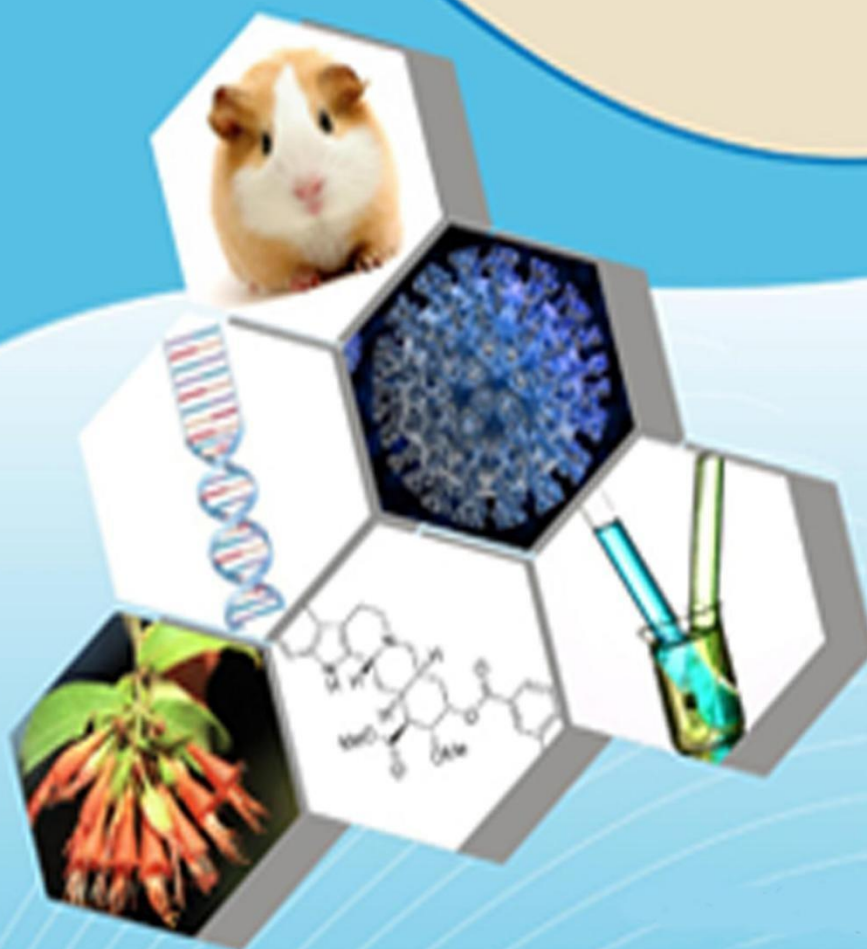




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Synergistic Polyherbal Blend for Improving Cognitive Function and Psychological Well-Being

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ABSTRACT

Anxiety is becoming more common in a world that is becoming faster. Polyherbal, LCMS, UV, and mental health issues emphasize the need of maintaining cognitive function, as shown by visible and FTIR spectroscopy. Although complex biological, psychological, antioxidant, molecular, and environmental connections might cause mental disease, its exact causes are yet unknown. Traditional conventional medicine provides a range of treatments, but complementary and alternative therapies, like as neurotonics, are gaining recognition for their inherent therapeutic value in bolstering the nervous system and encouraging calm. Neuragreen, a unique K.P. Safna Hussan polyherbal nervinetonic, is introduced in this research.

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Introduction

In today's fast-paced society, the majority of individuals struggle with mental health illnesses and anxiety. The key to living a good life is having a healthy mind. Some people prefer to ignore anxiety and sadness completely, while others use medicine to treat these conditions. It's critical to understand that mental health issues are serious issues that need proper attention. The exact causes of the majority of mental diseases are still unknown. Many of these disorders are the result of a complex interaction of biological, psychological, and environmental variables, according to research findings. Nervous weakness may result from a variety of conditions, including anxiety, sadness, and uneasiness. Mental health issues include a broad range of conditions that impact an individual's thoughts, feelings, and actions. A person's relationships, employment, and general well-being may all be significantly impacted by these problems. Anxiety disorders, mood disorders, psychotic disorders, personality disorders, eating disorders, drug abuse disorders, and post-traumatic stress

disorder are a few prevalent mental health issues. It's important to stress that there is a broad range of symptoms, severity levels, and durations associated with mental health disorders. Some people may struggle with modest symptoms that may be adequately treated, while others may have persistent and debilitating problems. Furthermore, physical symptoms like headaches,



soreness, and exhaustion may sometimes coexist with mental health

issues. There are many therapies for mental diseases available in conventional or Western medicine. Selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), mood stabilizers, stimulants (such as methylphenidate and amphetamine-based drugs), antipsychotics, antianxiety

drugs

(benzodiazepines), antidepressant augmentation agents, and anxiolytics are examples of antidepressants. To find the right drug and dose for a particular mental health problem, it's essential to speak with a psychiatrist or other mental health specialist. However, because of its potential benefits—such as their natural approach, possible therapeutic effects, cost and accessibility, and fewer side effects—herbal formulations for mental illness have drawn interest. Herbalism defines a tonic as a plant that strengthens a certain organ or system by toning it. A nervine tonic is a kind of herbal remedy intended to strengthen, maintain, and feed the human body's central nervous system. Nervinetonics may be used to boost general wellbeing, lessen stress and anxiety, and improve sleep. They are made especially to help the body regain its equilibrium and calm. This tonic helps you recover from stress and anxiety and keeps you feeling invigorated and active. 1. The central nervous system is thought to be calmed and



balanced by these nervine tonics. This medication increases blood flow by widening blood vessels, regenerating nerve cells, and having strong nootropic, anti-depressant, anti-oxidant, and anti-Alzheimer effects. 2.

Nerine tonics are often broken down by enzymes in the liver, which aid in the breakdown of the active ingredient in the herbs into smaller molecules that the body can more readily discard. While passionflower may be converted into compounds with a soothing effect, other constituents in nervine tonics, including valerian,

may be converted into compounds with an asedative effect. The metabolism of nervine tonics is influenced by the particular constituents in the tonic as well as the metabolic activity of the individual's age, sex, body weight, and general health. Since most nervine tonics have a modest impact, it is best to take them gradually for long-lasting results. 3.

Many herbs used to restore equilibrium and calm are classified as nerviness in naturopathic classifications. 4. A range of herbs that are historically used in herbal, naturopathic, and Ayurvedic medicine to strengthen the nervous system and encourage relaxation may also be used in a nerve tonic. 1. They are abundant in protein, calcium, silica, magnesium, and B vitamins. These herbs enhance the nervous system's activities by acting directly on it. The following are some instances of nervines (Fig. 1) and their purported advantages: The Ayurvedic book "Sarangdhar Samhita"

emphasizes the idea of combining many herbs to have better medicinal effects. By combining several herbs in a certain ratio, toxicity may be decreased and a more substantial therapeutic effect can be obtained. 5. Individual plants' phytochemical components are not enough to provide the intended therapeutic effects. A more significant therapeutic impact may be achieved while lowering toxicity by carefully balancing the ratios of many herbs and minerals. Plant-based pharmacological compounds included in polyherbal preparations have a variety of active ingredients that may have synergistic, potentiating, or antagonistic effects. These pharmacological concepts work in concert to provide the greatest possible therapeutic benefit with the fewest possible adverse effects. 6. It has been discovered that combining plants of different potencies rather than employing just one plant may result in superior outcomes because of a phenomena called synergism, in which the combined effects of the plants are more important than the total of their individual effects. Patients' convenience in not having to take many different herbal preparations enhances therapeutic results and compliance. As a result, the market now favors polyherbal formulations over single herbal formulations. Polyherbal formulations have a better risk-to-benefit ratio since they are often safe at large dosages and effective at low ones. Furthermore, compared to allopathic medications, polyherbal formulations often have less adverse effects 5.



FIG. 1: SOME EXAMPLES OF HERBAL PLANTS WITH NERVINE PROPERTIES. THESE INCLUDE (A) WITHANIA SOMNIFERA, (B) GINKGO BILOBA, (C) CENTELLA ASIATICA, (D) VALERIANA OFFICINALIS, (E) ST. JOHN'S



WORT, AND (F) BACOPA MONNIERI. EACH OF THESE PLANTS IS RENOWNED FOR ITS NERVINE, ANTIOXIDANT, AND NOOTROPIC PROPERTIES, CONTRIBUTING TO THE FORMULATION'S POTENTIAL THERAPEUTIC EFFECTS FOR COGNITIVE AND MENTAL HEALTH SUPPORT

Using specific plant parts from Ginkgo biloba, Centella asiatica, Bacopa monnieri, Withania somnifera, Mucuna pruriens, Pueraria tuberosa, Convolvulus pluricaulis, Celastrus paniculatus, beta-carotene, and B-complex, a new polyherbal formulation is presented here as a nervine tonic. By repairing nerve cells and expanding blood arteries, this medication increases blood circulation. Additionally, it has strong nootropic effects and anti-oxidant, anti-depressant, and anti-alzheimer effects

2. Active phytochemicals, which may increase the potency of polyherbal formulations because of the synergistic

interaction of active components from many plants, are known to display significant effectiveness. Plant products have been ingested since ancient times, but in order to identify the active chemical constituents and their effectiveness, the extracted and enhanced extracts and their novel formulations need to be validated.

RESOURCES AND METHODS: Bacopa monnieri, Withania somnifera, Mucuna pruriens, Pueraria tuberosa, Celastrus paniculatus, Centella asiatica, Convolvulus pluricaulis, and Ginkgo biloba are all used in the polyherbal formulation of Neuragreen,

an anervine tonic, which is designed to support cognitive function and encourage the health of the nervous system. Every herb in the mixture has a distinct combination of phytochemicals and therapeutic qualities that work together to provide a variety of therapeutic advantages. This nervine tonic reduces stress and anxiety, strengthens the nervous system, boosts cognitive function, and guards against inflammation and oxidative stress. The supplier of the Neuragreen Tablet by AyurgreenAyurveda Hospitals Pvt Ltd was Ayush Herbs Pvt Ltd, located in Himachal Pradesh, India, 176504.

The following plants were gathered from Green Health Herbs and Remedies Pvt. Ltd. in Alankode P.O., Kerala, India: Bacopa monnieri, Withania somnifera, Mucuna pruriens, Pueraria tuberosa, and Celastrus paniculatus. From the herbal garden of Ayurgreen Ayurveda Hospitals Pvt. Ltd. in Edappal, Malappuram district, Kerala, India, Centella asiatica and Convolvulus pluricaulis were purchased. Ayurgreen Ayurveda Hospitals Pvt. Ltd. Edappal, Malappuram district, Kerala, India, provided the B-complex, while AyushHerbs Pvt. Ltd. Nagrota Bagwan, Dist. Kangra, provided the ginkgobiloba. Figure 2 shows pictures of every component.





Figure 2: Supplements and medicinal plants used in herbal formulations. (A) BACOPA MONNIERI (BRAHMI): renowned for improving memory and cognition. (B) ASHWAGANDHA, or *Withania somnifera*, is used to promote vitality and relieve stress. (C) MUCUNA PRURIENS (VELVETBEAN): renowned for its mood-enhancing and neuroprotective properties.

