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(RESEARCH ARTICLE)

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Synthesis, in-silico design and spectral characterization, elucidation of *Cannabis sativa L. cannabaceae* containing phytoconstitutents demonstrating novel therapeutic efficacy against epilepsy

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Abstract

Cannabis sativa L. is a significant herbaceous species native to Central Asia that has been utilised for folk medicine and other industrial applications. It is a rich source of cellulosic and woody fibres as well as a treasure trove of phytochemicals. The pharmaceutical and construction industries are both very interested in this plant since its metabolites have powerful bioactivities on humans. *Cannabinoids* are the most researched class of chemicals, primarily because of the vast range of pharmacological effects they have on humans, including psychoactive effects. The phytoconstituents Apigenin and Alpha-bisabolol were synthesised in the current study, and *Cannabis sativa L.* phytoconstituent was further studied through *in silico* screening utilising (PDB ID: 6FYZ) along with spectral characterization NMR (^{1}H and ^{13}C) to confirm the presence of saturated and unsaturated moiety in *Cannabis sativa L.* phytoconstituents. Apigenin and Alpha-bisabolol, two phytoconstituents found in leaves and flower of *Cannabis sativa L.*, were found to have higher docking scores and glide energies when comapred with the standard drugs like Levetiracetam. *Cannabis sativa's* phytoconstituent has been shown in *in-silico* studies to have high anti-epileptic potency, making it a crucial source for novel anti-epileptic/anti-convulsant/anti-seizure medications that in the future will focus on a variety of neurological diseaeses and disorders.

Keywords: Levetiracetam; Spectral characterization; Epilepsy; Phytoconstitutents; In-silico design; *Cannabis sativa L.*; Apigenin; Alpha-bisabolol

1. Introduction

The cannabis plant has been used medicinally for over 6000 years as a treatment for autoimmune illnesses such rheumatoid arthritis [2], as well as for pain, diarrhoea, and inflammation [1]. Bhanga and Indrasana, sometimes known as Indra's hemp, are mentioned in Sanskrit alongside the Atharvavedic description of the magical and therapeutic Vedic plant Janjida. This herb is claimed to have been created three times by the gods. Hemp was known to the Greeks more than 2000 years ago. It was farmed by the Scythians, according to Herodotus, who utilised its fibre for clothing and seeds for vapour bath remedies. According to Dioscorides, seeds lose their virility potential if they are consumed in excess. Seeds are antiflatulent and carminative, according to Galen. Seeds are used to relieve constipation and internal heat of the intestines because they are a vermifuge and emollient with nutritional tonic effects.

* Corresponding author: Sudhanshu Kumar Jha

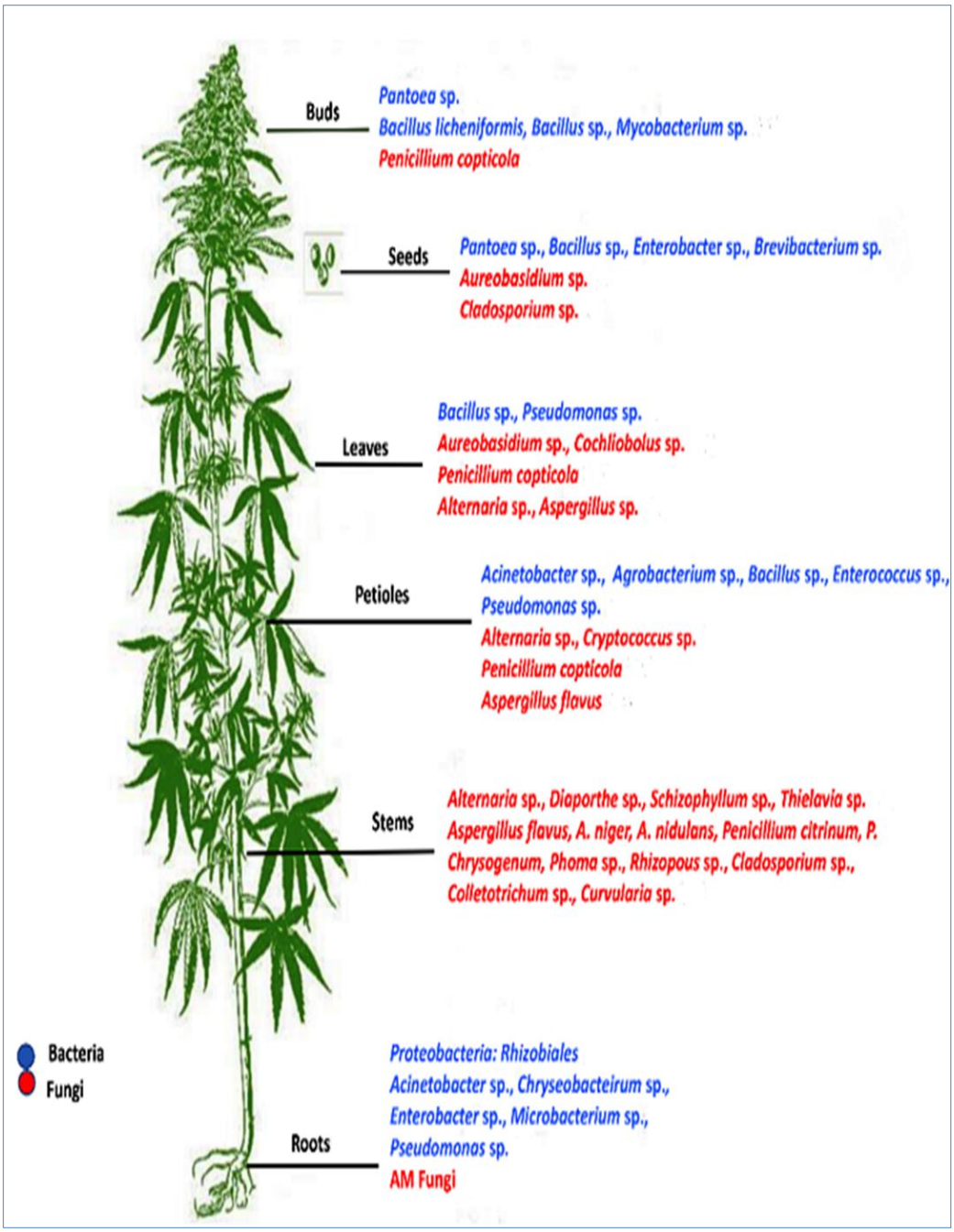
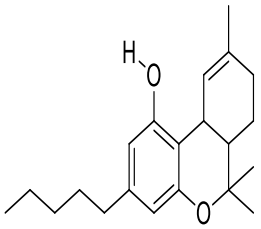
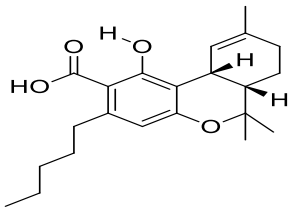
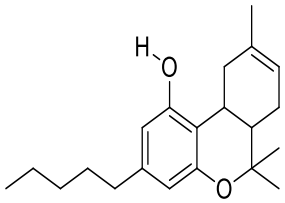
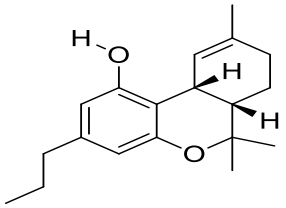
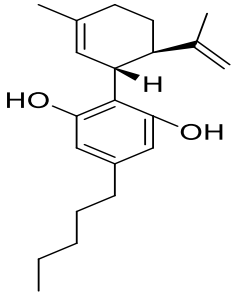
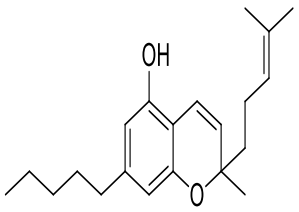
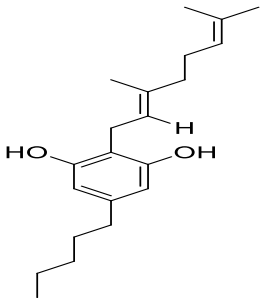
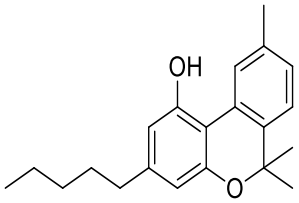
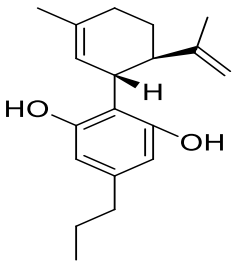
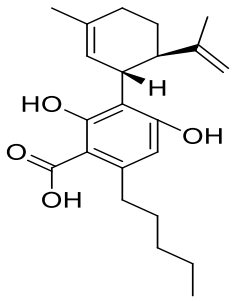
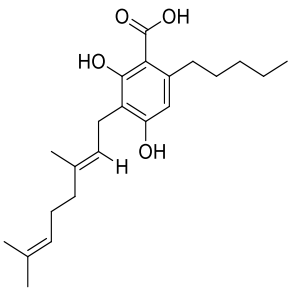
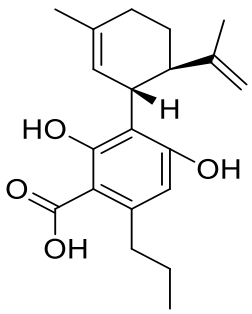


