants also boost the brain's natural healing system by providing proteins like brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which act like

fertilisers for neurones and help them grow. These proteins help nerve cells grow, repair, and stay flexible. This is really crucial when stress or genetic disorders might make them break down. When these proteins are combined with plant compounds, they work better. This helps fix and strengthen the networks in the brain. This makes them a powerful weapon against diseases that make the brain break down (Zeng et al., 2022).

Phytochemicals are also very good at stopping neurones from destroying themselves. In diseases like SMA, nerve cells may go through a process called apoptosis, which is when they cease operating altogether. Plant chemicals block the processes that kill and cease working on neurones, which makes them live and work longer (Yuan et al., 2017).

Lastly, these plant-based assistants take care of mitochondria, which are the little power plants in cells. When mitochondria stop working, neurones can't acquire the energy they require. This is particularly bad in SMA. Withanolides and ginsenosides are two examples of chemicals that assist mitochondria stay stable, generate more energy, and make fewer toxic byproducts. This keeps neurones healthy and full of energy (Huang et al., 2019).

These plants help preserve mitochondria, decrease cell death, soothe inflammation, and fight oxidative stress. They also help neurones flourish. All of these features provide a compelling argument for using them to treat SMA. This disease kills motor neurones, which makes muscles weak and lose strength. On the other hand, plant-based medicines that work in more than one manner seem to be able to help control symptoms and maybe even cure certain hard-to-treat diseases (Huang & Franklin, 2021) (Finkel et al., 2017).

2.9.4 Importance in genetic diseases such as SMA

Phytochemicals are natural molecules found in plants that might help us see things in a new manner.These compounds from plants have a number of bioactive qualities that might help cure hard-to-treat hereditary diseases like SMA (Giriwono et al., 2019). A lot of synthetic medications only work on one route, while phytochemicals can change a lot of biological processes at once.This makes them especially promising for SMA and other conditions when a lot of things go wrong at once.

For instance, phytochemicals can help with oxidative stress, which is a big problem in SMA

because reactive oxygen species (ROS) damage neurones (Joseph et al., 2021).They also help calm down an overactive immune system and lower inflammatory signals, which helps cool down the inflammation that causes motor neurone loss (Manczak et al., 2010).These chemicals can also stop cells from dying by stopping the mechanisms that cause cell death. This maintains motor neurones alive and working (Nabavi et al., 2015).

This strategy, which has a lot of aspects, is very important for SMA since no one issue can entirely explain the condition. Motor neurones can be affetced by things like protein misfolding, mitochondria that don't work, and inflammation that lasts for a long time.Phytochemicals are a superior strategy to slow down or control the disease's growth since they may work on more than one front at a time.Also, they could have less negative effects than man-made pharmaceuticals because they come from plants and animals. This might make them a better choice for those with long-term conditions like SMA (Calabrese et al., 2008) (Phani et al., 2012)

2.9.5 Bridging Traditional Knowledge with Modern Science

This vast range of plant-based knowledge, accumulated over decades, provides an important understanding of how individual plants may influence biological processes and bring about healing. Through the utilization of this indigenous knowledge, modern scientists are able to discover potential phytochemicals that can be the starting point for new therapeutic approaches (Kumar et al., 2017) (Uddin & Al Mamun, 2020).

High-throughput screening enables scientists to screen hundreds of plant extracts in a short time, assessing their bioactivity and potential medicinal properties. Molecular docking simulations explain the drug interactions with specific biological targets, such as receptors or enzymes involved in disease onset (Ma et al., 2021) (Brondino et al., 2013).

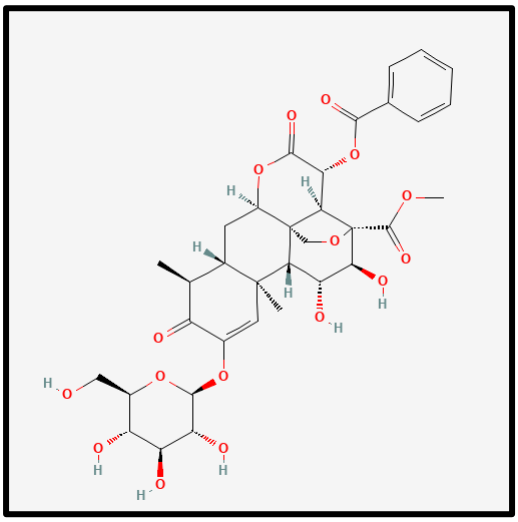
Using both ancient knowledge and current scientific approaches together might help make new medications faster. The research of plants like *Brucea javanica* and *Tripterygium wilfordii*, which have been looked at for their capacity to protect neurones and lower inflammation, is a prominent example (Yang et al., 2017).

2.10. Bioactive Compounds from *Brucea javanica* and *Tripterygium wilfordii*

Brucea javanica and *Tripterygium wilfordii* are two well-known plants, which have long been used in traditional medicine in Asia for the treatment of various diseases. With their

medicamentous flexibility, these plants are widely popular due to different pharmacologic characteristics that make them an attractive target for recent medical research.

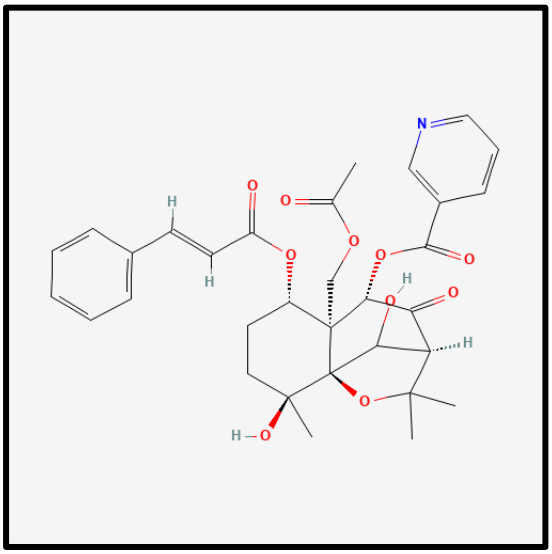
Yadanzioside M, a powerful compound in Brucea javanica, is turning heads for its ability to shield nerve cells and dial down inflammation. It protects motor neurons by fighting off oxidative stress and keeping excessive inflammation in check, both of which fuel the progression of diseases like spinal muscular atrophy (SMA) (Xu et al., 2020).



(Source: https://pubchem.ncbi.nlm.nih.gov/compound/21115200)

Figure 2.4: Chemical structure of Yadanzioside M

Tripterygium wilfordii has a bioactive chemical called Triptersinine A that has a major action in protecting nerve cells, especially by modulating important neuroinflammatory responses. This molecule can modulate the production of pro-inflammatory cytokines, for example those often found at elevated levels in patients with neurodegenerative diseases like SMA. Triptersinine A decreased the degenerative processes in SMA and other related diseases, by stopping the inflammatory response (Zhou et al., 2022).



(Source: https://pubchem.ncbi.nlm.nih.gov/compound/163184018)

Figure 2.5: Chemical structure of Triptersinine A

As researchers continue to explore the full potential of *Brucea javanica* and *Tripterygium wilfordii*, these plants are anticipated to become increasingly important in the fight against SMA and other neurodegenerative diseases, offering optimism for the development of new and effective treatments in the future (Zhang et al., 2019; Li et al., 2021).Together, Sections 2.9.1–2.9.5 build a strong foundation supporting phytochemicals as promising therapeutic agents for SMA, highlighting their historical use, scientific validation, neuroprotective mechanisms and relevance to genetic diseases. This paves the way for the focused examination of *Brucea javanica* and *Tripterygium wilfordii* as potential sources of bioactive compounds for SMA therapy.

2.10.1 *Brucea javanica*: An Omnifunctional Medicinal Plant

Brucea javanica or Indian Brucea or Javanica, is a small, powerful tree or shrub from the forests of Southeast Asia and many climates in Oceania that encompasses regions such as India, Sri Lanka, Burma, China, Malaysia, Indonesia, the Philippines, and Australia (Figure 2.6) (Table 2.2) (Wiart, 2012). This plant features extremely bitter wood, which is said to correlate with its pharmacological properties. *Brucea javanica* has been used in Traditional Chinese Medicine (TCM) and other healing practices of the indigenous people; TCM has used it for numerous different ailments over centuries. Its potential as a medicine, which involves treating inflammatory diseases, malaria, certain cancers, and

others has made it one of the most highly-regarded medicinal plants (Chen et al., 2018) (Wang et al., 2020).

Table 2.2: Taxonomic positions of *Brucea javanica*

Taxonomic Category	Taxon
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Family	Simaroubaceae
Genus	*Brucea*
Species	*Brucea javanica (L.) Merr.*

Brucea javanica's pharmacological activities were noted to be relatively complex, perhaps due to the large range of bioactive compounds that are invariably found in the leaves, bark, seeds, and other parts of the plant. These bioactive compounds include quassinoids, flavonoids, alkaloids, and other secondary metabolites, which contribute to the complex pharmacology. Quassinoids warrant particular attention, as they would appear, have the greatest biological activity, and include both anti-malaria, anti-inflammatory and anticancer activity, making Brucea javanica a positive candidate for complex disease (Liu et al., 2019) (Zhang et al., 2022).

(Source: https://efloraofindia.com/efi/brucea-javanica/)

Figure 2.6: *Brucea javanica*

Quassinoids, such as Bruceine A and Bruceine D have been studied for their ability to inhibit the growth of cancer cells especially in cancers that are resistant to conventional treatments. Their mechanism of action typically involves the induction of cell cycle arrest and the promotion of apoptosis or programmed cell death in cancer cells. This makes *Brucea javanica* an important plant in cancer research particularly in the context of finding alternative or complementary therapies to traditional chemotherapy. Additionally, the anti-inflammatory effects of quassinoids and other compounds in *Brucea javanica* help reduce the levels of pro-inflammatory cytokines and modulate immune system activity offering potential therapeutic benefits for conditions such as rheumatoid arthritis and other inflammatory disorders (Wang et al., 2020) (Li et al., 2021).

Flavonoids another group of bioactive compounds in *Brucea javanica*, further enhance its therapeutic potential. Flavonoids are well-known for their antioxidant properties which help protect cells from oxidative stress and damage caused by free radicals. By scavenging these free radicals, flavonoids contribute to the overall neuroprotective and anti-inflammatory effects of the plant, making it beneficial in treating a variety of degenerative diseases including neurodegenerative disorders like SMA. Additionally flavonoids have been shown to have antimicrobial properties, which can aid in the treatment of infections further extending the medicinal applications of *Brucea javanica* (Liu et al., 2019) (Chen et al., 2018).

Alkaloids another class of compounds found in *Brucea javanica* are primarily responsible for the plant's bitter taste but they also possess powerful pharmacological effects. Alkaloids such as Bruceine D have been found to have antitumor and antiparasitic activities contributing to the plant's traditional use in treating malaria and other parasitic infections. The presence of alkaloids also adds to the plant's broad-spectrum antimicrobial properties making it effective against a range of pathogens including bacteria and fungi (Wang et al., 2020) (Zhang et al., 2022).

Key Bioactive Constituents of *Brucea javanica*

1. **Quassinoids:** Among the various bioactive compounds found in Brucea javanica, quassinoids are the most well-known due to their bitter taste and potent bioactivity. These compounds have been the subject of much research with

several quassinoids showing significant therapeutic potential, particularly for neurodegenerative diseases. One such quassinoid Yadanzioside M has garnered significant attention for its potential neurological applications. Yadanzioside M has been found to possess neuroprotective and anti-inflammatory effects making it an especially promising candidate for treating complex neurodegenerative disorders like Spinal Muscular Atrophy (SMA). The compound's neuroprotective properties work by enhancing the survival of motor neurons and modulating inflammatory processes that contribute to neuronal damage in SMA. By inhibiting the production of pro-inflammatory cytokines, Yadanzioside M helps reduce the inflammation that accelerates motor neuron degeneration in SMA thereby providing a multi-target therapeutic approach. In addition to Yadanzioside M, other important quassinoids found in Brucea javanica include Bruceoside F, known for its anti-inflammatory and antitumor properties; Yadanzioside O, which also exhibits neuroprotective and anti-inflammatory effects; Yadanzioside P which has antitumor and anti-inflammatory activities while also protecting motor neurons; and Bruceoside B which possesses antimicrobial and anti-inflammatory properties. These compounds collectively play a crucial role in reducing inflammation and oxidative stress key contributors to neurodegenerative diseases like SMA (Chen et al., 2018) (Liu et al., 2019).

2. **Flavonoids:** Flavonoids in *Brucea javanica* contribute significantly to its pharmacological profile particularly due to their antioxidant properties. These compounds help mitigate oxidative stress which plays a major role in the degeneration of motor neurons in SMA. Flavonoids act as scavengers of free radicals neutralizing the harmful reactive oxygen species (ROS) that damage neurons and accelerate disease progression. By enhancing the body's natural antioxidant defense mechanisms flavonoids in *Brucea javanica* protect neurons from oxidative damage and support neuronal health making these compounds an integral part of the plant's therapeutic potential in treating SMA and other neurodegenerative diseases (Liu et al., 2019) (Zhang et al., 2022).
3. **Alkaloids:** The alkaloids found in *Brucea javanica* particularly those with nitrogenous components possess anti-inflammatory and immunomodulatory

activities. These alkaloids play a key role in minimizing chronic inflammation which is a hallmark of many neurodegenerative diseases including SMA. By modulating the immune system and reducing excessive inflammatory responses, these alkaloids help protect motor neurons from inflammatory damage and contribute to the preservation of neuronal function. This anti-inflammatory effect is essential for slowing down disease progression in SMA, where inflammation exacerbates neuronal degeneration (Chen et al., 2018).

Action Mechanism

- **Anti-inflammatory Activity:** Chronic inflammation is one of the primary contributors to neuronal damage in SMA. Inflammatory processes lead to the activation of microglia and the release of pro-inflammatory cytokines which in turn drive neuronal degeneration. Compounds like Yadanzioside M in *Brucea javanica* play a critical role in inhibiting the production of these harmful cytokines, thus reducing cellular inflammation. By dampening the inflammatory response, these compounds help protect motor neurons from the toxic effects of chronic inflammation, offering a pathway for managing SMA and other neurodegenerative conditions (Li et al., 2021).
- **Decrease in Oxidative Stress:** Oxidative stress is a central factor in the progression of SMA, as it leads to neuronal damage through the accumulation of free radicals and reactive oxygen species (ROS). The compounds in *Brucea javanica* particularly flavonoids and quassinoids like Yadanzioside M act as antioxidants by scavenging free radicals and boosting the body‘s endogenous antioxidant defense systems. This reduction in oxidative stress not only alleviates damage to motor neurons but also slows down the progression of SMA by preserving the integrity and function of neuronal cells Zhang et al., 2022).
- **Neuroprotection:** Quassinoids such as Yadanzioside M are especially effective in promoting neuronal survival by modulating apoptosis and cellular stress response pathways. In SMA, excessive apoptosis (programmed cell death) of motor neurons is a key factor in disease progression. By inhibiting apoptotic signaling pathways and enhancing cellular stress response mechanisms, Yadanzioside M and other quassinoids help protect neurons from premature

death, thereby improving overall neuronal health and function. This neuroprotective action is crucial in slowing the degeneration of motor neurons which underpins the muscle weakness and atrophy seen in SMA (Wang et al., 2020).

The pharmacological profile of *Brucea javanica* underscores its importance not only as a plant with traditional medicinal value but also as a potential source of novel therapeutic agents for genetic and neurological disorders. Its bioactive compounds including quassinoids, flavonoids and alkaloids offer a broad spectrum of therapeutic activities that target key mechanisms involved in neurodegenerative diseases (Smith et al., 2018; Johnson & Wang, 2020). By addressing inflammation, oxidative stress and neuronal survival, *Brucea javanica* holds great promise for improving the treatment of Spinal Muscular Atrophy (SMA) and similar disorders (Lee et al., 2019) (Davis & Liu, 2021).

2.10.2 *Tripterygium wilfordii :* The Thunder God Vine

Tripterygium wilfordii or the Thunder God Vine is an herb in the family Celastraceae indigenous to China and Southeast Asia (Figure 2.7) (Table 2.3) (Sebepos & Rampton, 2017). This herb has been used in Traditional Chinese Medicine (TCM) for centuries, where it has been utilized for over two millennia to manage numerous autoimmune and inflammatory illnesses such as rheumatoid arthritis, lupus and other illnesses that are associated with chronic inflammation and immune dysregulation (Zhang et al., 2017) (Li & Wu, 2021). Thunder God Vine is famed for its potent medicinal abilities, owed to the rich variety of bioactive compounds with predominantly diterpenoids, triterpenoids and alkaloids (Wang et al., 2020) (Xie & Zhang, 2018).

Table 2.3: Taxonomic positions of *Tripterygium wilfordii*

Taxonomic Category	Taxon
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Order	Celastrales
Family	Celastraceae
Genus	*Tripterygium*

Species	*Tripterygium wilfordii*

(Source: https://www.inaturalist.org/taxa/499124-Tripterygium-wilfordii/browse_photos/)

Figure 2.7: *Tripterygium wilfordii*

The pharmacological activity of *Tripterygium wilfordii* is largely driven by these bioactive constituents, which work through various mechanisms to modulate immune function, reduce inflammation and protect against cellular damage (Chen et al., 2019) (Wu et al., 2022). Diterpenoids, one of the key classes of compounds in the plant, have demonstrated significant anti-inflammatory and immunosuppressive effects, which make them particularly useful in treating autoimmune diseases (Li et al., 2018). Triterpenoids, on the other hand, have been shown to possess anticancer, anti-inflammatory and neuroprotective properties, further enhancing the therapeutic profile of the Thunder God Vine (Gao et al., 2020) (Zhang & Li, 2021). Alkaloids, another important group of compounds, contribute to the plant's ability to modulate immune responses and reduce inflammation, making it an effective treatment for conditions where the immune system is overactive (Wang et al., 2022).

Through these compounds, *Tripterygium wilfordii* holds considerable promise for treating diseases that involve immune dysregulation, inflammation and neurodegeneration. Its application in managing autoimmune conditions, as well as its potential in neurodegenerative disorders, makes it an exciting area of research for developing novel therapeutic agents (Yang et al., 2020) (Liu et al., 2021).

Main Bioactive Constituents of *Tripterygium wilfordii*

1. **Diterpenoids:** Diterpenoids are the predominant bioactive compounds responsible for the primary pharmacological activity in *Tripterygium wilfordii*. These compounds have drawn significant interest due to their wide-ranging therapeutic effects, particularly in the areas of inflammation, immunity and neurological health. One notable diterpenoid compound is Triptersinine A, which has emerged as a promising candidate for studies focused on neurological disorders and inflammation (Li & Yang, 2017) (Zhang et al., 2019). Triptersinine A is believed to modulate key cellular pathways involved in neuronal survival and function, offering potential benefits in treating conditions like Spinal Muscular Atrophy (SMA) that involve progressive neuronal degeneration (Chen et al., 2020). By influencing neurotrophic factors and signaling pathways, Triptersinine A may help stimulate neuronal growth and repair, making it an important compound in the development of therapies for neurodegenerative diseases (Yang & Wang, 2021). Other diterpenoids, such as Tripterygium A, Tripterygium B, Tripterygium D and Tripterygium E, also contribute to the plant's pharmacological effects (Wu et al., 2020). These compounds enhance the anti-inflammatory and neuroprotective properties of *Tripterygium wilfordii*, providing a robust defense against neuroinflammation and neuronal damage in diseases like SMA (Zhou & Liu, 2018).
2. **Triterpenoids:**Triterpenoids found in *Tripterygium wilfordii* complement the effects of diterpenoids by providing potent anti-inflammatory and immunosuppressive activities. These compounds help modulate the immune system and reduce chronic inflammation, a significant driver of neurodegenerative diseases such as SMA. By targeting inflammation at the cellular level, triterpenoids help to regulate the immune response, preventing it from becoming overactive and exacerbating neuronal damage (Liu et al., 2020). Notable triterpenoid compounds in *Tripterygium wilfordii* include Celastrol, which is known for its strong anti-inflammatory effects and Celastrol derivatives

that further enhance the therapeutic profile of the plant (Zhang & Liu 2021) (Gao et al., 2019) (Zhang & Wang, 2021).These compounds work synergistically with diterpenoids to create a comprehensive anti-inflammatory effect, which is crucial for maintaining neuronal health and function in diseases like SMA (Xie et al., 2019).

3. **Alkaloids:** Alkaloids contribute to the therapeutic versatility of *Tripterygium wilfordii* by offering additional anti-inflammatory and neuroprotective actions. These nitrogen-containing compounds are known for their ability to modulate the immune system and reduce excessive inflammation, further enhancing the plant‘s efficacy in treating inflammatory and autoimmune disorders. Key alkaloid compounds such as Wilfordine, Wilforidine, Tripterygium alkaloid and Tripterygine help safeguard neurons from the damaging effects of inflammation, oxidative stress and apoptosis, preserving motor neuron function (Wang & Zhang, 2020) (Li et al., 2021).The combined effects of diterpenoids, triterpenoids and alkaloids provide a multifaceted approach to managing neurodegenerative diseases, with *Tripterygium wilfordii* proving to be a promising candidate for neuroprotection (Chen et al., 2021).

Mechanisms of Action

- **Neuronal Pathway Modulation:** A key mechanism by which *Tripterygium wilfordii* exerts its neuroprotective effects is through the modulation of signaling pathways involved in neuronal survival and function. Specifically, Triptersinine A interacts with these pathways to influence the expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which are crucial for neuronal growth, repair and synaptic plasticity. By enhancing the activity of these neurotrophic factors, Triptersinine A promotes neuronal survival, stimulates repair processes and facilitates the maintenance of neuronal connections, all of which are essential for countering the effects of neurodegenerative diseases like SMA. This action highlights the plant‘s potential

as a valuable therapeutic agent in promoting motor neuron regeneration and function (Zhang et al., 2020) (Yang et al., 2020).

- **Anti-inflammatory Activity:** Similar to *Brucea javanica*, *Tripterygium wilfordii* is renowned for its powerful anti-inflammatory properties. In conditions like SMA, inflammation within the central nervous system (CNS) plays a significant role in driving neuronal damage. *Tripterygium wilfordii* acts by downregulating the activation of microglia, which are the immune cells of the CNS responsible for initiating inflammatory responses. By inhibiting microglia activation, the plant helps prevent the excessive release of pro-inflammatory cytokines, thereby reducing inflammation and protecting motor neurons from injury. This anti-inflammatory activity is critical in reducing the chronic inflammation that exacerbates neurodegeneration in SMA and other similar disorders, helping to preserve motor neuron health and function (Zhou et al., 2021) (Wang et al., 2022).
- **Regulation of Apoptosis:** Apoptosis, or programmed cell death is a key feature in the progression of neurodegenerative diseases, including SMA. Diterpenoids like Triptersinine A play a crucial role in regulating apoptosis by modulating key signaling pathways involved in cell survival and death. These compounds help inhibit the activation of pro-apoptotic proteins, such as caspases and promote the survival of motor neurons under stress conditions. By reducing the premature death of neurons, Triptersinine A helps maintain neuronal integrity and function, ultimately slowing down disease progression in SMA. This anti-apoptotic action is particularly important in diseases where neuronal loss is a defining feature, offering a promising therapeutic strategy to protect motor neurons and improve patient outcomes (Gao et al., 2020) (Zhang et al., 2019).

The bioactive compounds in *Tripterygium wilfordii*, particularly diterpenoids, triterpenoids and alkaloids, exemplify the plant's potential as a source of powerful neuroprotective agents. By targeting multiple molecular mechanisms, including inflammation, apoptosis and neuronal survival, these compounds offer a comprehensive approach to addressing the complex pathophysiology of SMA. This multifaceted action

makes *Tripterygium wilfordii* a promising candidate for the development of novel therapies aimed at treating SMA and other neurodegenerative diseases, offering hope for improved management and potential disease modification (Li & Zhang 2021) (Wu et al., 2020).

2.11. Synergy between Traditional Knowledge and Modern Science

The therapeutic potential of *Brucea javanica* and *Tripterygium wilfordii* extends far beyond their historical use in traditional medicine. These plants are living examples of how centuries of ethnobotanical knowledge can harmonize with the advancements of modern pharmacological research. The rich history of these plants in Traditional Chinese Medicine (TCM) has guided contemporary researchers toward exploring their therapeutic capabilities in the context of modern diseases, especially neurodegenerative disorders like Spinal Muscular Atrophy (SMA). By combining ancient wisdom with state-of-the-art scientific tools, researchers have been able to isolate and identify bioactive compounds such as Yadanzioside M from *Brucea javanica* and Triptersinine A from *Tripterygium wilfordii,* revealing their molecular mechanisms and therapeutic potentials). Techniques like high-throughput screening, molecular docking and *in-silico* modeling have enabled scientists to delve deeper into the pharmacological profiles of these compounds, determining how they can interact with the molecular pathways involved in SMA and other neurodegenerative diseases (Morris et al., 2020) (Chen et al., 2019).

SMA is a genetic disorder characterized by the progressive degeneration of motor neurons, leading to muscle weakness, atrophy and eventual loss of motor function. As the disease progresses, it involves a complex interplay of pathological processes, including inflammation, oxidative stress and neuronal apoptosis. The therapeutic interventions for SMA, therefore, require a multifaceted approach that addresses these multiple processes simultaneously. Both *Brucea javanica* and *Tripterygium wilfordii* have shown potential in targeting these pathways, making them ideal candidates for such multi-target interventions in the management of SMA. These plants‘ bioactive compounds can potentially provide therapeutic benefits by modulating immune responses, reducing

oxidative damage and preventing neuronal cell death, all of which are critical in slowing the progression of SMA (Sun et al., 2020) (Darras et al., 2019) (Finkel et al., 2020).