(D) PUERARIA TUBEROSE: A CLASSICAL USE FOR HEART HEALTH. (E) CELASTRUS PANICULATUS, INTELLECT TREE: IMPROVES MEMORY AND COGNITIVE FUNCTIONS. (F) CENTELLA ASIATICA (GOTU KOLA): USED TO SUPPORT BRAIN HEALTH AND SKIN HEALING. (G) CONVULVULUS PURICAULIS (SHANKHPUSHPI): ENERGIZES THE BRAIN AND DECLINES ANXIETY. (H) GINKGO BILOBA: RECOGNIZED FOR IMPROVING COGNITIVE FUNCTION AND CIRCULATION. (I) B-COMPLEX: A SUPPLEMENT THAT SUPPORTS THE FUNCTION OF THE BRAIN AND NERVES AS WELL AS OVERALL HEALTH

Herb Extraction: Using 100% ethanol as the solvent, the magnetic stirrer method is the extraction technique used in this investigation. Because it is effective and generates large amounts of the target components, this technique is often employed to extract phytochemicals from plant materials. In order to enhance the surface area for extraction, each medicinal plant is first pounded using a pestle and mortar to start the interaction process. After that, the ground medicinal herbs are weighed and put in a sterile conical flask. To guarantee full extraction of the phytochemicals, absolute ethanol is added to the conical flask at a ratio of 1:10 (plant material to solvent). After that, the conical flask is capped and set on a magnetic stirrer, which stirs the mixture all night long to make extraction easier. To get rid of any solid particles or contaminants, the mixture is filtered using filter paper and a funnel after being stirred for a full day. A sanitized glass jar is used to collect the resultant extract. To further purify the extract and eliminate any remaining solvent, the container is heated to between 50 and 60 degrees Celsius in a hot air oven. This temperature is kept constant for a number of hours until a concentrated crude extract is left behind after the ethanol has entirely evaporated. A polyherbal formulation may then be created by combining each crude extract that was extracted from the several medicinal plants.

Getting the Polyherbal Formulation Ready: In this work, extracts of several plant components were combined in certain ratios to create a polyherbal mixture. The following were the weights of the various plant extracts that were used: 300 mg of *Withania somnifera*, 240 mg of ginkgo biloba, 225 mg of *Pueraria tuberosa*, *Celastrus paniculatus*, and *Convolvulus pluricaulis*, 150 mg of *Mucuna pruriens*, 375 mg of *Bacopa monnieri* and *Centella asiatica*, and 25.5 mg of B-complex. To create the polyherbal formulation, the different plant extracts were first precisely weighed using a digital scale. To guarantee uniformity, the weighed amounts of each plant extract were then put together in a sterile container and well mixed.

Assessment of the Polyherbal Mixture: Using LCMS for phytochemical analysis: LC-MS/MS tests were conducted on Agilent 6520 mass accuracy Microsoft LC 1200 paired with MSQ-TOF. Dual AJSESI ion sources were used for the MS analysis in both positive and negative modes. The Mass spectrum data analysis was carried out using the Agilent molecular ion extraction technique. The following were the basic requirements for mass spectrometry: capillary voltage 3500V, fragment or volt 750 V, drying gas (nitrogen) flow 13 L/min, nebulizer pressure 35 psig, drying gas temperature 250°C, and Oct RF Vpp. The mobile phase for the ESI ionization mode was a gradient of water (95%) and acetonitrile (5%), with a steady flow rate of 0.3 ml/min. For the ESI ionization mode, the mobile phase was set as the gradient of the acidified methanol (A) and water (B) system. At a steady flow rate of 0.9 ml/min and pressure of 1200.00 bar, gradient elution was

carried out. 7.

Spectroscopic Examination: UV-VIS Spectroscopy: One often used analytical method for locating and measuring phytochemicals in plant extracts is UV-VIS (Ultraviolet-Visible) spectroscopy. It is based on how molecules absorb light, and the pattern of absorption may be used to determine which molecules are in the sample and how much of them there are. Its foundation is the Beer-Lambert law, which asserts that the absorbance (A) of a solution is proportional to the light's path length (b) through the solution and the concentration (c) of the absorbing species.

$$A = \epsilon b c$$

The UV-Vis spectra of a polyherbal formulation in ethanol were recorded using a Jasco UV-Visible spectrophotometer model V-550, where ϵ is the absorbing species' molar absorptivity or extinction coefficient. Prior to analysis, the baseline was adjusted using an ethanol solvent. The device and the sample being examined determine the operating conditions for UV analysis. In this instance, ethanol was used to dissolve the material, and UV spectroscopy was collected in the 200–800 nm range. By putting the sample solution in a quartz cuvette and using a UV-Visible spectrophotometer to measure the absorbance at various wavelengths, the absorption spectra were captured. 8.

Finding the peak absorbance wavelengths and comparing them to databases of known phytochemicals or standard compounds' recognized spectra are the steps involved in UV spectrum data analysis. The concentration of the phytochemical in the sample may be



estimated using the absorbance peak's strength. 9–12. Fourier-transform infrared spectroscopy, or FT-IR spectroscopy, is a potent analytical method for locating and measuring phytochemicals in plant extracts. By measuring how much infrared light is absorbed by molecules, FTIR spectroscopy may provide details about the functional groups and chemical bonds that are present in the molecules. The mathematical technique of turning the observed data into a spectrum is called FTIR spectroscopy. By converting the time domain into a frequency domain representation, this procedure facilitates data analysis and interpretation. Data from the extracted phytochemicals were obtained using an FTIR spectrometer fitted with Zn-Se crystal, ATR (Attenuated Total Reflection), and OPUS software (Bruker Co., Germany). Without any sample preparation, solid, liquid, and semi-solid materials might be analyzed non-destructively using the ATR accessory to get their spectra. The equipment and the material being examined determine the FTIR analysis's working conditions. In this instance, the spectra were obtained across the 4000-400 cm⁻¹ range 9, 11, 13, and 17 after the sample was put on the ATR crystal. Assays for Antioxidants: DPPH Radical Scavenging test: One popular technique for assessing a sample's antioxidant capacity is the

DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical scavenging test. It is predicated on the idea that an antioxidant's capacity to donate hydrogen may transform the stable free radical DPPH into a non-radical state. A compound's capacity to scavenge DPPH radicals, which causes a color shift from purple to yellow, is measured by the DPPH test. Figure 3: 18. After recording the absorbance value, the following formula was used to determine the percentage of scavenging activity:

$$= \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

$$= \% \text{ of Radical Scavenging}$$

where A sample is the absorbance of the test sample and A control is the absorbance of the control (which solely contains methanol and DPPH solution). To create a graph, the data is plotted as a percentage of radical scavenging against the concentration of plant extract. greater antioxidant activity is indicated by a greater rate of scavenging. The graph or the Hill Langmuir equation may be used to get the IC₅₀ value, or the concentration of the test substance needed to scavenge 50% of DPPH radicals. $I/I_{\text{max}} = 1/(1 + IC_{50}/A)^n$ where "I_{max}" is the highest percentage of inhibition, "IC₅₀" is the 50% of inhibition, "A" is the drug concentration, and "Hillcoefficient 10,13,19" is defined.

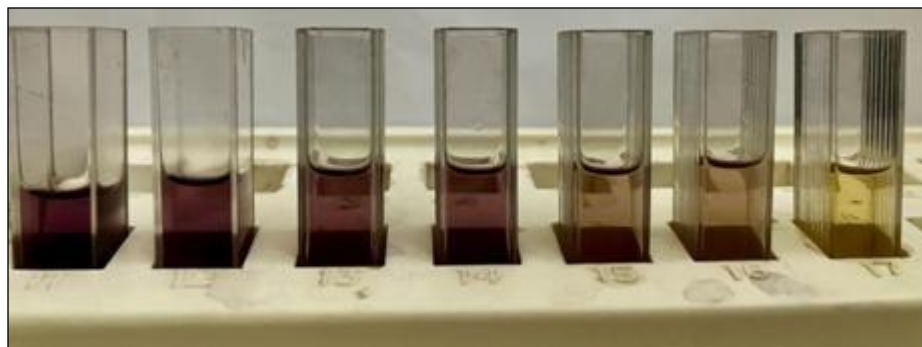


FIG.3:DPPHRADICALSCAVENGINGRESULTSINACOLORCHANGEFROMPURPLETOYELLOW

Superoxide radicals are very reactive oxygen species that have the ability to oxidatively harm cells and tissues. This is shown by the SOD Radical Scavenging Assay. The superoxide radical scavenging test may be used to determine a sample's capacity to scavenge or neutralize these radicals. The capacity of a medication to prevent superoxide radicals from reducing nitroblue tetrazolium (NBT) is the foundation of SOD activity. According to the SOD test, riboflavin produces superoxide anions when exposed to oxygen. Phosphate buffer, nitroblue tetrazolium, riboflavin, and NaCN/EDTA are the reagents used to assess the superoxide radical scavenging activity of a polyherbal formulation.

The polyherbal formulation extract was combined with phosphate buffer (pH=7.8), riboflavin, and nitrobluetetrazolium to create the reaction combination. To halt the reaction, NaCN/EDTA was added to the reaction mixture. A spectrophotometer was used to measure the absorbance of the reaction mixture at 560 nm. The formula for calculating the percentage of SOD inhibitory activity was $\% \text{ of Radical Scavenging} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$.

where the test sample's absorbance is the absorbance of the reaction mixture including sample 19, and the control's absorbance is the absorbance of the reaction mixture without the



sample.

The tremendous increase in computer power and availability, as well as the ease of access to small molecule and protein databases, have made molecular docking much easier. Automated molecular docking software seeks to comprehend and forecast molecular recognition both energetically (predicting binding affinity) and structurally (identifying probable binding modes). Typically, a small molecule and a target macromolecule engage in molecular docking. A common term for this is ligand-protein docking. Docking is a useful technique for lead optimization since it can be used to virtually screen vast libraries of compounds, rate the findings, and provide structural theories about how the ligands block the target. Analyzing the outcomes of stochastic search techniques may sometimes be confusing, and the configuration of the input structures for the docking is just as important as the docking itself. Structure-activity

studies, lead optimization, virtual screening for possible leads, generating binding hypotheses to help predict mutagenesis studies, helping x-ray crystallography fit substrates and inhibitors to electron density, chemical mechanism studies, and combinatorial library design are just a few of the many uses and applications of molecular docking in drug discovery. 10, 13, 16, 17, 19, and 22.

OUTCOMES AND CONVERSATIONS:
LCMS Analysis: ESI ionization was used in both positive and negative modes to perform LC/MS analysis on the generated Neuragreen sample. Eighty molecular ions were discovered and separated. Nine compounds with strong nervine activity were found from these 80 compounds; eight of them had positive ionization peaks, while one had negative ionization peaks. In this method, quadrupole mass analysis is used. Figures 4 and 5 showed the chromatogram with the positive and negative modes.