Figure 1 The most prevalent endophytes found in *Cannabis sativa* plants collected from various geographic areas and found in various tissues [7]

The herb has euphoric, stomachic, antispasmodic, analgesic, stimulant, aphrodisiac, and sedative effects in all parts. The potent narcotic resinous exudate known as charas is typically used to treat madness and hysteria. It is mostly used for sexual weakness and nocturnal discharges because it is analgesic, soporific, lowers excitation, and enhances sexual retention [3]. In India, Hakims and Vaids (practitioners of traditional Indian medicine) recommend bhang or ganja for intestinal ailments, as an aperitif, as a nervous stimulant, and as a source of tremendous stamina during periods of intense exertion or tiredness. The Tharu tribe of India's Gonda and Baharaich districts uses a piece of leaf wrap to treat piles. Seeds don't have any marijuana-like properties; instead, they are tonic and emollient. Numerous disorders, including multiple sclerosis, epilepsy, dystonia, chronic pain, inflammatory ailments, rheumatoid arthritis, and Crohn's disease are currently being treated with cannabis in clinical trials [1]. The "trip," as it is known, starts with an inner joy (euphoria) that is out of proportion to reality and is referred to as being high. As the toxicity grows, the user may "trip off" and become quiet and drowsy alone, or they may sit and see coloured illusions. Some users may also experience phantasmagoria, which is the sense that figures are racing at him at great speed and become larger as they get closer.

With company, the user might feel more at ease, unconstrained, and confident. They might also become talkative and funny, have altered time- and space-perceptions, and have diminished cognitive abilities. Some people may experience a "bring down," "downer," or "bummer" instead of exhilaration, during which the user may feel agitated, worried, slightly paranoid, and uneasy. Confusion, disorientation, and dread of dying could develop at a high enough dose. Three to five hours into the journey, mild fatigue and hunger set in [5]. After consumption, the person feels euphoric and exalted, has extravagant visions, and loses sense of time. The impact is temporary and disappears in 3–5 hours, though it might endure for 12 hours or longer without having any negative effects [6].