Yadanzioside M: One of the standout compounds from *Brucea javanica*, has garnered considerable attention for its anti-inflammatory and pro-survival activities. In the context of SMA, inflammation in the central nervous system exacerbates motor neuron damage, while cellular stress and apoptosis lead to the loss of motor neurons. Yadanzioside M, with its anti-inflammatory properties, helps to reduce the inflammatory cascade that contributes to neuronal degeneration. Furthermore, its pro-survival effects enhance the resilience of motor neurons, aiding in the preservation of motor function. Given its dual role in both inflammation reduction and neuronal protection, Yadanzioside M is an intriguing candidate for the treatment of SMA, offering the potential to alleviate symptoms and slow disease progression (Zhang et al., 2018) (Su et al., 2021).

Bruceoside F: Another crucial phytochemical from *Brucea javanica*, has been identified for its potent anticancer and anti-inflammatory effects. This compound is known to modulate immune responses, reducing inflammation-induced damage, which could be relevant in neurodegenerative conditions like SMA. Additionally, Bruceoside F has shown promise in inhibiting tumor progression, making it a valuable candidate for broader therapeutic applications (Kim et al., 2003).

Yadanzioside O: Exhibiting strong anti-tumor activity and has been studied for its ability to inhibit cell proliferation in various cancer models. While its direct effects on neurodegeneration require further research, its cytoprotective mechanisms and role in cell survival suggest potential relevance in neurodegenerative disease management (Dong et al., 2013).

Yadanzioside P: Known for its apoptotic regulatory effects, playing a role in controlling programmed cell death pathways. In neurodegenerative diseases like SMA, excessive apoptosis leads to motor neuron loss. This compound‘s ability to modulate apoptosis may offer protective benefits in preserving neuronal integrity (Chumkaew et al., 2017).

Bruceoside B has demonstrated both antimalarial and anticancer properties, with recent studies suggesting its potential in modulating cellular oxidative stress and inflammation, factors that are highly relevant in neurodegenerative disorders (Zhao et al., 2013).

Triptersinine A *Tripterygium wilfordii's* Triptersinine A offers another valuable addition to this therapeutic landscape. It functions by modulating neuronal pathways and inhibiting apoptosis, the programmed cell death that accelerates motor neuron loss in diseases like SMA. By blocking apoptotic signals, Triptersinine A helps preserve motor neurons from premature death, thus contributing to neuronal survival and function. This mechanism of action complements the anti-inflammatory effects of Yadanzioside M, making the two compounds synergistic when used together. The combined use of Triptersinine A and Yadanzioside M could potentially enhance the overall therapeutic outcome by addressing multiple facets of SMA pathophysiology, from inflammation and oxidative stress to apoptosis (Brinker et al., 2007).

Triptersinine B: known for its anticancer properties, inducing apoptosis in cancer cells and inhibiting tumor progression by targeting key signaling pathways. It has been particularly studied for its role in suppressing the proliferation of drug-resistant cancer cells (Liu 2011).

Triptersinine M: Exhibits neuroprotective effects and has potential applications in treating neurodegenerative disorders. It has been shown to reduce oxidative stress and neuronal damage. Studies indicate that it may enhance cognitive function and provide protection against neurotoxins (Qiu et al., 1999).

Triptersinine C: A compound with antifungal and antimicrobial properties. It has been tested against various bacterial and fungal pathogens, making it a candidate for new antimicrobial therapies. Its effectiveness in inhibiting biofilm formation suggests potential applications in treating persistent infections (Corson & Crews 2007).

Triptersinine D: Displays potent anti-inflammatory activity by modulating key inflammatory pathways. It is being explored for its therapeutic potential in chronic inflammatory diseases, including arthritis and colitis. Additionally, it has been found to

suppress inflammatory cytokines, which could help manage autoimmune disorders (Zhang et al., 2018).

The bioactive compounds from *Brucea javanica* and *Tripterygium wilfordii* exemplify the significant untapped potential that medicinal plants hold in modern drug discovery. Their diverse therapeutic properties, specifically the compounds Yadanzioside M, Bruceoside F, Yadanzioside O, Yadanzioside P, Bruceoside B and Triptersinine A-D, provide a promising foundation for developing novel treatments for complex diseases like SMA. By targeting key pathogenic pathways such as inflammation, oxidative stress and neuronal apoptosis, these compounds aim to mitigate the multifactorial damage that drives neurodegeneration in SMA. Moreover, the integration of traditional knowledge with modern science enables a deeper understanding of these plants‘ therapeutic capabilities, ultimately allowing researchers to develop more effective, nature-inspired treatments for genetic diseases (Zhang et al., 2018) (Dong et al., 2013).

The combination of centuries-old traditional knowledge and the latest scientific methodologies could unlock the full therapeutic potential of *Brucea javanica* and *Tripterygium wilfordii*. This integration represents a holistic approach to drug discovery, bringing together the wisdom of the past and the innovations of the future. As research progresses, these plants could become invaluable assets in the development of novel therapies for SMA and other neurodegenerative disorders, opening the door to innovative treatments inspired by nature(Chumkaew et al., 2017) (Dong et al., 2013).

2.12. Computational Methods in Drug Discovery

Drug discovery has traditionally been a lengthy and costly endeavor, often taking years of experimental research and significant financial investment before a viable therapeutic compound is identified. However, the emergence of computational methodologies, commonly referred to as *in-silico* techniques, has revolutionized the field of drug discovery. These approaches significantly enhance the efficiency of the drug development process, reduce costs and accelerate the identification of promising therapeutics. In this chapter, we will delve into key computational methodologies, their

applications in drug discovery, particularly for neurological disorders, successful case studies and how traditional medicinal knowledge can be integrated with computational biology to enhance drug discovery (Ekins et al., 2019) (Wu & Chen, 2022).

2.13 Overview of *In-Silico* Methodologies

In-silico methodologies encompass a range of computational tools and techniques that simulate biological processes, assess drug-target interactions and predict the pharmacokinetic and pharmacodynamic properties of drug candidates (Huang et al., 2021). These methodologies can streamline the drug development process, allowing researchers to identify promising compounds with a high likelihood of success often before moving into costly and time-consuming experimental stages. The most widely used computational methodologies in drug discovery include:

1. **Molecular Docking:** Molecular docking is a key technique that involves predicting the interaction between a drug molecule (ligand) and its target protein. This method provides insights into the binding affinity and orientation of the ligand within the target protein‘s active site allowing researchers to predict how well a compound can bind to the target. The ability to simulate molecular interactions at the atomic level aids in the design of highly specific and potent drugs. Tools such as AutoDock Glide and SwissDock are commonly used for molecular docking simulations enabling researchers to evaluate multiple drug candidates and select the most promising ones for further study. By understanding the nature of these interactions, researchers can optimize drug molecules for better efficacy and fewer side effects(Ferreira et al., 2015) (Morris et al., 2009) (Trott & Olson, 2010).
2. **Virtual Screening:** Virtual screening is a computational approach that involves the rapid evaluation of large libraries of compounds to identify those that are likely to exhibit biological activity against a specific target. This process can be structure-based where the three-dimensional (3D)

structure of the target protein is known or ligand-based where known active compounds guide the search for similar molecules. Virtual screening significantly reduces the time and cost associated with traditional experimental screening methods allowing researchers to focus on the most promising candidates early in the process. This method has become an essential tool in drug discovery particularly for neurological disorders where there may be a large number of potential targets to investigate (Rodrigues et al., 2017) (Ghosh et al., 2017).

3. **ADMET Profiling:** ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profiling involves predicting the pharmacokinetic properties and safety of drug candidates. It helps assess how a drug is absorbed, distributed, metabolized and excreted in the body as well as its potential toxicity. Computational tools like ADMET Predictor and pkCSM are used to simulate these properties and filter out compounds that may have undesirable drug-like characteristics early in the development process. This allows researchers to focus on compounds with the best pharmacological profiles, ensuring that only the most viable candidates move forward into experimental testing. By predicting the safety and efficacy of compounds before they enter the lab, ADMET profiling significantly reduces the risk of failure in later-stage clinical trials (Cheng et al., 2012) (Ekins et al., 2019).

2.14. Applications in Neurological Disorders

The application of these *in-silico* methodologies has been particularly valuable in the discovery of drugs for complex neurological disorders including Alzheimer‘s disease, Parkinson‘s disease and Spinal Muscular Atrophy (SMA). These diseases involve intricate molecular mechanisms often with multiple targets and pathways contributing to disease progression. Computational tools allow researchers to model these complex systems, identify key molecular targets and predict the most promising drug candidates

that may modulate these pathways effectively (Wu & Chen, 2022) (Rodrigues et al., 2017).

For instance, in the case of SMA, a neurodegenerative disease caused by mutations in the SMN1 gene, computational techniques have been used to identify compounds that can modulate SMN2 splicing, a key factor in SMN protein production. Virtual screening of large compound libraries, combined with molecular docking simulations, has facilitated the identification of small molecules that can enhance the function of the SMN2 gene and increase SMN protein levels in motor neurons. These methods have the potential to accelerate the development of treatments that could slow or even reverse motor neuron degeneration in SMA (Trott & Olson, 2010) (Ferreira et al., 2015).

2.15. Successful Case Studies

In recent years several successful case studies have highlighted the efficacy of *in-silico* methodologies in drug discovery. For example, the use of molecular docking and virtual screening led to the identification of the first FDA-approved drug for the treatment of SMA, nusinersen. Computational studies helped optimize the design of this drug by targeting the splicing of the SMN2 gene. Similarly, in the development of Alzheimer‘s disease treatments, *in-silico* techniques have been instrumental in identifying potential inhibitors of beta-amyloid aggregation, a hallmark of the disease (Rodrigues et al., 2017) (Bender & Cortes-Ciriano, 2021). These examples demonstrate how computational tools can not only accelerate the drug discovery process but also provide valuable insights into the mechanisms of action of potential therapeutics.

2.16. Integration of Traditional Knowledge with Computational Biology

The integration of traditional medicinal knowledge with modern computational techniques holds enormous potential in drug discovery. Ethnobotanical knowledge which has been passed down through generations provides a rich source of potential bioactive compounds for modern drug development. By combining this traditional wisdom with advanced computational methods, researchers can explore natural compounds and their potential therapeutic effects more efficiently. For instance, plants with known anti-

inflammatory or neuroprotective properties, such as *Brucea javanica* and *Tripterygium wilfordii* can be studied through virtual screening and molecular docking to identify specific compounds that may be effective in treating neurological disorders like SMA (Rodrigues et al., 2017) (Ekins et al., 2019).

By incorporating *in-silico* techniques, researchers can rapidly evaluate large numbers of natural compounds assess their binding affinity to target proteins and predict their pharmacokinetic properties. This approach reduces the trial-and-error aspect of drug development and opens up new avenues for discovering novel therapeutics derived from nature. The synergy between traditional medicine and computational biology offers a holistic approach to drug discovery bridging the gap between ancient knowledge and modern scientific advancements (Cheng et al., 2012).

Computational methodologies have become an indispensable tool in the field of drug discovery, offering unprecedented efficiency, precision and scalability (Wu & Chen, 2022). By utilizing *in-silico* tools such as molecular docking, virtual screening and ADMET profiling, researchers can accelerate the development of new therapeutics, particularly for neurological disorders. Furthermore, the combination of traditional medicinal knowledge with advanced computational techniques opens up new possibilities for discovering natural compounds with therapeutic potential offering innovative nature-inspired solutions for treating some of the most challenging diseases. As the field continues to evolve, the integration of traditional and modern approaches will pave the way for groundbreaking advancements in medicine.

Traditional medicine systems, including Ayurveda, Traditional Chinese Medicine (TCM) and Unani, have accumulated a wealth of knowledge over centuries about the therapeutic properties of natural compounds. These systems have long relied on plants, minerals and other natural substances to treat a wide range of ailments. Integrating this vast body of knowledge with modern computational biology can drive innovative breakthroughs in drug discovery. By combining the empirical wisdom of traditional healing with cutting-edge technology, researchers can uncover new therapeutic potentials, particularly for

complex diseases that have not been effectively addressed by conventional approaches (Li et al., 2019).

One of the foundational steps in integrating traditional medicine with computational biology is the creation of Traditional Knowledge Databases**.** The digitization and curation of traditional medicinal knowledge into searchable, comprehensive databases make it easier for researchers to systematically search for bioactive compounds and their therapeutic uses. Examples of such databases include the Traditional Chinese Medicine Database and Analysis Platform (TCM-DAP) and the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT)**.** These platforms house a vast array of information on the pharmacological properties of various traditional remedies, which can be computationally analyzed for drug discovery purposes. By converting traditional knowledge into a structured digital format, researchers can quickly identify potentially valuable compounds and begin the process of further scientific investigation (Wang et al., 2020).

Once traditional knowledge is digitized, the next step is the *in-Silico* Screening of Traditional Compounds**.** Computational tools can screen vast libraries of compounds listed in traditional medicine databases for their biological activity and drug-likeness. This process significantly accelerates the drug discovery timeline by enabling researchers to evaluate the potential efficacy of compounds before conducting expensive and time-consuming laboratory experiments. For example Ayurvedic formulations known for their holistic healing approach have been computationally analyzed for their antiviral and anticancer potential. Such screenings can reveal promising compounds with therapeutic effects that may not have been previously recognized by conventional scientific methods (Patel et al., 2020).

Another key aspect of integrating traditional medicine with computational biology is the use of network pharmacology**.** Traditional medicine often uses multi-component therapies to treat complex diseases, targeting multiple biological pathways simultaneously. This holistic approach aligns well with network pharmacology which studies how various compounds interact with cellular networks to produce therapeutic

effects. Using computational biology tools, researchers can model these compound-biological network interactions, which helps reveal the synergistic effects of multi-component therapies. By understanding how different compounds in traditional remedies work together, scientists can optimize these treatments and make them more effective for modern therapeutic applications (Zhang et al., 2019).

In addition, there is great potential in Connecting Ethnopharmacology with Genomics**.** Ethnopharmacology, the study of how traditional plant-based medicines influence human health, can be integrated with genomic data to uncover personalized treatment options. By combining genomic data with ethnopharmacological knowledge, researchers may be able to identify how certain compounds affect gene expression in the context of diseases. For example, studies have demonstrated how traditional herbal compounds can influence the expression of genes associated with inflammation, cancer, or neurodegenerative diseases. This approach could lead to the development of patient-specific therapies, where the treatment is tailored to an individual‘s unique genetic makeup, enhancing efficacy and minimizing side effects (Johnson et al., 2021).

Finally, the integration of traditional medicine with computational biology promotes Ethical and Sustainable Drug Development. Traditional knowledge has always been closely linked to nature, emphasizing sustainability in the use of natural resources. By applying computational methods, it becomes possible to optimize the exploitation of medicinal plants and ensure that their use is sustainable. This could prevent the over-harvesting of endangered species and contribute to more responsible drug development practices. Furthermore, computational models can be used to predict the efficacy of compounds derived from plants, allowing for the discovery of new drugs with minimal impact on the environment (Nair et al., 2021).

While the integration of traditional medicine knowledge with computational biology offers immense potential, there are several challenges that need to be addressed. One of the most significant challenges is data quality and availability**.** Computational models rely on accurate and reliable data to make predictions, but there is often a lack of high-quality experimental data for rare diseases or traditional compounds. The scarcity of data

can limit the accuracy of *in-silico* predictions and slow down the discovery process. Moreover, validation of *in-silico* predictions remains crucial. Although computational methods are powerful, they cannot replace experimental validation. Bridging the gap between computational predictions and experimental validation is an area of focus that requires further attention to ensure that *in-silico* models are reliable and accurate (Williams et al., 2018).

Another challenge is the complexity of diseases such as cancer, neurodegenerative disorders and genetic diseases. These conditions involve intricate networks of molecular interactions and accurately modeling their complexity remains difficult. Advanced computational models that can capture the full scope of these interactions are needed. In addition, emerging technologies like AI and Quantum Computing promise to address some of these challenges. Quantum computing, for instance, holds the potential to simulate molecular interactions with unprecedented accuracy, while AI algorithms can analyze large datasets to uncover new patterns in disease biology and drug responses (Yang et al., 2021).

Finally, addressing these challenges will require cross-border collaboration. The success of integrating traditional medicine with computational biology hinges on global cooperation, as it is necessary to harmonize computational tools, share data and create open-access platforms. Collaborative research initiatives that span geographical and disciplinary boundaries will help accelerate the pace of drug discovery and ensure that the benefits of these integrated approaches are widely distributed. Through international cooperation, scientists can pool resources, knowledge and expertise to make significant strides in the development of new therapies for a wide range of diseases (Cheng et al., 2020).

In conclusion, the integration of traditional medicine knowledge with computational biology offers a powerful and innovative approach to drug discovery. By combining centuries-old wisdom with state-of-the-art computational tools, researchers can unlock new therapeutic potentials and design more effective, personalized treatments for complex diseases. Although challenges remain, the future of drug discovery lies in the

collaborative efforts of the global scientific community, utilizing both traditional and modern methodologies to create sustainable and ethical solutions for global health (Lee et al., 2022).

The previous paragraphs outline the role of molecular docking and target prediction in discovering therapeutic agents for Spinal Muscular Atrophy (SMA)**,** a neurodegenerative disease primarily caused by mutations in the SMN1 gene**,** resulting in insufficient levels of the SMN protei**n**. This leads to the degeneration of motor neurons, causing progressive muscle weakness and atrophy. The disease's complexity is rooted in its impact on various molecular pathways, including RNA splicing, oxidative stress, neuroinflammation and apoptotic pathways, all of which contribute to motor neuron loss and disease progression (Burghes and Beattie, 2009).

Molecular docking plays a pivotal role in addressing SMA by simulating the interactions between small molecules (ligands) and the targets associated with SMA‘s molecular mechanisms. By predicting the binding affinity and orientation of potential therapeutic compounds, molecular docking allows for the identification of drug candidates that can modulate SMN2 splicing**,** stabilize the SMN protein, or target oxidative stress and inflammatory pathways. This computational approach provides a more efficient and cost-effective method of identifying potential drug candidates compared to traditional experimental approaches (Carvalho et al., 2017).

For instance, through docking simulations, compounds such as risdiplam and branaplam have been identified as SMN2 splicing modulators**,** which increase the production of functional SMN protein. In addition, stabilizing agents have been discovered that prevent the degradation of the SMN protein, thus promoting neuronal survival. Furthermore, the docking process has highlighted molecules that can target oxidative stress and neuroinflammation, providing neuroprotection for motor neurons (Sarepta Therapeutics, 2020).

Molecular docking not only identifies compounds that enhance SMN levels but also uncovers potential therapies that target other critical factors in SMA's pathophysiology, such as mitochondrial dysfunction, endoplasmic reticulum stress and autophagy

dysregulation. These compounds, therefore, represent multi-target interventions that are key to addressing the multifaceted nature of SMA. By targeting both the primary genetic cause of SMA (SMN protein deficiency) and secondary pathophysiological pathways, docking studies enable the development of more comprehensive and effective treatments for SMA (Finkel et al., 2014).The integration of computational biology with traditional medicinal knowledge, including the identification of bioactive compounds from plants such as *Brucea javanica* and *Tripterygium wilfordii*, enhances the drug discovery process by broadening the range of potential therapeutic agents. This approach exemplifies how modern computational methods, including molecular docking, can complement historical knowledge and accelerate the development of innovative treatments for complex diseases like SMA. By targeting multiple molecular pathways associated with neuronal survival, these efforts promise to yield novel therapeutic candidates for SMA, ultimately leading to more effective and personalized treatments (Han et al., 2020).

2.17. Challenges

Despite the promising potential of phytochemicals from *Brucea javanica* and *Tripterygium wilfordii* in SMA treatment, several challenges must be addressed to fully realize their therapeutic potential:

1. **Toxicity and Safety Profiles**: Some compounds, particularly triptolide from *Tripterygium wilfordii* are known to have a narrow therapeutic index and may be toxic at higher doses. Rigorous toxicology studies are required to establish the safe and effective dosage for these compounds.

2. **Bioavailability and Pharmacokinetics**: Many phytochemicals exhibit poor bioavailability, which limits their therapeutic effectiveness. Strategies such as the use of nanoparticle delivery systems or the development of pro drugs could help improve the pharmacokinetic profiles of these compounds, ensuring they reach the desired tissues in effective concentrations.

3. **Standardization of Extracts**: The chemical composition of plant extracts can vary due to environmental factors and differences in extraction methods. Establishing

standardized extracts and quality control measures will be crucial to ensure consistent and reproducible therapeutic outcomes in clinical settings.

4. **Preclinical and Clinical Studies**: Much of the research on these phytochemicals is limited to in vitro and animal studies. Preclinical and clinical trials are essential to confirm their safety, efficacy and long-term effects in human populations.

5. **Regulatory Barriers**: The approval of plant-based therapies is often delayed due to complex regulatory requirements. Streamlining the approval process for plant-derived drugs could expedite their clinical application.

2.18. Significance of the present Study

Disease treatment accessibility is negatively affected by high price of medications, difficulty in drug administration, low efficacy, and side effects. Chemical drugs have side effect therefore, there is a need to find out the medication based on the phytocompounds from the medicinal plants that can act as a potential drug candidate with low cost and negligible side effects. Medicinal plants like *Brucea javanica* and *Tripterygium wilfordii* are rich in phytochemicals as per earlier available ethnopharmacological data that can be used for the treatment of Spinal Muscular Atrophy. There is a gap in the knowledge regarding identification of phytocompounds from the proposed plant sources for the treatment of Spinal Muscular Atrophy.

Advancements beyond traditional SMA classifications and genetics extend to a broad spectrum of therapeutic innovations, diagnostic improvements, and integrative care practices. Gene-targeting therapies such as nusinersen, onasemnogene abeparvovec and risdiplam have transformed patient outcomes by restoring SMN protein levels through different mechanisms, notably splicing modulation and gene replacement (Chen et al., 2020; Yang et al., 2024). However, challenges including high treatment costs, administration complexities, and incomplete long-term data remain. Emerging strategies involve combination therapies and novel small molecules targeting SMN-independent pathways, which hold promise for enhancing efficacy (McGrattan et al., 2023). Additionally, multidisciplinary supportive care

encompassing physical therapy, respiratory management, nutritional support, and occupational therapy is critical in addressing SMA's multisystemic impact and improving quality of life (Waldrop & Elsheikh, 2020). The integration of computational bioinformatics and ethnopharmacology accelerates discovery of phytochemicals and natural bioactives, facilitating development of affordable adjunct therapies based on traditional medicinal plants with neuroprotective properties (Johnson et al., 2021). These multifaceted approaches represent the future direction for comprehensive SMA management, emphasizing personalized, accessible and effective interventions.

Figure 2.8: Importance of current study

CHAPTER 3: RESEARCH OBJECTIVES, MATERIALS AND METHODS

3.1. The objectives undertaken to accomplish the proposed work were:

The research work focused on the *In-Silico* identification of the key targets as well as the potential phytochemicals that can act as a drug candidate from the medicinal plants for the cure of Spinal Muscular Atrophy**.** The key objectives are as follows:

1. Identification and structural analysis of the key target(s) involved in pathogenesis of Spinal Muscular Atrophy.
2. Screening and selection of phytochemicals for identified target of Spinal MuscularAtrophy.
3. Interaction study between phytochemicals and target for the proposal of potential drug candidate.

This section or chapter also presented the different software, tools, server database used and parameters utilized during analysis as well as the methodology followed to conduct this research work.

3.2. Methodology for Identification and structural analysis of the key target(s) involved in pathogenesis of Spinal Muscular Atrophy (SMA)

This research is concerned with the identification of target protein/s involved in the pathogenesis of SMA. SMN1 protein is a key protein involved in the pathogenesis of Spinal Muscular Atrophy.

3.2.1 Sequence Information

The sequences of the selected targets were retrieved from the National Center for Biotechnology Information (NCBI) GenBank /UniProt database. The Cytogenetic location of SMN1 was validated using Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/https://www.omim.org/), the NCBI Gene

(https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve) and The University of California Santa Cruz (UCSC) Genome Browser (https://genome.ucsc.edu/). The sequences were retrieved only from human sources.

3.2.2 Motif and Domain

The motif, domain, signature, pattern of the target sequence and their role were predicted by using Prosite (https://prosite.expasy.org/), InterPro Scan (https://www.ebi.ac.uk/interpro/) and SMART (http://smart.embl-heidelberg.de/) databases.

3.2.3 Secondary Structure Prediction

The Secondary structure elements and disorder amino acids of the SMN1 protein were predicted by using the PSIPRED server. PSIPRED was used to assess the proportion of alpha helices in the targeted SMN1 protein sequence, the corresponding amino acid residues, and disrupted amino acids in the entire protein sequence (http://bioinf.cs.ucl.ac.uk/psipred/). The secondary structure elements were also predicted by the Self-Optimized Prediction method with Alignment (SOPMA) (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html).

3.2.4 Protein structure identification

The crystal structure of SMN has four homologue chains and in this study, A chain with a sequence length of 66 aa (from 82 to 147) and 1.8 A^{o} resolution was retrieved from the Protein Data Bank (PDB)database (PDB ID 7W30) (https://www.rcsb.org/) (Liu et al., 2022).