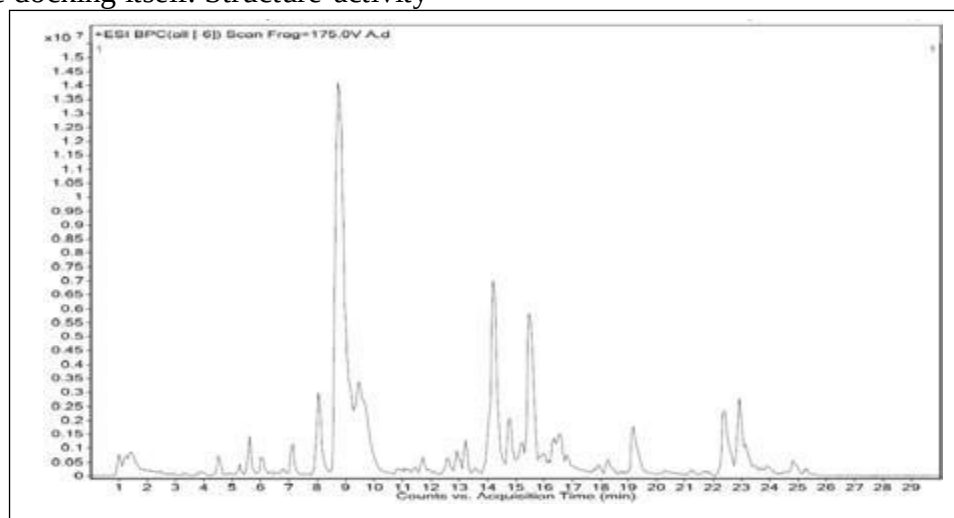
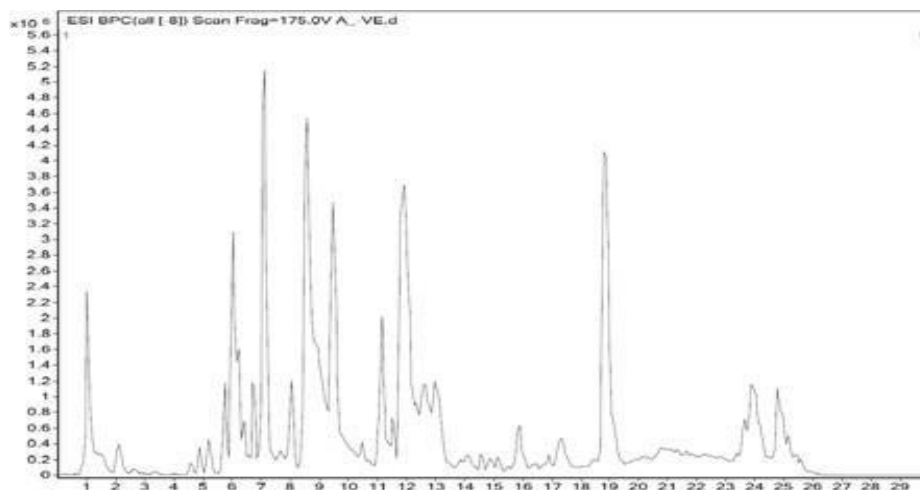


FIG. 4: CHROMATOGRAM OF THE ETHANOL EXTRACT OF THE SAMPLE NEURAGREEN AND OBTAINED IN POSITIVE MODE



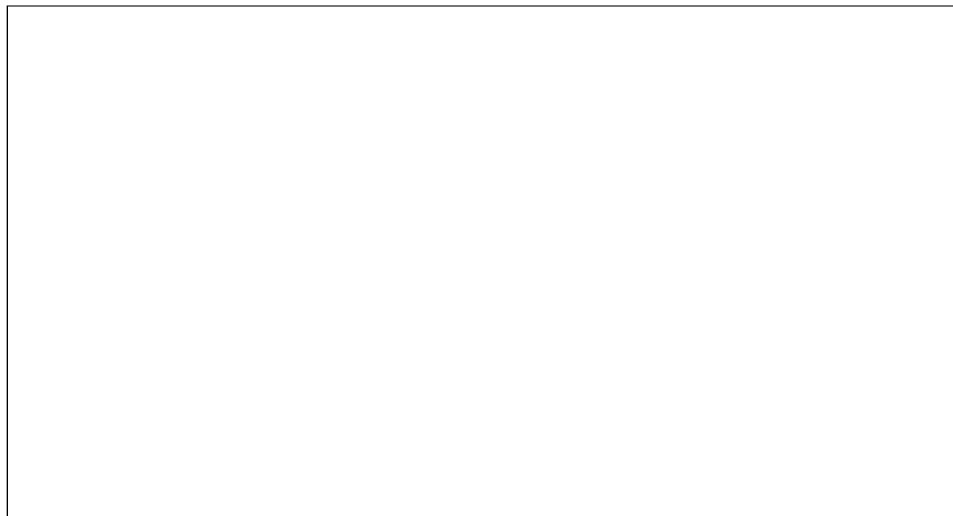


FIG. 5: CHROMATOGRAM OF THE ETHANOL EXTRACT OF THE SAMPLE NEURAGREEN AND OBTAINED IN NEGATIVE MODE

TABLE1:COMPOUNDSFROMPOSITIVEMODEOFLC/MS

Sl.no.	m/zvalue	Tentativeidentification	Molecularformula
1	141.114	Pseudotropine	C ₈ H ₁₅ NO
2	223.0815	Bendiocarb	C ₁₁ H ₁₃ NO ₄
3	143.1318	Pseudoconhydrine	C ₈ H ₁₇ NO
4	201.1725	11-amino-undecanoicacid	C ₁₁ H ₂₃ NO ₂
5	286.0441	Isoscutellarein	C ₁₅ H ₁₀ O ₆
6	248.0676	Propanoicacid,2-hydroxy-3-[(4-hydroxy-1-naphthalenyl)oxy]-	C ₁₃ H ₁₂ O ₅
7	430.1185	N-Ethylmaleimide-S-glutathione	C ₁₆ H ₂₂ N ₄ O ₈ S
8	403.1814	ArgAsnAsp	C ₁₄ H ₂₅ N ₇ O ₇
9	386.1549	NNAL-N-glucuronide	C ₁₆ H ₂₄ N ₃ O ₈
10	302.0387	Quercetin	C ₁₅ H ₁₀ O ₇
11	232.1548	Norfentanyl	C ₁₄ H ₂₀ N ₂ O
12	324.2159	9a-Fluoro-B-hydroxyandrosterone	C ₁₉ H ₂₉ FO ₃
13	262.1174	Ornaline	C ₁₀ H ₁₈ N ₂ O ₆
14	465.3055	Glycocholicacid	C ₂₆ H ₄₃ NO ₆
15	459.2434	Cerivastatin	C ₂₆ H ₃₄ FN ₂ O ₅
16	422.2155	1-Octen-3-ol-3-o-beta-D-xylopyranosyl(1->6)-beta-D-glucopyranoside	C ₁₉ H ₃₄ O ₁₀
17	506.2087	13-Hydroxypergolideglucuronide	C ₂₅ H ₃₄ N ₂ O ₇ S
18	252.1714	3(4->5)-Abeo-4,11:4,12-diepoxy-3-eudesmanol	C ₁₅ H ₂₄ O ₃
19	192.151	4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one	C ₁₃ H ₂₀ O
20	424.2066	ArgTyrSer	C ₁₈ H ₂₈ N ₆ O ₆
21	164.1564	5-Ethyl-3-methyl-2E,4E,6E-nonatriene	C ₁₂ H ₂₀
22	496.2008	TyrTrpGlu	C ₂₅ H ₂₈ N ₄ O ₇
23	495.2424	Dihydratetrabenazineglucuronide	C ₂₅ H ₃₇ NO ₉
24	516.2291	Perindoprilatglucuronide	C ₂₃ H ₃₆ N ₂ O ₁₁
25	511.2739	AFN911	C ₂₉ H ₃₃ N ₇ O ₂



26	174.1408	1,2,3,4-tetrahydro-2,5,8-trimethylnaphthalene	C ₁₃ H ₁₈
27	434.2272	ProArgTyr	C ₂₀ H ₃₀ N ₆ O ₅
28	252.1717	3(4->5)-Abeo-4,11:4,12-diepoxy-3-eudesmanol	C ₁₅ H ₂₄ O ₃
29	192.1511	4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one	C ₁₃ H ₂₀ O
30	492.2318	Caryoptin	C ₂₆ H ₃₆ O ₉
31	448.3515	6-Deoxodolichosterone	C ₂₈ H ₄₈ O ₄
32	626.2278	Tetrahydropteroyltri-L-glutamate	C ₂₄ H ₃₄ N ₈ O ₁₂
33	453.2689	Delcosine	C ₂₄ H ₃₉ N ₇ O
34	604.259	6-Hydroxysandoricin	C ₃₁ H ₄₀ O ₁₂
35	599.3039	LividomycinB	C ₂₃ H ₄₅ N ₅ O ₁₃
36	522.257	(23E)-26,26,26,27,27,27-hexafluoro-1 α ,25-dihydroxy-23,24-didehydrovitaminD ₃	C ₂₇ H ₃₆ F ₆ O
37	606.2763	Trandolaprilglucuronide	C ₃₀ H ₄₂ N ₂ O ₁₁
38	598.3096	PI(18:1(9Z)/0:0)	C ₂₇ H ₅₁ O ₁₂ P

TABLE2:COMPOUNDSFROMNEGATIVEMODEOFLC/MS

Sl.no.	M/Z	Tentativeidentification	Molecularformula
1	332.0936	TosyllysineChloromethylKetone	C ₁₄ H ₂₁ ClN ₂ O ₃ S
2	342.1226	DictyoquinazolC	C ₁₈ H ₁₈ N ₂ O ₅
3	696.2013	GnemonolA	C ₄₂ H ₃₂ O ₁₀
4	326.1051	6-O-p-Coumaroyl-D-glucose	C ₁₅ H ₁₈ O ₈
5	564.157	Protoaphinaglucone	C ₃₀ H ₂₈ O ₁₁
6	424.1437	Eprosartan	C ₂₃ H ₂₄ N ₂ O ₄ S
7	680.2061	Copalliferol B	C ₄₂ H ₃₂ O ₉
8	440.1388	GinkgolideC	C ₂₀ H ₂₄ O ₁₁
9	418.1336	2-N,6-N-Bis(2,3-dihydroxybenzoyl)-L-lysine	C ₂₀ H ₂₂ N ₂ O ₈
10	430.109	Bispyribac	C ₁₉ H ₁₈ N ₄ O ₈
11	564.1571	Protoaphinaglucone	C ₃₀ H ₂₈ O ₁₁
12	534.1468	Aprepitant	C ₂₃ H ₂₁ F ₇ N ₄ O ₃
13	936.598	1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-3-(Galactosyl-alpha-1-6-Galactosyl-beta-1)-glycerol	C ₅₁ H ₈₄ O ₁₅
14	326.1971	Hydroquinidine	C ₂₀ H ₂₆ N ₂ O ₂
15	388.0869	Dopaxanthinquinone	C ₁₈ H ₁₆ N ₂ O ₈
16	578.1732	eq-4"-Hydroxymaysin	C ₂₇ H ₃₀ O ₁₄
17	432.1129	Genistein8-C-glucoside	C ₂₁ H ₂₀ O ₁₀
18	614.1485	CMP-N-acetylneuraminicacid	C ₂₀ H ₃₁ N ₄ O ₁₆ P
19	516.1344	b-D-Glucuronopyranosyl-(1->3)-a-D-galacturonopyranosyl-(1->2)-L-rhamnose	C ₁₈ H ₂₈ O ₁₇
20	422.1282	Bifenthrin	C ₂₃ H ₂₂ ClF ₃ O ₂
21	788.2664	5,10-Methylenetetrahydromethanopterin	C ₃₁ H ₄₅ N ₆ O ₁₆ P
22	786.2496	Estradiolmustard	C ₄₂ H ₅₀ Cl ₄ N ₂ O ₄
23	408.1496	Marmesingalactoside	C ₂₀ H ₂₄ O ₉
24	304.1373	Nopaline	C ₁₁ H ₂₀ N ₄ O ₆
25	414.1138	Asperuloside	C ₁₈ H ₂₂ O ₁₁
26	302.0477	5,6,7,3',4'-Pentahydroxyisoflavone	C ₁₅ H ₁₀ O ₇
27	210.0363	Galactaricacid	C ₆ H ₁₀ O ₈
28	328.2307	Auxina	C ₁₈ H ₃₂ O ₅
29	330.2466	9S,12S,13S-trihydroxy-10E-octadecenoicacid	C ₁₈ H ₃₄ O ₅
30	914.5009	TragopogonsaponinL	C ₅₀ H ₇₄ O ₁₅
31	490.3389	7',8'-Dihydro-8'-hydroxyreticulataxanthin	C ₃₃ H ₄₆ O ₃
32	1008.7071	Undecaprenylphosphatemannose	C ₆₁ H ₁₀₁ O ₉ P
33	444.3327	N-Nitrosotomatidine	C ₂₇ H ₄₄ N ₂ O ₃
34	932.4602	ScopolosideI	C ₄₅ H ₇₂ O ₂₀
35	456.333	Citranaxanthin	C ₃₃ H ₄₄ O
36	488.3587	GanoderiolD	C ₃₀ H ₄₈ O ₅
37	440.3371	17-[[3-(1-Pyrrolidinyl)propyl]imino]androst-5-en-3beta-olacetate	C ₂₈ H ₄₄ N ₂ O ₂
38	252.16	Hexazinone	C ₁₂ H ₂₀ N ₄ O ₂
39	456.3685	Oleanolicacid	C ₃₀ H ₄₈ O ₃
40	272.24	16-Hydroxyhexadecanoicacid	C ₁₆ H ₃₂ O ₃
41	454.3528	GanoderolB	C ₃₀ H ₄₆ O ₃



Nine of the seventy-nine chemicals that were tracked from LCMS had higher neural activity. There are six of them with negative ionization peaks and three with positive ionization peaks.