Table 1 Some Important Cannabis (*Cannabis sativa L.*) cannabinoids' chemical structure [8]

 **Δ⁹-Tetrahydrocannabinol**	 **Δ⁹-Tetrahydrocannabinolic acid**	 **Δ⁸-Tetrahydrocannabinol**	 **Δ⁹-Tetrahydrocannabivarin**
 **Cannabidiol**	 **Cannabichrome**	 **Cannabigerol**	 **Cannabinol**
 **Cannabidivarin**	 **Cannabidiolic acid**	 **Cannabigerolic acid**	 **Cannabidivarinic acid**

1.1. Phytoconstitutents present in Cannabis (*Cannabis sativa L.*)

It has been shown that *C. sativa* contains more than 20 flavonoids, the majority of which are flavone (apigenin and luteolin) and flavonol (kaempferol and quercetin) aglycones and glycosides prseent in leaves of *C. sativa* [9,10]. Although they are often far less numerous than compounds like myrcene, beta-caryophyllene, or d-limonene, alpha-bisabolol and camphene are among the top 20 most abundant tepenes discovered in 240 distinct cannabis cultivars of *C. sativa* flower [11].Evidence on the therapeutic potential of apigenin, including its antioxidant activity and potential function as a neuroprotective agent, as well as its chemistry, pharmacokinetics, and metabolism in the context of depression, Alzheimer's disease, and Parkinson's disease, has come from a variety of animal models and human clinical trials [12]. Depending on the dosage, apigenin can cause muscular relaxation and drowsiness. It also has interesting potential for use in the treatment or prevention of Alzheimer's disease and is active as an antioxidant, anti-inflammatory, anti-amyloidogenic, neuroprotective, and cognition-enhancing chemical [13]. In order to avoid oxidative stress, inflammatory disorders, infections, neurological illnesses, malignancies, and metabolic disorders, -bisabolol has been shown to have a variety of therapeutic and biological qualities [14].

1.2. Epilepsy

Around 50 million people worldwide suffer from epilepsy, a chronic noncommunicable brain condition. Recurrent seizures are its defining feature. Seizures are short bursts of involuntary movement that might affect either a portion of the body (partial) or the full body (generalised), and they can occasionally be followed by loss of awareness and control over bowel or bladder function. Excessive electrical discharges in a cluster of brain cells cause seizure episodes. Such discharges can occur in many areas of the brain. The smallest muscular twitches or concentration lapses can be seizures, as well as violent convulsions that last for a long time. The frequency of seizures can also vary, from fewer than one per year to many per day. Up to 10% of individuals worldwide experience one seizure in their lives, thus having one does not always indicate epilepsy. Two or more unprovoked seizures are considered to be an epileptic seizure. Written accounts of epilepsy date as far back as 4000 BCE, making it one of the earliest recognised medical diseases in the world. Epilepsy has been shrouded in fear, misinformation, prejudice, and social shame for millennia. The quality of life for those who have the condition and their family may be negatively impacted by this stigma, which persists in many nations today [15].

2. Methodology

2.1. Synthesis of Alpha-bisabolol

(-)-Alpha by steam distilling the essential oils obtained from *Brazilian candeia* (Eremanthus erythropappus), bisabolol is chemically created [17].

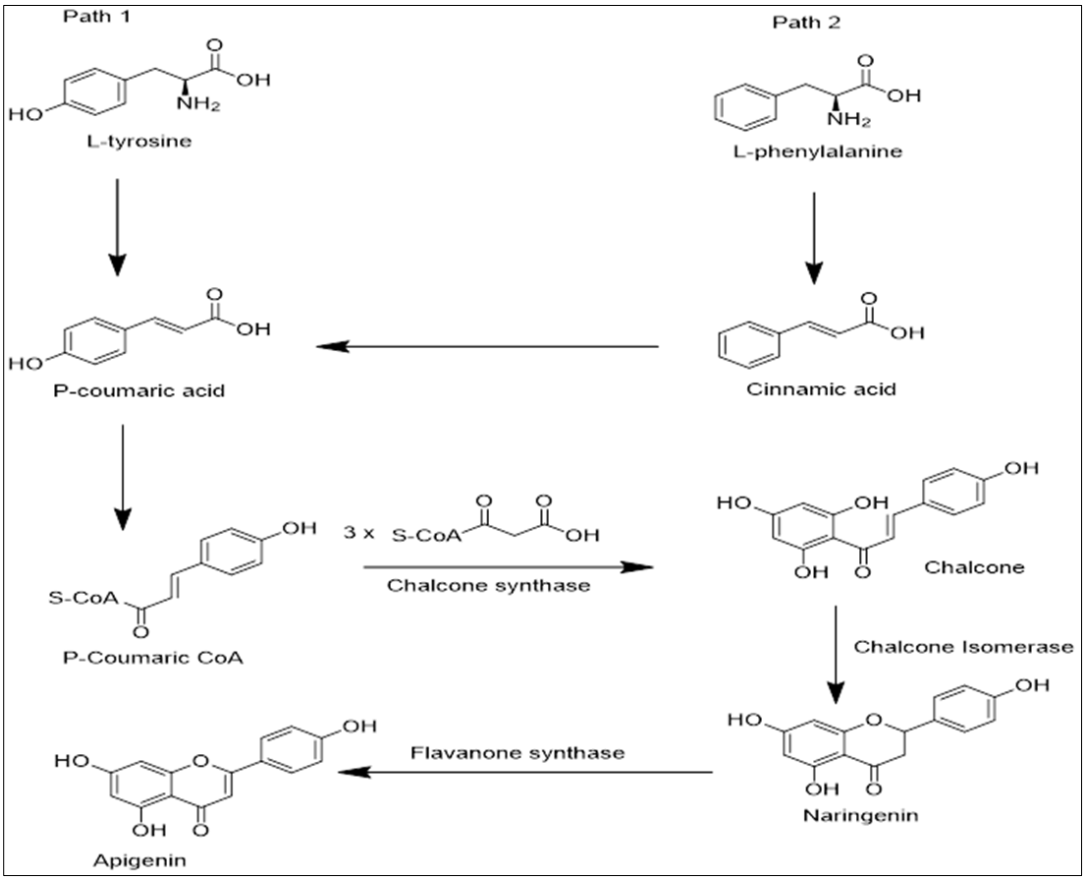


Figure 2 Apegenin is produced by the phenylpropanoid pathway [16]

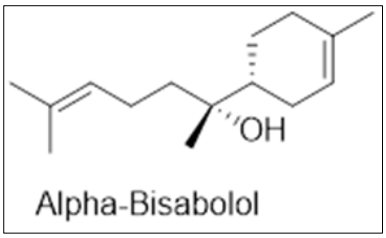


Figure 3 Chemical structure of alpha-bisabolol

2.2. Molecular docking study

For virtual derivative screening, the structures of substituted syringic acid derivatives were sketched using ChemDraw 19.1. For molecular docking, Schrodinger suite v 13.1 was employed.