3.2.5 Structural Analysis and Validation

The Protein Structure Validation Software (PSVS) suite v1.5 (https://montelionelab.chem.rpi.edu/PSVS/PSVS/) was used to evaluate the quality of protein structure. To evaluate the stereo-chemical quality of the protein; the Ramachandran plot of the target protein (SMN1) was constructed using PROCHECK .The Z-score was calculated using the ProSA-web program in order to evaluate the entire protein structure (https://prosa.services.came.sbg.ac.at/prosa.php).

3.2.6 Structural Simulation

The stability and consistency of SMN1 structure were assessed using a molecular dynamics (MD) simulation using GROMACS 2022.3 (Groningen Machine for Chemical Simulations) software at 1000 ns (Spoel et al.,2005) (Praveena et al.,2022).

3.2.7 Active/binding Site prediction

The potential binding amino acids had been determined by the reported binding site in protein crystal structure from literature and CASTp (http://sts.bioe.uic.edu/castp/index.html?2r7g) and PDBsum (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) binding site prediction tools.

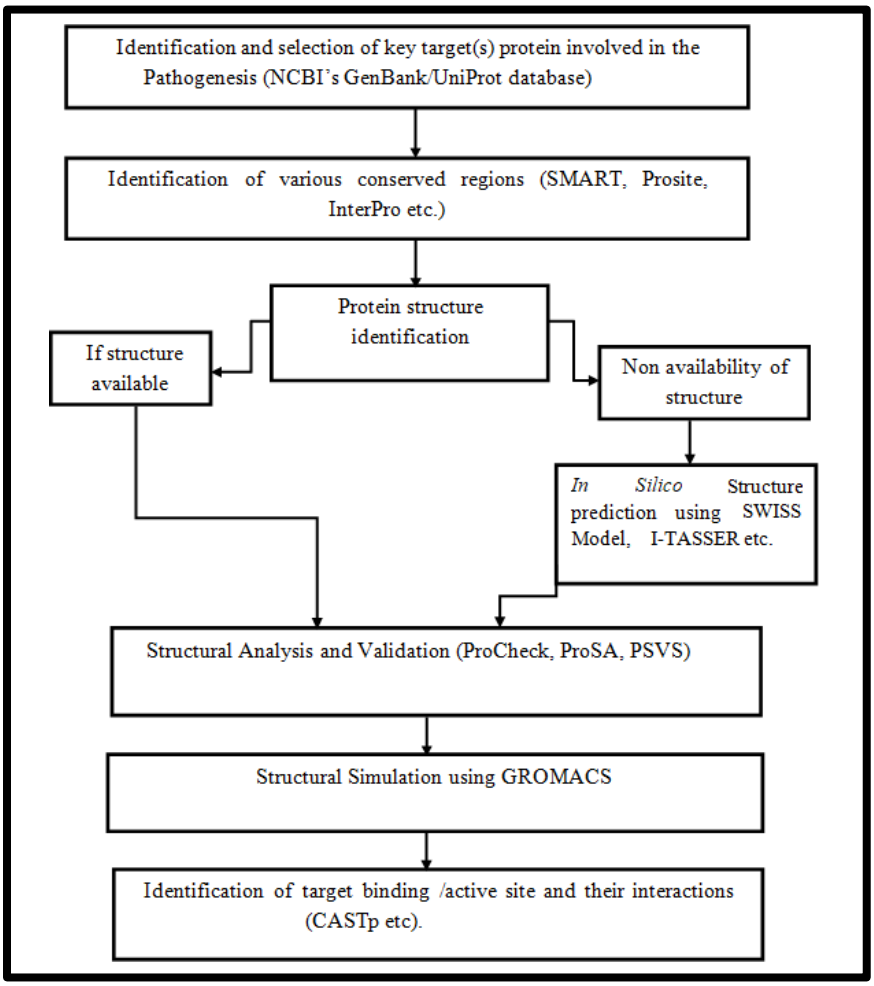


Figure 3.1: Flow chart for Identification and structural analysis of the key targets involved in pathogenesis of Spinal Muscular Atrophy.

3.3. Methodology for Screening and selection of phytochemicals for identified target of Spinal Muscular Atrophy.

3.3.1 Identification of ligand(s) from selected medicinal plant(s)

To ensure rational screening, phytochemicals were chosen based on neuroprotective, anti-inflammatory or antioxidant efficacy, structural suitability for docking and drug-like characteristics. A total of 115 phytochemical compounds of *Brucea javanica* and *Tripterygium wilfordii* were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to generate a dataset for molecular docking analysis (Kim et al., 2023).The SMILE format of each compound was converted into a PDB using the OpenBabel web server. Avogadro tool was used to optimize the compounds, and after that, they were transferred to pdbqt format by applying the proper torsions for proper binding with protein during the docking.

3.3.2 Virtual screening of phytochemicals based on docking methods

In total 115 ligands (Two Datasets: 73 and 42) were submitted to OpenBabel (O'Boyle, 2011) and the ligands were prepared for further docking protocol. From each dataset, top five ligands had high binding affinities score were considered and docked into the SMN1 protein using AutoDock Tools 4.2.6 (Trott et al., 2010) (Peanlikhit et al.,2024) packages. AutoDock creates the grid maps automatically and clusters the results in a way that is transparent to the user. Various stochastic global optimization approaches, such as genetic algorithms, particle swarm optimization, simulated annealing, and others were investigated in the development of this docking tool and they were combined with various local optimization procedures and special tricks to speed up the optimization. In order to find out the best ligand molecule that can act as a suitable drug candidate against SMN1 active site amino acids were performed by using AutoDock tool.In the present study, the AutoDock Tool was employed to make PDBQT files of protein and ligands and grid was generated among the active site residues with dimensions of 25 X 25 X 25. Hydrogen atoms and Kollman charges were added to the protein followed by the removal of water molecules and heteroatoms for protein preparation using AutoDock tool (Morris et al., 2009). The docked structures were explored with the help of PyMOL and UCSF Chimera (DeLano, 2002) (Pettersen et al., 2004). The protein-ligand interactions of the

docked complexes were analysed by LigPlot+ v2.2.8 (Laskowski & Swindells, 2011).

3.3.3 Identification and analysis of pharmacological properties of phytochemical compounds

The selection was further refined using ADMET properties including as oral bioavailability, absorption, metabolism and toxicity estimations were also used as filters to choose the best candidates. To be successful as a drug, a powerful molecule must reach its target in the body in sufficient concentration and stay there in a bioactive form long enough for the predicted biological processes to occur. In the medication development process, absorption, distribution, metabolism, excretion and toxicity (ADMET) testing is used. ADMETlab3.0 is a web service that gives us free access to comprehensively and accurately measure the physicochemical properties of molecules, alongside their pharmaceutical chemical friendliness. It characterizes the phytochemicals according to their Physicochemical Properties (Molecular Weight, logP, TPSA, logS, etc.), medicinal chemistry (Pfizer Rule etc), Absorption (MDCK Permeability etc.), Distribution (PPB, BBB etc.), Metabolism (HLM Stability etc.), Excretion (C_L plasma) and Toxicity (hERG Blockers, Respiratory etc.) (Fu et al., 2024).

3.4. Methodology for the Interactions study between phytochemicals and target for the proposal of potential drug candidate.

3.4.1 Molecular dynamics simulations of virtually selected Phytochemicals

To investigate the mechanism of interactions between the SMN1 Tudor domain, Triptersinine-A and Yadanzioside-M compound was studied using molecular dynamics (MD) simulations by GROMACS 2022.3 (Hess et al., 2008) to explore the structural flexibility of the apo-protein and protein-phytochemical complexes obtained from the virtual screening and ADMET property analysis. Both protein and protein-ligand complexes were simulated by an OPLS-AA(Optimized Potential For Liquid Simulations-All Atom) force field for 1000 ns. The apo-protein SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex were immersed in a simple point charge water model in a cube box of 1 nm. The topologies of ligands were obtained from OpenBabel to form the protein-ligand complex. To neutralise the system of apo-protein, protein-Tripersinine-A and protein-Yadanzioside-M, Na+ ions were added to the respective protein and protein-

ligand complexes. Energy minimization of the three systems was carried out using the steepest descent and conjugate gradient method. The systems were equilibrated 1000ps at 300 k and 1 bar pressure in NVT (Constant number of Particles, Volume and Temperature) and NPT (Constant number of Particles, Pressure and Temperature). Final MD production was carried out for apo-protein and two SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex for a 1000ns time. The root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, -bond interactions and solvent-accessible surface area (SASA) of each protein-ligand complex considered to estimate the behaviour of native protein molecule and after its binding to the phytochemical compounds.

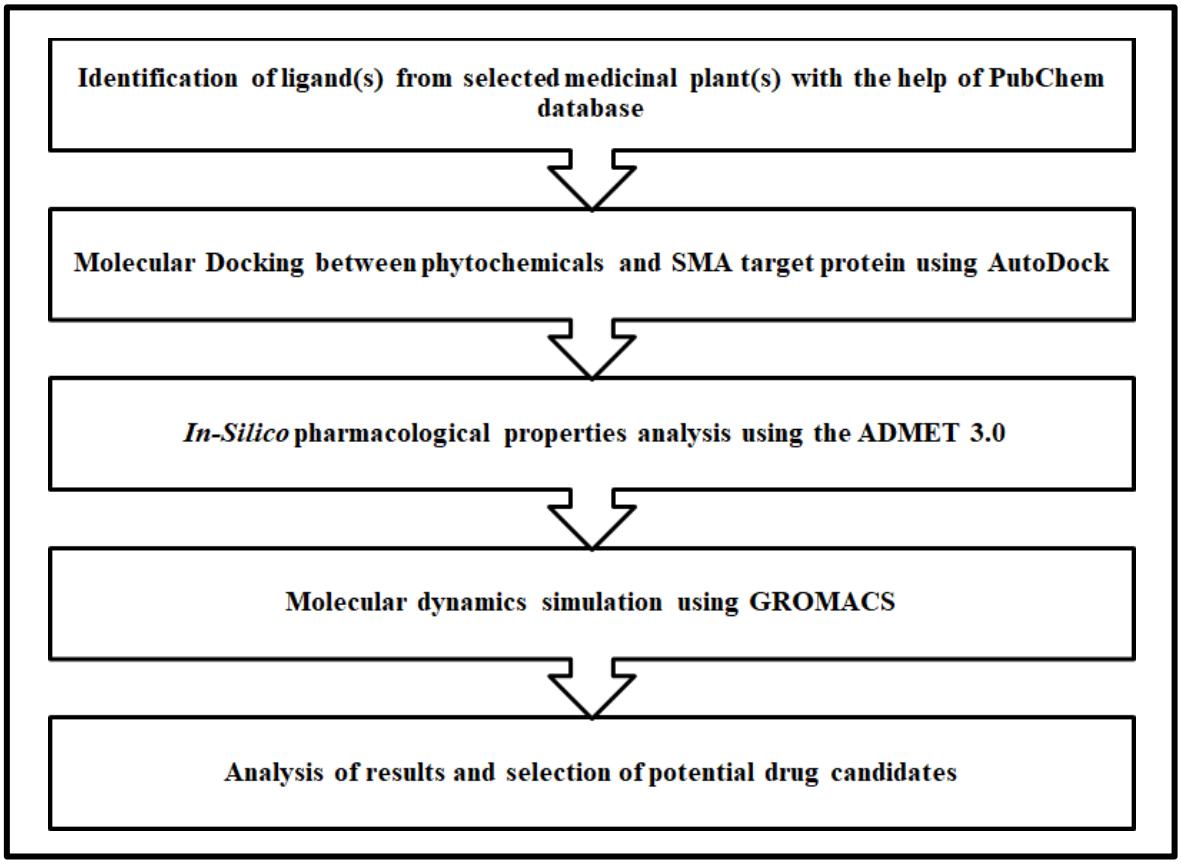


Figure 3.2: Flow chart for Screening and selection of phytochemicals for identified target of Spinal Muscular Atrophy and Interaction study between phytochemicals and target for the proposal of potential drug candidate.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Identification and structural analysis of the key target(s) involved in pathogenesis of Spinal Muscular Atrophy (SMA)

4.1.1 Sequence Information

Spinal muscular atrophies (SMAs) are defined by the deterioration of the anterior horn cells in the spinal cord, which results in progressive symmetrical paralysis of the limbs and trunk, along with muscle wasting. SMA is passed down through an autosomal recessive pattern. In the majority of instances, it is triggered by mutations in the survival motor neuron 1 gene located on chromosome 5q13.2 (**Figure 4.1**).The primary sequence of SMN1 protein had accession number NP_000335.1 and sequence length 294 aa (Lefebvre et al.,1995) (Nurputra et al.,(2024).

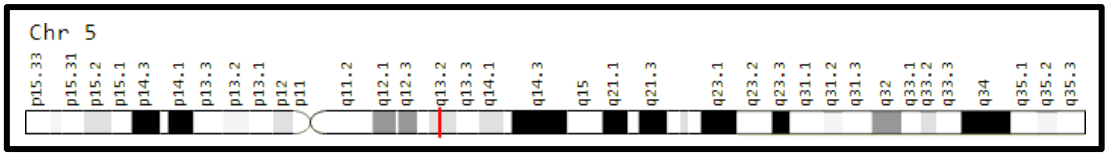


Figure 4.1: Cytogenetic location of SMN1 protein

Our target protein‘s UniProt ID was Q16637. Name and taxonomy, subcellular location, disease and variations, post-translational modifications (PTM)/Processing, expression, interaction, structure, family and domains, sequence and isoforms, and similar proteins related to or target protein SMN1 were all studied using UniProt (**Table 4.1**).

Table4.1: Basic information about the SMN1 protein

Gene Name	Survival of motor neuron 1, telomeric [*Homo sapiens* (human)]
NCBI Gene ID	6606
HGNC(HUGO Gene Nomenclature Committee) Approved Symbol	SMN1
HGNC Approved Name	Survival of motor neuron 1, telomeric
HGNC ID	11117
GenBank/Refseq Accession Number	NM_000344.4

Number of Nucleotides	1482 bp
Protein Accession Number	NP_000335.1
Number of Amino Acids	294 aa
UniProt ID	Q16637
Cytogenetic Location	5q13.2
OMIM ID	600354
UCSC ID	uc003kak.4

4.1.2 Motif and domain

The InterProScan, PROSITE and SMART results clearly shows that the domain present in our query protein was TUDOR domain. The domain in our query sequence begins at positions 90 and end at151 (**Figure 4.2 a, 2 b, 2 c**). The Tudor domain is a domain of approximately 50-70 amino acids that was first identified as a repeat present in 10 copies in the *Drosophila* Tudor protein (Glenn & Searles, 2001). Many eukaryotic proteins have one or more copies of the Tudor domain, which colocalizes with ribonucleoprotein or single-stranded DNA-associated complexes in the nucleus, mitochondrial membrane and at kinetochores. The Mammalian survival of motor neuron protein (SMN) were also had Tudor domains. SMN helps to assemble small nuclear ribonucleoprotein (snRNP) particles in the cytoplasm and may potentially have a direct effect on splicing in the nucleus. In humans, SMN protein abnormalities are linked to spinal muscular atrophy (SMA), a disorder in which the anterior horn cells in the spinal cord die, resulting in increasing muscle weakening and, in some cases, the inability to breathe and swallow (Selenko et al., 2001).

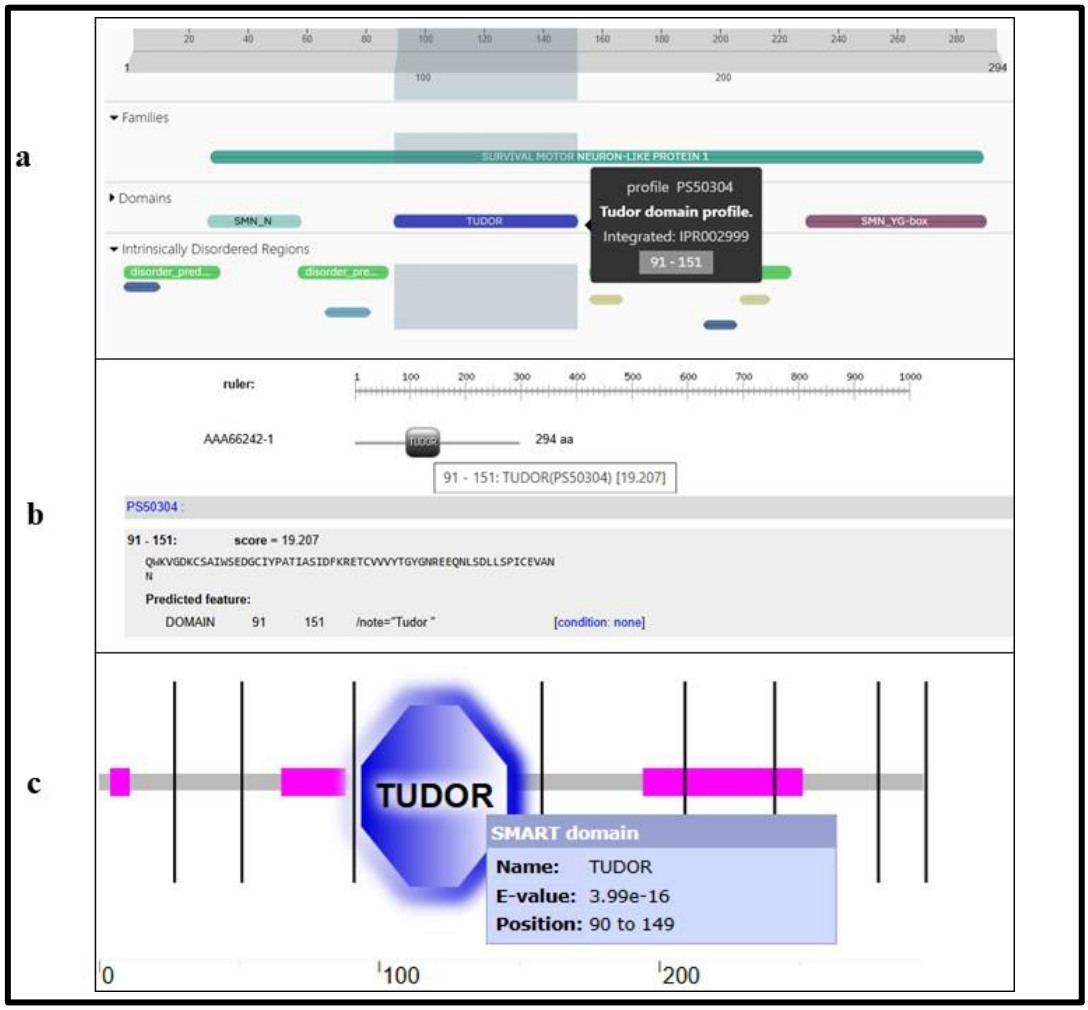


Figure 4.2: SMN1 domain prediction results **a.** InterProScan **b.**PROSITE **c.** SMART database

4.1.3 Secondary structure Prediction

Using specific color codes—yellow for strands, pink for helices, orange for extracellular and gray for coils—the PSIPRED result depicts the secondary structural elements of SMN1 **(Figure 4.3a).** Furthermore, out of 294 amino acids, the SOPMA analysis shows the amounts of several structural components in the SMN1 protein, such as 58-α helices, 39-extended strands, 15-β twists, and 182-random coils **(Figure 4.3b).**

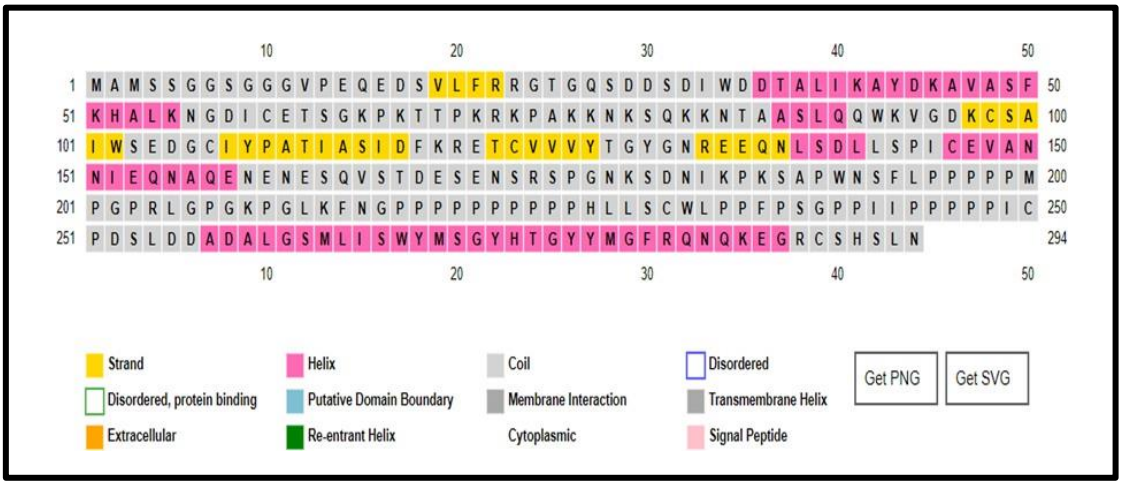


Figure 4.3 (a): The PSIPRED outcome illustrates the secondary structural components of SMN1 protein.

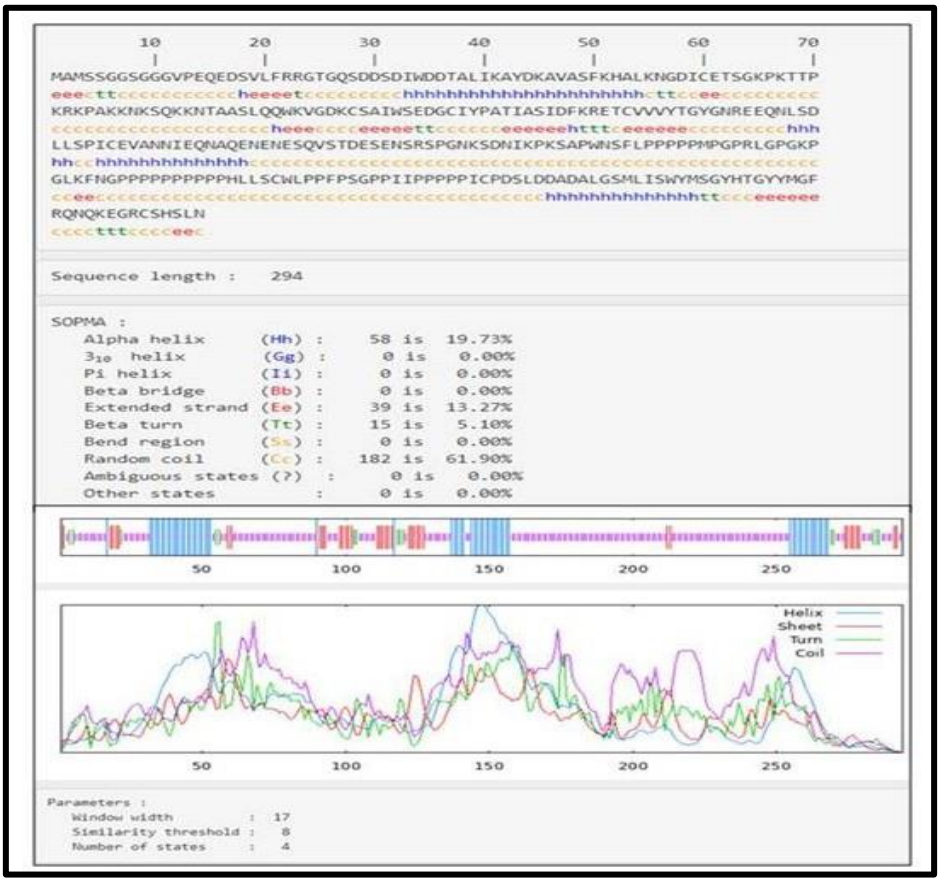


Figure 4.3 (b): Secondary Structure Prediction using SOPMA.

4.1.4 Protein structure identification

The virtual screening and molecular dynamics simulations analysis was carried out on the available crystal structure of SMN1 protein PDB ID 7W30 (**Figure 4.4**) (Liu et al., 2022). The monomeric form of SMN was considered for this study instead of the dimeric form, as the functional unit of the protein is a homodimer where the equilibrium of the dimer–monomer structure was mainly affected by the ligand molecules, and the monomeric form is more reliable than the dimeric form (Tam et al., 2021; Silvestrini et al., 2021). The structure has three domains, out of which two domains are comprised of antiparallel beta-barrels and the other domain has a cluster of five helices (Morris et al., 2009).