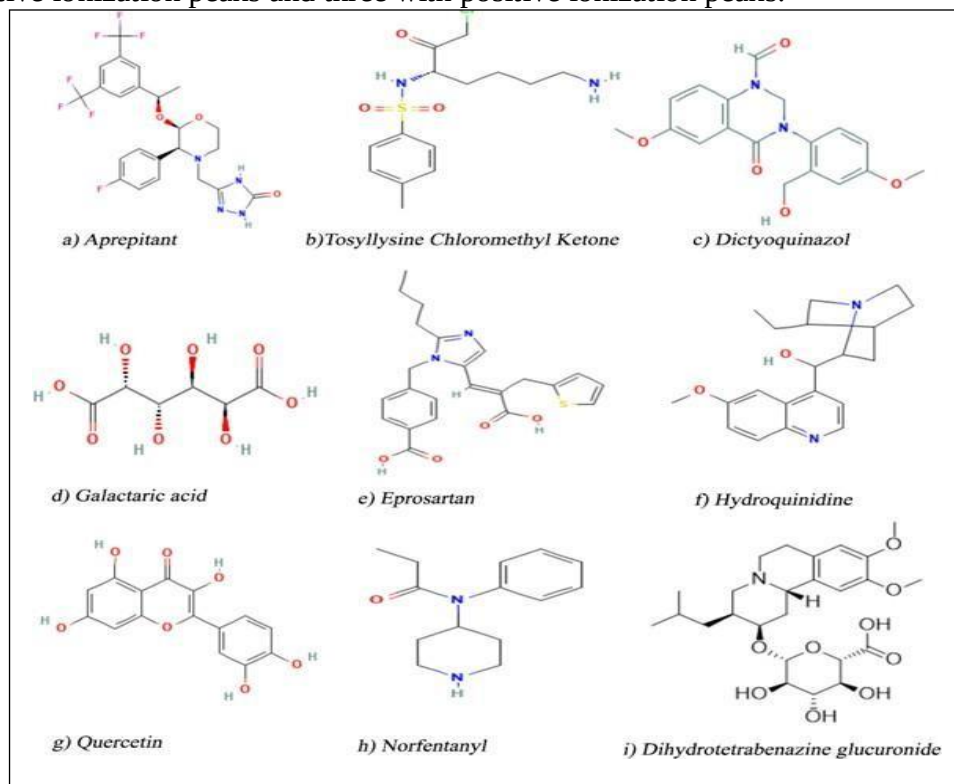


FIG. 6:

The chemical structures of the several bioactive compounds that are included in the prepared polyherbal formulation that exhibits nerve action are shown in Figure 6. A) DICTYOQUINAZOL B) TOSYLLYSINE CHLOROMETHYL KETONE D) GALACTARIC ACID F) HYDROQUINIDINE E) EPROSARTAN G) QUERCETIN H) NORFENTANYLI) GLUCURONIDE DIHYDROTETRABENAZINE

The nine substances exhibiting nervine effect are tosyllysine chloromethyl ketone, eprosartan, aprepitant, galactaric acid, norfentanyl, cis-dihydrotetrabenazine glucuronide, quercetin, hydroquinidine, and dictyoquinazol c. The molecular weights of tosyl lysine chloromethyl ketone (332.0936), eprosartan (424.1437), aprepitant (534.1468), galactaric acid (210.0363), nor fentanyl (232.1548), cis-dihydro tetrabenazine glucuronide (495.2424), quercetin (302.0387), hydroquinone (326.1971), and dictyoquinazol (342.1226).

These substances are all neuroprotective. Additionally, these substances exhibit biological characteristics including anti-inflammatory and antioxidant qualities. Fig. 6 shows the structures of these nine compounds. Spectroscopic Examination: Spectroscopy of UV-VIS: UV-Vis spectroscopy analysis may be utilized as a quality control in Ayurvedic formulations as spectra can be thought of as a fingerprint of each verified standard composition. The ethanolic extract of the polyherbal formulation seems to include molecules with C=O and C=C functional groups, according to the UV-VIS spectroscopy examination. Typically, these functional groups are present in a variety of phytochemical classifications, including as alkaloids, terpenes, and flavonoids. Its foundation is the electronic absorption brought about by the chemicals found in plants and those created during chemical processes employed in manufacturing. UV or visible light is absorbed as a consequence of electronic changes in molecules. The



excitation of electrons from their ground state to higher energy levels explains why molecules absorb UV-Vis rays. The computed UV-visible spectrum of neurogreen polyherbal formulation is shown in Figure 7.

different phytochemicals that have been shown to have a range of potential medicinal uses.

The electronic transitions linked to the C=O and C=C functional groups, respectively, are represented by the electronic absorptions seen in the ethanolic extract of the polyherbal formulation at 205 and 260 nm (Fig. 7). The strong peak in the UV-Vis spectrum at 205 nm shows that the electronic transition is associated with the excitation of electrons from the oxygen atom's non-bonding π -electron lone pairs to the carbonyl group's antibonding π^* orbitals. This transition, called the $\pi \rightarrow \pi^*$ transition, is typical of substances like flavonoids, coumarins, or quinones that have a carbonyl group. The presence of flavonoids, such as flavones or flavonols, or other substances, such as coumarins, quinones, or steroids, may be the cause of the strong peak seen at 205 nm, which denotes the presence of a C=O functional group. Numerous biological actions, such as antioxidant, anti-inflammatory, and neuroprotective qualities, have been found for these substances. The large peak in the UV-VIS spectrum at 260 nm shows that the excitation of π -electrons from the double bond of the C=C functional group to the π^* antibonding orbitals is what causes the electronic transition. This transition, often referred to as the $\pi \rightarrow \pi^*$ transition, is typical of molecules with double bonds, such as lignans, stilbenes, and terpenes. The existence of C=C functional groups is indicated by the wide spectrum seen at 260 nm, which may be related to the presence of terpenes or other substances like lignans or stilbenes. Terpenes are known to have a number of biological actions, such as sedative, analgesic, and anxiolytic effects, which may help to support the health of the nervous system. It is crucial to remember that a number of variables, including solvent polarity, pH, and molecular environment, influence the absorption peak seen in UV-Vis spectroscopy. As a result, the particular functional groups and chemicals included in the polyherbal formulation might affect the electronic transitions seen in the UV-Vis spectrum.

FTIR Spectroscopy: The polyherbal formulation's FTIR spectrum (Fig. 8) showed the presence of many functional groups, indicating the presence of

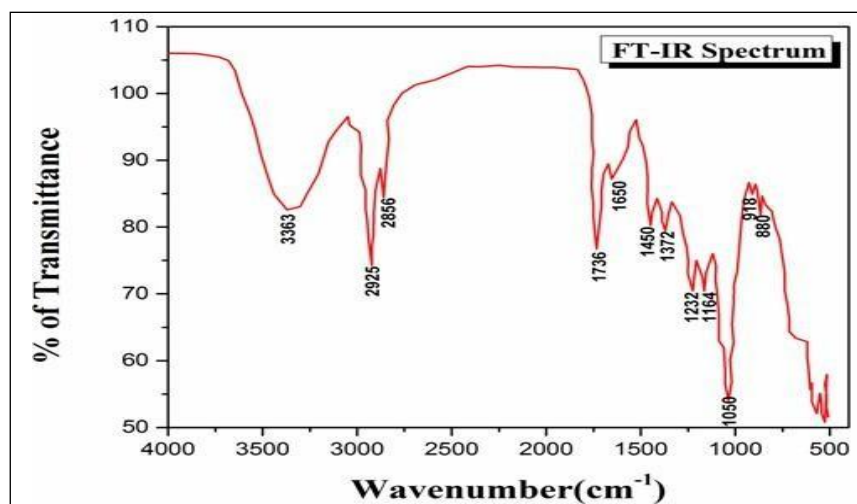


FIG.8:FTIRSPECTRUMREPRESENTINGPOTENTIALBANDSINTHEETHANOLICEXTRACTOF THE FORMULATION

The polyherbal formulation comprises a variety of functional groups, including quinone oximes, cellulose, C=C conjugated with C=CC=O, R-CH₃-C(CH₃)₂, -OC(CH₃)₂, and P-CH₃, according to the FTIR spectrum. The wide OH stretching vibration (linked to quinone oximes) is responsible for the peak at 3353 cm⁻¹, while the C-H stretching vibration of the acyclic -CH₂-group is responsible for the peaks at 2925 cm⁻¹ and 2855 cm⁻¹. The C=O stretching vibration of oxidized cellulose is shown by the peak at 1736 cm⁻¹. The C=O stretching vibration of oxidized cellulose is shown by the peak at 1736 cm⁻¹. The existence of C=C conjugated with C=C C=O is shown by the signal at 1650 cm⁻¹. The asymmetric and symmetric C-H deformation vibrations of the R-CH₃ group are represented by the peaks at 1456 cm⁻¹ and 1372 cm⁻¹, respectively. The C-H deformation vibration of cyclobutene is responsible for the peak at 1232 cm⁻¹, while the C-C stretching vibration of the -C(CH₃)₂ group is responsible for the peak at 1164 cm⁻¹. The presence of ethanolic extract in the polyherbal formulation may be indicated by the peak at 1050 cm⁻¹, which indicates the C-O stretching vibration of primary alcohols, notably R-CH₂-CH₂-OH. The C-C skeletal vibration of cyclobutane and the C-H rocking vibration of the P-CH₃ group are shown by the peaks at 918 cm⁻¹ and 880 cm⁻¹, respectively. The polyherbal formulation seems to include a diverse variety of substances with different functional groups, according to the FTIR spectra. Polyphenolic compounds with possible antioxidant action are suggested by the presence of cellulose, quinoneoximes, and conjugated C=C, C=O. Terpenoids are indicated by the presence of cyclobutane.