2.2.1. Protein preparation

For the molecular docking research of a chosen data set of *Cannabis sativa L.* phytoconstitutes like Apigenin & Alpha-bisabolol, transcriptional regulation (PDB Id: 6FYZ) was picked from the Protein Data Bank. The typical structure file downloaded from the PDB is not suitable for immediate use in calculations for molecular modelling. Among other things, a typical PDB structure file includes co-crystallized ligands, water molecules, metal ions, and cofactors. The protein preparation wizard preprocessed, optimised, and reduced protein before creating it. A refined, hydrogenated ligand and ligand-receptor complex structure is the end product, which can be applied to various Schrodinger modules [18].

2.2.2. Ligand Preparation

The Maestro v 13.1 LigPrep module is used to prepare the ligands for the optimum docking outcomes. The docked structures need to be accurate representations of the real ligand structures as they would appear in a protein-ligand complex. The structures must therefore comply with the following Glide docking software criteria. Three dimensions are required. Glid solely modifies the ligand's internal torsional coordinates; hence, the other geometric parameters must be modified in advance. Each of them must consist of a single molecule, without any covalent receptor attachments or other pieces like counter ions or solvent molecules. They must be hydrogen-filled (valences). They need to be properly protonated for physiological pH levels, which are around 7 [19,20].

2.2.3. Grid generation

The grid is produced by Maestro version 13.1's receptor grid generation module. The co-crystallized ligand's binding site is surrounded by a grid that makes it possible for other molecules to bind there while keeping the co-crystallized ligand out [21].

2.2.4. Molecular docking

After creating the glide grid zip file and preparing the ligands, docking was carried out using the maestro v 13.1 ligand docking module. The XP module conducts more accurate molecular docking of certain phytoconstitutes of *Cannabis sativa L.*, such as molecules of apigenin and alpha-bisabolol. The size of the data gathering decreases as the level of precision increases. In Maestro v 13.1 [22,23], the XP parameters docking score, glide energy, and glide model value were estimated. Apigenin and Alpha-bisabolol, two phytoconstitutes of *Cannabis sativa L.*, were subjected to in silico screening. We next create a library of 7 compounds, dock *Cannabis sativa L.* phytoconstitutets with the necessary parametric compounds, and select Levetiracetam as the standard medication, out of which we select our top two docked score phytoconstitutets of *Cannabis sativa L.* for synthesis. When compared to common drugs like levetiracetam, the top 2 phytoconstituents ligands of *Cannabis sativa L.* demonstrate higher docking scores and glide energies, respectively. Fig. 7,8,9,10 depict a 2D and 3D ligand-protein interaction.

2.3. Phytoconstitutets of *Cannabis sativa L.* and standard drug chemical structure composition

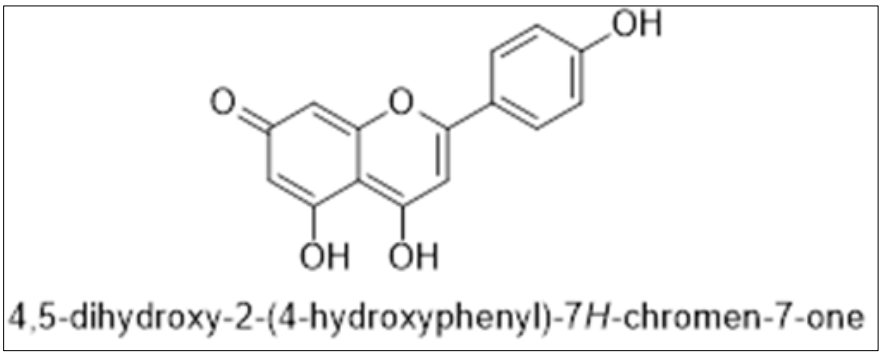


Figure 4 Chemical structure of Apigenin ($C_{15}H_{10}O_5$)

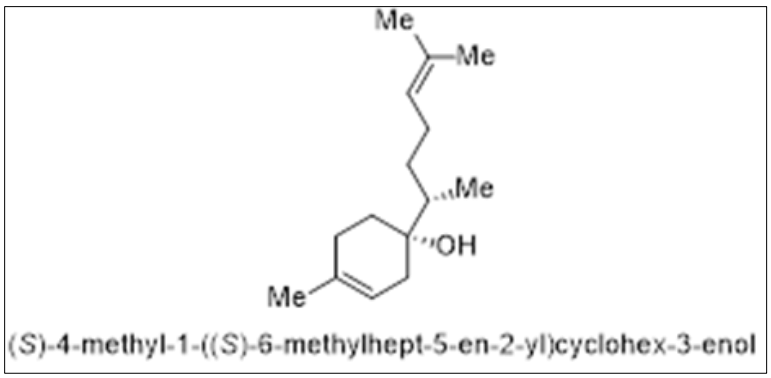


Figure 5 Chemical structure of Alpha-bisabolol ($C_{15}H_{26}O$)

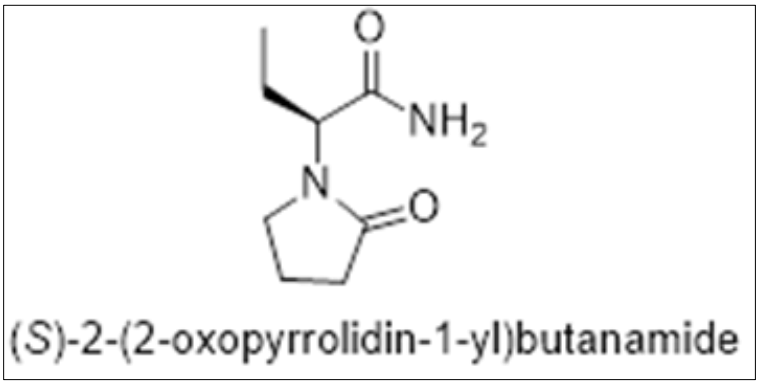
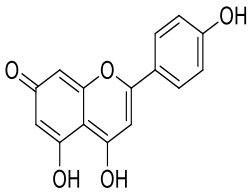
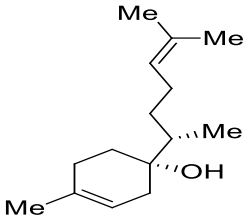
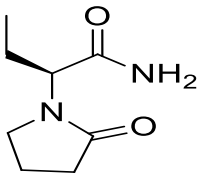