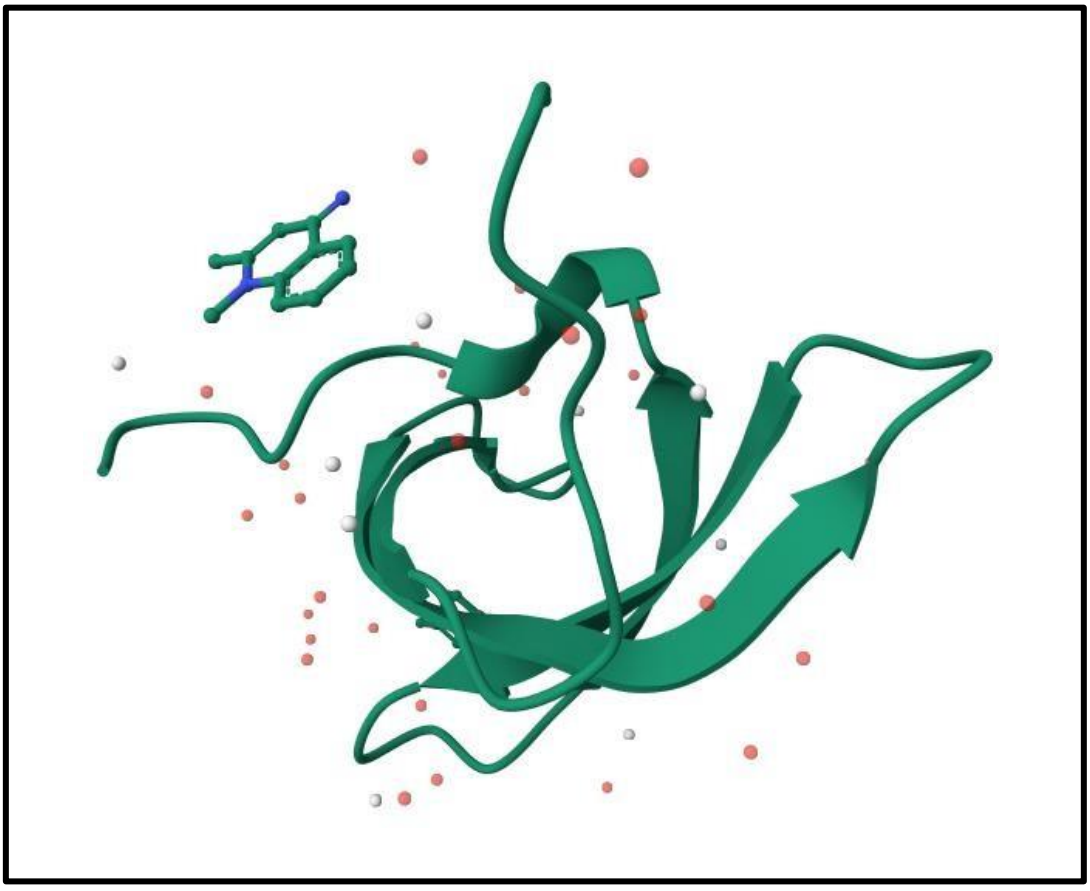


Figure 4.4: Tudor domain of SMN1 in complex with a small molecule.

4.1.5 Structural Analysis and Validation

The Ramachandran plot was made with PROCHECK, and out of 60 residues, it showed that there were 52 (100%) residues in the most favored regions, 0 (0.0%) in the additional allowed regions, 0 (0.0%) in the generously allowed regions, and 0 (0.0%) in the disallowed regions. There were also 0 (0.0%) non-glycine and non-proline residues, 2 end residues (excluding Gly and Pro), 4 glycine residues (shown in a triangle), and 2 proline residues (**Figure 4.5**).

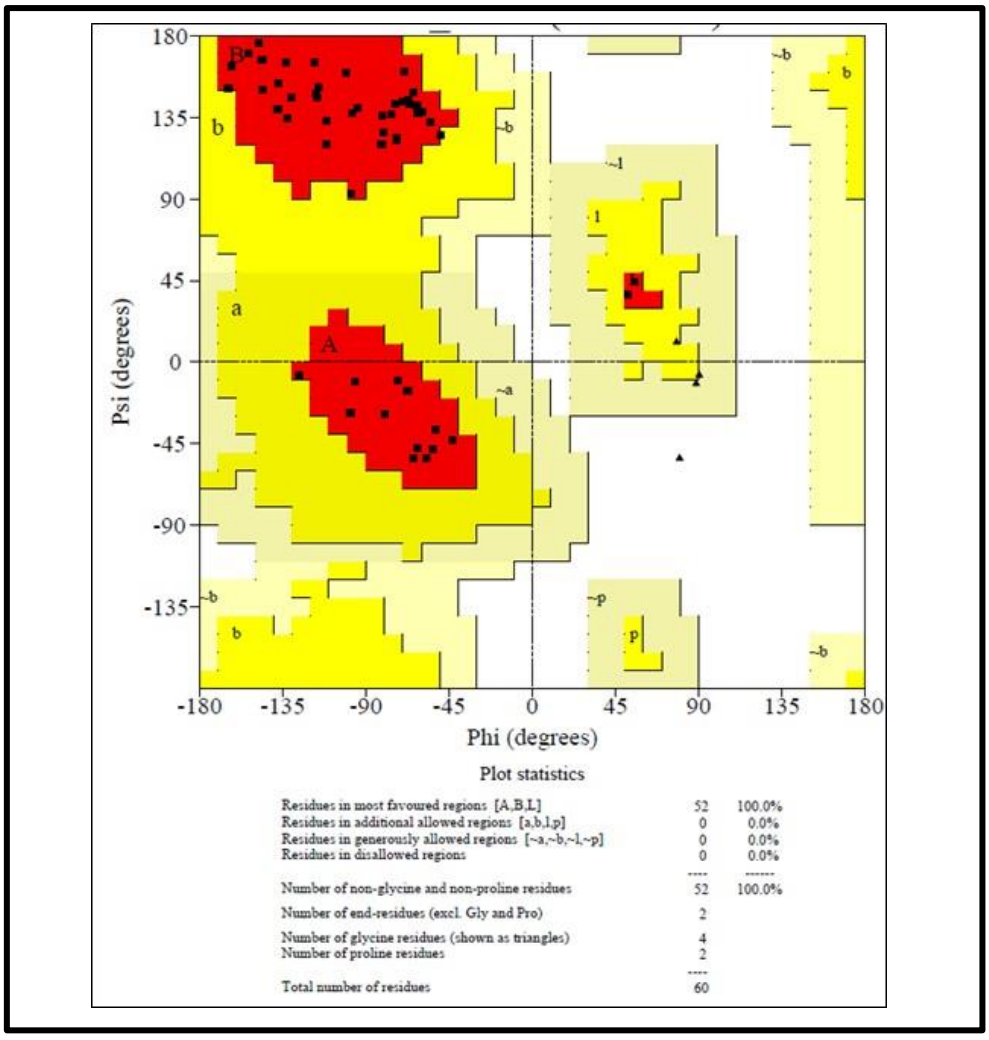


Figure 4.5: Structural validation using Ramachandran Plot

The Z-score acquired from ProSA-web servers was used to further validate the structure of the SMN1 protein, confirming its structural quality (a) The SMN1 structure was within the permissible range for X-ray and NMR investigations based on the curated data (the total score was -5.04) (b) Two distinct window sizes were used to depict the sequence position and knowledge-energy graph: light green denotes a window size of 10 and deep green denotes a window size of 40. This plot shows that all of the SMN1 protein's residues have energy values that are noticeably less than zero **(Figure 4.6)**.

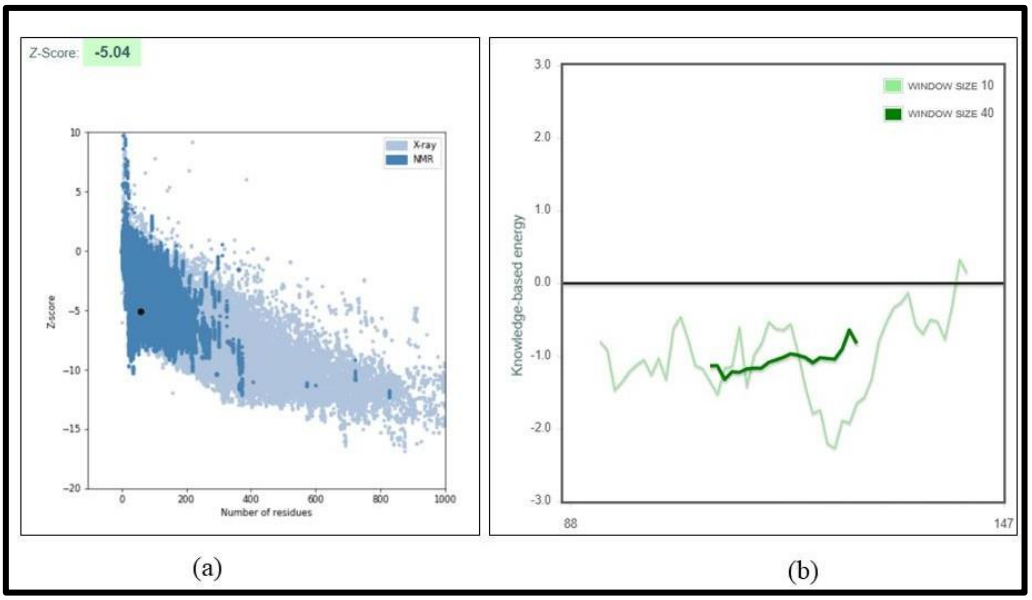


Figure 4.6: Structure validation by using ProSA-Web **a.** Overall model quality **b.** Local model quality.

4.1.6 Active/binding Site prediction

The crystal structure of SMN has four similar chains and in this study, A chain with a sequence length of 66 (from 82-147) and 1.8 A^{o} resolution was retrieved from the PDB database (PDB ID 7W30) (Liu et al., 2022). The monomeric form of SMN was considered for this study instead of the dimeric form, as the functional unit of protein is a homodimer where the equilibrium of dimer–monomer structure was mainly affected by the ligand molecules and monomeric form is more reliable than the dimeric form (Anjum et al., 2023) (Silvestrini et al., 2021). The structure has three domains out of which two

domains are comprised of antiparallel beta-barrels and the other domain has a cluster of five helices. CASTp server was used to predict the binding site residues (**Figure 4.7**). PDBsum tool was also used find forecasting active sites by detecting surface grooves and binding regions, typically linked to active sites, and facilitates the alignment of structures with homologous proteins to highlight conserved residues.The binding site residues are considered from crystal protein structure article (Gln90, Trp92, Asp96, Lys97, Ile101, Trp102, Ser103, Tyr109, Asp117, Thr122, Cys123, Tyr127, Thr128, Gly129, Tyr130, Asn132, Arg133, Glu134, Glu135, Leu138, Ser143, Pro144, Cys146 and Glu147) (Liu et al., 2022).

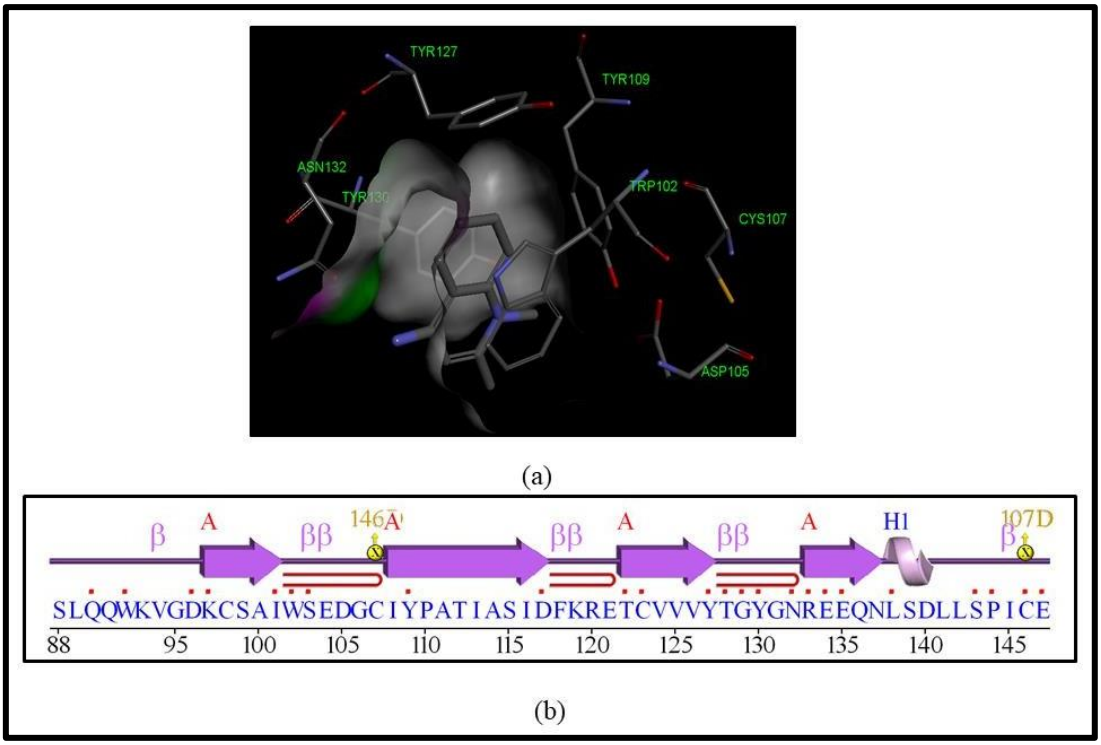


Figure 4.7: Active Sites residue analysis of SMN1 using **a.** CASTp server **b.** PDBsum

4.2 Screening and selection of phytochemicals for identified target of Spinal Muscular Atrophy.

4.2.1 Identification of ligand(s) from selected medicinal plant(s)

Brucea javanica and *Tripterygium wilfordii* represent valuable sources of bioactive compounds with significant therapeutic potential for treating SMA (Khan et al., 2020).). Their anti-inflammatory, neuroprotective, oxidative stress-mitigating and anti-apoptotic properties make them promising candidates for alternative and adjunctive treatments for SMA. When combined, the phytochemicals from these plants could offer a synergistic and holistic approach to managing SMA by addressing the multiple molecular pathways involved in disease progression (Hsu et al., 2012) (Baek et al.,2019). The details of phytochemicals of *Brucea javanica* and *Tripterygium wilfordii* considered for virtual screening is provided in the tables (4.2 and 3) below.

Table 4.2: List of phytochemicals from *Brucea javanica*

S. No	Compound Name	Pubchem ID	SMILES(Simplified Molecular Input Line Entry System) Notation	Compound type	Location
1	Bruceine A	160006	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)CC(C)C)(OC5)C(=O)OC)O)O)C)O	Quassinoids	Seeds
2	Bruceine B	161496	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C)(OC5)C(=O)OC)O)O)C)O	Quassinoids	Seeds
3	Bruceine C	5315509	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)O)(OC5)C(=O)OC)O)O)C)O	Quassinoids	Seeds
4	Bruceine D	441788	CC1=CC(=O)C(C2(C1CC3C45C2C(C(C(C4(C(C(=O)O3)O)O)(OC5)C)O)O)C)O	Quassinoids	Seeds
5	Bruceine E	5315510	CC1=CC(C(C2(C1CC3C45C2C(C(C(C4(C(C(=O)O3)O)O)(OC5)C)O)O)C)O)O	Quassinoids	Seeds
6	Bruceine F	76315043	CCC12C(C(C3C4(C(CC5C3(C1(C(C(=O)O5)O)O)CO2)C(=CC(C4O)O)C)C)O)O	Quassinoids	Seeds
7	Bruceine G	102059835	CC1=CC(=O)CC2(C1C(C3C	Quassinoids	Seeds

			45C2C(C(C(C4C(C(=O)O3)O)(OC5)C)O)O)O)C		
8	Bruceine I	21126551	CCOC(=O)C12C3CC(=O)OC4C3(CO1)C(C(C2O)O)C5(CC(=O)C(=C(C5C4)C)O)C	Quassinoids	Seeds
09	Bruceine J	23656476	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)CC(C)C)(OC5)C(=O)O)O)O)C)O	Quassinoids	Fruits
10	Bruceine K	101549286	CC1=CC(C(C2(C1CC3C4C25COC(C5O)C(C4(C(C(=O)O3)O)O)(C)O)C)O)O	Quassinoids	Dried ripe fruits
11	Bruceine L	101549287	CC1=CC(C(C2(C1CC3C4C25COC(C4C(C(=O)O3)OC(=O)C=C(C)C)(C(C5O)O)C(=O)OC)C)O)O	Quassinoids	Dried ripe fruits
12	Bruceanic Acid E	70681802	CC(=CC(=O)OC1C2C34COC2(C(C(C3C(C(CC4OC1=O)C(=O)C)(C)CC(=O)O)O)O)C(=O)OC)C	Quassinoids	Seeds
13	Bruceanic Acid F	70681803	CC(=CC(=O)OC1C2C34COC2(C(C(C3C(C(CC4OC1=O)C(=O)C)(C)CC(=O)O)O)O)C(=O)O)C	Quassinoids	Seeds
14	Bruceanic Acid E Methyl Ester	70688171	CC(=CC(=O)OC1C2C34COC2(C(C(C3C(C(CC4OC1=O)C(=O)C)(C)CC(=O)OC)O)O)C(=O)OC)C	Quassinoids	Seeds
15	Yadanzioside A	72956	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)CC(C)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Fruits
16	Yadanzioside B	72952	CC1=C(C(=O)C(C2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)CC(C)C)(OC5)C(=O)OC)O)O)C)O)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Fruits
17	Yadanzioside C	6436227	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)O)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Fruits

18	Yadanzioside E	460522	CC1=CC(C(C2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C)(OC5)C(=O)OC)O)O)C)O)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Fruits
19	Yadanzioside F	72955	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Fruits
20	Yadanzioside G	6436228	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)OC(=O)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Fruits
21	Yadanzioside I	134715120	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
22	Yadanzioside K	14060345	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)OC(=O)C)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
23	Yadanzioside L	6436225	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)O)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
24	Yadanzioside M	21115200	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C6=CC=CC=C6)(C(C(C5C2(C=C(C1=O)OC7C(C(C(C(O7)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Seeds
25	Yadanzioside N	6451089	CC1C=C(C(=O)C2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)C)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
26	Yadanzioside O	10417797	CCC(=CC(=O)OC1C2C34COC2(C(C(C3C5(C=C(C(=O)C(C5CC4OC1=O)C)OC6C(C	Quassinoids	Seeds

			(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC)C(C)(C)OC(=O)C		
27	Yadanzioside P	11765370	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)C)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
28	Yadanziolide C	10409280	CC1=CC(=O)C(C2(C1CC3C45C2C(C(C(C4(C(C(=O)O3)O)O)(OC5)C)O)O)C)O	Quassinoids	Seeds
29	Yadanzigan	76307878	CC1=CC(C(C2(C1CC3C45C2C(C(C(C4(C(C(=O)O3)O)O)(OC5)C)O)O)C)O)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
30	Brusatol	73432	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C)(OC5)C(=O)OC)O)O)C)O	Quassinoids	Seeds
31	Bruceantin	5281304	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)C)(OC5)C(=O)OC)O)O)C)O	Quassinoids	Fruits
32	Bruceantinol	5281305	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)OC(=O)C)(OC5)C(=O)OC)O)O)C)O	Quassinoids	Seeds
33	Bruceantinosides A	54604526	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C(C)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Fruits
34	Bruceoside B	3000796	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
35	Bruceoside C	73122	CC1=C(C(=O)CC2(C1C(C3C45C2C(C(C(C4CC(=O)O3)(OC5)C(=O)OC)O)O)OC(=O)C=C(C)C)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
36	Bruceoside D	460525	CC1C2CC3C45COC(C4C(C(	Quassinoids	Seeds

			=O)O3)OC(=O)C=C(C)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)O		
37	Bruceoside E	460524	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)CC(C)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)O	Quassinoids	Seeds
38	Bruceoside F	6451123	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)OC(=O)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)O	Quassinoids	Seeds
39	Bruceene	101600137	CC12C(CC3C45C1C(C(C(C4(C(C(=O)O3)O)O)(OC5)C)O)O)C(=C)C=CC2O	Quassinoids	Fruits
40	Brujavanol A	132491878	CC1C2C(C(=O)OC3C2(C(C(C1O)O)C4(C(C3)C(=CC(C4O)O)C)C)C)O	Quassinoids	Roots
41	Dihydrobrucea javanin A	10076750	CC(=O)OC1CC2C(C(=O)CCC2(C3C1(C4=CCC(C4(CC3)C)C5CC(OC5OC(=O)C)C6C(O6)(C)C)C)C)(C)C	Quassinoids	Stems
42	Javanicolide B	10071101	CC1=CC(=O)C(C2(C1CC3C45C2C(C(C(C4(C(C(=O)O3)O)O)(OC5O)C)O)O)C)O	Quassinoids	Seeds
43	Javanicolide C	11214625	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C)(C(C(C5C2(CC(C1O)O)C)O)O)C(=O)OC	Quassinoids	Seeds
44	Javanicolide D	11455840	CC1=CC(C(C2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)O)(OC5)C(=O)OC)O)O)C)O)O	Quassinoids	Seeds
45	Javanicolide E	70686049	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C)(C(C(C5C2(CC(=O)C1O)C)O)O)C(=O)OC	Quassinoids	Seeds
46	Javanicolide H	70683917	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C)(C(C(C5C2(CC(C1=O)O)C)O)O)C(=O)OC	Quassinoids	Seeds

47	Javanicoside B	11169907	CC1C=C(C(=O)C2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)CC(C)C)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
48	Javanicoside C	11169890	CC1=C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Seeds
49	Javanicoside E	11274270	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)CC(C)C(C)(C)OC(=O)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC	Quassinoids	Seeds
50	Javanicoside F	11445415	CCC(=CC(=O)OC1C2C34COC2(C(C(C3C5(C=C(C(=O)C(C5CC4OC1=O)C)OC6C(C(C(C(O6)CO)O)O)O)C)O)O)C(=O)OC)C	Quassinoids	Seeds
51	Javanicoside G	12086835	CC1C2CC3C45COC(C4C(C(=O)O3)OC(=O)C=C(C)C)(C(C(C5C2(C=C(C1=O)OC6C(C(C(CO6)O)O)O)C)O)O)C(=O)OC	Quassinoids	Seeds
52	Javanicoside I	11445340	CC1C=C(C(=O)C2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C)(OC5)C(=O)OC)O)O)C)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
53	Javanicoside K	11479645	CC1=CC(C(C2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)C=C(C)C(C)(C)O)(OC5)C(=O)OC)O)O)C)O)OC6C(C(C(C(O6)CO)O)O)O	Quassinoids	Seeds
54	3′-hydroxybrucei n A	132570107	CC1=C(C(=O)CC2(C1CC3C45C2C(C(C(C4C(C(=O)O3)OC(=O)CC(C)(C)O)(OC5)C(=O)OC)O)O)C)O	Quassinoids	Seeds
55	Bruceolline H	56833856	CC1(C2=C(C3=C(N2)C=C(C=C3)O)C(=O)C1=O)C	Alkaloids	Stems
56	Bruceolline I	56833857	CC1(C(C(=O)C2=C1NC3=C2C=CC(=C3)O)O)C	Alkaloids	Stems

57	Bruceolline J	56833858	CC1(C(C(=O)C2=C1NC3=CC=CC=C32)O)C	Alkaloids	Stems
58	Bruceolline K	56833859	CC1(C(C(=O)C2=C1NC3=CC=CC=C32)OC4C(C(C(C(O4)CO)O)O)O)C	Alkaloids	Stems
59	Bruceolline L	56833860	CC(C)C(C(=O)C1=CNC2=CC=CC=C21)O	Alkaloids	Stems
60	Bruceolline M	56833861	CC(C)(C(=O)CC1=CN(C2=CC=CC=C21)C3C(C(C(C(O3)CO)O)O)O)O	Alkaloids	Stems
61	Bruceolline N	56833961	CC(C)(C(CC1(C2=CC=CC=C2N(C1=O)C3C(C(C(C(O3)CO)O)O)O)O)O)O	Alkaloids	Stems
62	Brujavanone A	102196212	CC(=O)OC1CC2C(C(=O)C=CC2(C3C1(C4=CCC(C4(CC3O)C)C5CC(OC5OC(=O)C)C6C(O6)(C)C)C)C)(C)C	Triterpenoids	Twigs
63	Brujavanone B	102196213	CC(=O)OC1CC2C(C(=O)C=CC2(C3C1(C4=CCC(C4(CC3O)C)C5CC(OC5OC)C6C(O6)(C)C)C)C)(C)C	Triterpenoids	Twigs
64	Brujavanone D	102196215	CC(=O)OC1CC2C(C(=O)C=CC2(C3C1(C4=CCC(C4(CC3O)C)C5CC(OC5OC)C(C(C)(C)O)O)C)C)(C)C	Triterpenoids	Twigs
65	Brujavanone E	102196216	CC(=O)OC1CC2C(C(=O)C=CC2(C3C1(C4=CCC(C4(CC3O)C)C5CC(OC5O)C(C(C)(C)O)O)C)C)(C)C	Triterpenoids	Twigs
66	Brujavanone F	102196217	CC(=O)OC1CC2C(C(=O)C=CC2(C3C1(C4=CCC(C4(CC3O)C)C5CC(OC5OC)C(C(C)(C)OC)O)C)C)(C)C	Triterpenoids	Twigs
67	Brujavanone G	102196218	CC(=C)C(C1CC(C(O1)OC)C2CC=C3C2(CC(C4C3(C(CC5C4(C=CC(=O)C5(C)C)C)OC(=O)C)C)O)C)O	Triterpenoids	Twigs
68	Brujavanone H	102196219	CC(=O)OC1CC2C(C(=O)C=CC2(C3C1(C4=CCC(C4(CC3)C)C5CC(OC5OC)C(C(C)(C)O)O)C)C)(C)C	Triterpenoids	Twigs
69	Brujavanone I	102196220	CC(=O)OC1CC2C(C(=O)CCC2(C3C1(C4=CCC(C4(CC3)	Triterpenoids	Twigs

			C)C5CC(OC5OC)C(C(C)(C)O)O)C)C)(C)C		
70	Brujavanone J	102196221	CC(=O)OC1CC2C(C(=O)CCC2(C3C1(C4=CCC(C4(CC3)C)C5CC(OC5OC)C(C(C)(C)O)O)C)C)(C)C	Triterpenoids	Twigs
71	Brujavanone K	102196222	CC(=O)OC1CC2C(C(=O)CCC2(C3C1(C4=CCC(C4(CC3)C)C5CC(OC5O)C(C(C)(C)O)O)C)C)(C)C	Triterpenoids	Twigs
72	Brujavanone L	102196223	CC(=O)OC1CC2C(C(=O)CCC2(C3C1(C4=CCC(C4(CC3)C)C5CC(OC5O)C6C(O6)(C)C)C)C)(C)C	Triterpenoids	Twigs
73	Brujavanone M	102196224	CCCCCC(=O)OC1CC2(C(CC=C2C3(C1C4(C(CC3OC(=O)C)C(C(=O)CC4O)(C)C)C)C)C5CC(OC5OC)C(C(C)(C)O)O)C	Triterpenoids	Twigs