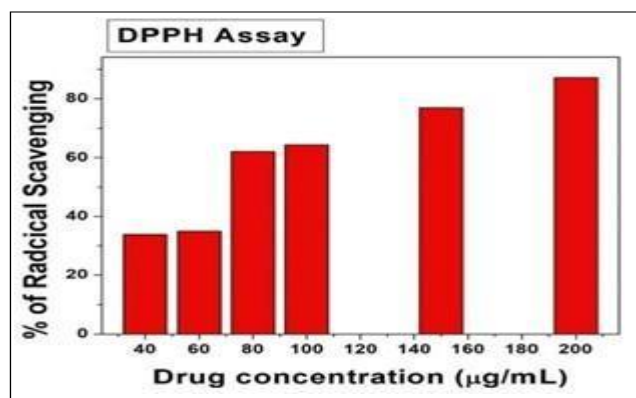
TABLE 3: OBSERVED VIBRATIONAL FREQUENCIES FOR THE POLYHERBAL FORMULATION AND THEIR TENTATIVE ASSESSMENT

Peak number	Band Range (literature) Wavenumber (cm ⁻¹)	Band Range (experimental) Wavenumber (cm ⁻¹)	Band Interactions	Band Assignment	Functional Groups	Possible Compounds
1	3540-2700	3353	OH stretching (broad, associated)	Quinone oximes	Hydroxyl(-OH)	Quinone oximes
2	2940-2915	2925	C-H stretching (acyclic), symmetric	-CH ₂ - (acyclic)	Alkane(-CH ₂ -)	Fatty acids, esters, aldehydes
3	2870-2840	2855	C-H stretching (acyclic), symmetric	-CH ₂ - (acyclic)	Alkane(-CH ₂ -)	Fatty acids, esters, aldehydes
4	1750-1725	1736	C=O stretching	Cellulose (after oxidation)	Carbonyl(C=O)	Cellulose derivatives



5	1660-1580	1650	C=C stretching	C=C conjugate with C=CC=O	Alkene(C=C), carbonyl(C=O)	Carotenoids, flavonoids
6	1440-1465	1456	C-H deformation (assymmetric)	R-CH ₃	Methyl(-CH ₃)	Fattyacids, esters, aldehydes
7	1390-1370	1372	C-H deformation (symmetric)	R-CH ₃	Methyl(-CH ₃)	Fattyacids, esters, aldehydes
8	1245-1220	1232	C-H deformation	Cyclobutane	Alkane(-CH ₂ -)	Cyclobutane derivatives
9	1175-1165	1164	C-C stretching	-C(CH ₃) ₂	Alkane(-CH ₂ -)	Alkanes, alkenes
10	1050	1050	C-O stretching	R-CH ₂ -CH ₂ - OH	Primary alcohols	Alcohols, glycols, or derivatives
11	930-890	918	C-C skeleton vibration	Cyclobutane	Alkane(-CH ₃ -)	Cyclobutane derivatives
12	960-830	880	C-H rocking	P-CH ₃	Methyl(-CH ₃)	Phospholipids, phosphates

Assays for antioxidants: DPPH Assay: One popular technique for figuring out the antioxidant activity of natural compounds is the DPPH radical scavenging test. The test relies on the electron transfer principle, which states that an antioxidant molecule's electron or hydrogen radical may be accepted by the DPPH radical, a stable free radical, to create a stable diamagnetic molecule. A spectrophotometer may be used to quantify the drop in DPPH absorbance at 517 nm caused by this reaction. In this experiment, an antioxidant molecule reduces the DPPH radical, which lowers the absorbance at 517 nm. The test sample's antioxidant activity is closely correlated with the degree of DPPH radical reduction. The DPPH radical scavenging test was used in this work to assess the antioxidant activity of a polyherbal mixture used as a nervine tonic. A microplate reader is used to measure the absorbance at 517 nm after the control and all test samples are incubated in the dark for 15 to 20 minutes. To create a graph, the data is plotted as a percentage of radical scavenging against the plant extract concentration. With increasing concentrations of the polyherbal formulation, the percentage of DPPH radical scavenging increased, as seen by the rising trend in graph Fig. 9. This implies that there are a lot of antioxidant molecules in the polyherbal formulation that are capable of efficiently scavenging the DPPH radical. The IC₅₀ value, which indicates the concentration of the plant extract needed to scavenge 50% of the DPPH radical, was determined using the Hill Langmuir Equation. The polyherbal formulation exhibited high antioxidant activity, as shown by its IC₅₀ value of 7.3 µg/ml. A stable diamagnetic molecule is formed by the polyherbal formulation, which results in a colorless methanol solution, as shown by the reduction in absorbance at 517 nm (Fig. 9).



The polyherbal formulation's DPPH RADICAL SCAVENGING activity at varying concentrations is shown in Figure 9. Dose-dependent antioxidant activity is shown by the rising proportion of radical scavenging with



medication concentration. 200 $\mu\text{g/ml}$ exhibits the maximum radical scavenging (about 80%), while 40 $\mu\text{g/ml}$ exhibits the lowest (approximately 30%).

Assay for SOD: By using McCord and Fridovich's (1969) nitroblue tetrazolium (NBT) reduction technique, one may assess the significant antioxidant activity of superoxide scavenging. The polyherbal formulation extract was combined with phosphate buffer, riboflavin, and nitroblue tetrazolium to create the reaction mixture for this investigation. To halt the reaction, NaCN/EDTA was added to the reaction mixture. At 560 nm, the reaction mixture's absorbance was measured.

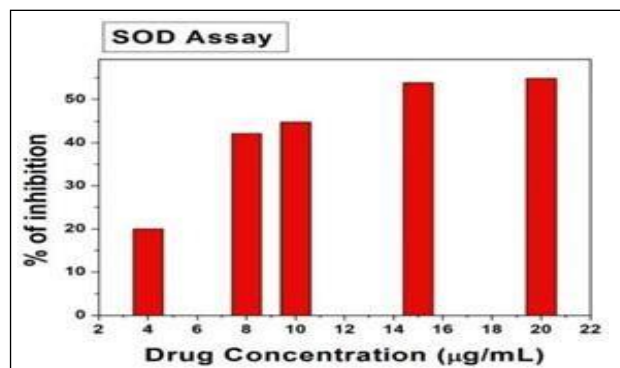
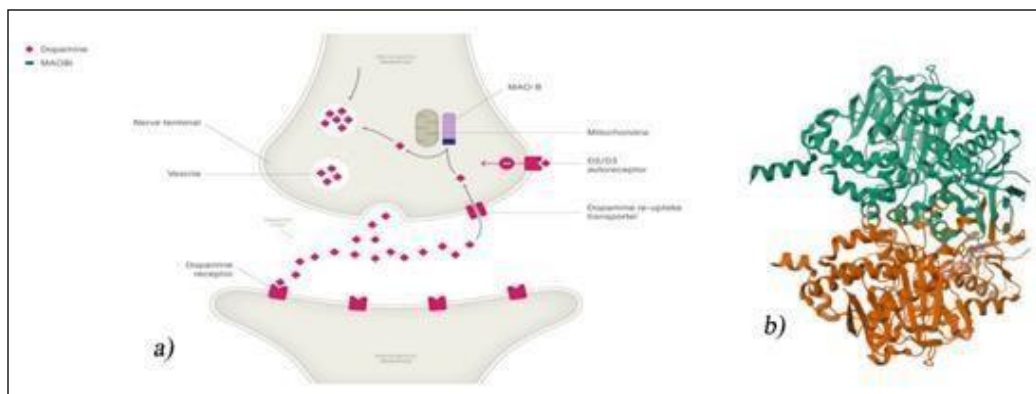


Figure 10: POLYHERBAL FORMULATION AT VARYING CONCENTRATIONS' SOD RADICAL SCAVENGING ACTIVITY. As medication concentration rises, the proportion of radical scavenging increases as well, suggesting dose-dependent antioxidant action. The assay's findings were shown as a graph showing the formulation's concentration in relation to the percentage of radical scavenging. Using the Hill Langmuir equation, the IC_{50} value was determined to be 14.9 $\mu\text{g/ml}$. Stronger SOD activity and, thus, a stronger antioxidant capacity are indicated by a larger percentage of inhibition activity. According to the study's IC_{50} value, the polyherbal formulation exhibits a strong antioxidant capacity and substantial SOD radical scavenging activity. The study's findings are in line with earlier research that demonstrated the polyherbal formulation's strong antioxidant properties. Flavonoids and phenolic compounds, two phytochemicals included in the polyherbal mixture, have been shown to exhibit antioxidant properties. The synergistic action of these phytochemicals may be responsible for the polyherbal formulation's antioxidant efficacy. The results of this research indicate that the polyherbal formulation may be used as a possible therapeutic agent for the prevention and treatment of illnesses linked to oxidative stress as well as a natural source of antioxidants.

Docking

Research:

Using MAO to dock: Nervine tonics are often used to enhance mental clarity, ease anxiety, and encourage relaxation. The brain's neurotransmitter modulation is responsible for these effects. Monoamine oxidase B, or MAO-B, is an enzyme that breaks down dopamine and serotonin, among other neurotransmitters. The breakdown of dopamine is slowed down by blocking MAO-B activity, which raises dopamine levels in the brain. Furthermore, it has been shown that MAO-B inhibitors have neuroprotective properties, which means they may aid in preventing damage or degeneration to nerve cells. This is especially important when it comes to neurological conditions like Parkinson's disease, where MAO-B inhibitors are often utilized as a treatment strategy to slow the illness's development. Maintaining a proper balance of neurotransmitters is the primary function of MAO-B in the context of nervine tonics, which are drugs that support and sustain the health and functioning of the nervous system. As a result, MAO-B aids in controlling dopamine levels, a neurotransmitter linked to pleasure, reward, and motivation. This may help people feel happier and more content 23. The action mechanism may be seen in Figure 11A.



The structure of monoamine oxidase-beenzyme derived from protein data bank 2V5Zisa2-chain structure with sequences from Homosapiensis employed in the dosing procedure is shown in FIG.11:THEMECHANISMOFMAO-B(REF)B). The protein data bank's monoamine oxidase B enzyme structure is shown in Fig. 11B. The MAO-B protein docks with the nine chemicals that were chosen, as shown in Fig. 11. Figure 12 illustrates the three-dimensional protein-ligand interaction, whereas Figure 13 shows the two-dimensional interaction of every molecule chosen in the MAO-B dynamic site that was retrieved using the Maestro graphical user interface. It is discovered that all of the chosen active ligands are indistinguishably buried in MAO-B's deep binding pocket. Red and dotted lines represent the H-bonding and π -bonding interactions that all of the ligands have with the protein's amino acids at the active site.

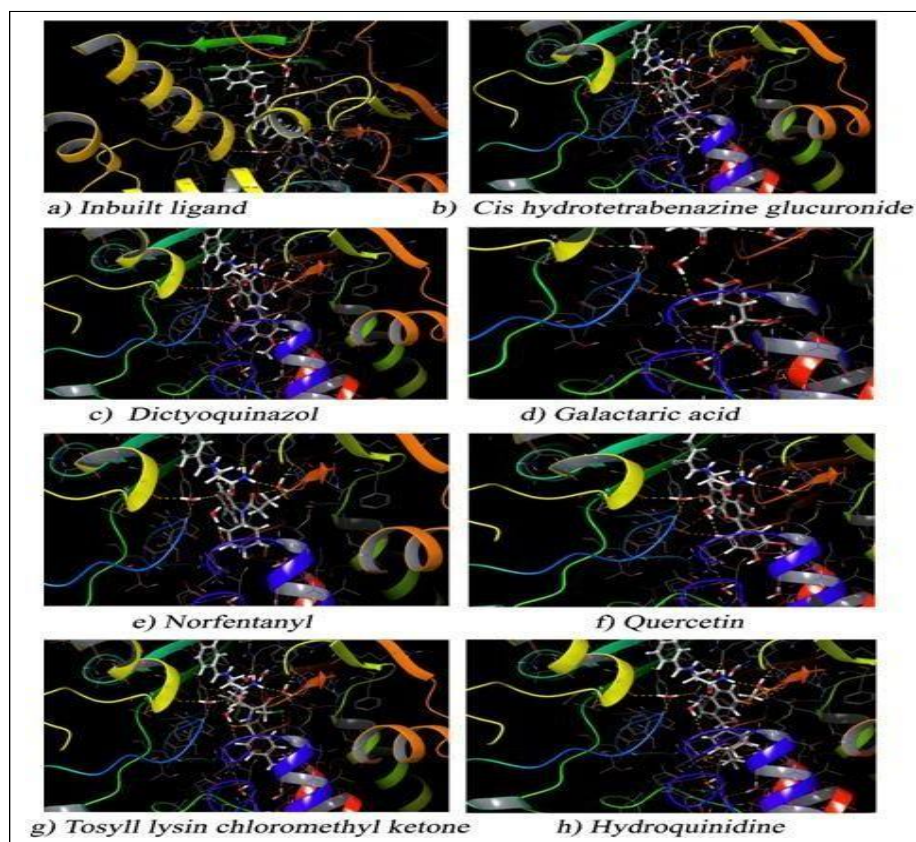
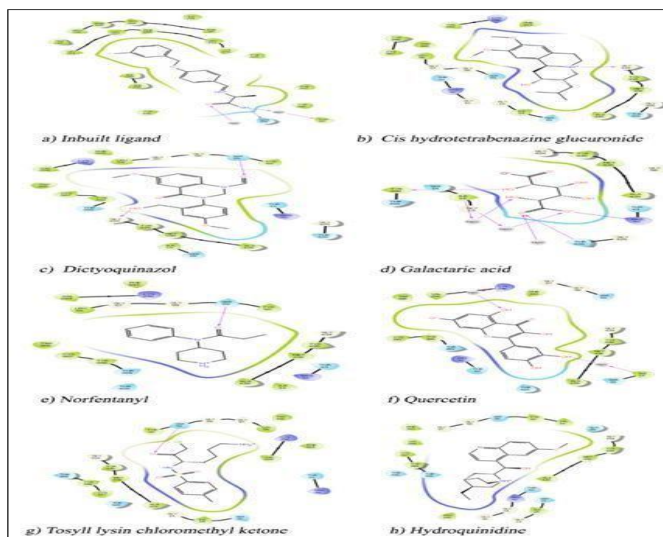