Figure 6 Chemical structure of Levetiracetam ($C_8H_{14}N_2O_2$)

3. Results and discussion

Table 2 Phytoconstitutets of *Cannabis sativa* L molecular docking screening in comparison to antiepileptic standard medication

S.No	Name of Compounds	Chemical Structure	Docking score (PDB ID: 6FYZ)	Glide energy	Molecular Weight	cLogP
1.	Apigenin		-6.38	-41.806	270.24	1.91
2.	Alpha-bisabolol		-7.396	-32.567	222.37	4.78
3.	Levetiracetam (Standard drug)		-6.08	-33.432	170.21	-0.344

3.1. PDB ID 6FYZ

Class IIa histone deacetylase inhibitor of benzhydryl hydroxamic acid that is CNS-penetrant has been developed and characterised [24].

- Classification: HYDROLASE
- Organism(s): Homo sapiens
- Expression system: Escherichia coli
- Mutation(s): No

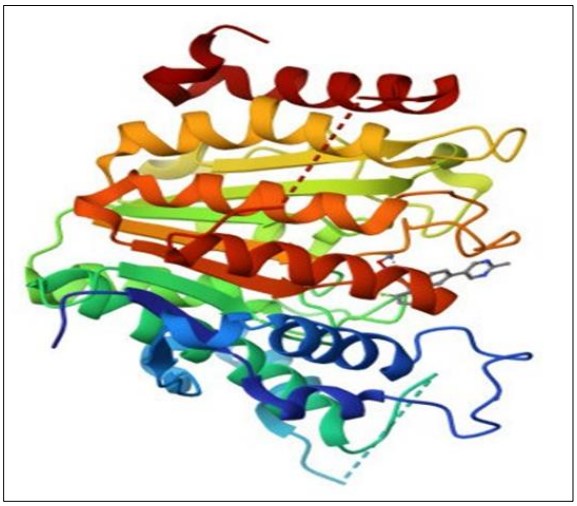


Figure 7 3D- Structure of protein (6FYZ)

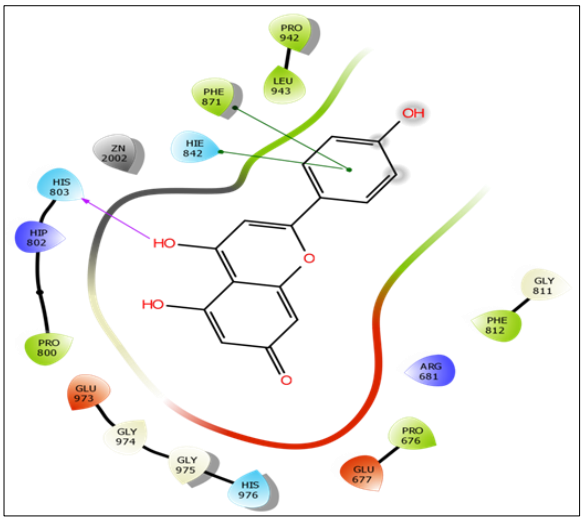


Figure 8 Apigenin 2D diagrams of docked conformation compound

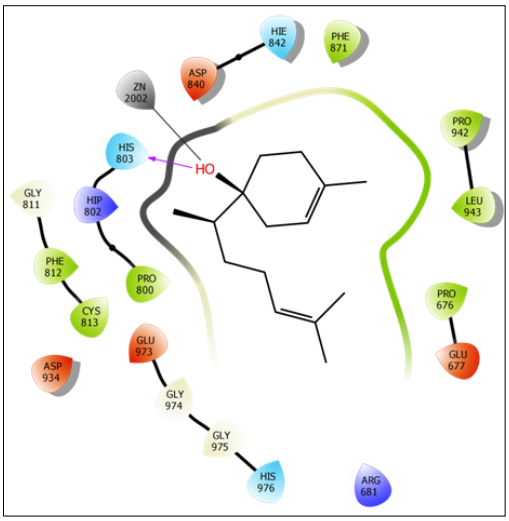


Figure 9 Alpha-bisabolol 2D diagrams of docked conformation compound

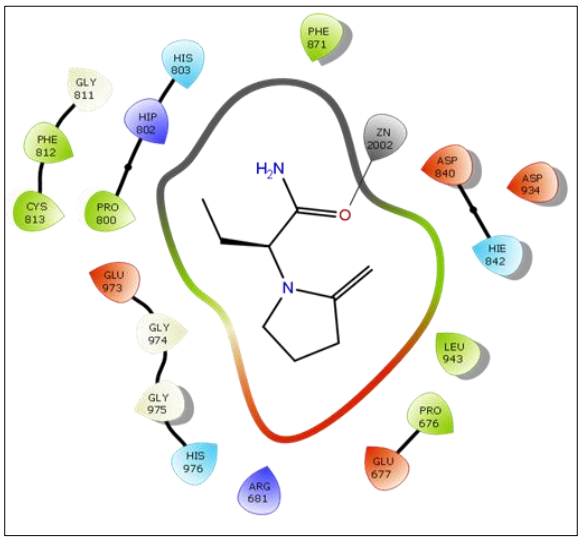
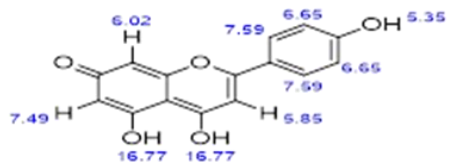