Table 4.3: List of phytochemicals from *Tripterygium wilfordii*

S. No	Compound Name	PubChem ID	Smile Notation	Compound Type	Location
1	Tripterygiumine C	163183995	CC1C(C(=O)OC2C(C(C3(C(C(C4C(C3(C2(C)O)OC4(COC(=O)C5=C1N=CC=C5)C)OC(=O)C6=CC=CC=C6)OC(=O)C)OC(=O)C)COC(=O)C)OC(=O)C)OC(=O)C7=CC=CC=C7)C	Nitrogen-containing sesquiterpenoids	Root
2	Tripterygiumine D	163183994	CC1C(C(=O)OC2C(C(C3(C(C(C4C(C3(C2(C)O)OC4(COC(=O)C5=C1N=CC=C5)C)OC(=O)C6=CC=CC=C6)O)O)CO)O)O)C	Nitrogen-containing sesquiterpenoids	Root
3	Tripterygiumine E	163183993	CC1C(C(=O)OC2C(C(C3(C(C(C4C(C	Nitrogen-containing	Root

			3(C2(C)O)OC4(CO C(=O)C5=C1N=C C=C5)C)O)OC(=O)C)OC(=O)C)COC(=O)C)OC(=O)C)O C(=O)C6=COC=C 6)C	sesquiterpenoids	
4	Tripterygiumine F	163183992	CC1C(C(=O)OC2C (C(C3(C(C(C4C(C 3(C2(C)O)OC4(CO C(=O)C5=C1N=C C=C5)C)OC(=O)C 6=COC=C6)OC(= O)C)OC(=O)C)CO C(=O)C)OC(=O)C) OC(=O)C7=CC=C C=C7)C	Nitrogen-containing sesquiterpenoids	Root
5	Tripterygiumine G	163183991	CC1C(C(=O)OC2C (C(C3(C(C(C4C(C 3(C2(C)O)OC4(CO C(=O)C5=C1N=C C=C5)C)OC(=O)C 6=CC=CC=C6)OC(=O)C)OC(=O)C)C OC(=O)C)OC(=O) C)OC(=O)C7=CO C=C7)C	Nitrogen-containing sesquiterpenoids	Root
6	Tripterygiumine H	163183990	CC1C(C(=O)OC2C (C(C3(C(C(C4C(C 3(C2(C)O)OC4(CO C(=O)C5=C1N=C C=C5)C)OC(=O)C) O)O)CO)O)O)C	Nitrogen-containing sesquiterpenoids	Root
7	Tripterygiumine K	163183989	CC1C(C(=O)OC2C (C(C3(C(C(C4C(C 3(C2(C)O)OC4(CO C(=O)C5=C1N=C C=C5)C)O)OC(=O)C)OC(=O)C)COC(=O)C6=CC=CC=C 6)OC(=O)C)O)C	Nitrogen-containing sesquiterpenoids	Root
8	Tripterygiumine L	163183988	CC1C(C(=O)OC2C (C(C3(C(C(C4C(C 3(C2(C)O)OC4(CO	Nitrogen-containing sesquiterpenoids	Root

			C(=O)C5=C1N=CC=C5)C)O)OC(=O)C)OC(=O)C)COC(=O)C)OC(=O)C6=CN=CC=C6)OC(=O)C)C		
9	Tripterygiumine M	163183987	CC1CCC2=C(C=CC=N2)C(=O)OCC3(C4C(C5(O3)C(C(C(C(C5(C(C4=O)OC(=O)C)COC(=O)C)OC(=O)C)OC(=O)C6=CC=CC=C6)OC1=O)(C)O)O)C	Nitrogen-containing sesquiterpenoids	Root
10	Tripterygiumine N	163183986	CC(=O)OCC12C(C(C3C(C14C(C(C(=O)C2OC(=O)C)C(O4)(COC(=O)C5=C(CCC(C(=O)O3)(C)OC(=O)C6=COC=C6)N=CC=C5)C)O)(C)O)OC(=O)C7=CC=CC=C7)OC(=O)C	Nitrogen-containing sesquiterpenoids	Root
11	Tripterygiumine O	163183985	CC(=O)OCC12C(C(C3C(C14C(C(C(C2OC(=O)C)OC(=O)C5=COC=C5)OC(=O)C(CCC6=C(C=CC=N6)C(=O)OCC3(O4)C)(C)OC(=O)C7=CC=CC=C7)(C)O)O)OC(=O)C)OC(=O)C	Nitrogen-containing sesquiterpenoids	Root
12	Tripterygiumine P	163183984	CC(=O)OC1C2C(C(C3(C14C(C(C(C3O)O)OC(=O)C(CCC5=C(C=CC=N5)C(=O)OCC2(O4)C)(C)OC(=O)C6=CC=CC=C6)(C)O)CO)O)O	Nitrogen-containing sesquiterpenoids	Root
13	Tripterygiumine S	163183982	CC(=O)OCC12C(C(C3C(C14C(C(C(=	Nitrogen-containing	Root

			O)C2OC(=O)C)C(O4)(COC(=O)C5=C(CCC(C(=O)O3)(C)OC(=O)C6=COC=C6)N=CC=C5)C)OC(=O)C)(C)O)O)OC(=O)C	sesquiterpenoids	
14	Tripterygiumine T	163183981	CC(=O)OCC12C(C(C3C(C14C(C(C(=O)C2OC(=O)C)C(O4)(COC(=O)C5=C(CCC(C(=O)O3)(C)O)N=CC=C5)C)O)(C)O)O)OC(=O)C	Nitrogen-containing sesquiterpenoids	Root
15	Tripterygiumine V	163183980	CC(=O)OCC12C(C(C3C(C14C(C(C(C2OC(=O)C)OC(=O)C)C(O4)(COC(=O)C5=C(CCC(C(=O)O3)(C)OC(=O)C6=CC=CC=C6)N=CC=C5)C)OC(=O)C)(C)O)O)OC(=O)C	Nitrogen-containing sesquiterpenoids	Root
16	Wilfornine H	163183964	CC1C(C(=O)OC2C(C(C3(C(C(C4C(C3(C2(C)O)OC4(COC(=O)C5=C1N=CC=C5)C)OC(=O)C6=CC=CO6)OC(=O)C)OC(=O)C)COC(=O)C)OC(=O)C7=CC=CO7)OC(=O)C)C	Nitrogen-containing sesquiterpenoids	Root
17	Tripterygiumine R	163183983	CC1(C2CC(C3(C(CCC(C3(C2O)O1)(C)O)OC(=O)C4=CN=CC=C4)C)OC(=O)C5=CC=CC=C5)C	Nitrogen-containing sesquiterpenoids	Root
18	Triptersinine A	163184018	CC(=O)OCC12C(CCC(C13C(C(C(=O)C2OC(=O)C4=CN=CC=C4)C(O3)(C)	Nitrogen-containing sesquiterpenoids	Leaf

			C)O)(C)O)OC(=O)C=CC5=CC=CC=C5		
19	Triptersinine B	163184017	CC(=O)OCC12C(CCC(C13C(C(C(=O)C2OC(=O)C4=CN=CC=C4)C(O3)(C)C)O)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-containing sesquiterpenoids	Leaf
20	Triptersinine C	163184016	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)O)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-containing sesquiterpenoids	Leaf
21	Triptersinine D	163184015	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)O)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-containing sesquiterpenoids	Leaf
22	Triptersinine M	163184013	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)OC(=O)C)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-containing sesquiterpenoids	Leaf
23	Triptersinine N	163184012	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=COC=C4)OC(=O)C)C(O3)(C)C)OC(=O)C5=COC=C5)(C)O)OC(=O)C6=CN=CC=C6	Nitrogen-containing sesquiterpenoids	Leaf
24	Triptersinine O	163184011	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)OC(=O)C	Nitrogen-containing sesquiterpenoids	Leaf

			5=COC=C5)(C)O)OC(=O)C6=COC=C6		
25	Triptersinine P	163184010	CC=C(C)C(=O)OC1CCC(C23C1(C(C(C(C2OC(=O)C)C(O3)(C)C)OC(=O)C4=CN=CC=C4)OC(=O)C5=CN=CC=C5)COC(=O)C)(C)O	Nitrogen-containing sesquiterpenoids	Leaf
26	Triptersinine Q	163184009	CC=C(C)C(=O)OC1C(C2C(C3(C1(C(CCC3(C)O)OC(=O)C4=COC=C4)COC(=O)C)OC2(C)C)OC(=O)C)OC(=O)C5=CN=CC=C5	Nitrogen-containing sesquiterpenoids	Leaf
27	Triptersinine S	163184008	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)OC(=O)C5=COC=C5)(C)O)OC(=O)C	Nitrogen-containing sesquiterpenoids	Leaf
28	Triptersinine T	163184007	CC1CCC(C2(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)O)COC(=O)C)OC(=O)C	Nitrogen-containing sesquiterpenoids	Leaf
29	Triptersinine Z5	163184000	CC1CCC(C2(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)OC(=O)C5=CC=CO5)COC(=O)C)OC(=O)C	Nitrogen-containing sesquiterpenoids	Leaf
30	Triptersinine Z6	163183999	CC1CCC(C2(C13C(C(C(C2OC(=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)OC(=O)C5=CC=CO5)COC(=O)C)OC	Nitrogen-containing sesquiterpenoids	Leaf

			(=O)C6=COC=C6		
31	Triptersinine I	163184014	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=COC=C4)OC(=O)C)C(O3)(C)C)O)(C)O)OC(=O)C5=COC=C5	Nitrogen-free sesquiterpenoids	Leaf
32	Triptersinine V	163184006	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CC=CO4)O)C(O3)(C)C)OC(=O)C)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-free sesquiterpenoids	Leaf
33	Triptersinine W	163184005	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CC=CO4)OC(=O)C)C(O3)(C)C)O)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-free sesquiterpenoids	Leaf
34	Triptersinine X	163184004	CC(=O)OCC12C(CCC(C13C(C(C(C2OC(=O)C4=CC=CO4)OC(=O)C)C(O3)(C)C)O)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-free sesquiterpenoids	Leaf
35	Triptersinine Y	163184003	CC(=O)OC1C2C(C(C3(C1(C(CCC3OC(=O)C=CC4=CC=CC=C4)(C)O)OC2(C)C)CO)OC(=O)C5=CC=CO5)O	Nitrogen-free sesquiterpenoids	Leaf
36	Triptersinine Z1	163184002	CC(=O)OCC12C(CCC(C13C(C(C(=O)C2OC(=O)C4=CC=CO4)C(O3)(C)C)O)(C)O)OC(=O)C=CC5=CC=CC=C5	Nitrogen-free sesquiterpenoids	Leaf
37	Triptersinine Z2	163184001	CC(=O)OCC12C(CCC(C13C(C(C(=O)C2OC(=O)C4=CC=CO4)C(O3)(C)C)	Nitrogen-free sesquiterpenoids	Leaf

			O)(C)O)OC(=O)C=CC5=CC=CC=C5		
38	Tripterycoside A	163183998	CC(C)C1=CC(=C2C(=C1OC3C(C(C(C(O3)CO)O)O)O)CCC4C2(CCC5=C4COC5=O)C)O	Diterpenes	Root
39	Tripterycoside B	163183997	CC(C)C1=CC(=C2C(=C1O)CCC3C2(CCC4=C3COC4=O)C)OC5C(C(C(C(O5)CO)O)O)O	Diterpenes	Root
40	Tripterycoside C	163183996	CC(C)C1=C(C(=C2C(=C1)C(=O)CC3C2(CCC4=C3COC4=O)C)OC5C(C(C(C(O5)CO)O)O)O)O	Diterpenes	Root
41	15-hydroxytriptonide	163184084	CC12CCC3=C(C1CC4C5(C26C(O6)C7C(C5=O)(O7)C(C)(C)O)O4)COC3=O	Diterpenes	Root bark
42	5-hydroxymethylmellein	14807793	CC1CC2=C(C=CC(=C2C(=O)O1)O)CO	Other compounds	Root

4.2.2 Molecular docking provided phytochemicals with the highest binding affinity for SMN1 protein.

The 24 binding sites from the SMN1 protein were considered for docking-based virtual screening with the 115 phytochemicals .The binding affinity score of 115 phytochemicals with SMN1 were shown in **table 4.4 and 4.5**.

Table 4.4: Binding affinity scores of phytochemicals from ***Brucea javanica*** with SMN1 protein after virtual screening.

S.No	Phytochemicals	Binding affinity score (kcal/mol)	S.No	Phytochemicals	Binding affinity score (kcal/mol)
1	Yadanzioside M	-11.4	**38**	Brujavanone E	-7.6
2	Bruceoside F	-10.5	**39**	Brujavanone G	-7.5
3	Yadanzioside O	-10.1	**40**	Brujavanone K	-7.5
4	Yadanzioside P	-10.0	**41**	Brujavanone B	-7.4
5	Bruceoside B	-9.8	**42**	BruceanicAcid E	-7.3
6	Yadanzioside K	-9.8	**43**	Bruceolline M	-7.3
7	Bruceoside C	-9.6	**44**	Brujavanone A	-7.2
8	Javanicoside B	-9.6	**45**	Dihydrobruceajav-anin A	-7.2
9	Javanicoside F	-9.6	**46**	Bruceine A	-7.1
10	Yadanzioside B	-9.6	**47**	Yadanzigan	-7.1
11	Brujavanone M	-9.4	**48**	Brujavanol A	-7.0
12	Yadanzioside A	-9.3	**49**	Brujavanone L	-7.0
13	Yadanzioside N	-9.3	**50**	Javanicolide D	-6.8
14	Yadanzioside E	-9.2	**51**	Javanicolide E	-6.8
15	Bruceine L	-9.0	**52**	Bruceine J	-6.7
16	Javanicoside L	-9.0	**53**	Bruceolline K	-6.7
17	Bruceoside E	-8.9	**54**	Bruceantinol	-6.6
18	Javanicoside J	-8.9	**55**	Bruceine C	-6.6
19	Javanicoside I	-8.8	**56**	Bruceolline L	-6.5
20	Yadanzioside I	-8.7	**57**	Brusatol	-6.5
21	Javanicoside E	-8.4	**58**	Bruceine I	-6.4

22	Yadanzioside C	-8.4	**59**	Bruceine E	-6.1
23	Bruceantinosides A	-8.3	**60**	Javanicolide H	-6.1
24	Javanicoside K	-8.3	**61**	Brujavanone J	-6.0
25	Yadanzioside L	-8.3	**62**	Bruceine B	-5.8
26	Bruceanic Acid E Methyl Ester	-8.2	**63**	Javanicolide B	-5.8
27	Bruceolline N	-8.2	**64**	Bruceine D	-5.7
28	Yadanzioside G	-8.2	**65**	Bruceine F	-5.7
29	Brujavanone F	-8.1	**66**	Bruceine K	-5.7
30	Brujavanone I	-8.1	**67**	Bruceolline H	-5.5
31	Javanicoside C	-8.1	**68**	Bruceolline I	-5.5
32	Yadanzioside F	-8.1	**69**	Bruceolline J	-5.5
33	Brujavanone H	-8	**70**	Javanicolide C	-5.5
34	BruceanicAcid F	-7.9	**71**	Bruceene	-5.4
35	Brujavanone D	-7.7	**72**	Bruceine G	-5.3
36	Javanicoside G	-7.7	**73**	3′-hydroxybrucein A	-5.3
37	Bruceoside D	-7.6			

Table 4.5: Binding affinity scores of phytochemicals from ***Tripterygium wilfordii*** with SMN1 protein after virtual screening.

S.No	Phytochemical name	Binding affinity score (Kcal/mol)
1	Triptersinine A	-10.5
2	Triptersinine B	-10.1
3	Triptersinine M	-10.0
4	Triptersinine C	-9.8
5	Triptersinine D	-9.4

6	Triptersinine I	-9.3
7	Triptersinine W	-9.0
8	Triptersinine O	-8.8
9	Tripterygiumine M	-8.8
10	Tripterygiumine N	-8.8
11	Triptersinine Z2	-8.6
12	Tripterygiumine P	-8.5
13	Triptersinine S	-8.4
14	Triptersinine T	-8.4
15	Triptersinine Z1	-8.3
16	Tripterygiumine V	-8.2
17	Triptersinine Z6	-8.1
18	Tripterygiumine C	-8.1
19	Tripterygiumine S	-8.1
20	Triptersinine N	-7.9
21	Wilfornine H	-7.8
22	Triptersinine Y	-7.7
23	Tripterygiumine V	-7.6
24	Tripterygiumine R	-7.6
25	Tripterygiumine K	-7.5
26	Tripterycoside A	-7.4
27	Tripterygiumine D	-7.4
28	Tripterygiumine E	-7.4
29	Tripterygiumine L	-7.3
30	Tripterycoside B	-7.2
31	Tripterygiumine G	-7.2
32	Tripterygiumine H	-7.2
33	Triptersinine Q	-7.1

34	Triptersinine P	-7.0
35	Tripterygiumine O	-7.0
36	Triptersinine Z5	-6.9
37	Tripterycoside C	-6.9
38	Tripterygiumine F	-6.1
39	Triptersinine X	-5.8
40	Tripterygiumine T	-5.8
41	5-hydroxymethylmellein	-5.0
42	15-hydroxytriptonide	-5.0

The top 10 phytochemicals that have shown the highest binding affinity with SMN1 protein from the two datasets (Top five from each dataset) were further considered for virtual screening (**Table 4.6**).

Table 4.6: Binding affinity scores of phytochemicals (top 10) with SMN1 protein after virtual screening

S.No	**Phytochemical**	**Binding affinity score (Kcal/mol)**
1	Yadanzioside M	-11.4
2	Bruceoside F	-10.5
3	Triptersinine A	-10.5
4	Triptersinine B	-10.1
5	Yadanzioside O	-10.1
6	Yadanzioside P	-10
7	Triptersinine I	-10
8	Bruceoside B	-9.8
9	Triptersinine C	-9.8
10	Triptersinine D	-9.4

The protein-ligand interactions were analysed by LigPlot for all ten docked protein-phytochemical complexes. SMN1 interactions with Bruceoside F shown Hydrogen Bond Interactions with Trp102, Ser103, Glu104, Tyr109, Tyr130 and Residue involved in electrostatic interactions were Cys107, and Glu134 (**Figure 4.8**).

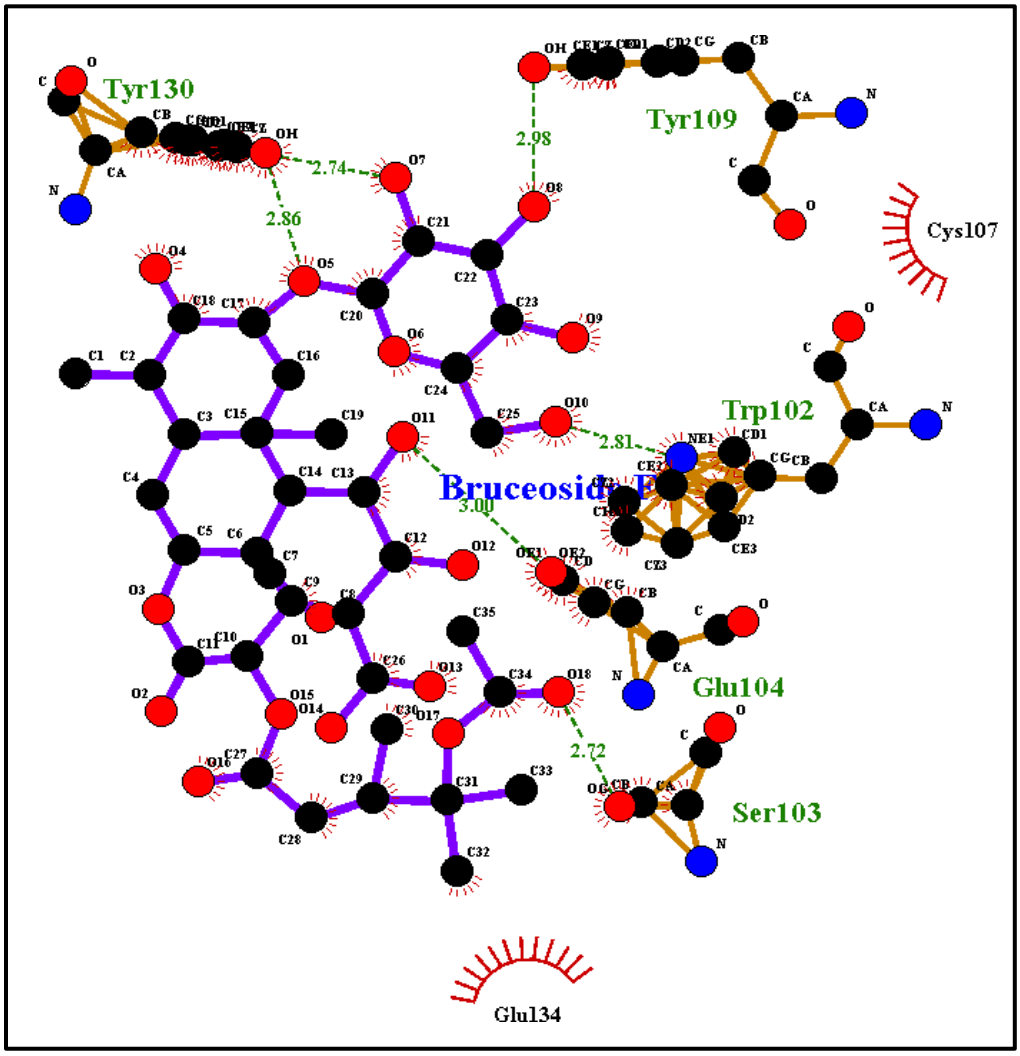


Figure 4.8: SMN1 interactions with Bruceoside F

Similarly interactions of SMN1 with Yadanzioside O shown electrostatic interactions were Trp102,Glu104,Gly129,Asn132 and Hydrogen Bond Interactions with Ser103,Tyr130 and Glu134 (**Figure 4.9**).