Figure 12: 3D INTERACTION PICTURE OF SCREENED BIOACTIVE COMPOUNDS IN POLYHERBAL FORMULATION NEURAGREEN WITH MAO-B TARGET PROTEIN (A) INBUILT LIGAND, (B) CIS HYDROTETRABENAZINE GLUCURONIDE, (C) DICTYOQUINAZOL, (D) GALACTARIC ACID, (E) NORFENTANYL, (F) QUERCETIN, (G) TOSYLL LYSIN CHLOROMETHYL KETONE, (H) HYDROQUINIDINE



The docking analysis of screened bioactive compounds with Monoamine Oxidase B (MAO-B) is shown in Fig. 13. A number of important interactions with amino acid residues in the active site were found, offering information on the compounds' binding mechanisms and possible inhibitory effects. In order to stabilize the ligand within the binding pocket, the preexisting ligand, acting as a reference, demonstrated hydrophobic interactions with ILE199 and TYR326 as well as π - π stacking interactions involving TYR60 and TYR398. The baseline for comparison with the other ligands is established by this interaction pattern. In addition to hydrophobic interactions with TYR398 and MET60, *cis* hydrotetrabenazine glucuronide demonstrated significant hydrogen bonding with SER59 and GLY434, indicating a well-suited location inside the MAO-B active site. Dityoquinazol's capacity to establish persistent binding via non-polar contacts was further shown by its strong hydrophobic interactions with LEU171 and TYR398 as well as its π - π interactions with TYR60. In addition to π - π stacking with TYR398 and TYR60, galactaric acid's many hydroxyl groups established significant hydrogen bonds with GLY434, SER59, and ARG58, suggesting strong polar interactions that might increase its inhibitory potential. Quercetin, a well-known flavonoid, formed hydrogen bonds with SER59, GLY434, and ARG58, with additional π - π stacking involving TYR398 and TYR60, highlighting its effective interaction through both polar and non-polar mechanisms. Norfentanyl's interaction profile was dominated by hydrophobic interactions with ILE199, TYR398, and LEU171, as well as hydrogen bonding with SER59 and GLY434. In addition to interacting inhydrophobically and hydrogen-bondingly with LEU171 and GLY434, the reactive molecule tosyl lysin chloromethyl ketone most likely created covalent connections with SER59, suggesting a powerful inhibitory potential via covalent modification. Last but not least, hydroquinidine showed a balanced pattern of interactions, establishing π - π stacking with TYR398 and TYR60 and hydrogen bonds with SER59 and GLY434.



All ligands exhibit hydrogen bonds and hydrophobic interactions, indicating a multifaceted inhibitory strategy in which molecules use both polar and non-polar residues to maximize binding.

FIG. 13: 2D INTERACTION DIAGRAMS OF MOLECULAR DOCKING STUDIES INVOLVING THE SCREENED BIOACTIVE COMPOUNDS FROM THE POLYHERBAL FORMULATION NEURAGREEN WITH NERVINE ACTION AND THE ENZYME MONOAMINE OXIDASE B (MAO-B). (A) INBUILT LIGAND, (B) CIS HYDROTETRA-BENZAZINE- GLUCURONIDE, (C) DICTYOQUINAZOL, (D) GALACTARIC ACID, (E) NORFENTANYL, (F) QUERCETIN, (G) TOSYLL LYSIN CHLOROMETHYL KETONE AND (H) HYDROQUINIDINE

Table 4 presents the docking scores and binding energies, which provide important information about the effectiveness of the chosen bioactive substances. As a basis for comparison, the inherent ligand (2V5Z) had the greatest interaction with MAO-B, with a docking score of -21.250 and a binding energy of -350.664 kcal/mol. With a docking score of -9.542 and a binding energy of -84.404 kcal/mol, quercetin showed the greatest affinity among the chemicals tested, indicating significant binding stability. With a binding energy of -51.615 kcal/mol and a docking score of -9.385, dictyoquinazol came in second, suggesting that it might be an effective inhibitor. With docking scores of -7.469 and -7.268, respectively, tosyll lysin chloromethyl



ketone and norfentanyl also demonstrated moderate binding affinities. Although not as strong as the built-in ligand or quercetin, galactaric acid, cis-dihydrotetrabenazine glucuronide, and hydroquinidine nonetheless showed binding energy values that indicate considerable interactions in spite of lower docking scores. Given their capacity to inhibit MAO-B, which increases neurotransmitter availability and provides neuroprotective advantages, quercetin and dictyoquinazol have the potential to be developed as nervine tonics, as shown by their comparatively higher docking scores and binding energies. These substances' potential as nervine tonic options is highlighted by their capacity to interact with important MAO-B residues. These ligands may raise dopamine and other monoamine levels in the brain by blocking MAO-B, which would assist to calm mood, enhance cognitive performance, and provide neuroprotection. Furthermore, a number of these substances, like quercetin, have antioxidant qualities that reinforce their function in lowering oxidative stress, a major contributing cause to neurodegenerative illnesses.

TABLE4:DOCKINGSCORESANDBINDINGENERGYOFTHELIGANDSWITHMAO-BPROTEIN

Sample	DockingScore	BindingEnergyKcal/mol
Inbuiltligand2V5Z	-21.250	-350.664
Quercetin	-9.542	-84.403
Dictyoquinazol	-9.385	-51.615
Tosyllysinchloromethylketone	-7.469	-60.221
norfentanyl	-7.268	-51.462
Cis-dihydrotetrabenazineglucuronide	-7.030	-46.778
hydroquinidine	-6.622	-38.369
Galactaricacid	-6.416	-65.294

Through the use of an H₂O molecule, MAO-B's built-in ligand coordinates N from NH₂ and TYR 60 coordinate to GLN 206. Its binding energy is -350.664 kcal/mol, and its docking score is -21.250. The cis hydrotetrabenazine glucuronide ligand has a docking score of -7.030 and a binding energy of -46.778 Kcal/mol. It connects with GLY 434 via N of the NH⁺ group. Dictyoquinazol has a docking score of -9.385 and a binding energy of - 51.615 Kcal/mol. It coordinates at two sites: OH to GLY 434 and carbonyl oxygen to SER 59. Through H₂O, galactaric acid coordinates at three different locations. Carbonyl oxygen to THR 426, O- to ARG 42. ILE and OH coordinate via H₂O from OH to TYR 98. With a binding energy of -65.294 K cal/mol and a docking score of -6.416, one H₂O is directly coordinated to carbonyl oxygen. SER 59 is coordinated to carbonyl oxygen by the norfentanyl ligand, which has a binding energy of -51.465 Kcal/mol and a docking score of -7.030. Through H₂O, quercetin ligands have two coordination sites. LYS296 to OH via H₂O. ILE14 to another OH via H₂O. The binding energy is -84.403 kcal/mol and the docking score is -9.542. One coordination of SER59 to carbonyloxygen is present in the tosyllysin chloromethylketone ligand. The binding energy is -60.221 Kcal/mol, and the docking score is -7.469. The hydroquinidine ligand and the protein are coordinated by an electrostatic force. It has a binding energy of 38.369 kcal/mol and a docking score of 6.622. The quercetin-9.542 has the highest docking score.

Gamma-aminobutyric acid (GABA) is a significant inhibitory neurotransmitter in the spinal cord and the brain's principal inhibitory neurotransmitter. It docks with GABA. One neurotransmitter that is essential to the neurological system is GABA. GABA often plays a role in nervine tonics by encouraging relaxation, lowering anxiety, calming and balancing brain activity, and enhancing the quality of sleep. Numerous nervine tonics work to raise GABA levels or improve the effects of GABA in the brain. They also bind to GABA receptors on nerve cells, which lowers neuronal excitability and nerve impulse firing. They could raise GABA levels and extend its effects by blocking the enzymes that break it down. Additionally, these tonics could interact with GABA receptors to increase GABA binding. A feeling of relaxation and serenity may arise from this. It is impossible to undervalue GABA's therapeutic importance. Numerous neurological and behavioral disorders are linked to abnormalities in GABA signaling. Numerous pharmaceutical therapies in neurology, psychiatry, and anesthesia are based on the modification of GABA signaling 24. GABA (gamma-aminobutyric acid) interneurons are the main inhibitory neurons in the central nervous system (CNS), and they play a critical role in a variety of pathophysiological processes,



including modulation of cortical and hippocampal neural circuitry and activity, cognitive function-related neural oscillations (e.g., gamma oscillations), and information integration and processing. The excitatory/inhibitory (E/I) balance in the cortex may be upset by dysfunctional GABA interneuron activity, which may be a key pathophysiological mechanism behind cognitive failure in schizophrenia. According to recent studies, a possible therapy for cognitive impairments linked to schizophrenia is specific manipulation of the GABA-ergic system [25]. The action mechanism may be seen in Figure 14.

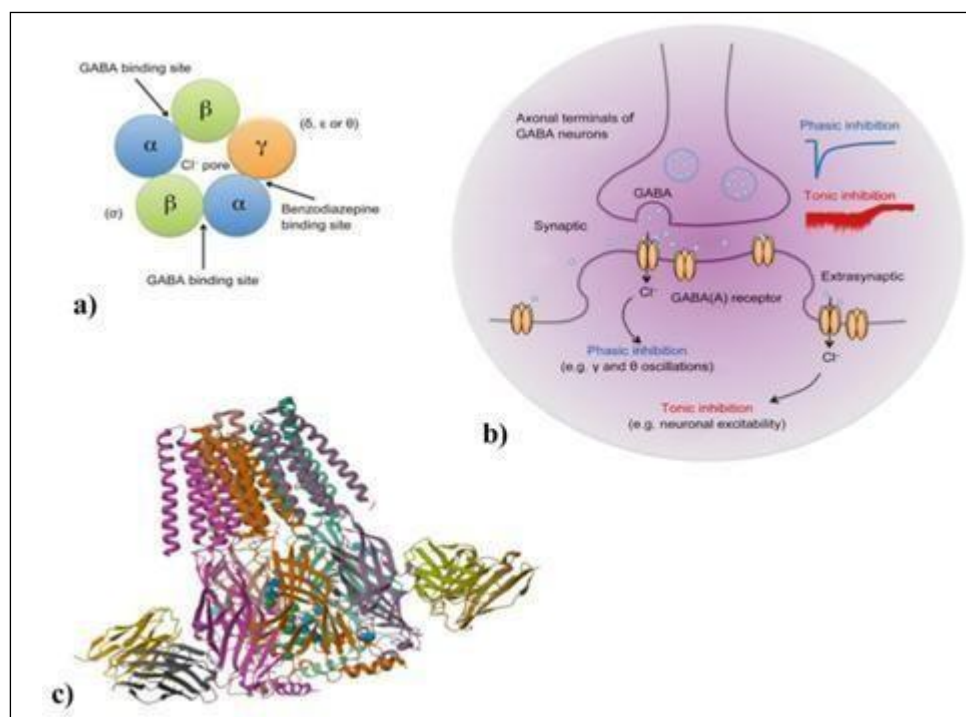
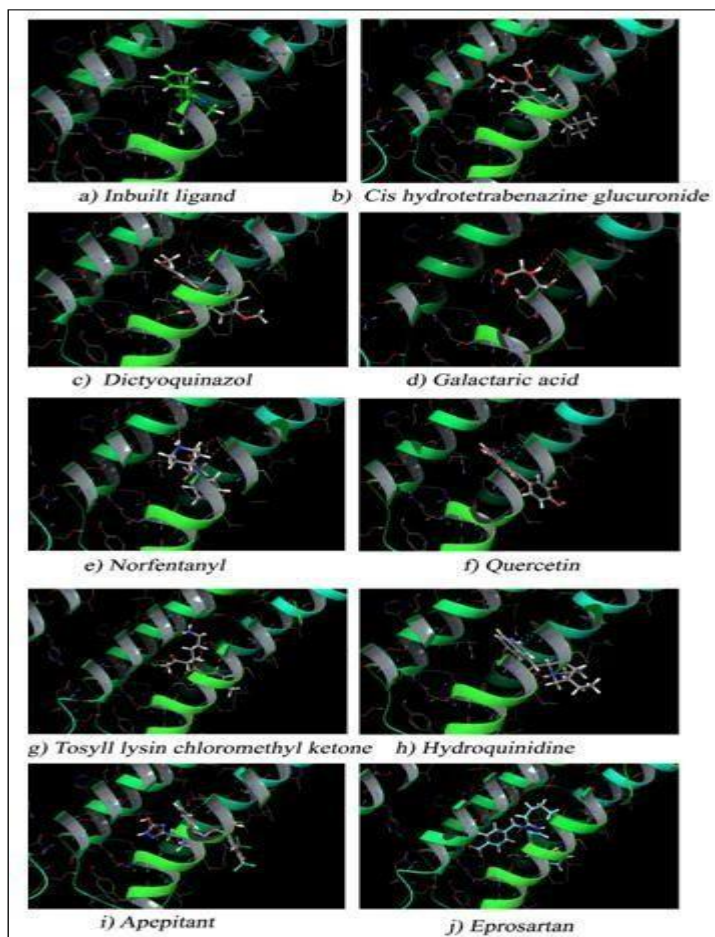