Figure 10 Levetiracetam 2D diagrams of docked conformation compound

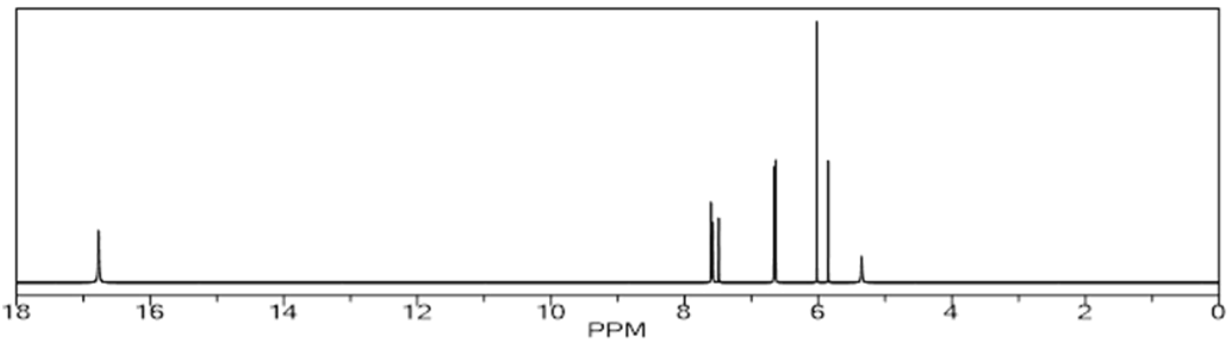
3.2. Spectral characterization

The analytical method of nuclear magnetic resonance (NMR) spectroscopy provides details on the local magnetic field surrounding atomic nuclei. Since characteristics of the molecular structure, such as constitution, configuration, conformation, intermolecular interactions, etc., directly affect the local magnetic field of the nucleus, NMR is uniquely able to provide comprehensive information on the chemical structure. Beginning in the 1950s, NMR spectroscopy transformed organic chemistry and established itself as a vital technique for determining the structures of tiny, soluble compounds. NMR quickly dominated other fields of chemical sciences as the technique advanced. The method became a mainstay in materials characterisation as well as it became possible to analyse macromolecules and solids as well. Since its inception, NMR spectroscopy has grown dramatically in all areas, including scientific and technological advancement and its applications in the natural sciences. As a result, it would be hard to discuss all pertinent issues relating to this comprehensive analytical instrument. We demonstrate some cutting-edge methods pertinent to materials characterisation. We demonstrate the enormous scope of the technique in the examination of materials using a small number of examples from various areas of materials research. An extensive list of resources should aid the reader in exploring NMR spectroscopy further after our necessarily constrained introduction [25]. We forecast the 1H and 13C NMR data of phytoconstituents, apigenin and alpha-bisabolol, found in *Cannabis sativa L.*, in the current work.

ChemNMR ^{1}H Estimation



Estimation quality is indicated by color: good, medium, rough



Protocol of the H-1 NMR Prediction:

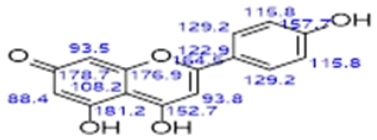
Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
OH	16.77	15.00	enol
		1.77	general corrections
OH	16.77	15.00	enol
		1.77	general corrections
OH	5.35	5.00	aromatic C-OH
		0.35	general corrections
CH	6.65	7.26	1-benzene
		-0.53	1 -O
		-0.05	1 -C-C
		-0.03	general corrections
CH	7.59	7.26	1-benzene
		-0.17	1 -O
		0.04	1 -C-C
		0.46	general corrections
CH	6.65	7.26	1-benzene
		-0.53	1 -O
		-0.05	1 -C-C
		-0.03	general corrections
CH	7.59	7.26	1-benzene
		-0.17	1 -O
		0.04	1 -C-C
		0.46	general corrections
H	6.02	5.25	1-ethylene
		-0.05	1 -C-C trans
		-0.60	1 -O-C=C cis
		1.42	1 -C(=O)-C=C gem
H	5.85	5.25	1-ethylene
		0.36	1 -1:C*C*C*C*C*C*1 cis
		-1.00	1 -O-C=C trans
		1.24	1 -C=C gem
H	7.49	5.25	1-ethylene
		-0.05	1 -C-C trans
		?	1 unknown substituent(s)
		1.42	1 -C(=O)-C=C gem
		0.87	general corrections

1H NMR Coupling Constant Prediction

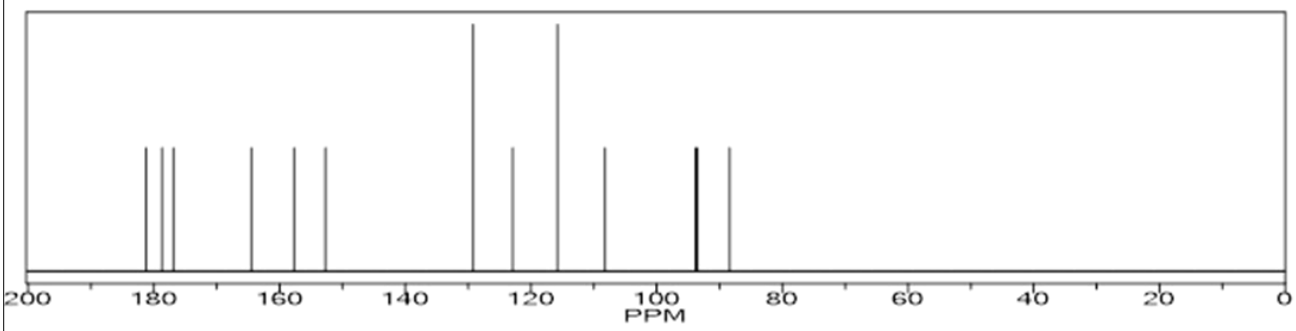
shift	atom index	coupling partner, conatant and vector		
16.77	13			
16.77	12			
5.35	20			
6.65	16			
		15	7.5	H-C*C-H
		18	1.5	H-C*C*C-H
7.59	15			
		16	7.5	H-C*C-H
		19	1.5	H-C*C*C-H
6.65	18			
		19	7.5	H-C*C-H
		16	1.5	H-C*C*C-H
7.59	19			
		18	7.5	H-C*C-H
		15	1.5	H-C*C*C-H
6.02	21			
5.85	22			
7.49	23			