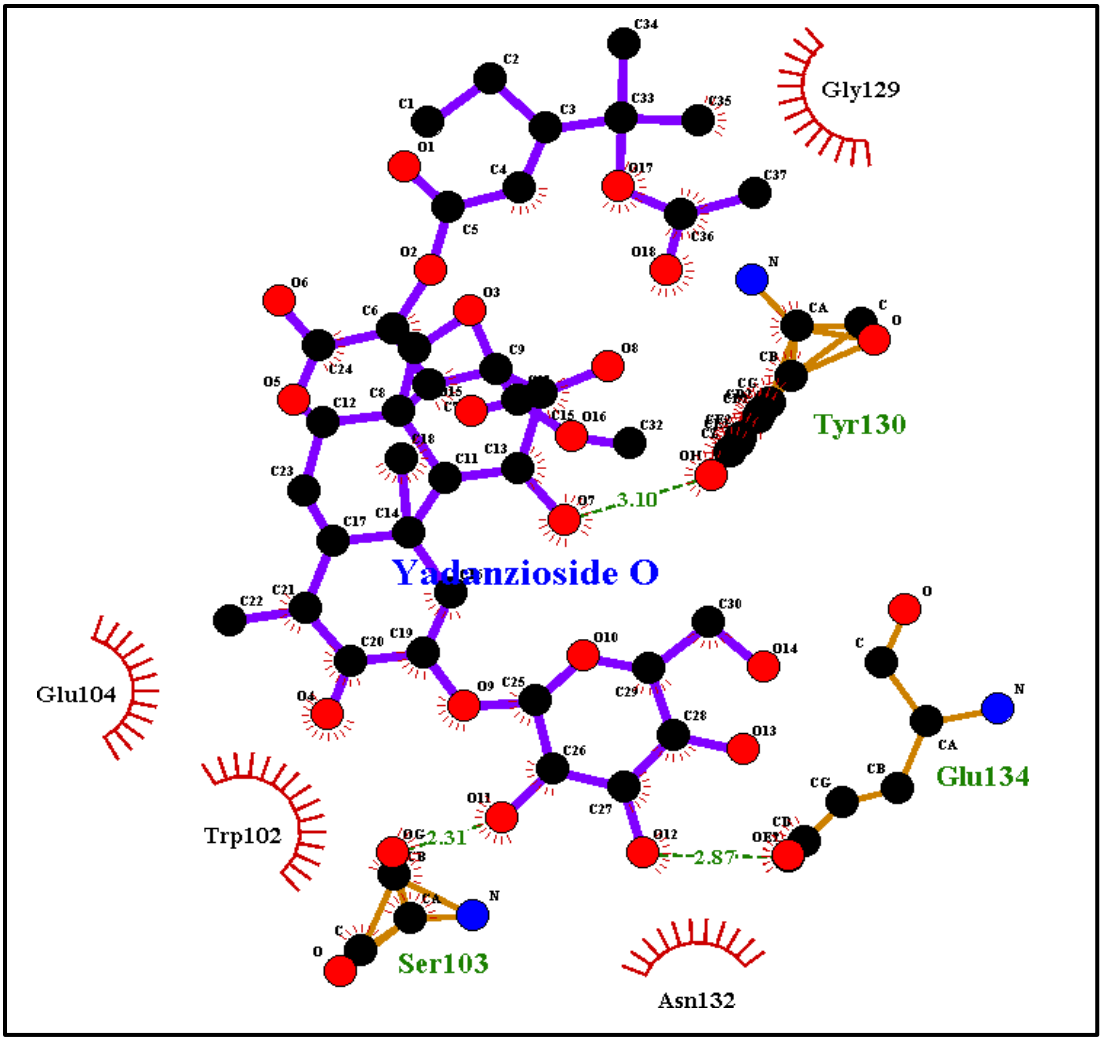


Figure 4.9: SMN1 interactions with Yadanzioside O

The amino acid residues Tyr130 ,Asn132 of SMN1 showed hydrogen bond interactions with Yadanzioside P and electrostatic interactions with Trp102, Ser103, Glu104 and Asp105(**Figure 4.10)**

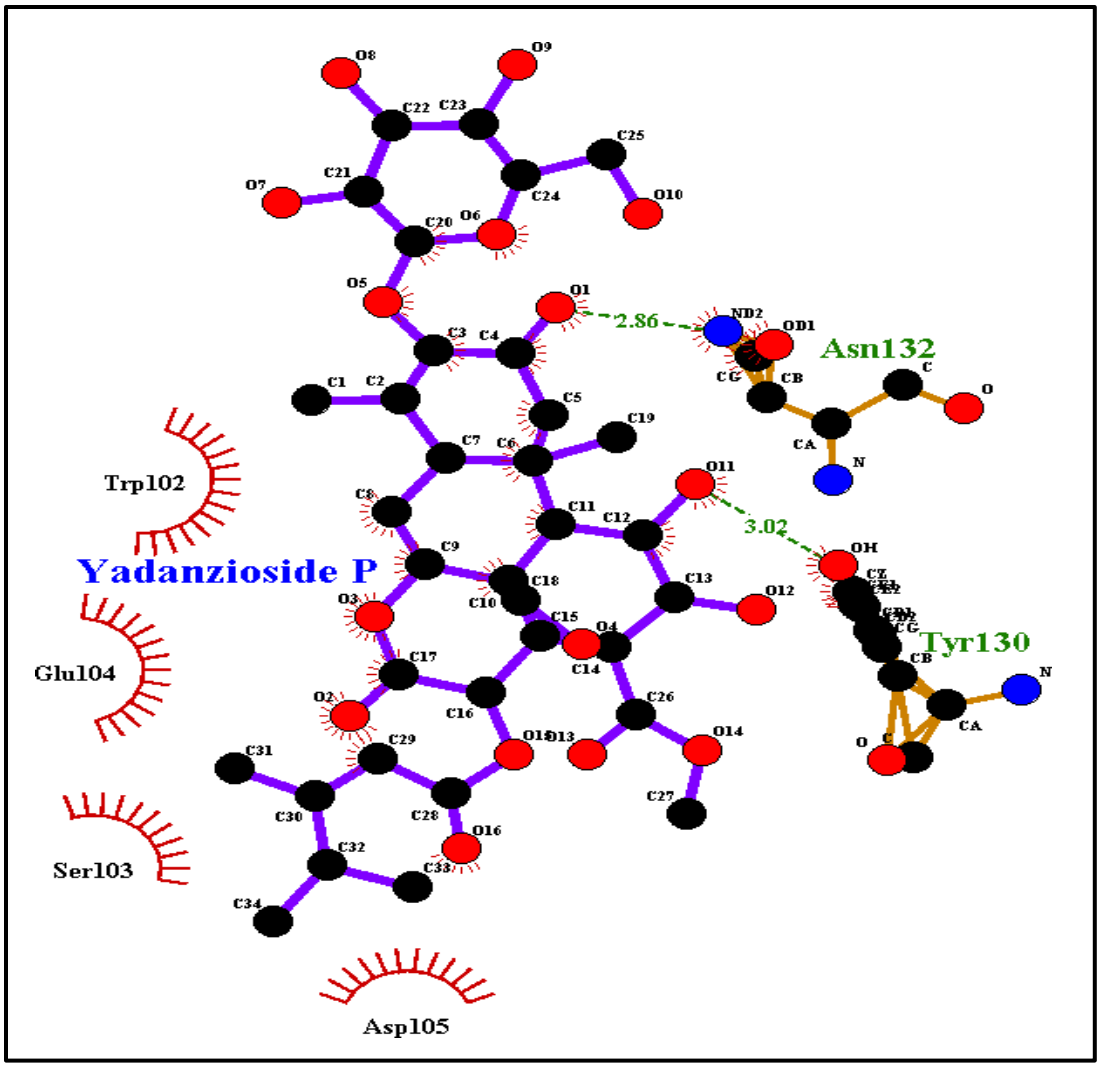


Figure 4.10:SMN1 interactions with Yadanzioside P

Bruceoside B interacted with SMN1 and shown Hydrogen Bond Interactions with Tyr130 and residues involved in electrostatic interactions were Trp102, Ser103, Glu104, Cys107, Gly129,Gly131, Asn132 (**Figure 4.11).**

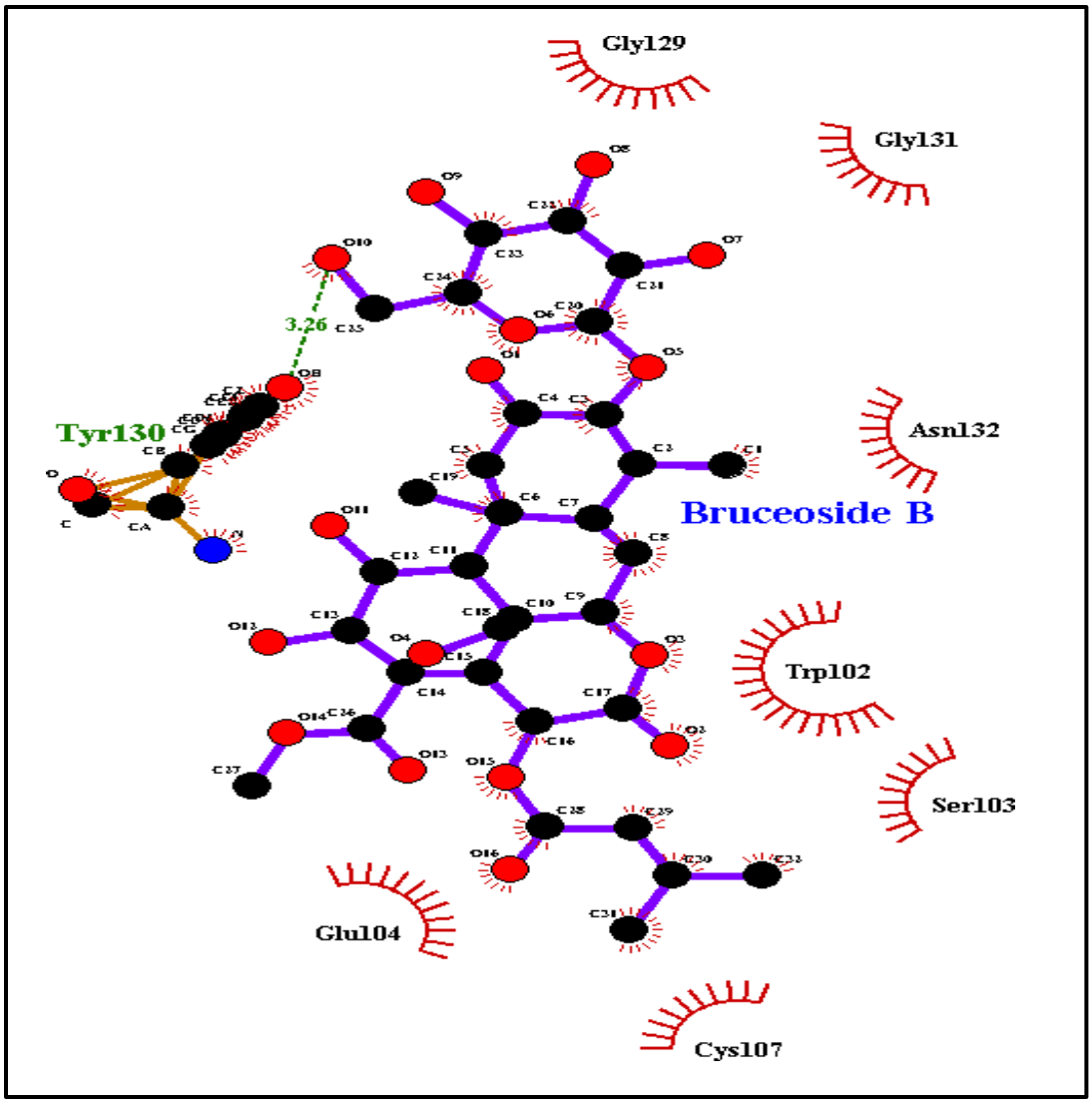


Figure 4.11. SMN1 interactions with Bruceoside B

Interactions of SMN1 with Triptersinine B has shown Hydrogen bond interactions with Trp102, Asn132 and Residue involved in electrostatic interactions were Ser103, Glu104, Cys107, Tyr130, Glu134 (**Figure 4.12**).

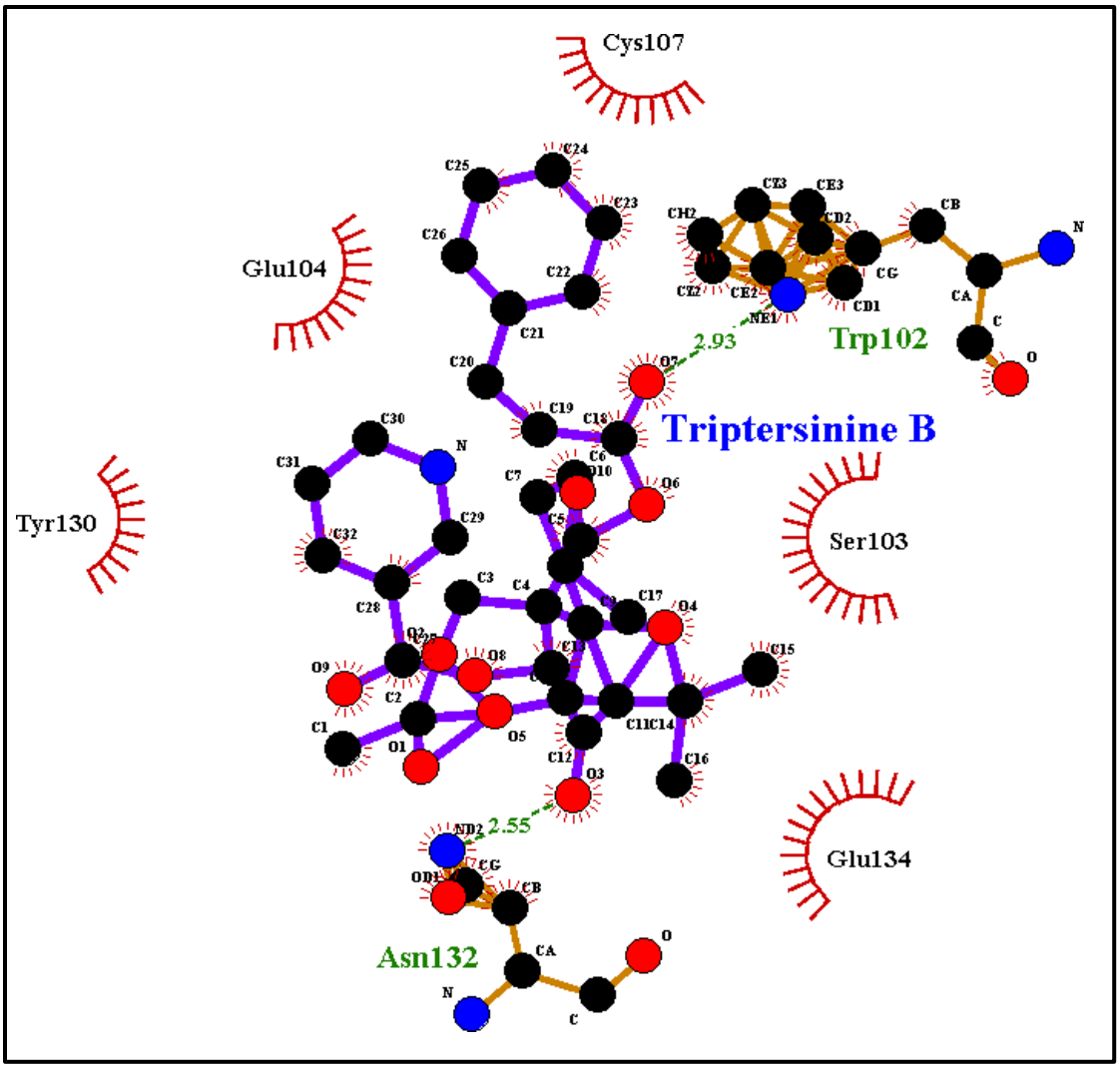


Figure 4.12: SMN1 Interactions with Triptersinine B

SMN1 interactions with Triptersinine M shown electrostatic interactions with Ser103, Glu104, Tyr109, Thr128,Gly129.Asn132 and Hydrogen Bond Interactions with Trp102,Tyr130 (**Figure 4.13**).

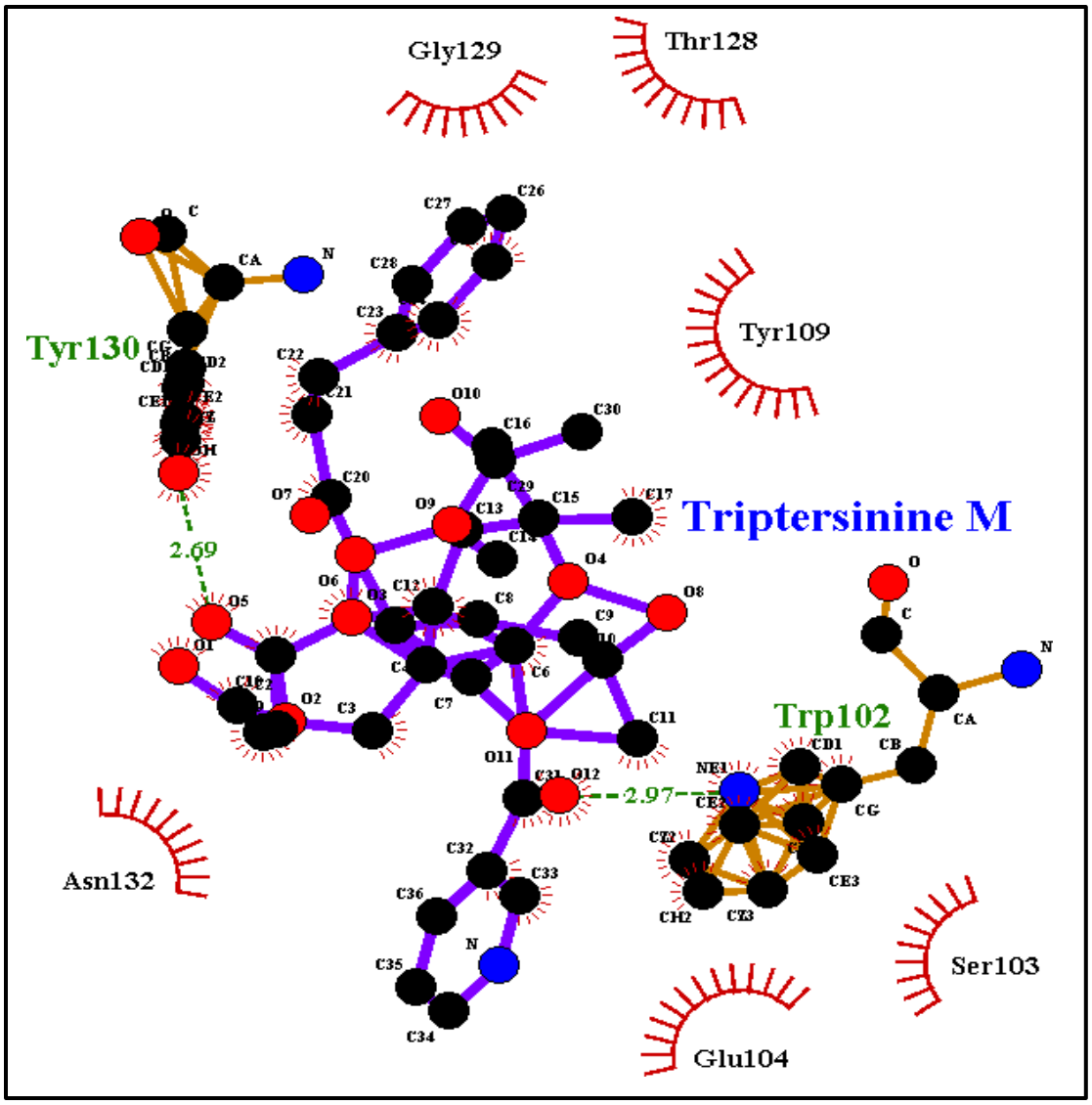


Figure 4.13. SMN1 interactions with Triptersinine M

Triptersinine C interacted with SMN1 and shown hydrogen bond interactions with Asn132 and residues involved in electrostatic interactions were Trp102, Ser103, Glu104,Cys107, Tyr109, Tyr130 and Glu131 (**Figure 4.14)**

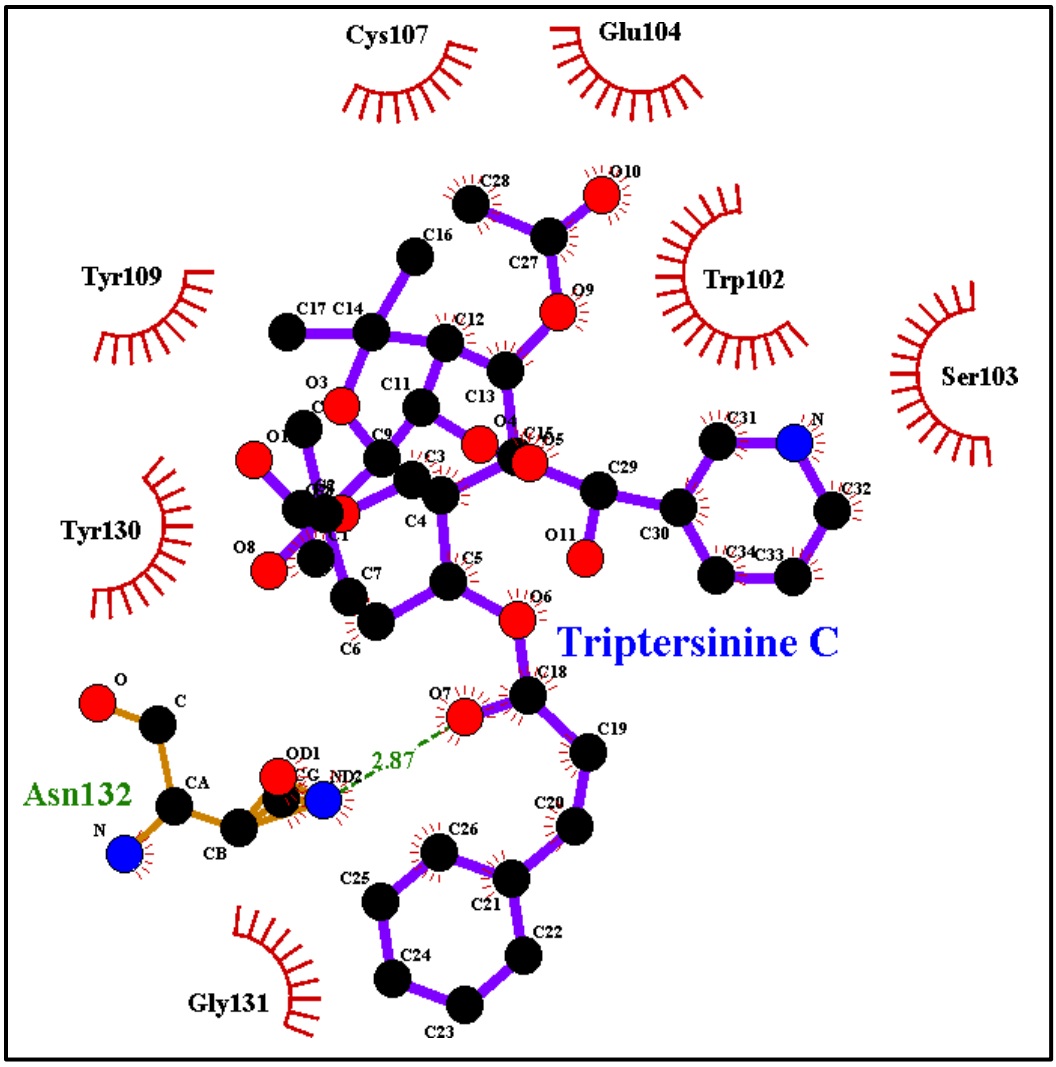


Figure 4.14: SMN1 interactions with Triptersinine C

SMN1 interacted with Triptersinine D and shown hydrogen bond interactions with Tyr130, Asn132 and residues involved in electrostatic interactions were Trp102, Ser103 and Glu104 (**Figure 4.15).**

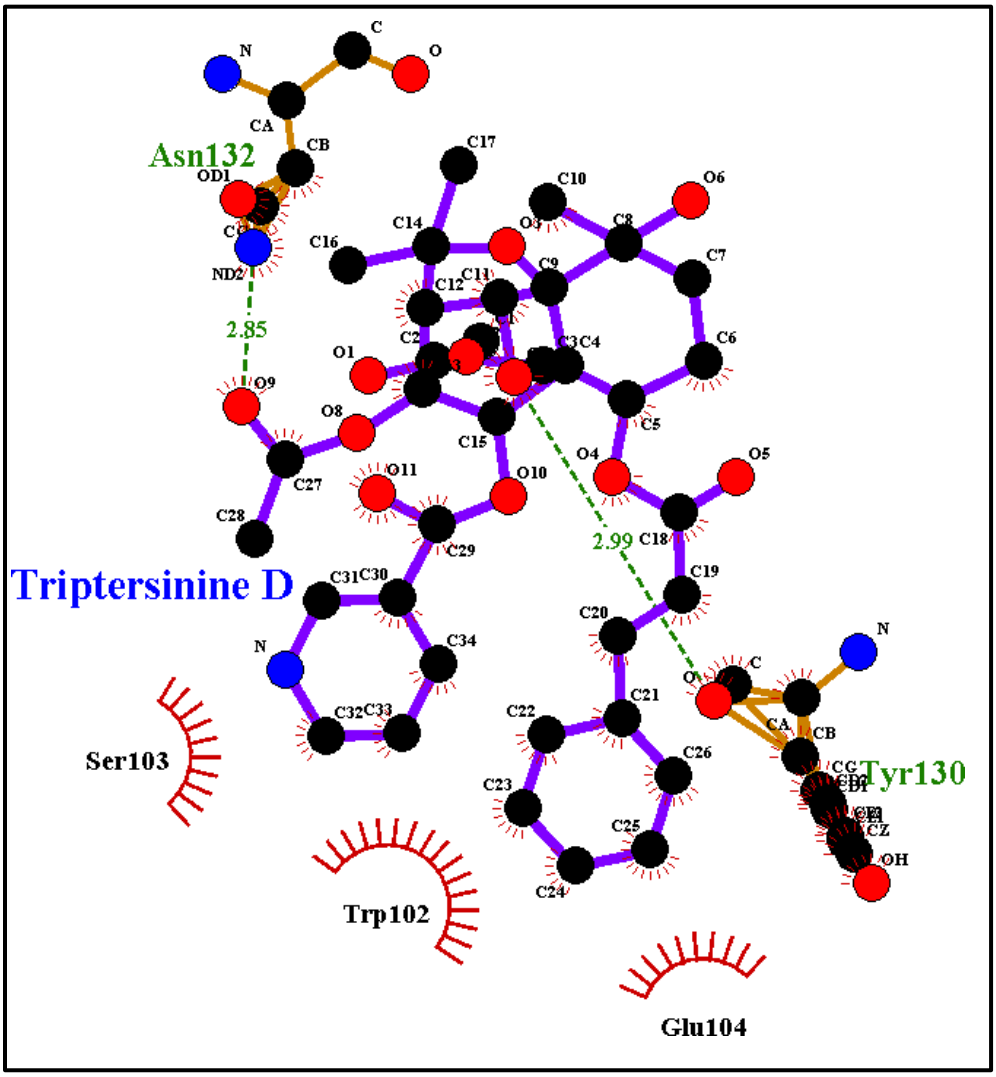


Figure 4.15: SMN1 interactions with Triptersinine D

All the phytochemicals interacted consistently with aromatic amino acid and amino acid residues reported in earlier studies. Among these 10 phytochemicals, we have considered one top ligand from two datasets i.e. Yadanzioside M and Triptersinine A for MD analysis.

After molecular docking of Yadanzioside -M with SMN1, hydrogen bond interactions were observed between the amino acid residues C107, Y130, and N132 and ionic interactions were observed between the protein and amino acid residues W102, S103, E104, D105 and G131. The interactions between the SMN1-Tripersinine-A

complex after molecular docking were hydrogen bonds between the protein and amino acid residue Y130 and W102, S103, E104, and D105 were involved in ionic interactions (**Figure 4.16**).