FIG.14:MECHANISMOFGABA(REF)C STRUCTUREOFGAMMA-AMINOBUTYRICACIDENZYMETAKEN FROM PROTEIN DATA BANK

The three-dimensional protein-ligand interaction is shown in Figure 15, and the two-dimensional interaction of all the chosen compounds in the GABA protein's dynamic region, which was acquired using the Maestro graphical user interface, is shown in Figure 16. It is discovered that all of the chosen active ligands are indistinguishably buried in GABA's deep binding pocket. Red and dotted lines show how each ligand interacts with the protein's active site amino acids via H- and π -bonds. Figure 16 illustrates the interactions between the screened bioactive compounds in the polyherbal formulations and the GABA receptor, which offer crucial information about their potential as modulators of GABA neurotransmission and the nervous system's balance between excitatory and inhibitory signals. These interactions are very important for investigating possible therapy options since GABA receptors are a primary target for medications that treat disorders including anxiety, sleeplessness, and epilepsy.



benefits.

FIG. 15: PICTURE OF 3D INTERACTION BIOACTIVE COMPOUNDS SCREENED IN A POLYHERBAL FORMULATION INBUILT LIGAND, CIS HYDROTETRABENAZINE GLUCURONIDE, DICTYOQUINAZOL, GALACTARIC ACID, NORFENTANYL, FQUERCETIN, G TOSYLLLYSINCHLOROMETHYLKETONE, H HYDROQUINIDINE, I APEPITANT, and J EPROSARTAN NEURAGREEN WITH GABA TARGET PROTEIN

Strong hydrophobic interactions between the built-in ligand and important residues such as LEU259, ASN265, PHE289, and MET236 are shown. Through nonpolar interactions, especially with aromatic rings, these residues often help stabilize ligands in the binding pocket of GABA receptors. These interactions may entail hydrophobic interactions and π - π stacking. A substantial inhibitory impact on the receptor is suggested by such high binding, which may help to modulate inhibitory transmission in the brain. ASN265, THR267, and PHE289 are among the residues with which cis hydrotetrabenazine glucuronide demonstrates both hydrophobic contacts and hydrogen bonding. Fluorine may increase the ligand's binding affinity by forming more halogen bonds or van der Waals forces in its structure. Cis

hydrotetrabenazine glucuronide may stabilize the receptor in a manner that might possibly improve

GABAergic activity, which makes it a viable option for adjusting the inhibitory effects of the receptor. Dictyoquinazol interacts hydrophobically with LEU259, PRO255, and ASN265; it may also create a hydrogen bond with ASN265. These interactions demonstrate how well the ligand binds to the active region of the GABA receptor, perhaps altering its activity. A desired property for drugs meant to relax the nervous system is the ability of dictyoquinazol to affect the receptor's inhibitory signaling pathways, which may be shown by the hydrophobic stabilization inside the receptor. Strong polar contacts are shown by the many hydrogen bonds that galactaric acid makes with residues like ASN265 and THR267.



These hydrogen bonding are probably facilitated by the presence of several hydroxyl groups in galactaric acid, which further stabilizes the ligand in the binding pocket of the receptor. This may indicate that it may play a part in regulating GABA receptor activity, which could result in increased inhibitory neurotransmission. This would be advantageous in lowering excitatory imbalances that cause anxiety or seizures. In addition to hydrophobic interactions with LEU259 that provide persistent binding inside the active site, interactions with residues such as ASP230 and LEU259 for norfentanyl imply the creation of salt bridges or ionic contacts. The receptor may be effectively modulated by these interactions, and the ligand may affect the receptor's capacity to mediate inhibitory signals, which might make it a viable option for applications involving the nervous system.

Along with hydrophobic interactions with LEU259 and PHE289, quercetin exhibits a number of significant hydrogen bonds with THR267, ASN265, and LEU236. The polyphenolic structure of quercetin suggests that these interactions entail both hydrogen bonding and π - π stacking, resulting in a stable ligand-receptor complex. Given its well-established anti-inflammatory and antioxidant qualities as well as its possible GABA receptor modulation, quercetin may have a dual function in mood regulation and neuroprotection. Chloromethyl ketone of tosyllysine exhibits hydrophobic interactions with LEU259 and MET236 as well as possible covalent bond formation with THR267. These interactions

suggest that the receptor may be irreversibly inhibited, which may result in long-term GABAergic signaling regulation. Drugs that need long-term receptor inhibition, such as those for persistent anxiety or seizure disorders, are particularly susceptible to this kind of interaction. Hydroquinidine has hydrophobic interactions with LEU259 and PHE289 and forms a hydrogen bond with ASP230, indicating a stable binding conformation. Hydroquinidine may be an effective modulator of the GABA receptor, perhaps improving inhibitory neurotransmission and fostering a calming impact on the nervous system, according to the balanced mix of polar and nonpolar interactions.

Aprepitant interacts hydrophobically with residues such as LEU259, MET236, and PHE289, and forms hydrogen bonds with ASN265 and ARG254. According to this interaction profile, aprepitant may be able to stabilize the GABA receptor, improving its inhibitory function and offering treatment potential for diseases including anxiety disorders and epilepsy that involve excessive neuronal excitability. Lastly, Eprosartan reveals interactions with LEU259 and ARG234, indicating the establishment of ionic connections, along with hydrophobic contacts with ILE262. These interactions presumably fix the ligand inside the receptor's binding pocket, leading to efficient control of GABAergic neurotransmission. Eprosartan's ability to modulate the receptor might give neuroprotective or calming benefits, making it a possible option for treating anxiety or other excitatory imbalances in the nervous system.

TABLE 5: THE DOCKING SCORES AND BINDING ENERGY OF THE LIGANDS WITH GABA PROTEIN

Sample	Docking	Binding Energy kCal/Mol
Inbuiltligand6x3x	-9.003	-67.749
Dictyoquinazol	-7.904	-54.191
Quercetin	-7.398	-50.023
Eprosartan	-6.909	-47.572
Tosyllysine	-6.851	-63.102
chloromethylketone		
Norfentanyl	-6.807	-47.432
Hydroquinidine	-6.784	-35.336
Cisdihydrotetrabenzine	-6.618	-29.040
glucuronide		
Aprepitant	-7.051	-39.725
Galactaricacid	-3.921	-27.454

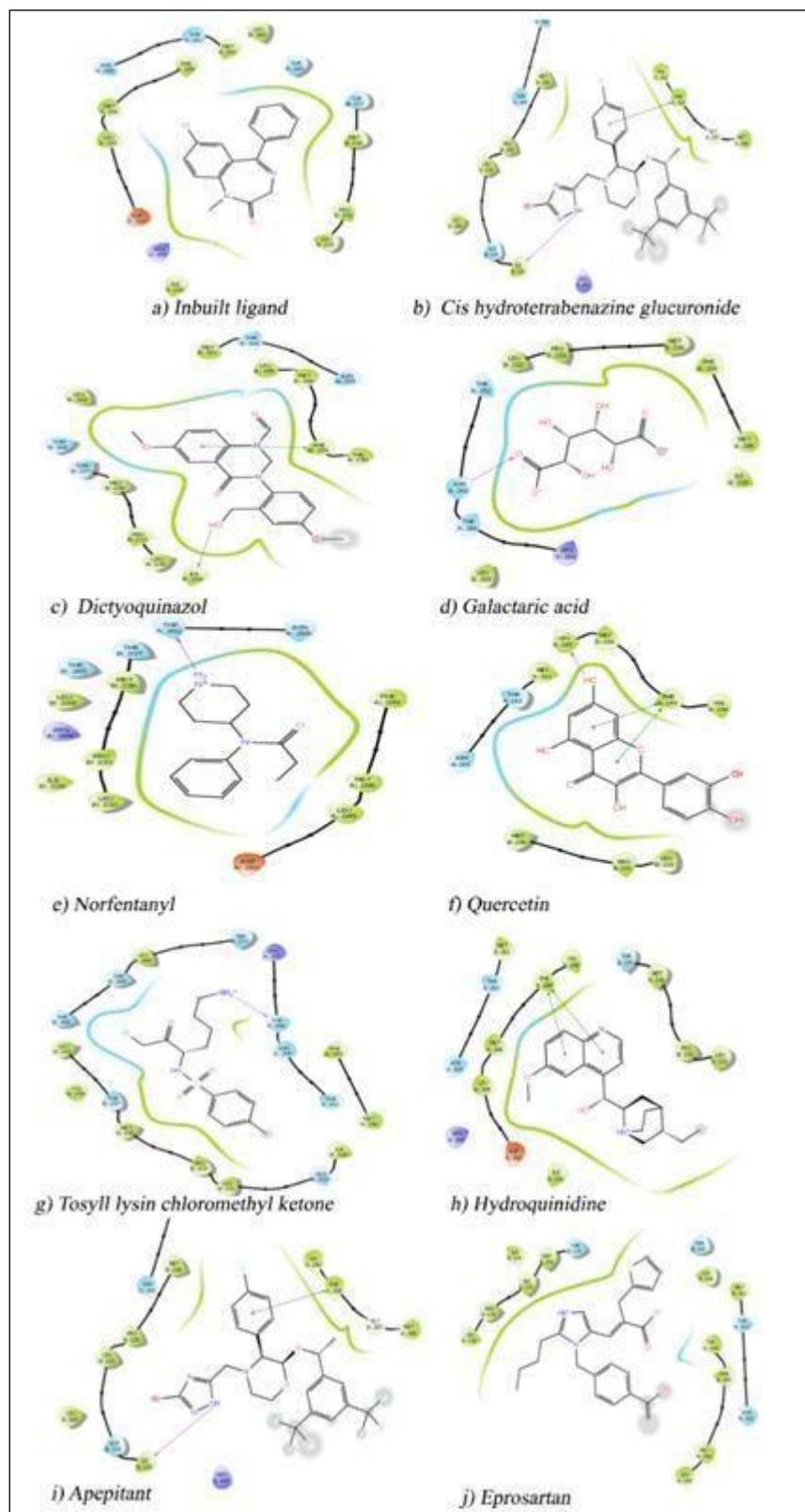


FIG. 16: 2D INTERACTION PICTURE OF SCREENED BIO ACTIVE COMPOUNDS IN POLYHERBAL FORMULATION NEURAGREEN WITH GABA TARGET PROTEIN (A) INBUILT LIGAND, (B) CIS HYDROTETRABENAZINE GLUCURONIDE, (C) DICTYOQUINAZOL, (D) GALACTARIC ACID, (E) NORFENTANYL, (F) QUERCETIN, (G) TOSYLL LYSIN CHLOROMETHYL KETONE, (H) HYDROQUINIDINE, (I) APEPITANT AND (J) EPROSARTAN