Figure 11 Spectral characterization of Apigenin ^{1}H NMR Result

ChemNMR ^{13}C Estimation



Estimation quality is indicated by color: good, medium, rough

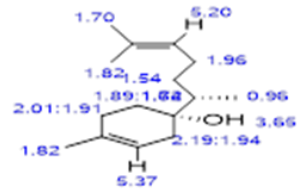


Protocol of the C-13 NMR Prediction:

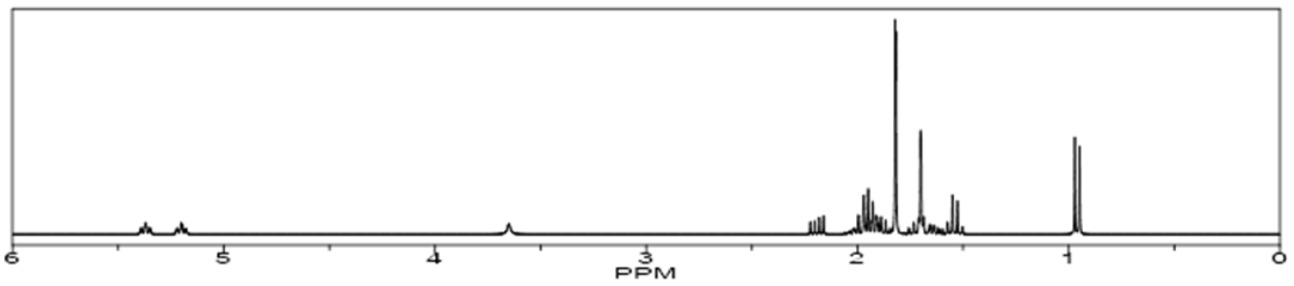
Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
C	176.9	123.3	1-ethylene
		13.6	1 -C=C
		25.5	1 -OC-C
		14.5	1 -C-O
C	164.5	123.3	1-ethylene
		12.5	1 -1:C*C*C*C*C*C*1
		25.5	1 -OC-C
		-7.0	1 -C=C
		10.2	general corrections
C	178.7	193.0	1-carbonyl
		-6.0	2 -C-C
		-8.3	general corrections
C	152.7	123.3	1-ethylene
		13.6	1 -C-C
		52.7	1 -O
		-14.0	2 -C=C
		-22.9	general corrections
C	181.2	123.3	1-ethylene
		13.6	1 -C-C
		52.7	1 -O
		14.5	1 -C-O
		-22.9	general corrections
C	157.7	128.5	1-benzene
		28.8	1 -O
		-0.8	1 -C=C
		1.2	general corrections
C	108.2	123.3	1-ethylene
		-7.0	1 -C=C
		-35.3	1 -O
		27.2	2 -C-C
C	122.9	128.5	1-benzene
		-7.4	1 -O
		6.4	1 -C-C
		-4.6	general corrections
CH	93.5	123.3	1-ethylene
		-7.0	1 -C-C
		-30.2	1 -OC-C
		17.3	1 -C-O
		-9.9	general corrections
CH	93.8	123.3	1-ethylene
		-11.0	1 -1:C*C*C*C*C*C*1
		-30.2	1 -OC=C
		13.6	1 -C-C
		-1.9	general corrections
CH	88.4	123.3	1-ethylene
		-7.0	1 -C-C
		-35.3	1 -O
		17.3	1 -C=O
		-9.9	general corrections
CH	115.8	128.5	1-benzene
		-12.8	1 -O
		-0.1	1 -C-C
		0.2	general corrections
CH	129.2	128.5	1-benzene
		1.4	1 -O
		-2.3	1 -C-C
		1.6	general corrections
CH	115.8	128.5	1-benzene
		-12.8	1 -O
		-0.1	1 -C-C
		0.2	general corrections
CH	129.2	128.5	1-benzene
		1.4	1 -O
		-2.3	1 -C-C
		1.6	general corrections

Figure 12 Spectral characterization of Apigenin ^{13}C NMR Result

ChemNMR ^{1}H Estimation



Estimation quality is indicated by color: good, medium, rough



Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
OH	3.65	2.00	alcohol
		1.65	general corrections
CH2	2.19,1.945000	1.96	cyclohexene
		0.15	1 beta -O from methylene
		-0.04	1 beta -C from methylene
CH2	1.89,1.635000	1.65	cyclohexene
		0.15	1 beta -O from methylene
		-0.04	1 beta -C from methylene
CH2	2.01,1.910000	1.96	cyclohexene
CH	1.72	1.50	methine
		0.17	1 alpha -C
		0.08	1 beta -O
		-0.02	2 beta -C
		-0.01	1 beta -C
CH3	1.82	0.86	methyl
		0.85	1 alpha -C=C
		0.11	general corrections
CH2	1.54	1.37	methylene
		-0.08	2 beta -C
		0.00	1 beta -C=C
		0.25	general corrections
CH2	1.96	1.37	methylene
		0.63	1 alpha -C=C
		-0.04	1 beta -C
CH3	0.96	0.86	methyl
		0.20	2 beta -C-R
		-0.10	general corrections
CH3	1.82	0.86	methyl
		0.85	1 alpha -C=C
		0.11	general corrections
CH3	1.70	0.86	methyl
		0.85	1 alpha -C=C
		-0.01	general corrections
H	5.37	5.59	cyclohexene
		-0.22	1 -C cis from 1-ethylene
H	5.20	5.25	1-ethylene
		-0.50	2 -C c + t
		0.45	1 -C gem