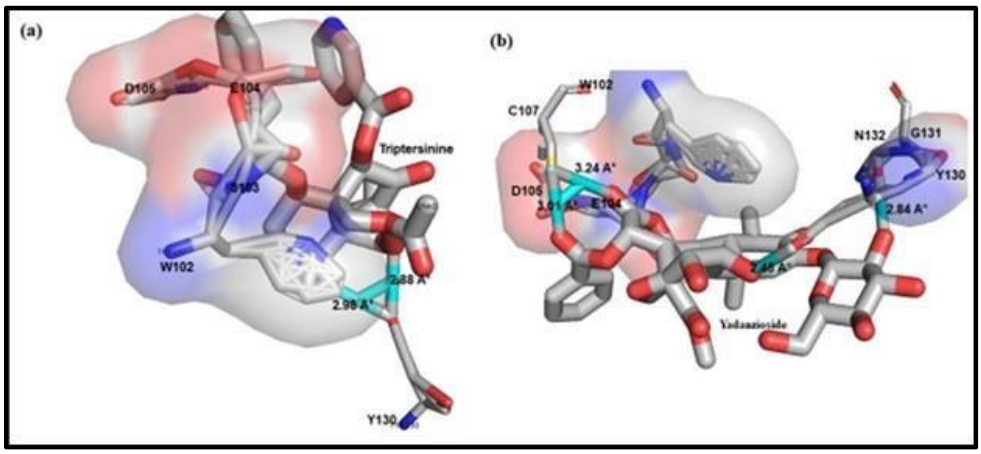


Figure 4.16. Amino acid residues of SMN1 interacting with the ligands (A) Triptersinine-A are Tryptophan (W102), Serine (S103),Glutamic Acid (E104),Aspartic Acid(D105), Tyrosine(Y130) (B) Yadanzioside-M are Tryptophan (W102), Glutamic Acid (E 104), Aspartic Acid(D105), Cysteine(C107), Tyrosine(Y130), Glycine(G131), Asparagine(N132). All the above interacting residues are present in the functional domain of SMN1 i.e Tudor domain

4.2.3 Identification and analysis of pharmacological properties of phytochemicals

Adsorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analysis is more important to understand drug nature of the compounds. The ADMETlab 3.0 was used to predict the ADMET properties like Physicochemical (Molecular Weight (MW), Number of hydrogen bond acceptors (nHA), Number of hydrogen bond donors (nHD), The logarithm of aqueous solubility value (logS), The logarithm of the n-octanol/water distribution coefficient (logP), Topological polar surface area (TPSA)),medicinal chemistry (Pfizer Rule), Adsorption (Madin−Darby Canine Kidney cells (MDCK) permeability), Distribution (Plasma protein binding (PPB) , blood–brain barrier (BBB)), Metabolism(The human liver microsomal(HLM) stability), Excretion(Plasma clearance ($C_L plasma$)),Toxicity(respiratory toxicity, hERG Blockers(human ether-a-go-go related gene)) of the considered ligand molecules (Pettersen et al., 2004) (**Table 4.7**). In

General, use of natural products and medicinal plants gives a most advantageous road map to discover novel compounds and sometimes-natural extracts used directly to cure the disease condition because of the lack of efficacy of purified compounds without any rule of three or rule of five. Because of their chemical diversity, natural compounds bypass the ADMET predictions through computer-based software‘s because of the stringent filtering approaches (Banjari et al., 2017) (Thomford et al., 2016a) (Thomford et al., 2016b) (Thomford et al., 2016c) (Yatoo et al., 2017). Most of the countries, Plant extracts, and natural compounds are used in a combination with other prescribed drugs (Ji et al., 2017) (Ruhsam & Hollingsworth, 2017). The most important part is having the right ADMET properties may not have strong biological activity and balance of the natural products requires more analytical and biological process (Schneider, 2017). In the present considered phytochemicals bypassed the few ADMET properties.

Table 4.4: ADMET properties of phytochemicals considered for molecular docking

Phytochemical Name	MW	nHA	nHD	logP	TPSA	logS	logD	Pfizer Rule	MDCK Permeab-ility	PPB	BBB	HLM Stabili-ty	CL_{plasma}	Respira-tory	hERG Blockers
Triptersinine-A	593.23	11.0	2.0	2.422	158.55	-4.382	2.612	Accepted	0.0	77.4%	0.05	0.004	1.371	0.028	0.142
Yadanzioside-M	704.23	16.0	6.0	0.281	245.04	-2.259	1.124	Accepted	0.0	67.3%	0.003	0.535	2.024	0.019	0.035
Bruceoside F	754.27	18.0	7.0	-0.396	282.34	-2.257	1.018	Accepted	0.0	50.7%	0.0	0.0	1.006	0.018	0.004
Triptersinine B	593.23	11.0	2.0	2.422	158.55	-4.382	2.612	Accepted	0.0	77.4%	0.055	0.004	1.371	0.028	0.142
Yadanzioside O	782.3	18.0	6.0	0.849	271.34	-3.203	1.693	Accepted	0.0	58.3%	0.0	0.0	1.308	0.017	0.013
Yadanzioside P	710.28	16.0	6.0	0.911	245.04	-3.024	1.849	Accepted	0.0	52.7%	0.005	0.0	1.673	0.026	0.013
Triptersinine I	590.2	13.0	12.0	1.97	181.17	-2.805	2.095	Accepted	0.0	33.3%		0996	1.61	0.053	0.142
Bruceoside B	682.25	16.0	6.0	-0.062	245.04	-2.184	0.965	Accepted	0.0	56.1%	0.144	0.0	1.817	0.019	0.012
Triptersinine C	637.25	12.0	2.0	2.95	167.78	-4.619	2.936	Accepted	0.0	71.3%	0.006	0.122	0.803	0.017	0.135
Triptersinine D	637.25	12.0	2.0	2.95	167.78	-4.619	2.936	Accepted	0.0	71.3%	0.006	0.122	0.803	0.017	0.135

4.3 Interactions study between phytochemicals and target for the proposal of potential drug candidate.

4.3.1 Molecular dynamics simulation

To understand the interactions between protein and phytochemicals, MD simulations were carried out and the results were discussed in the following section.

Molecular dynamics simulation of SMN1 apo-protein, SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex systems production MD run for 1000ns timescale were carried out for better understanding of the structural flexibility and behaviour of the SMN1 Apo-protein and with inhibitors (**Figure 4.17**). MD results of apo-protein; protein-Ttriptersinine-A and protein-Yadanzioside-M complex was represented with black, red and green colour for Figure 4.17. The structural stability of the respective system was compared by RMSD of the backbone of protein from the MD trajectories that showed the changes in conformational properties in the simulation box. RMSD values for SMN1 apo-protein, SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex varied around the average ~3, ~3 and ~1.5 nm respectively.

The SMN1 apo-protein, SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex attained stability after 100 ns. SMN1-Yadanzioside-M complex has shown fewer deviations as compared to apo-protein and SMN1-Triptersinine-A complex throughout the 1000ns MD simulations. The initial points of deviations of the apo-protein, SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex were observed from 0.3, 0.2 and 0.1 nm, respectively. The maximum deviation for apo-protein, SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex were ~0.48nm at 380 ns, ~0.52 nm at 390 ns and ~0.18 nm at 580 ns. At the end of simulations, the RMSD values of apo-protein, SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M Complex were ~0.35, 0.3 nm and 0.16 nm respectively.

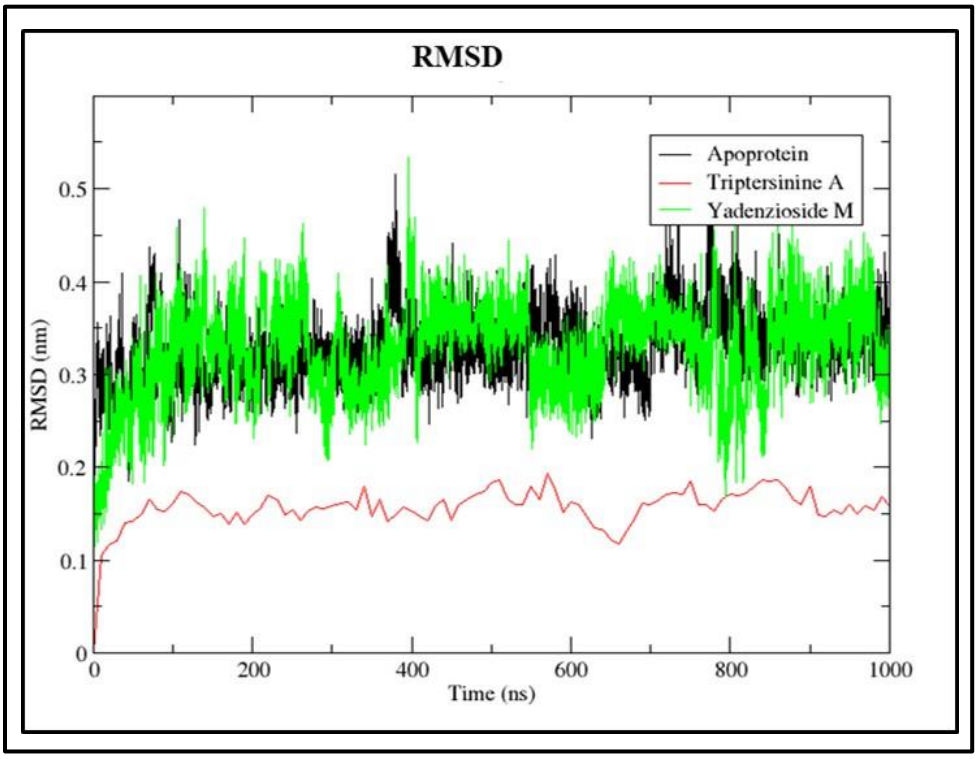


Figure 4.17. Root-mean-square deviation (RMSD) values of the backbone represented structural stability of SMN1 apo-protein (black), SMN1-Triptersinine-A complex (Red) and SMN1-Yadanzioside-M complex (Green).

The radius of gyration (Rg) for apo-protein, SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex was observed for protein, the backbone of the protein and ligands to depict the compactness of protein and the ligand (**Figure 4.18**).

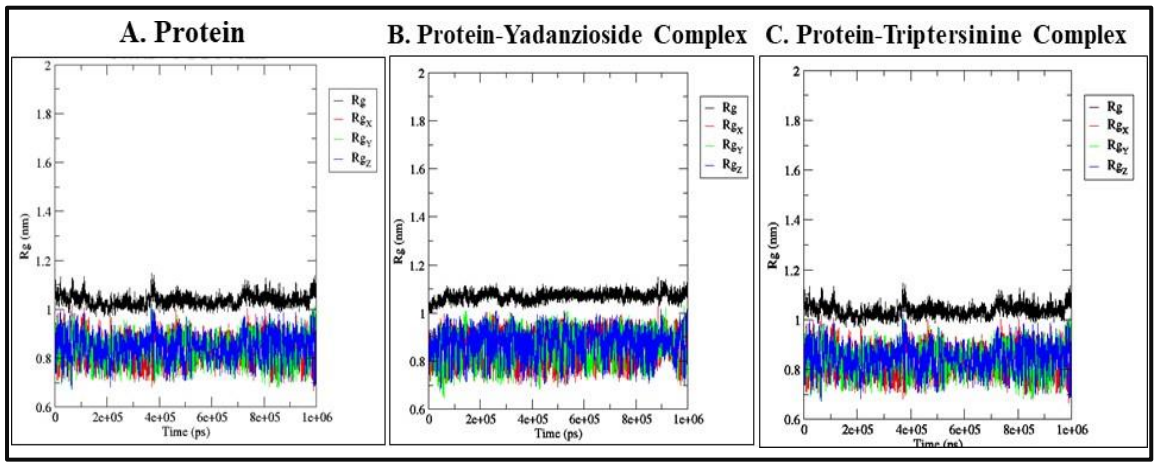


Figure 4.18. Radius of gyration values illustrated the compactness of (a) Apo-protein, (b) SMN1-Triptersinine-A complex and (c) SMN1-Yadanzioside-M complex.

The Rg showed changes until 400 ns in the case of Apo-protein, however afterwards the proteins showed fewer fluctuations and protein structure compactness was maintained. While the protein-Triptersinine A complex has shown less fluctuations as compared to apo-protein and the protein structure was more compact as compared to apo-protein. Protein-Yadanzioside-M has shown fluctuations until 350 ns and after that, there are fewer fluctuations observed as compared to apo-protein indicating better compactness of protein-ligand complex in comparison to apo-protein. Rg values of Triptersinine-A were observed between 0.8 to 3 nm however in case of Yadanzioside-M showed Rg between 0.25 to 0.55 nm during the protein-ligand simulations (**Figure 4.19**).

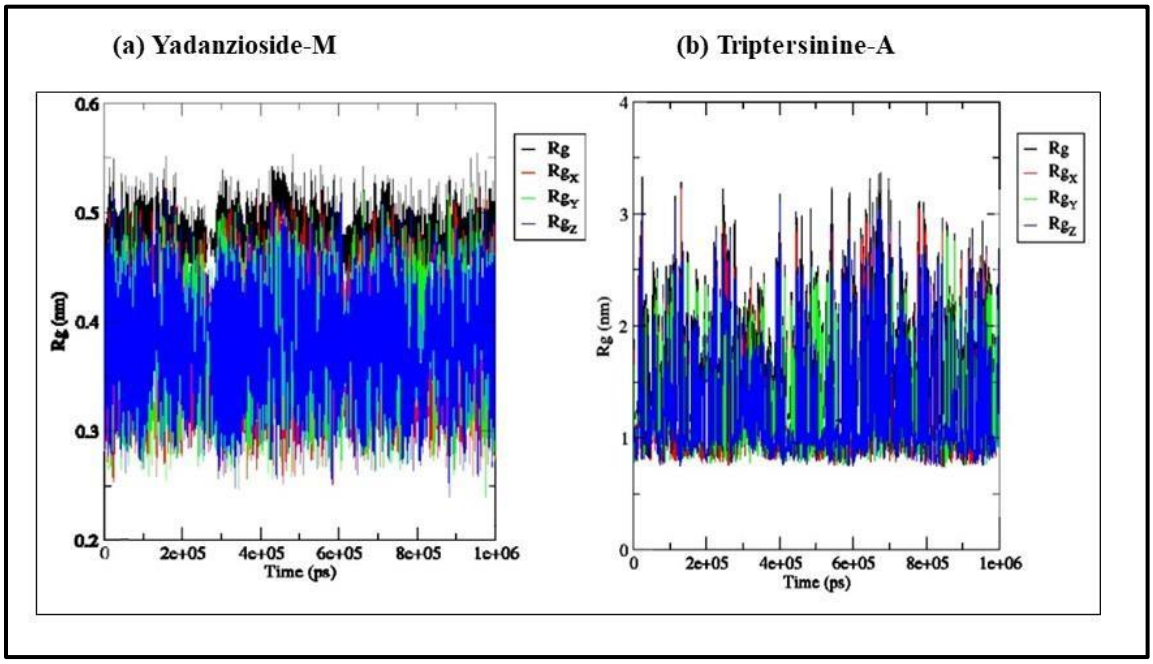


Figure 4.19. Radius of gyration values illustrated the compactness of ligands i.e. (a) Yadanzioside-M and (b) Triptersinine-A

Solvent Access Surface Area (SASA) provided the details of the available surface of protein and protein-phytochemical complexes by the measurement of exposed hydrophobic residues. Apo-protein has shown 35 nm SASA at the beginning of MD and 44 nm at the end of MD simulations, which shows lesser area exposed to solvent. While

in the case of protein-ligand complexes, SASA was 38 nm and 45 nm for protein-Ttriptersinine-A and protein-Yadanzioside-M complex at the beginning of the simulations (**Figure 4.20**) and it increased to 52.5 nm and 57.5 nm for protein-Ttriptersinine-A and protein-Yadanzioside-M complex respectively towards the end of simulations. It indicates the flexibility of the whole system and better interactions among protein-ligand complexes.

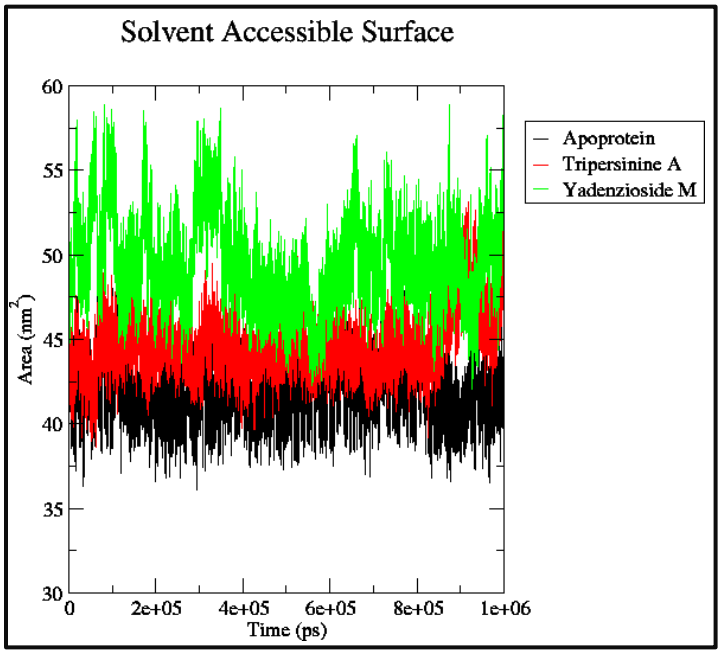


Figure 4.20. Solvent accessible surface area (SASA) estimated the solvent accessibility of SMN1 apo-protein (black), SMN1-Triptersinine-A complex (Red) and SMN1-Yadanzioside-M complex (Green).

Root Mean Square Fluctuations (RMSF) of individual amino acid residues in three systems were observed representing the variation in the protein stability. It was observed that residues of SMN1-Triptersinine-A complex and SMN1-Yadanzioside-M complex were having lower fluctuations as compared to apo-protein (**Figure 4.21**).

The amino acid residues that have formed hydrogen bond interactions and van der Waals interactions in the protein-Ttriptersinine-A complex have been represented in red colour and for the protein-Yadanzioside-M complex, the interacting amino acid residues have been represented in green colour. The fluctuations of the interacting amino acid

residues show that the interactions are stable during the evolution of the MD trajectory.The binding site of SMN1 protein has been reported to have a tricyclic scaffold that is sandwiched between W102 and Y130 that forms pi–pi interactions with ligands (Vorreiter et al., 2024). The intermolecular hydrogen bond interactions of protein-ligand complexes were observed indicating the possible mechanism of interactions at the molecular levels.

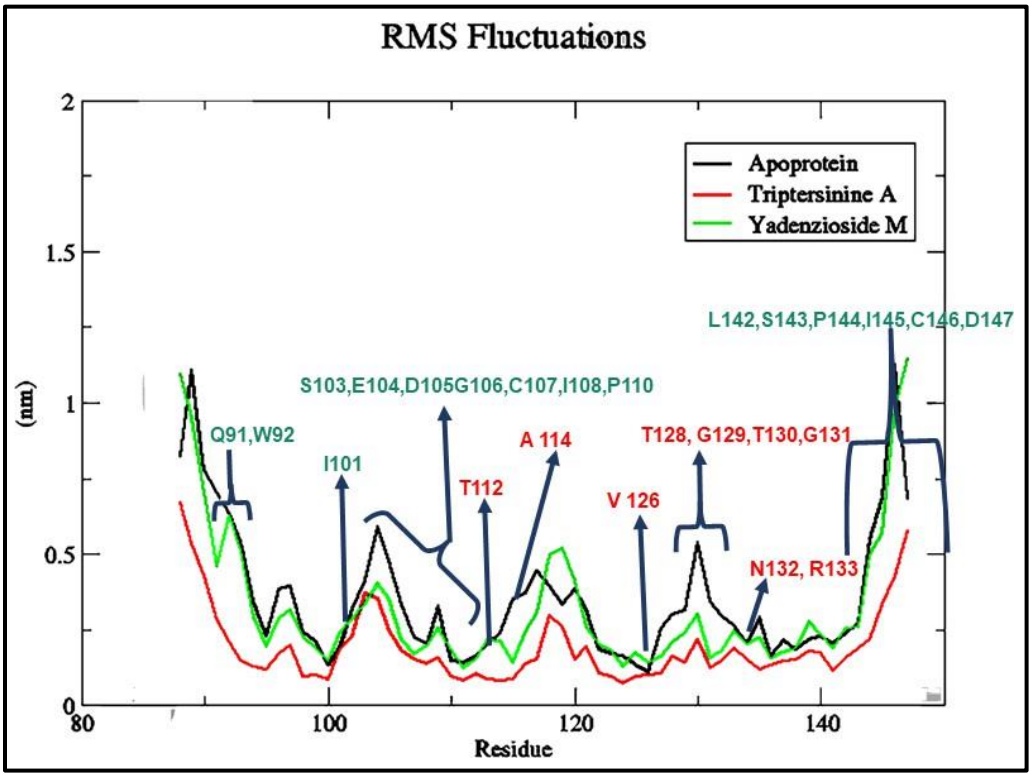


Figure 4.21. Root-mean-square fluctuation (RMSF) measured the flexibility of all the amino acid residues of SMN1 apo-protein (black), SMN1-Triptersinine-A complex (Red) and SMN1-Yadanzioside-M complex (Green).

Protein- Triptersinine-A and protein-Yadanzioside-M complex systems formed intermolecular hydrogen bond interactions almost uniformly till 1000 ns (**Figure 4.22**). Hydrogen bond formation was commenced from 1 ns onwards in the case of Ttriptersinine-A till the end of the MD simulations. However, hydrogen bond was observed after 2 ns in the case of the Yadanzioside-M ligand. While the number of hydrogen bond formations and Vander Waals interactions were higher in Yadanzioside-M.

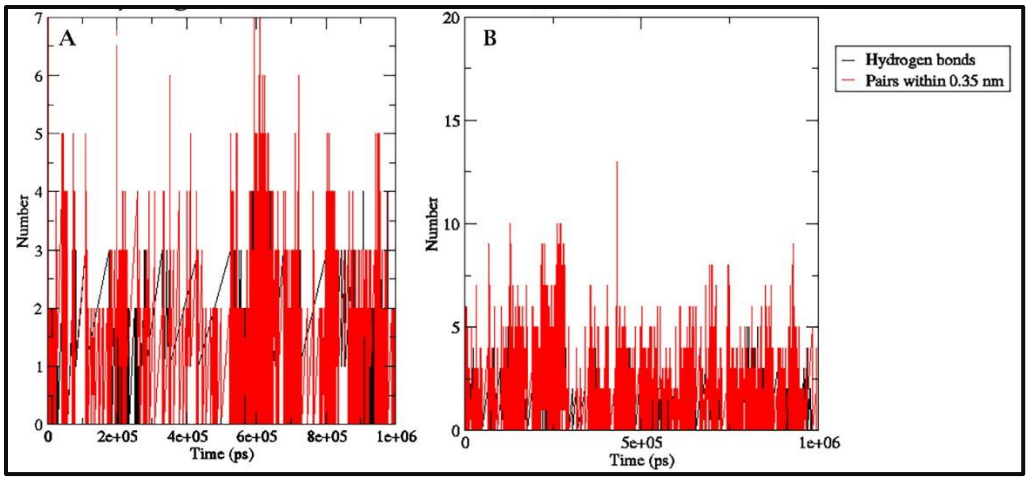


Figure 4.22. Intermolecular hydrogen bonds analysis between protein and ligand molecules illustrated structural stability of the complexes: (a) SMN1-Triptersinine-A complex and (b) SMN1-Yadanzioside-M complex.

4.3.2 Interactions between SMN1 and Yadanzioside-M compound

During hydrogen bond analysis in the protein- Yadanzioside-M complex, it was observed that the ligand has interacted with Q91, W92, I101, S103, E104, D105, G106, C107, I108, P110, D134, Q136, L142, S143, P144, I145, C146 and, D147 amino acid residues and formed either hydrogen bond or Vander Waals interactions (**Figure4. 23**).

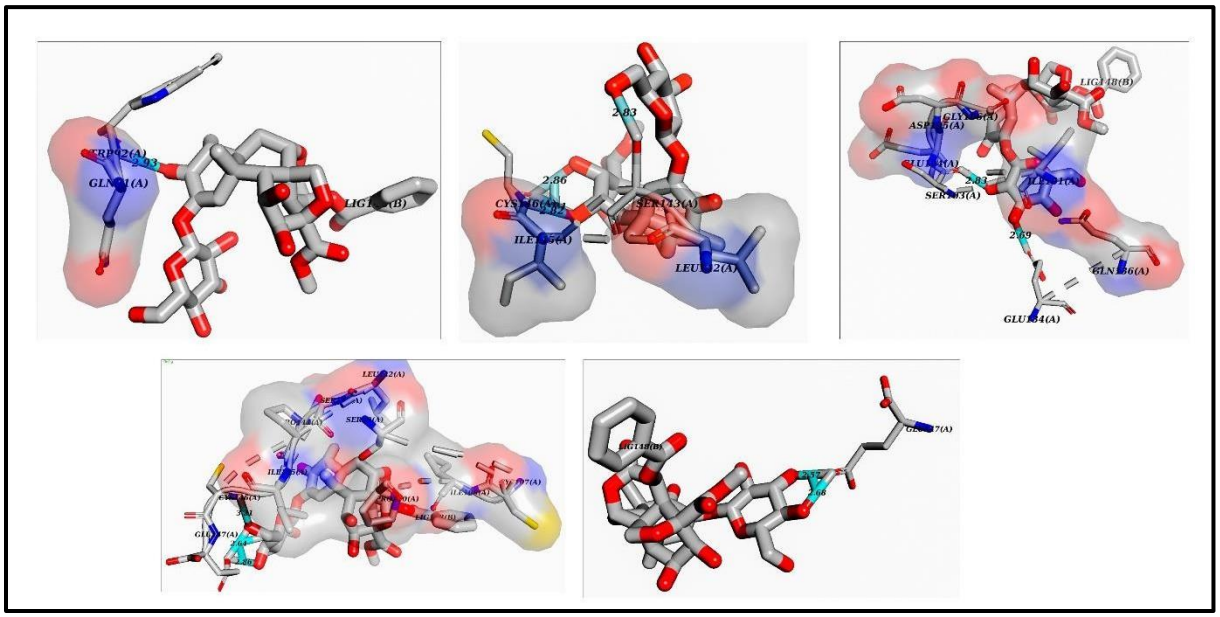


Figure 4.23. The evolution of molecular interactions between SMN1 protein and Yadanzioside-M has been represented in the figure. It was observed that the ligand formed hydrogen interactions with W92, D134, S143, C146 and D147 during the time evolution of the MD trajectory.