Table 5 lists the docking scores and binding energies for the tested ligands that interacted with the GABA receptor. With a binding energy of -67.749 kcal/mol and the greatest negative docking score of -9.003, the inbuilt ligand has the strongest contact, suggesting a high binding affinity and stability in the binding pocket of the receptor. This establishes the baseline for comparison. With a docking score of -7.904 and a binding energy of -54.191 kcal/mol, dictyoquinazol also exhibits strong binding, suggesting that it may successfully regulate the activity of the receptor. Likewise, quercetin has an excellent binding profile with a docking score of -7.398 and a binding energy of -50.023 kcal/mol, indicating that it may interact effectively with the receptor, maybe as a result of its polyphenolic structure and hydrogen bonding capabilities. With docking scores of -6.909 and -6.851 respectively, eprosartan and tosyllysine chloromethylketone exhibit moderate binding; however, the high binding energy of -63.102 kcal/mol for tosyllysine chloromethylketone indicates stronger and more persistent interactions, most likely as a result of covalent or irreversible binding. With a docking score of -6.807 and a binding energy of -47.432 kcal/mol, norfentanyl also exhibits moderate binding, suggesting that it may still be useful in regulating the receptor, but not as strongly as the leading ligands. With docking scores of -6.784 and -6.618, respectively, hydroquinidine and cis dihydrotetrabenazine glucuronide exhibit poorer binding; lower binding energies signify fewer persistent and advantageous interactions with the receptor. Aprepitant has considerable binding but less stability than some other ligands, with a docking score of -7.051 and a binding energy of -39.725 kcal/mol. Galactaric acid has the weakest interaction, indicating limited potential as a GABA receptor modulator, with the lowest docking score of -3.921 and binding energy of -27.454 kcal/mol.

The findings of the docking research show how several substances in the polyherbal formulation may interact with the GABA receptor, which is essential for controlling neuronal excitability via inhibitory neurotransmission. This is particularly pertinent to nervine tonics, which are meant to soothe and bring the nervous system back into

equilibrium.

CONCLUSION: Using a mix of cutting-edge analytical methods and molecular docking investigations, this work offers a thorough examination of the unique polyherbal nervinetonic formulation, Neuragreen. The presence of important bioactive substances, such as flavonoids, terpenoids, saponins, and alkaloids, which are recognized for their antioxidant and neuroprotective qualities, was validated by the LC-MS, UV-visible, and FTIR spectroscopy studies. The formulation's therapeutic potential against illnesses linked to oxidative stress was further supported by the significant antioxidant activity shown by the antioxidant tests (DPPH and SOD).

The polyherbal formulation seems to include a diverse variety of substances with different functional groups, according to the FTIR spectra. Conjugated C=C C=O, cellulose, and quinone oximes all point to the existence of polyphenolic compounds with possible antioxidant properties. Terpenoids are suggested by the presence of cyclobutane and the C(CH₃)₂ group. While the polyherbal formulation's IC₅₀ value for DPPH radical scavenging activity was found to be 7.3µg/mL, indicating a strong antioxidant activity, the IC₅₀ value for SOD enzyme activity was found to be 14.9µg/mL, suggesting that the polyherbal formulation has a potent antioxidant capacity and significant SOD radical scavenging activity.

The complex mechanisms of nine phytochemicals that were found to be responsible for nervine activity—Cishydrotetrabenazineglucuronide, Dictyoquinazol, Galactaric acid, Norfentanyl, Quercetin, Tosyll lysine chloromethyl ketone, Hydroquinidine, Aprepitant, and Eprosartan—were examined using molecular docking analysis based on LCMS chromatogram analysis. Important information about the possible action of the polyherbal formulation is revealed by the docking investigations with the MAO-B and GABA proteins. Tosylllysine chloromethyl ketone, quercetin, and dictyoquinazol were among the chemicals that showed substantial binding affinities for MAO-B, suggesting that they may be able to suppress the enzyme's activity.



Increased levels of neurotransmitters like dopamine, which are essential for treating neurological conditions, elevating mood, and promoting cognitive function, may result from MAO-B inhibition. However, docking experiments with the GABA receptor indicate that substances such as quercetin, tosyllysine chloromethyl ketone, and dictyoquinazol may also modify inhibitory neurotransmission, which can help to relax and anxiolytically affect the nervous system. These results together imply that the polyherbal formulation may function as a nervine tonic via two different pathways: GABA receptor modulation to encourage relaxation and lessen anxiety, and MAO-B inhibition to increase neurotransmitter levels. The formulation may be especially useful in treating diseases relating to the nervous system, stress, and anxiety because of its dual action. Numerous folk cultures have traditionally used the nervines in the suggested innovative polyherbal formulations to enhance mood, sleep, and mental performance. The majority of botanical practitioners still use these herbs extensively and in a similar way, and they seem to be quite safe and effective for treating mild-to-moderate anxiety and all of its symptoms. Therefore, before using this composition, a thorough research is required.

REFERENCES:

1. NervineTonics_IncredibleHealthBenefits,Usesand Side Effects.
2. Abascal K and Yarnell E: Nervine herbs for treating anxiety. *Altern Complement Ther* 2004; 10(6): 309-315. doi:10.1089/act.2004.10.309
3. William A. Harrison and ND P: Herbal Nervines by Magnificent Rector Dr. William A. Harrison, ND, PhD.
4. Shashi A, Jain SK, Verma A, Kumar M and Mahor AMS: Plant profile, phytochemistry and pharmacology of *Asparagus racemosus* (Shatavari): A review. *Asian Pacific J Trop Dis* 2013; 3(3): 242-251.
5. Parasuraman S, Thing GS and Dhanaraj SA: Polyherbal formulation: Concept of ayurveda. *Pharmacogn Rev* 2014; 8(16): 73-80. doi:10.4103/0973-7847.134229
6. Karole S, Shrivastava S and Thomas S: Polyherbal formulation concept for synergic action: a review. *J Drug Deliv Ther* 2019; 9(1): 453-466. doi:10.22270/jddt.v9i1-s.2339
7. Kn S, Mv H, Dev J, Tv J, Nk H and Pv MK: Chemical profiling, spectroscopic characterization and biological evaluation of a novel polyherbal formulation with natural binders- *Ayurgreen Natura Pain Gel* 2022; 8(2): 72-84. doi:10.31254/jahm.2022.8204
8. Alekhya M, Jyothi M and Ramya M: R review a rticle a review of hydrocephalus. *Rev Lit Arts Am.* 2023; 18(3): 216-220. doi:10.5281/zenodo.10232708
9. Safna Hussan KP, Pareeth CM, Muraleedharan K, Shahin Thayyil M and Babu TD: Synthesis, spectroscopic, chemical reactivity, molecular docking, DFT calculations and in-vitro anticancer activity studies of a novel ionic liquid; Metforminium ibuprofenate. *J Mol Liq* 2022; 364: 120046. doi:10.1016/j.molliq.2022.120046
10. Safna Hussan KP, Babu TD and Pareeth CM: Antioxidant activity of erlotinib and gefitinib: theoretical and experimental insights. *FreeRadicRes* 2022; 56(2): 196-208.
11. Safna Hussan KP, Thayyil MS, Deshpande SK, Jinitha T V, Rajan VK and Ngai KL: Synthesis and molecular dynamics of double active pharmaceutical ingredient- benzalkonium ibuprofenate. *J Mol Liq* 2016; 223: 1333-1339. <http://dx.doi.org/10.1016/j.molliq.2016.09.054>
12. SHK P, Shahin M and Deshpande SK: Studies of ionogel structure and its electronic and optical characterization by oniom and other hybrid computational approaches. *Mater Today Proc* 2018; 5(8): 16272-16279. doi:10.1016/j.matpr.2018.05.119
13. Sakkariya KN, Dev J and Moidu HH: Chemical profiling, spectroscopic characterization thereafter molecular docking studies of phytochemical constituents of a polyherbal formulation- biogymnema. *Int J Green Herb Chem* 2023; 12(4): 301-323. doi:10.24214/ijghc/hc/12/4/30123
14. Safna Hussan K P, Mohamed Shahin Thayyil, Vijisha K Rajan and Muraleedharan K: Experimental and density functional theory studies on benzalkonium ibuprofenate, a double active pharmaceutical ingredient. *Comput Biol Chem* 2018; 72: 113-121. doi:10.1016/j.compbiolchem.2017.12.004
15. Jinitha T V, P SHK, Subair N, Shaniba V, Balan AK and Purushothaman E: Polyaniline modified lignocellulosic fibers from sago seed shell powder for electrochemical. *RSC Adv* 2018; 8: 34388-34396. doi:10.1039/C8RA05774E
16. Pareeth CM, Hussan KPS, Anu D, Meera N, Mathew D and Valsalan R: Antiproliferative effect of *Saraca asoca* methanol bark extract on triple negative breast cancer (TNBC). *Futur J Pharm Sci. Published online* 2024. doi:10.1186/s43094-024-00623-3
17. Hussan KPS, Thayyil MS, Rajan VK and



- Muraleedharan K: DFT studies on global parameters, antioxidant mechanism and molecular docking of famlodypinebesylate. *Comput Biol Chem* 2019;80(1):46-53. doi:10.1016/j.compbiolchem.2019.03.006
18. Safna SH, Babu TD, C. M P, Joshy G, Mathew D and Thayyil MS: Antioxidant activity of ferulic acid and gefitinib: theoretical and experimental insights. *Free Radic Res* 2022; 56(2): 196-208. Nahala NM, Pareeth CM, Hussan KPS, Babu TD, Soni KB and Amritha VS: Anticancerous Properties of Tetragonula travancorica (stingless bee) Honey on Reproductive Cancer Cells. *Int J Agro Nutr Pract* 2023; 3(4): 1-9. doi:10.36647/ijanp/03 04.a001
 19. Torres PHM, Sodero ACR and Jofily P: Key Topics in Molecular Docking for Drug Design 1-29.
 20. K.P SH, K.A AR, Medammal Z, Thayyil MS and Babu TD: Theoretical insights into the radical scavenging activity of glipizide: dft and molecular docking studies. *Free Radic Res* 2022;0(0):1-19. doi:10.1080/10715762.2022.2034803
 21. Girija D, Deepa K and Chubicka T: Structural and functional validation of a cloned parasporin from *Bacillus thuringiensis* isolate KAU 41 native to Western Ghats of India. *Proteins Struct Funct Bioinforma* 2023; (5): 2022: 1-9. doi:10.1002/prot.26544
 22. Finberg JPM and Rabey JM: Inhibitors of MAO-A and MAO-B in psychiatry and neurology. *Front Pharmacol* 2016; 7(10): 1-15. doi:10.3389/fphar.2016.00340 Jin Z, Mendu SK and Birnir B: GABA is an effective immunomodulatory molecule. *Amino Acids* 2013;4 5(1): 87-94. doi:10.1007/s00726-011-1193-7
 23. Xu MY and Wong AHC: GABAergic inhibitory neurons as therapeutic targets for cognitive impairment in schizophrenia. *Acta Pharmacol Sin* 2018; 39(5): 733-753. doi:10.1038/aps.2017.172