1H NMR Coupling Constant Prediction

shift	atom index	coupling partner, constant and vector		
3.65	12			
2.07	4	diastereotopic	-12.4	H-C-H
	17		6.2	H-CH-C(sp2)-H
1.76	6	diastereotopic	-12.4	H-C-H
	1		7.1	H-CH-CH-H
1.96	1	diastereotopic	-12.4	H-C-H
	6		7.1	H-CH-CH-H
	17		-1.0	H-CH2>C=C>H
1.72	7			
	8		7.0	H-C-CH-H
	16		6.8	H-C-CH2-H
1.82	13			
	17		-1.0	H-CH2>C=C<H
1.54	8			
	7		7.0	H-CH-C-H
	9		7.1	H-CH-CH-H
1.96	9			
	18		6.2	H-CH-C(sp2)-H
	8		7.1	H-CH-CH-H
0.96	16			
	7		6.8	H-CH2-C-H
1.82	14			
	18		-1.0	H-CH2>C=C<H
1.70	15			
	18		-1.0	H-CH2>C=C>H
5.37	17			
	4		6.2	H-C(sp2)-CH-H
	1		-1.0	H>C=C>CH-H
	13		-1.0	H>C=C<CH2-H
5.20	18			
	9		6.2	H-C(sp2)-CH-H
	14		-1.0	H>C=C<CH2-H
	15		-1.0	H>C=C>CH2-H

Figure 13 Spectral characterization of Alpha-bisabolol ^{1}H NMR Result

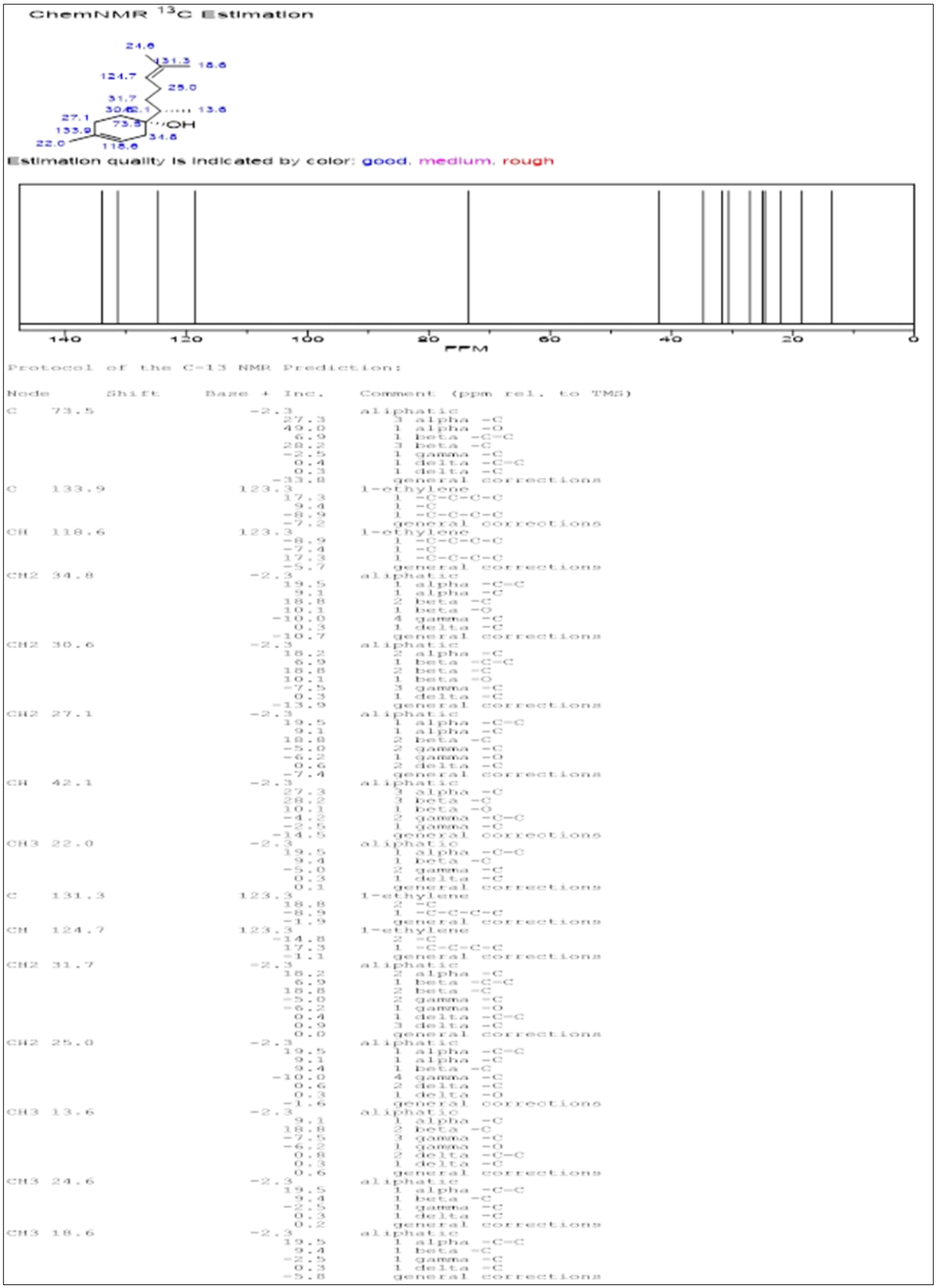


Figure 14 Spectral characterization of Alpha-bisabolol ^{13}C NMR Result

4. Conclusion

Even the foundation of our study is computational molecular docking, it's critical that the scientific tool Maestro 12.8 employed for molecular docking research proves its validity. According to our research, the drug shows strong and potent anti-neurodegenerative diseases and disorder characteristics, particularly for epilepsy (seizure). It is based on the synthetic scheme of phytoconstituents such as apigenin and alpha-bisabolol found in leaves and flower of *C.sativa L.,* and further *in silico* study of *Cannabis sativa L.* phytoconstitutent was investigated using (PDB ID: 6FYZ), and then for confirmation the presence of saturated and unsaturated moiety's spectral characterization NMR(Nuclear magnetic resonance);^{1}H &^{13}C were done. When compared to standard drug like Levetiracetam (-6.08) in a computer simulation, Apigenin (-6.38) and Alpha-bisabolol (-7.396) have greater docking scores and glide energies, especially Alpha-bisabolol. Consequently, it has been shown in *in silico* studies that the phytoconstituent present in *Cannabis sativa L.*, such as Apigenin & Alpha-bisabolol, has significant potency against a number of neurodegenerative diseases and disorders, making it a crucial source for noval anti-epileptic/anti-convulsant/anti-seizure drugs that focus on treatment of various CNS diseases/disorder in the future.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare there is no conflict of interest in this study.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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