At the beginning of the MD simulation, Yadanzioside-M formed a hydrogen bond with W92 followed by S143, C146 and D147. Towards the end of simulations, the ligand interacted with D134 and S103. The multi-cyclic scaffold of Yadanzioside-M interacted with aromatic amino acids like tryptophan, which led to the formation of pi-pi interactions and resulted in better protein-ligand interactions.

4.3.3 Interactions between SMN1 and Triptersinine-A compound

During hydrogen bond analysis in the protein- Triptersinine-A complex, it showed interactions with T112, A114, V126, Y127, T128, G129, T130, G131, N132 and, R133 amino acid residues that resulted in hydrogen bond and Vander Waals interactions (**Figure 4.24**).

At the beginning of the MD simulation, Triptersinine-A formed a hydrogen bond with R133 followed by interaction with Y130, G129 and G131. The interactions between aromatic amino acid and the cyclic scaffold of the compound may have led to the pi-pi interactions with the ligand as reported in earlier studies (Vorreiter et al., 2024). The

overall MD studies have shown that the phytochemical compounds considered in the study have better binding affinity. These inhibitors bind to the tudor domain of the SMN1 protein and form stable pi-pi interactions in the aromatic cage of the protein. Therefore, these compounds could be potential candidates for the treatment of spinal muscular atrophy.

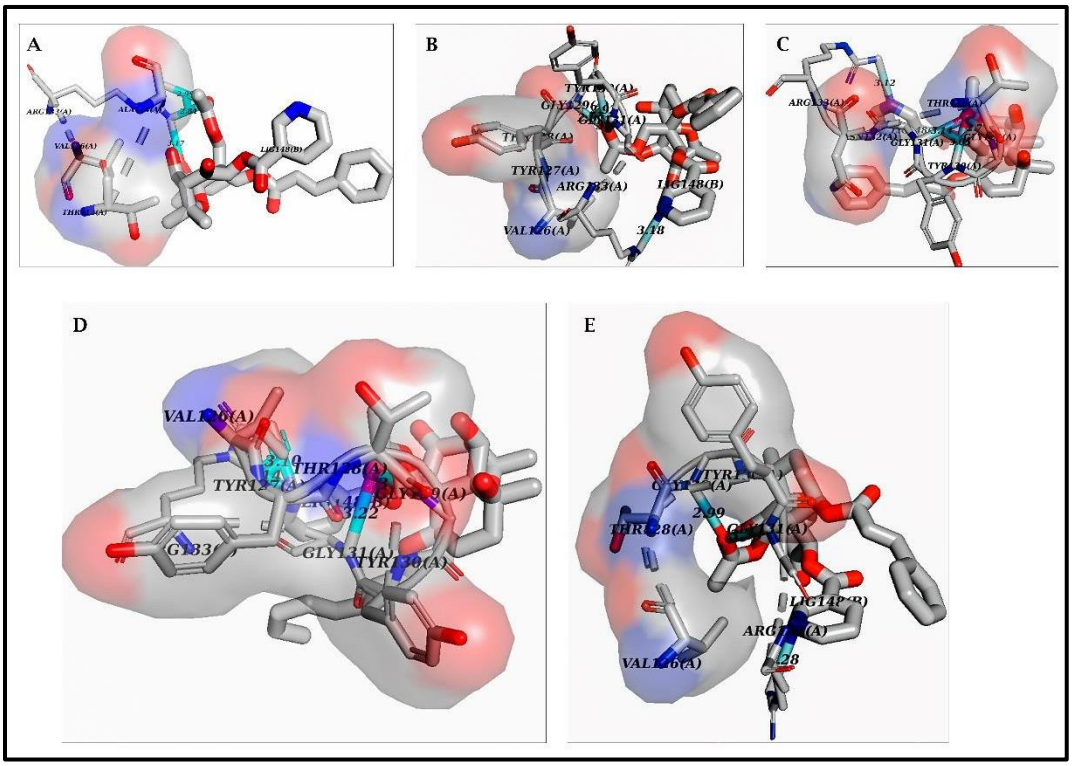


Figure 4.24. The interactions between SMN1 protein and Triptersinine-A were analysed throughout the 1000 ns trajectory of MD simulation. It was observed that the ligand has formed a hydrogen bond with G129, Y130, G131 and R133 amino acid residues of the protein.

These interaction analyses, including hydrogen bonding evaluation and aromatic stacking explanation, follow well-established computational methods commonly applied in protein-ligand studies. While there is no previous detailed hydrogen bond or molecular dynamics simulation studies specifically on phytochemical ligands with SMN1 in SMA, similar approaches have provided valuable insights into ligand binding in related proteins (Li et al., 2017) (Liu et al., 2022). In our study, residues such as W92, D134 and other aromatic

amino acids were found to play important roles in ligand recognition and stabilization. The stable interactions observed in molecular dynamics simulations support the potential of Yadanzioside-M and Triptersinine-A as SMN1 modulators. This work therefore extends current understanding by applying these computational techniques to SMA targets for the first time. The computationally predicted binding interactions were investigated in the context of SMA pathology. The role of SMN1 in snRNP assembly and motor neurone survival demonstrates the potential of the identified phytochemicals to alter disease-relevant pathways. While the *in-silico* findings give strong evidence of molecular binding, more experimental validation is required to validate their roles in SMA pathogenesis.

CHAPTER 5: CONCLUSIONS AND FUTURE PERSPECTIVES

Spinal muscular atrophy (SMA) is a group of hereditary (inherited) neuromuscular illnesses that cause certain muscles to weaken and waste away (atrophy). SMA is caused by the death of lower motor neurons, also known as anterior horn cells, in the spinal cord. These cells regulate muscular movement. Without these motor neurons, muscles do not receive the nerve signals that cause them to move. The weakness in SMA is more severe in the muscles closest to the center of your body (proximal muscles) than in the muscles farther away from the center. Muscle weakness tends to worsen over time. Although SMA is considered uncommon, it is the second most prevalent severe inherited disease of infancy and childhood, following cystic fibrosis. It affects between 1 in 6,000 and 1 in 10,000 live births. Mutations (changes) in the *SMN1* (survivor motor neuron 1) gene result in all types of spinal muscular atrophy. The number of copies of the SMN2 gene determines the severity of the illness. A healthy *SMN1* gene generates SMN protein. Motor neurons require this protein to survive and function appropriately. If you have SMA, your body does not produce enough SMN protein, which causes your motor neurons to shrink and die. As a result, your brain cannot control voluntary motions, particularly those in your head, neck, chest, and legs. Normally, most of proteins produced by *SMN1* genes are full-length and functional; however, mutations result in little or no full-length, functional SMN protein production. This loss can be partially mitigated by the presence of nearby *SMN2* genes, which are 99% identical to *SMN1* genes. The number of *SMN2* gene copies varies by person. The majority of proteins produced by *SMN2* genes are short and non-functional, although some are full-length and useful. We inherit SMA in an autosomal recessive manner, which means that both of your biological parents carry mutations in the *SMN1* gene. In most cases, the biological parents of someone with an autosomal recessive disorder each have one copy of the mutated gene. However, these carriers often do not exhibit signs of the illness. The *SMN1* gene is often mutated in the general population. SMA is categorized into five different types based on the onset of disease i.e. type 0, I, II, III and IV. Between 2016 and 2020, the United States Food and Drug Administration (FDA) approved therapies that can

They include Disease-modifying therapy: These drugs increase the production of SMN2 protein. The FDA has approved the first drug Nusinersen (Spinraza®) in 2016 to treat both children and adults with SMA. Nusinersen is administered via intrathecal injection, which delivers medicines directly to the cerebrospinal fluid (CSF) surrounding the spinal cord, where motor neurons degenerate in SMA patients due to low levels of SMN protein.Risdiplam (Evrysdi®) is a different drug that can help those with SMA who are two months or older. Risdiplam is taken orally once day. Gene replacement therapy: Children under the age of two may benefit from a single intravenous (IV) infusion of the medicine onasemnogene abeparvovec-xioi (Zolgensma®). This therapy replaces a missing or faulty *SMN1* gene with a functional one. The SMN1structure was obtained from PDB and its active site was predicted using CASTp and PDBsum. Currently, there are limited therapeutic options and there is an imminent medical need for new medications. This study focuses on the potential of phytochemicals derived from *Brucea javanica* and *Tripterygium wilfordii* as alternative agents for the treatment of SMA. Using molecular docking, a dataset of 115 phytochemicals from two plants was used to determine binding affinities with the SMN1 protein. Docking studies revealed Yadanzioside-M (–11.4 kcal/mol) and Triptersinine-A (–10.5 kcal/mol) as the most promising SMN1 binders. Subsequent 1000 ns molecular dynamics simulations demonstrated that the initial RMSD deviations for the apo-protein, SMN1–Triptersinine-A, and SMN1–Yadanzioside-M complexes were 0.3, 0.2, and 0.1 nm, respectively. The maximum deviations reached ~0.48 nm, ~0.52 nm, and ~0.18 nm, while final RMSD values stabilized at ~0.35 nm (apo-protein), ~0.3 nm (Triptersinine-A) and ~0.16 nm (Yadanzioside-M), confirming superior structural stability of the ligand-protein complexes during the simulation.Yadanzioside-M and Triptersinine-A were two leading phytochemicals that developed sustained interactions with the SMN1 protein.Yadanzioside-M initially formed hydrogen bonds with W92, followed by interactions with S143, C146, and D147. By the end of the simulations, the ligand established interactions with D134 and S103. The multi-cyclic scaffold of Yadanzioside-M also interacted with aromatic amino acids like tryptophan, forming pi-pi interactions,

which contributed to enhancing protein-ligand stability. Similarly, Triptersinine-A formed stable hydrogen bonds with critical residues (R133, Y130, G129, and G131) of the SMN1 protein and displayed pi-pi interactions within the aromatic cage of the tudor domain. The identified phytochemical ligands demonstrated strong binding affinities validated by extensive molecular docking and 1000 ns molecular dynamics simulations, confirming the stability of ligand-receptor interactions. These plant-derived phytochemicals neuroprotective qualities and enduring binding interactions make them promising therapy choices for SMA. This comprehensive *in-silico* validation supports their potential as drug candidates for Spinal Muscular Atrophy. The growth of computer methods and the explosion of biomedical data have created new avenues for the development of SMA treatments.

In the future, research is needed to address challenges related to toxicity, bioavailability and clinical validation.These studies will include various docking engines, diverse simulation platforms, larger compound databases and through experimental validation would improve reproducibility and confidence optimization process. Future optimization can be furthered by focused structure-activity relationship studies and pharmacokinetic profiling to enhance selectivity and drug-likeness, guiding experimental validation and lead development. This study is the first *in-silico* exploration of phytochemical-based therapies for Spinal Muscular Atrophy (SMA), providing a novel framework for identifying possible leads and investigating molecular interactions with the SMN1 protein. To ensure robustness, all analyses were carried out with well-established and reliable bioinformatics tools, despite the limited availability of SMA-specific computational and pharmacological datasets for extensive cross-validation.While the work provides useful findings, molecular dynamics simulations and ADMET predictions were used as reliability measures to foster confidence in the findings. Interestingly, this research was able to identify potential phytochemical leads and examine their binding interactions with SMN1, the discussion did not extend far enough to examine the underlying biological processes of SMA. This was partly because experimental data and mechanistic research were scarce in the literature. The discovered

compounds have the potential to modulate oxidative stress, apoptosis and neuro inflammation pathways, offering a sound connection between *in-silico* predictions and disease pathophysiology.It is translating computational prediction into therapeutics of clinical utility that poses inherent challenges. With continued research and innovation, these plants may play an integral role in the next generation of SMA treatments, providing hope for patients affected by this devastating disease.

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APPENDIX

1. LIST OF PUBLICATIONS:

1. Bagga, P., Singh, S., Ram, G., Kapil, S., & Singh, A. (2024). Diving into progress: A review on current therapeutic advancements in spinal muscular atrophy. *Frontiers in Neurology*, *15*, 1368658.

2. Bagga, P., Singh, S., Kumari,I., & Swargam,S.(2025). Molecular docking and dynamics simulation studies to identify the phytochemicals as lead molecules against the survival motor neuron 1 (SMN1) protein of spinal muscular atrophy. *Medicinal Plants,17* (3), 584-593.

3. Bagga, P., Singh, S. ., Ram, G. ., Kapil, S. ., Eerapagula, R. ., Puri, P., Kumar, J. ., & Sheikh, I. (2025). Molecular Dynamics Simulation and Structural Insights into The SMN1 Protein Involved in the Pathogenesis of Spinal Muscular Atrophy. *Journal of Neonatal Surgery*, *14*(13S), 1066–1077.Retrieved from https://www.jneonatalsurg.com/index.php/jns/article/view/3935.

2. LIST OF PATENT

1. Granted Design Patent

Design Title – Spinal Muscular Atrophy Activity Monitoring Device, Registered under the Designs Act, 2000, Registration Number: 426463-001, Granted by Intellectual Property India. Date of Registration: 10/08/2024.

3. COPYRIGHT (GRAPHICAL ABSTRACT)

1.Graphical abstract copyright for the work titled *"In-Silico Identification of Phytochemical Ligands as Drug Candidates for the Treatment of Spinal Muscular Atrophy"* was submitted and accepted (Request ID: 22368) on 08/04/2025.

LIST OF CONFERENCES:

1. Oral presentation on *"In Silico* Study of Spinal Muscular Atrophy: Insights into Molecular Mechanisms" at the International Conference on One Health Initiative: Harmonizing Human, Animal, and Environmental Health (OHI-2023) held at GLA University Mathura on Jan. 18-20, 2024 and got best oral presentation award.

2. Oral presentation on "Deciphering the structural dynamics of SMN1 Protein : A computational Exploration for Functional Revelation '' at the International Conference on Recent trends and innovations for a Greener Future in collaboration with The Indian Science Congress Association (ISCA) Shimla Chapter, held at GC Hamirpur on Feb. 09-10, 2024.

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Diving into progress: a review on current therapeutic advancements in spinal muscular atrophy

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Spinal muscular atrophy (SMA) is an uncommon disorder associated with genes characterized by the gradual weakening and deterioration of muscles, often leading to substantial disability and premature mortality. Over the past decade, remarkable strides have been made in the field of SMA therapeutics, revolutionizing the landscape of patient care. One pivotal advancement is the development of gene-targeted therapies, such as nusinersen, onasemnogene abeparvovec and risdiplam which have demonstrated unprecedented efficacy in slowing disease progression. These therapies aim to address the root cause of SMA by targeting the survival motor neuron (SMN) gene, effectively restoring deficient SMN protein levels. The advent of these innovative approaches has transformed the prognosis for many SMA patients, offering a glimmer of hope where there was once limited therapeutic recourse. Furthermore, the emergence of small molecule compounds and RNA-targeting strategies has expanded the therapeutic arsenal against SMA. These novel interventions exhibit diverse mechanisms of action, including SMN protein stabilization and modulation of RNA splicing, showcasing the multifaceted nature of SMA treatment research. Collective efforts of pharmaceutical industries, research centers, and patient advocacy groups have played an important role in expediting the translation of scientific discoveries into visible clinical benefits. This review not only highlights the remarkable progress achieved in SMA therapeutics but also generates the ray of hope for the ongoing efforts required to enhance accessibility, optimize treatment strategies, rehabilitation (care and therapies) and ultimately pave the way for an improved quality of life for individuals affected by SMA.

KEYWORDS

spinal muscular atrophy, survival motor neuron, rehabilitation, nusinersen, onasemnogene abeparvovec, risdiplam

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Research Article

Molecular docking and dynamics simulation studies to identify the phytochemicals as lead molecules against the survival motor neuron 1 (SMN1) protein of spinal muscular atrophy

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ABSTRACT

Spinal Muscular Atrophy is an autosomal recessive neuromuscular disorder resulting from deletions or mutations in the survival motor neuron (SMN1) gene. Loss of lower motor neurons (anterior horn cells) in the brainstem and spinal cord nuclei is a characteristic of SMA, which causes gradual symmetrical muscular weakening and atrophy. Currently, there are limited therapeutic options and there is an imminent medical need for new medications. This study focuses on the potential of phytochemicals derived from *Brucea javanica* and *Tripterygium wilfordii* as alternative agents for the treatment of SMA. Using molecular docking, a dataset of 115 phytochemicals from two plants was used to determine binding affinities with the SMN1 protein. 1000ns molecular dynamics (MD) simulation were performed on the high-binding affinities compounds to elucidate their stability and interaction dynamics in a physiological state. Yadanzioside-M and Triptersinine-A were two leading phytochemicals that developed sustained interactions with the SMN1protein. Specifically, Yadanzioside-M formed hydrogen bonds with W92, S143, C146, D147, and finally D134 and S103. Its multi-cyclic structure exhibited pi-pi interactions with aromatic amino acids that enhanced the stability of protein-ligand. Likewise, Triptersinine-A established hydrogen bonds with R133, Y130, G129, and G131, as well as pi-pi interactions inside the Tudor domain's aromatic cage, indicating a high binding affinity. These findings highlight the utility of the phytochemicals as neuroprotective agents with a prolonged bind to the target protein, suggesting that these are potential therapeutic agents for SMA. Further experimental and clinical research is needed to validate their efficacy and safety for therapeutic application.

Keywords: Spinal muscular atrophy, Molecular docking, molecular dynamics (MD) simulations, *Brucea javanica*, *Tripterygium wilfordii*

INTRODUCTION

Spinal Muscular Atrophy (SMA) is a genetic disease affecting the motor neurons of the spinal cord, which results in progressive muscle weakness and atrophy. This rare autosomal recessive neuromuscular disease, though having intense effects on the quality of life of the afflicted persons

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Molecular Dynamics Simulation and Structural Insights into The SMN1 Protein Involved in the Pathogenesis of Spinal Muscular Atrophy

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ABSTRACT:

Spinal muscular atrophy (SMA) is an an autosomal recessive inherited neuromuscular condition distinguished by the deterioration of alpha motor neurons within the spinal cord. This degeneration leads to a gradual onset of muscle weakness and paralysis primarily affecting muscles close to the body's center. The SMA is categorized into four severity grades (SMA I, SMA II, SMA III and SMA IV) determined by the age of onset and the level of motor function attained. This condition arises from homozygous mutations in the survival motor neuron 1 (SMN1) gene, with diagnostic tests typically revealing homozygous deletion of SMN1 exon 7 in the majority of patients. Herein, we have applied bioinformatics approaches to predict the structure of SMN1 protein (using AlphaFold, I-TASSER and RoseTTAFold), structure validation (PSVS v1.5, PROCHECK and ProSA-web) and molecular dynamics (MD) simulation using GROMACS 2022.3 at 100 ns (nanoseconds) to analyze the Root Mean Square Deviation, Root Mean Square Fluctuation, and Radius of Gyration. MD results clearly indicate that RoseTTAFold predicted structure of SMN1 is highly stable and consistent.

Keywords: *Spinal Muscular Atrophy; Survival Motor Neuron1; Molecular Dynamics Simulation; Root Mean Square Deviation; Root Mean Square Fluctuation; Radius of Gyration*

1. INTRODUCTION

Spinal muscular atrophy (SMA) is a genetic disorder that affects the nerves responsible for muscle movement. Over the time, these nerves deteriorate, leading to the muscle weakness and loss of function, results in significant and gradual decrease in muscle tone and strength (Younger and Mendell (2023). With a prevalence rate of about 1 in 10,000 live births, SMA is considered the second most common autosomal recessive disorder (Nilay *et al*., 2021). Most cases of SMA are a result of

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डिजाइन के पंजीकरण का प्रमाण पत्र | Certificate of Registration of Design

डिजाइन सं. / Design No.	:	426463-001
तारीख / Date	:	10/08/2024
पारस्परिकता तारीख / Reciprocity Date*	:	
देश / Country	:	

प्रमाणित किया जाता है कि संलग्न प्रति में वर्णित डिजाइन जो *SPINAL MUSCULAR ATROPHY ACTIVITY MONITORING DEVICE* से संबंधित है, का पंजीकरण, श्रेणी 24-01 में 1.Pankaj Bagga 2. Dr. Sudhakar Singh 3.Dr. Gobind Ram 4.Dr. Subham Kapil 5.Dr. Jitender Kumar 6.Dr. Neha 7.Prof. Punit Puri 8.Dr. Sonika 9.Ankush के नाम में उपर्युक्त संख्या और तारीख में कर लिया गया है।

Certified that the design of which a copy is annexed hereto has been registered as of the number and date given above in class 24-01 in respect of the application of such design to *SPINAL MUSCULAR ATROPHY ACTIVITY MONITORING DEVICE* in the name of 1.Pankaj Bagga 2. Dr. Sudhakar Singh 3.Dr. Gobind Ram 4.Dr. Subham Kapil 5.Dr. Jitender Kumar 6.Dr. Neha 7.Prof. Punit Puri 8.Dr. Sonika 9.Ankush.

डिजाइन अधिनियम, 2000 तथा डिजाइन नियम, 2001 के अध्यधीन प्रावधानों के अनुसरण में।
In pursuance of and subject to the provisions of the Designs Act, 2000 and the Designs Rules, 2001.

उन्नत की पंडित
महानियंत्रक पेटेंट, डिजाइन और व्यापार चिह्न
Controller General of Patents, Designs and Trade Marks

जारी करने की तिथि :
Date of Issue : 25/10/2024

*पारस्परिकता तारीख (यदि कोई हो) जिसकी अनुमति दी गई है तथा देश का नाम। डिजाइन का स्वत्वाधिकार पंजीकरण की तारीख से दस वर्षों के लिए होगा जिसका विस्तार, अधिनियम एवं नियम के निबंधनों के अधीन, पाँच वर्षों की अतिरिक्त अवधि के लिए किया जा सकेगा। इस प्रमाण पत्र का उपयोग विधिक कार्यवाहियों अथवा विदेश में पंजीकरण प्राप्त करने के लिए नहीं हो सकता है।

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This is to certify that Prof./Dr/Mr/Ms PANKAJ BAGGA, LPU JALANDHAR, PUNJAB has Participated in the OHI-2023 and Presented Oral/ ~~Poster~~ Paper entitled IN SILICO STUDY OF SPINAL MUSCULAR ATROPHY : INSIGHTS INTO MOLECULAR MECHANISMS and received Best ~~Poster~~/Oral Award.

Prof. A K Gupta
Pro Vice-Chancellor

Dr. Jagdip Singh Sohal
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This is to certify that Dr/Mr/Ms Pankaj Bagga, Deptt of Bioinformatics, LPU Jalandhar Punjab. Professor/Associate Professor/Assistant Professor/Research Scholar/UG/PG Student has participated as a Keynote Speaker/Resource Person/Invited Speaker/Presented Oral Paper/Poster entitled Deciphering the Structural Dynamics of SMN1 Protein : A Computational Exploration for Functional Revelation in the International Conference held on 9th-10th February, 2024. He/She has **Chaired/Co-chaired the Technical Session.**

He/She has been awarded with **Best Poster Presentation.**

Dr. N. Deepika Khanna	Dr. Ratan C. Sharma	Dr. Shashi Sharma	Dr. Pramod S. Patial
Organising Secretary	**Convenor**	**Chairman**	**Patron/Principal)**

List of Other Publications during Ph.D work

1. Anuradha, Bagga, P., Seth, R. K., Kumar, P., & Kumar, S. (2025). Insights into the structural, chemical and optical properties of hematite/rGO nanocomposites for dye decolorization via adsorption and photocatalysis. *Journal of Materials Science: Materials in Electronics*, *36*(9), 566.

2. Kumar, S., Bagga, P., Verma, S., & Kumar, S. (2025). Study of transition metal Zr-doped hematite nanoparticles: structural, chemical, optical, magnetic and photocatalytic properties. *Research on Chemical Intermediates*, *51*(1), 157-182.

Book Cahpter

1. Kapil, S., Bagga, P., Puri, P. and Katnoria, N. (2025) Transcription Factor Databases. In: Ranganathan, S.,Cannataro, M., Khan, A. M. (ed.) *Encyclopedia of Bioinformatics and Computational Biology, 2nd Edition*, vol. 3,
pp. 341–350. US: Elsevier.

2. BAGGA, P., KAPIL, S., SINGH, S., & PURI, P. Evaluating Contemporary Approaches to COVID-19 Pandemic Management.(biothink.in)

3. Lata, H., Kumari,A.,Chauhan,R.,Prashar,N.,Thakur,I.,Kapil,S.,Bagga,P.Bronchitis and COPD: Advances in Diagnosis, Treatment, and Prevention.(biothink.in)

Accepted for Publication

1. Subham Kapil,Pankaj Bagga,Isha Thakur. Climate Change and Infectious Diseases (being published by Elsevier).

2. Subham Kapil , Pankaj Bagga. In-Silico Validation of AI-Assisted Drugs in Healthcare (being published by Springer)